JOINT INFLAMMATIONS AND EXACERBATIONS IN MICE BY CLONED HELPER T CELLS

GEWRICHTSONTSTEKINGEN EN EXACERBATIES IN MUIZEN DOOR GEKLONEERDE HELPER T CELLEN

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. A.H.G. Rinnooy Kan
en volgens besluit van het college van Dekanen.
De openbare verdediging zal plaatsvinden op
vrijdag 2 december 1988 om 15.00 uur.

door

Ina Sylvia Klasen
geboren te Voorburg (Z.H.)

Gedrukt bij
Offsetdrukkerij Kanters B.V., Alblasserdam
1988

Promotiecommissie

Promotoren	:	Prof. Dr. R. Benner
		Prof. Dr. O. Vos
Overige leden	:	Prof. Dr. R.O. van der Heul
		Prof. Dr. J.F. Koster

Dit proefschrift werd bewerkt binnen de instituten Immunologie en Celbiologie II van de vakgroep Celbiologie en Genetica van de Erasmus Universiteit Rotterdam. Het onderzoek werd mede mogelijk gemaakt door financiële steun van de Nederlandse Vereniging voor Reumabestrijding. Deze vereniging droeg ook bij in de drukkosten van dit proefschrift.

Aan mijn ouders
voor Ad

CONTENTS

Chapters 2-7 represent the appendix papers of this thesis.

PREFACE

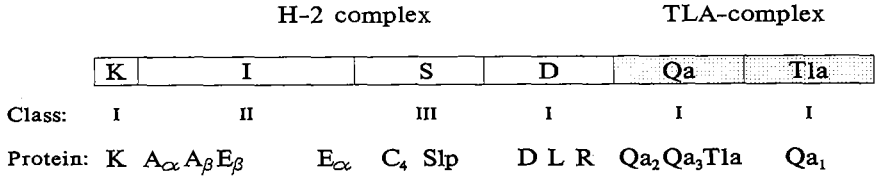
The immune system is the part of the defense system that is able to react specifically to intruding infectious agents. Cells of the immune system can co-operate with the non-specific part of the defense system, which consists of polymorphonuclear granulocytes and cells of the mononuclear phagocyte system (e.g. monocytes and macrophages). The immune system can react by two types of responses, the humoral and the cellular responses. Humoral responses are mediated by the B lymphocytes. These lymphocytes can, after having encountered a structure that is foreign for the body, an antigen, produce antibodies. These antibodies can spread throughout the body by the circulation. After an antibody has bound an antigen, the antigen can be eliminated efficiently by the mononuclear cell system. Foreign cells can be lysed by antibodies when they bind molecules of the complement system. Antibodies and antigens, in certain ratios, can form complexes, so-called immune complexes (IC), that can precipitate in blood vessels and tissues and thereby can cause damage.

The cellular immune system consists of the T lymphocytes. T lymphocytes are unable to recognize separate antigen molecules. Molecules of the major histocompatibility complex (MHC) are of great importance for the recognition of antigens by T cells. This histocompatibility complex is called HLA in man and H-2 in mice. A schematic representation of the genes coding for these MHC molecules is given in Fig. 1.

T cells have a receptor that can only recognize an antigen when it is associated with molecules of the individuals' own MHC complex, on the surface of cells of the mononuclear phagocyte system (these are called 'syngeneic' MHC molecules).

Within the T cell population at least two subpopulations can be discriminated based on their cell surface molecules, namely the helper T cell (Th) population that has CD4 molecules on its surface and the cytotoxic/suppressor T cell (Tc/s) population that has CD8 molecules on its surface. The Th cells recognize antigens in the context of class II molecules of the MHC complex. They are said to be class II restricted in their antigen

Chromosome 17 mouse



Chromosome 6 human

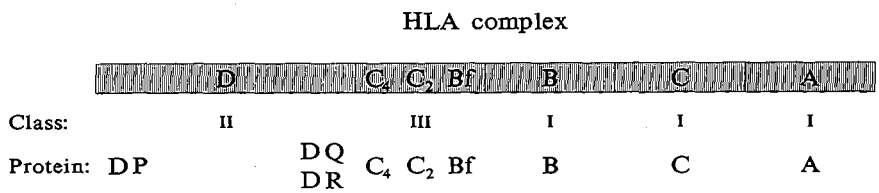


Fig. 1. Genes and proteins of the human and mouse major histocompatibility complex.

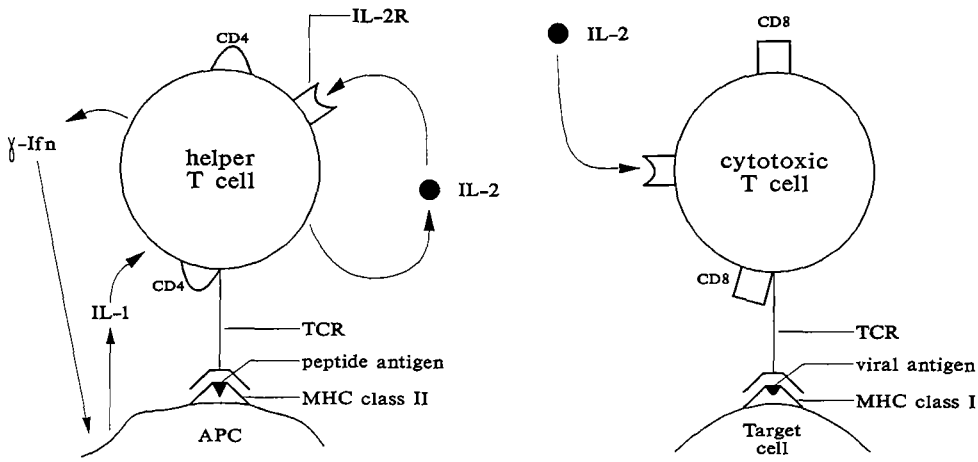


Fig. 2. Activation of T cells. After binding of antigen to the antigen presenting cell (APC) the latter is induced to produce IL-1. Binding of IL-1 and recognition of the antigen in the context of class II molecules by helper T cells activate the latter to express IL-2 receptors and to secrete IL-2, γ-IFN and other lymphokines. When IL-2 binds to the IL-2 receptor, the T cell starts to proliferate. The cytotoxic T cell recognizes viral antigens in the context of class I molecules. Proliferation of the cytotoxic T cells is dependent on IL-2 produced by helper T cells.

recognition. After having recognized an antigen, they react by the production of soluble factors, so-called lymphokines, that can induce B cells to produce antibodies and activate non-lymphoid cells. Furthermore, by the release of chemotactic factors they can recruit non-lymphoid cells to produce an inflammation. Tc cells can lyse target cells which are recognized to contain non-self molecules in the context of class I molecules. Ts cells, finally, can suppress humoral as well as cellular responses. The cell surface molecules and soluble factors that are involved in the activation of the T cells are shown in Fig. 2.

Under normal conditions immune responses against 'self' molecules are prevented. This is thought to be regulated in the thymus. Consequently the organism is 'self-tolerant'. In most autoimmune diseases the immune system probably recognizes self structures that should not be recognized like foreign antigens, leading to disease. Rheumatoid arthritis is one of the autoimmune diseases. It is clear that T lymphocytes play an important role in this disease. In this thesis we show that cloned T cell populations with the helper phenotype together with the (here artificial) antigen they recognize, can induce joint inflammations in mice. Moreover, these joint inflammations can, after waning, show exacerbations after the second administration of the same antigen. This resembles the course of the disease as this is observed in rheumatic diseases. These results emphasize the importance of T lymphocytes in the origin as well as in the exacerbations of these diseases. The structures that are recognized by the T cells and that induce the T cells to cause inflammatory reactions, however, are still under debate.

CHAPTER 1

ARTHRITIS

1. GENERAL INTRODUCTION

One out of every five people of the Dutch population suffers from one or another form of rheumatic disease during his or her lifetime. In this name several diseases of the joints, the spinal cord and the connective tissues are gathered. One of the most frequently occurring diseases is rheumatoid arthritis (RA) which is an inflammatory joint disease. About three percent of the Dutch population is suffering from this disease. Depending on age it occurs two to three times more often in females than in males. Rheumatoid arthritis is considered to belong to the collective term of 'autoimmune diseases'. Autoimmune diseases are the diseases that are assumed to be caused by a reaction of the immune system to the own body structures.

Table 1

Pathogenesis by T cells

Multiple sclerosis	McFarlin and McFarland, 1982
Demyelinisating polyradiculoneuritis	Korn-Lubetzki and Abramsky, 1980
Diabetes mellitus	Eisenbarth, 1986
Chronic polyarthritis	Decker et al., 1984
Hashimoto-thyreoiditis	Wick et al., 1985
Forms of uveitis	Kaplan and Waldrep, 1984
Forms of interstitial nephritis	McCluskey and Bhan, 1982
Psoriasis	Valdimarsson, 1986

After Fierz, 1987.

Many investigators suspect T lymphocytes to play a pivotal role in the pathogenesis of these autoimmunities (Table 1). In spite of the word 'auto'-immunity it has been often suggested that infectious agents might play a role in the etiology of these diseases. Especially with regard to joint inflammations there are many indications that bacterial infections are involved. For example a correlation has been found between the appearance of ankylosing spondylitis and Klebsiella infections (Keat, 1986). In the so-called reactive arthritis, infections have been found of Yersinia, Salmonella, Shigella etc. (Phillips and Christian, 1985; Toivanen et al.,

1985). Moreover, it is known that patients that suffer from Crohn's disease, which is a chronic gut inflammation, have a higher risk of developing joint inflammations (Greenstein et al., 1976). Obesitas patients that have undergone intestinal bypass surgery have a higher risk to develop rheumatic disease (Lancet editorial, 1983). A role of micro-organisms in the etiology of RA has been suggested by Bennett (1978). With regard to viral infections there are indications for a possible role of Epstein-Barr virus (EBV). RA patients have been shown to exhibit higher antibody titers against EBV nuclear antigen (Alspaugh and Tan, 1976). Recently a parvovirus has been found in high frequency in synovial extracts of RA patients (Simpson et al., 1984).

The inflammatory diseases of the joints often occur with exacerbations and remissions. The reason for this is unknown.

Many factors that influence the etiology and the pathology of the joint inflammatory diseases such as genetic predisposition, hormonal influence, cellular and humoral immunity and production of lymphokines and other mediators have been studied. This is schematically presented in Fig. 1.

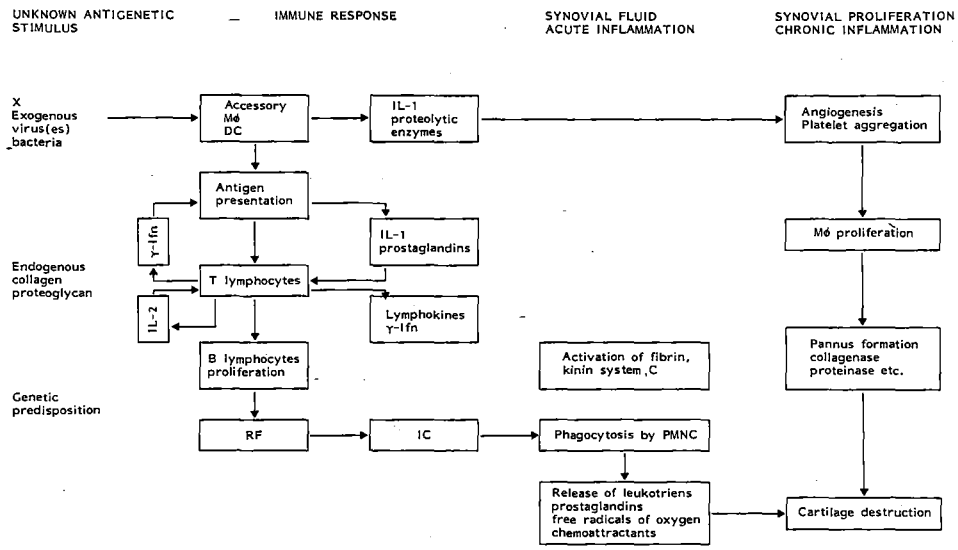


Fig. 1. A model for pathophysiology of RA. Adapted after Harris (1985).

The genetic predisposition is apparent in individuals that are HLA-DR4 in their major histocompatibility (MHC) antigens. They have a 4-6 times higher risk of developing RA. Individuals that are HLA-B27 positive have a 140 times higher risk for ankylosing spondylitis (Harris, 1986). Reactive arthritis also occurs prevalently in HLA-B27 positive individuals (Toivanen et al., 1985). Hormonal influence is apparent from the two to three times higher risk of women to develop RA, whereas ankylosing spondylitis occurs three times more often in men.

Much research has been performed to investigate the cellular and humoral responses that occur in joint inflammations. In recent years also the role of lymphokines has got much attention. Such studies have been done in patients suffering from rheumatic diseases as well as in several animal models. These investigations will be discussed in the following paragraphs.

2. HUMAN ARTHRITIS

2.1. Cellular responses

Many contradictory results have been presented concerning the phenotype, degree of activation and ability to be stimulated by antigen or polyclonal stimulators of T cells in peripheral blood (PB), synovial fluid (SF) and synovial tissue (ST) of patients suffering from joint inflammations. These differences are partly due to the fact that different authors study different diseases such as RA, reactive arthritis (ReA), juvenile rheumatoid arthritis (JRA) or Reiter's syndrome. There is also a difference in the source of the cells studied. Some authors study PB lymphocytes, others study the lymphocytes that are derived from the actual place of inflammation, the SF or ST. Much attention has been paid to the ratio of the expression of the surface antigens CD4 and CD8, the CD4/CD8 ratio, which represents the ratio between helper T (Th) and cytotoxic/suppressor T (Tc/s) cells.

Several authors have found that in the PB of patients suffering from rheumatic diseases the CD4/CD8 ratio has increased (Luyten et al., 1986; Raeman et al., 1981; Veys et al., 1982). In the SF often a lower CD4/CD8 ratio has been found than in the PB (Duke et al., 1983; Jahn et al., 1986; Lasky et al., 1988; Thoen et al., 1987). The $CD8^+$ T cells have been found to include the 'spontaneously' proliferating cells in the SF (Bergroth et al., 1985). In the ST predominantly $CD4^+$ T cells were found (Johnell et

al., 1985; Poulter et al., 1985). Others have described an increased $CD8^+$ population in the ST as compared to the PB (Burmester et al., 1981). Possibly, these results depend upon the site of the joint examined (Kurosaka and Ziff, 1983)

Functional aberrations have also been described in the suppressor system (Lasky et al., 1985). An increased CD8 expression has been reported in association with a decreased suppressor activity which might be due to a defect in the T suppressor inducer cell (Tsi) population, which is thought to have the CD4 phenotype (Lasky et al., 1985). A decrease in the Thi/Tsi ratio can be detected by the use of the recently discovered monoclonals 2H4 and 4B4 (Morimoto et al., 1985a, 1985b).

Generally large numbers of activated T cells have been found in the SF (Ford et al., 1985; Jahn et al., 1986; Nykanen et al., 1986; Poulter et al., 1985) and the PB (Keystone et al., 1986), but contradictory results have been found here as well (Lautenschlager et al., 1987). Also a partial activation has been found in which the T cells exhibit an increased MHC class II expression but a normal interleukin-2 (IL-2) receptor expression and transferrin receptor expression (Konttinen et al., 1985; Pitzalis et al., 1987).

Also with regard to IL-2 changes have been found, e.g. a higher than normal turnover (Waalen et al., 1987a; Salmon and Bacon, 1988). Several authors investigated the possibility to stimulate the PB or SF cells of patients suffering from joint diseases using polyclonal stimulators. Also the autologous mixed lymphocyte culture reaction (AMLR) has been employed. In the AMLR the proliferative response is due to T lymphocytes stimulated by autologous non-T mononuclear cells (Macdermott and Stacey, 1981; Smolen et al., 1981). The MHC class II antigens are thought to initiate these responses (Palacios and Moller, 1981). Responses to polyclonal stimulators like pokeweed mitogen (PWM) are often low in the SF (Reynolds and Abdou, 1973; Abrahamsen et al., 1978; Nilsson et al., 1986), which may be due to previous stimulation of the T cells *in vivo*. Responses against 'recall' antigens as PPD (protein purified derivative from *Mycobacterium tuberculosis*) have been reported to be lower in the PB of RA patients than in normal controls. Apparently there is an anergy for these responses (Wahl et al., 1983; Malone et al., 1984; Kingsley et al., 1987). The AMLR in the PB has also been shown to be lower than normal (Forre et al., 1982; Kalden et al., 1983; Kingsley et al., 1987; Smith and DeHoratius, 1982).

T cell clones and/or lines derived from the SF have been produced by various groups of investigators. Such clones and lines may express various phenotypes (Schlesier et al., 1984; Ofosu-Appiah et al., 1986; Bishop et al., 1986; Abdel-Nour et al., 1986; Schlesier et al., submitted). These T cell clones have been found with specificity for autologous MHC antigens (Schlesier et al., 1984, submitted), proteoglycans (Mikecz et al., 1988) and collagen (Londei et al., 1988).

A noteworthy finding has been done by Holoshitz et al. (1986) and Res et al. (1988), who have found that lymphocytes from patients with RA seem to be sensitized for a fraction of Mycobacterium tuberculosis (MT) which contains epitopes that display immunochemical resemblance to cartilage (this is discussed further in section 3.2). The proliferation of the lymphocytes upon stimulation with this antigen was first noted in the SF and in a later stage of the disease in the PB. Moreover it has been found that individuals having HLA-DR4 histocompatibility antigens exhibit stronger responses upon cutaneous testing with MT (Ottenhoff et al., 1986). This is of interest as HLA-DR4$^+$ persons have a higher risk of developing RA. Other authors argue that the higher proliferation of lymphocytes from RA patients to MT is not due to the disease but to the fact that this group of patients involves a relatively high percentage of HLA-DR4 positive individuals (Palacios-Boix et al., 1988).

With regard to T cell receptor (TCR) expression it has been found that T cells of RA patients exhibit a dominant rearrangement of TCR β chain genes. Distinct rearrangements have been found in SF T cells, which were not found in the PB T cells (Savill et al., 1987; Stamenkovic et al., 1988). In the SF of JRA patients a rather frequent usage of TCR$\gamma\delta$ has been found (De Maria et al., 1987).

2.2. Humoral responses

In studies on humoral responses much attention has been paid to the occurrence of auto-antibodies. One of the most frequently investigated antibodies are the rheumatoid factors (RF). The RF mainly occur in RA, but patients with other autoimmunities like primary Sjögren's syndrome, systemic lupus erythematosus and scleroderma can also express RF (Carson, 1985). RF are antibodies directed against the constant region of IgG molecules. Most of the RF in human are of the IgM isotype, but IgA, IgG and IgE RF can also occur (Carson, 1985). In a large percentage of the patients

suffering from RA, RF are not found in the serum. This makes the role of RF in the etiology of RA doubtful. It has been suggested that the relationship that is found between RA and HLA-DR4 in fact is a relationship between the ability to make RF and HLA-DR4 (Panayi et al., 1987).

Possibly, the occurrence of RF is a reflection of perturbations of the immune system e.g. by continuous antigenic stimulation, increased immune complex (IC) formation or immune regulatory dysfunctions (Fong et al., 1986). B cells able to produce RF are reported to be present in all individuals, and the production of RF can be induced in all individuals. Part of the RF have a common germline Vk gene (Fong et al., 1986).

Three possible stimuli for the production of RF are mentioned in the literature:

1. Stimulation by aggregated IgG (Nemazee, 1985) or IC (Goldstein and Karsh, 1986);
2. Induction by exogenous antigens that crossreact with IgG. Striking are the publications that describe that human IgG RF have structural homology with bacterial Fc receptor proteins (Weisbart et al., 1987);
3. Polyclonal B cel stimulators (Koopman and Schrohenloher, 1980; Goldstein and Karsh, 1986).

The glycosylation of the serum IgG in patients with RA and osteoarthrosis is abnormal (Parekh et al., 1985). The amount of galactose is decreased. This might be responsible for the production of RF, since abnormal glycosylation may account for new antigenic determinants or lectinlike activity as a result of complex formation or autoaggregation. Galactose deficiency has also been found in patients suffering from SLE or Crohn's disease (Tomana et al., 1988).

Autoantibodies have also been described directed against several other autologous structures e.g. collagen type II (Trentham, 1985; Choi et al., 1988) and DNA (Hahn, 1985; Rowley et al., 1988).

Concerning the relationship between ankylosing spondylitis and Klebsiella infections a crossreactivity at the antibody level between Klebsiella pneumonia and a determinant of HLA-B27 has been described (Keat, 1986). In reactive arthritis caused by a Yersinia infection crossreactivity has been shown between Yersinia and HLA-B27 (Chen et al., 1987).

2.3. Antigen presenting cells

The rheumatoid synovial tissue contains a large number of different

MHC class II (HLA-DR) expressing cells, some of which have a high antigen presenting capacity. The rheumatoid tissue often contains infiltrates that strongly resemble a delayed type hypersensitivity (DTH) reaction, showing Th cells in close contact with class II positive cells (Janossy et al., 1981; Klareskog et al., 1982b). The class II positive cells are involved in the activation of the T cells by presenting the antigen. Various cell types have been described that can be class II positive (Burmester et al., 1983):

1. Macrophages. A part of the synovial cells is macrophage like. They are phagocytic and express macrophage markers (Burmester et al., 1983).
2. Dendritic cells (DC). These cells express class II molecules very strongly, are unable to process particulate antigens, but can efficiently perform accessory functions in antigenic and mitogenic stimulation of T cells and the induction of AMLR (Steinman and Nussenzweig, 1981). DC are present in increased numbers in the rheumatoid synovium (Klareskog et al., 1981) and express HLA-DR, -DP and -DQ molecules (Waalen et al., 1987b). DC derived from the synovial fluid have been shown to be able to produce interleukin-1 (Waalen et al., 1986). The synovial mononuclear cells have been claimed to be 10 times more active as antigenpresenting cells than PB cells (Zvaifler et al., 1985). Burmester et al. (1984), however, have found that the non-lymphoid synovial cells do not induce T cell proliferation and even inhibit polyclonal T cell activation.
3. Chondrocytes. Also chondrocytes have been shown to be able to present antigen (Tiku et al., 1985). After stimulation with interferon-γ they can express HLA-DR antigens, whereas HLA-DP is still negative (Jahn et al., 1987).

2.4. Lymphokines

The role of lymphokines in the inflamed joint has been studied by several authors. Some of these studies will be summarized here.

1. Interleukin-1 (IL-1). This lymphokine is produced by macrophages and dendritic cells. IL-1 is needed for the activation of resting T cells to express receptors for interleukin-2 (IL-2) and for the release of IL-2. IL-1 activity has been shown to be present in the SF (Fontana et al., 1982; Hopkins et al., 1988). IL-1 can induce various changes including the proliferation of fibroblasts and synovial cells, secretion

of collagenase, production of collagen, prostaglandin E2 (PGE2), lysosomal enzymes and proteases by chondrocytes, inhibition of chondrocyt proliferation and stimulation of bone resorption (Danis et al., 1987). The low response of synovial mononuclear cells upon mitogenic stimulation (Reynolds and Abdou, 1973; Abrahamsen et al., 1978; Nilsson et al., 1986) might to be caused by an IL-1 inhibitor that interferes with the effects of IL-1 on the T cells (Lotz et al., 1986).

2. Interleukin-2 (IL-2). This lymphokine is produced by activated T cells and induces, after binding to the IL-2 receptor, proliferation. According to Combe et al. (1987), the PHA induced IL-2 production by SF and PB lymphocytes from RA patients is decreased as compared to the production by control lymphocytes. A radiosensitive suppressor cell is reported to be involved in this decreased IL-2 production (Combe et al., 1987). Contradictory results have been presented by Lemm and Warnatz (1986) and Nouri and Panayi (1987).

   In SF only small amounts of IL-2 have been detected (Egeland and Lund, 1987), possibly as a result of absorption of the IL-2 by the activated T cells (Knobloch et al., 1985). Recently, elevated soluble IL-2 receptors have been reported in the synovial fluids of RA patients (Keystone et al., 1988). This might influence these results. High levels of IL-2 and IL-2 receptor transcripts have been found in the mononuclear cell fraction in RA lesions. The expression of these messages is prolonged as compared to the expression in normal, stimulated PB mononuclear cells (Buchan et al., 1988).

3. Interleukin-6 (IL-6). This is a recently discovered interleukin that has been investigated under several other names, e.g. Ifn-β2, hybridoma growth factor (HGF) and B cell stimulatory factor-2 (BSF-2). IL-6 is often found in association with IL-1. IL-6 is increased in many inflammatory reactions and has been found to be significantly elevated in SF from RA patients (Houssiau et al., 1988).

4. Interferon-γ (Ifn-γ). This lymphokine is produced by a subclass of activated helper T cells (Mossman et al., 1986) and can induce MHC class II expression on various types of non-lymphoid cells (e.g. Geppert and Lipsky, 1985). Ifn-γ has been found in the SF of RA patients (Cesario et al., 1983; Degré et al., 1983).

Moreover, lymphokine-like factors have been reported to occur in the SF, such as migration inhibition factor (MIF) (Egeland et al., 1981), B

cell differentiation factor (BCDF)(Al-Balaghi et al., 1984) and B cell colony stimulating factor (Fay et al., 1985).

2.5. Histology

As reported in 1. General introduction, rheumatic diseases include several diseases of the joints, the spinal cord and the connective tissues. Therefore the histopathology of these diseases is also heterogeneous. In RA the types of inflammatory cells seen are in part dependent on the chronicity of the lesion. In early cases of RA a synovitis is seen, together with capillary and venular congestion and oedema. Polymorphonuclear (PMNC) cells migrate through the venous endothelial intercellular gaps, pass the synovial lining cells and escape into the synovial fluid. In early RA they are the predominant cell type. They release proteolytic enzymes (elastase and collagenase), prostaglandins and free radicals of oxygen. Subsequently, the PMN cells are joined by macrophages, later on followed by plasma cells and again in a later stage by a diffuse infiltrate of T lymphocytes. In this stage fibrin accumulates and sometimes lymphoid follicles with germinal centers occur. Several days after the onset of the disease granulation tissue and fibrosis are evident. In later stages also destruction of the hyaline cartilage occurs (Zvaifler, 1983; Gardner, 1986).

3. ANIMAL MODELS

To investigate the various processes involved in the etiology and pathogenesis of rheumatic diseases, several animal models have been developed. These models will be discussed hereafter, with emphasis on the role of T lymphocytes.

3.1. Antigen induced arthritis

The antigen induced arthritis (AIA) model has been developed initially in rabbits (Dumonde and Glynn, 1962), but the arthritis can also be induced in mice (Brackertz et al., 1977a). This form of arthritis is induced by immunization with an antigen in Complete Freund's Adjuvant (CFA), several weeks later followed by a challenge dose of the antigen in one of the knee joints. After the latter antigen injection a joint inflammation develops. Not all mouse strains are equally susceptible to the induction of

this type of arthritis (Brackertz et al., 1977a, 1977b).

The induction of AIA is T cell dependent. This has been shown in adoptive transfer studies (Brackertz et al., 1977c). Van den Berg et al. (1984, 1986) have shown that the charge of the antigen used is of great importance for the antigen handling within the joint and that the development of chronic arthritis is a.o. dependent on the amount of antigen that is retained in the joint. Cationic antigens are retained in a joint as the result of charge interactions with the negatively charged cartilage structures.

Retention of antigen in joints has been shown by autoradiography (van den Berg et al., 1982). Antigen-antibody complexes are unable to penetrate the cartilage. Antibodies directed against the cationic proteins only recognize these antigens on the surface of the cartilage and they do not penetrate the cartilage (Zatarain-Rios and Mannik, 1987). Therefore, antibodies directed against these antigens seem to be less important for retention of the antigen than the charge interactions of the cationic antigens with the cartilage. The higher the cationisation of the antigen, the better the retention of the antigen. Cationic antigens with a very high pI, however, induce a weaker T cell response and therefore the arthritis is less chronic (van Lent et al., 1987).

An interesting feature of the AIA model is the possibility to induce exacerbations by a rechallenge with the original antigen. This can be achieved by a repeated local injection of the antigen (van Beusekom et al., 1981), but also by a systemic administration of the antigen, either by the intravenous (iv) (van de Putte et al., 1983; Lens et al., 1983) or by the oral route (Lens et al., 1984). These exacerbations, also called flare-up reactions, can be inhibited by in vivo administration of anti class II antibodies (van den Broek et al., 1986).

Intra-articular administration of the antigen can induce an acute type of inflammation histologically characterized by exudation and deposition of fibrinlike material. Initially PMNC are predominant, later on followed by an increase of the proportion of mononuclear cells. Synovial lining cell hyperplasia is also regularly observed. Van den Berg et al. (1981) and Kruijsen et al. (1983) described that in the AIA the muscles nearby the joint became intensively infiltrated by PMNC that spread out of the synovial tissue. Proliferation of the fibroblasts of the fibrous capsule was seen that was most extensive at the site of attachment to the bone. There is subperiosteal new bone formation, but there is also loss of subchondral bone. Chondrocyte death was found a.o. in the center of the patella, and

the formation of new cartilage was seen at the margins of the patella. In an immunohistochemical study the presence of macrophages and DC in the joints of rats with AIA was established (Dijkstra et al., 1987). Classical macrophages were found superficially in the synovium, while the DC occurred in clusters around the small vessels in the synovium.

3.2. Adjuvant arthritis

Adjuvant arthritis (AA) is a chronic arthritis that develops in rats after injection of Mycobacterium tuberculosis (MT) in mineral oil (CFA) (Pearson and Wood, 1959). Already 25 years ago it was shown that this disease can be transferred to naive animals by passive transfer of lymphoid cells (Waksman and Wennersten, 1963; Pearson and Wood, 1964). Passive transfer of the disease has also been shown using thoracic duct lymphocytes (Whitehouse et al., 1969). Later it has been shown that the AA could also be passively transferred to naive rats by concanavalin A stimulated lymph node cells (Taurog et al., 1983a). This population of cells appeared to consist predominantly of $W3/25^+$ (helper phenotype) T cells (Taurog et al., 1983b). However, transfer of cultured T cells can also induce suppression of subsequently induced AA (Ogawa and Tsunematsu, 1986).

Recently the group of Cohen succeeded in isolating T cell clones from Lewis rats with AA. Clone A2b, which is specific for MT, transferred arthritis to gamma irradiated naive Lewis rats without antigen (Holoshitz et al., 1983; 1984). Another clone, A2c, isolated from the same culture, was not arthritogenic. On the contrary, rats that had received A2c clone had acquired resistance to the active induction of AA (Cohen et al., 1985). The A2b clone, after treatment with hydrostatic pressure, was also able to induce resistance to AA (Lider et al., 1987). The rationale for the arthritogenicity of the A2b clone likely is that this clone does not only recognize MT but also cartilage proteoglycans (van Eden et al., 1985; 1987) as there exists a crossreactive epitope between MT and these proteoglycans. This is shown in Fig. 2. The epitope that is involved here is situated on a 65 kD protein of MT, is cloned and consists of nine amino acids (van Eden et al., 1988). The 65 kD protein can also protect rats against AA (van Eden et al., 1988). This 65 kD protein is reported to be a heatshock protein (Shinnick et al., 1988), which is a protein that is very common in bacteria and is released after a heat shock (Polla, 1988).

Histologically, the arthritis induced by active immunization using MT, the arthritis induced by systemic transfer of MT specific T cell clones

and the bystander arthritis by an ovalbumin specific T cell clone in combination with local administration of ovalbumin have been compared. All three forms of arthritis were characterized by the same pathologic changes, though the day of onset was different. Marked hyperplasia of the synovium was

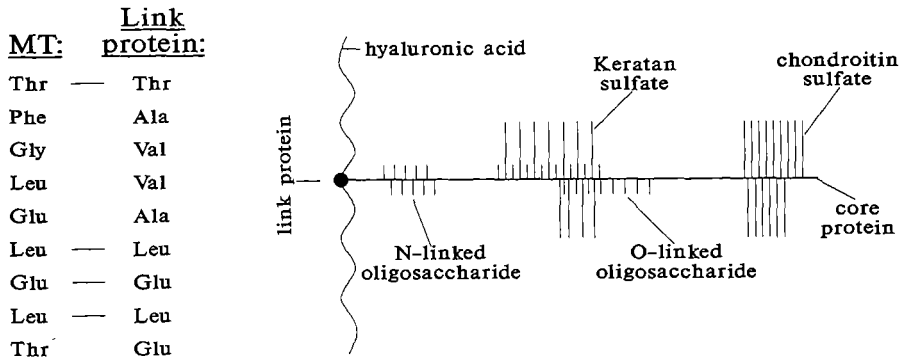


Fig. 2. Molecular basis of the antigenic mimicry between Mycobacteria and proteoglycans. After Cohen 1988.

observed together with infiltration of inflammatory cells, predominantly lymphocytes and plasma cells and a smaller number of PMN cells, histiocytes and fibroblasts. Pannus formation and bone erosion also occurred (Stanescu et al., 1987).

3.3. Bacterial cell wall induced arthritis

A single intraperitoneal (ip) injection of cell wall fragments of Streptococcus group A bacteria can induce a chronic arthritis in susceptible rat strains (Cromartie et al., 1977). These cell wall fragments consists of the type of peptidoglycan-polysaccharide (PG-PS) complexes shown in Fig. 3. These PG-PS complexes of Streptococci have been reported to be able to activate the alternative complement pathway (Greenblatt et al., 1978), to activate macrophages and to polyclonally stimulate lymphoid cells (Stewart-Tull, 1980). The immunized rats first develop an acute arthritis that is dependent on the complement system (Schwab et al., 1982), followed by a chronic joint inflammation that can persist for many months.

Athymic, T cell deficient nude rats only developed the acute joint

inflammations but not the chronic form (Ridge et al., 1985). This is indicative for the importance of T cells in the chronic joint inflammation. However, Hunter et al. (1980) reported that cell mediated immunity against these bacterial cell walls might not be the major pathogenic factor in this experimental disease. Not all rat strains are susceptible to the induction of joint inflammations by PG-PS extracts (Wilder et al., 1983). Possibly suppressor mechanisms play a role in the protection of these insusceptible rat strains (van den Broek et al., in press a).

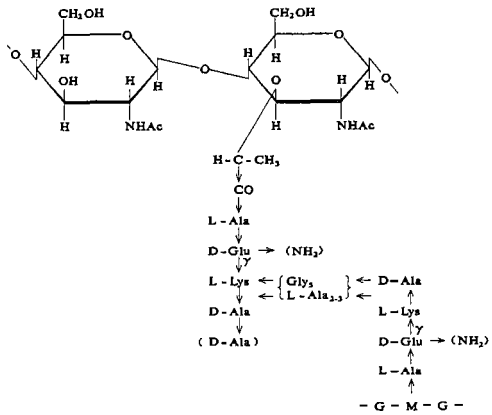


Fig. 3. Structural representation of the peptidoglycan molecule. The basic unit consists of an alternating N-acetylglucosamine and N-acetylmuramic acid, to which a peptide side chain is attached. After Bennett (1978).

The arthritogenicity of the PG-PS extracts is dependent on several factors such as the particle size (Esser et al., 1986; Fox et al., 1982), the rhamnose content and the resistance for lysozyme (Lehman et al., 1985). Acetylation of the PG-PS extracts caused an earlier waning of the disease with little evidence for recurrent disease (Stimpson et al., 1987a). This indicates that the persistence of the PG-PS in the body is obligatory for chronicity of the arthritis.

Local injection of S. pyogenes extracts only induces transient arthritis which can show exacerbations after systemic administration of subarthritogenic doses of homologous or heterologous PG-PS complexes, muramyldipeptide (Esser et al., 1985), lipopolysaccharide (Stimpson et al., 1987b) or IL-1

(Stimpson et al., 1988).

Acute joint inflammations after systemic administration of PG-PS complexes of various bacteria has also been found in mice (Koga et al., 1985). Furthermore, in mice arthritis induced by local administration of Streptococcal cell walls has been shown to exhibit flare-up reactions after systemic rechallenge with homologous or heterologous cell walls (van den Broek et al., in press b).

Not only cell wall extracts of Streptococci group A are able to induce arthritis in rats but also extracts derived from Lactobaccilus casei (Lehman et al., 1983) and extracts of strictly anaerobic gut bacteria such as Peptostreptococcus productus (Stimpson et al., 1986) and Eubacterium aerofaciens, which occur in very high numbers in the human gut (Severijnen et al., 1988; submitted).

The histology of the arthritis induced by Streptococcus group A has been described by Cromartie (1977). The acute inflammation is characterized by vascular congestion, oedema, fibrin deposition and infiltration by neutrophils and mononuclear phagocytes predominantly in the synovial membrane. After two weeks this reaction subsides and is replaced by a chronic inflammation characterized by proliferation of the connective tissue, blood vessels and the stroma of the synovial villi, infiltration by mononuclear phagocytes and lymphocytes, continued hyperplasia of the synovial lining cells, and destruction of the cartilage and subchondral bone.

3.4. Collagen type II induced arthritis

Collagen type II induced arthritis (CIA) is a model that is based upon intradermal injection of collagen type II. The model was first described in rats (Trentham et al., 1977) and later in mice (Courtenay et al., 1980) and monkeys (Cathcart et al., 1986). The susceptibility for CIA in mice is restricted to mouse strains with particular H-2 class II haplotypes (H-2 I-A^q) (Wooley et al., 1981). Results have been presented that suggest a role for humoral as well as cellular immunity in the pathogenesis of this experimental disease.

The role of T cells in CIA is apparent from the observation that athymic nude rats do not develop CIA (Takagishi et al., 1985) and that treatment with anti-thymocyte serum decreases the incidence of the arthritis (Brahn and Trentham, 1984). Trentham et al. (1978) have shown that the CIA can be passively transferred by pooled LN cells from actively immunized rats. This does not give, however, a decisive answer about the role of T and B cells

in the inoculum. Others have made T cell clones and lines with specificity for collagen type II (Dallman and Fathman, 1985; Hom et al., 1986a; 1986b; Holmdahl et al., 1985a, 1986; Brahn and Trentham, 1987; Kakimoto et al., 1988). In mice such clones can transfer the joint inflammations to naive recipients (Holmdahl et al., 1985a, 1986).

In mice (Kakimoto et al., 1988) and rats (Brahn and Trentham, 1987) passive transfer of collagen type II specific T cell lines can protect against subsequent induction of CIA. Antigen specific suppression was also obtained by iv (Cremer et al., 1983) or intragastrical (Nagler-Anderson et al., 1986; Thompson and Staines, 1985) pretreatment with collagen type II. The importance of humoral immunity in this model is apparent from the observation that passive transfer of serum can also transfer the arthritis (Stuart et al., 1982, 1983; Stuart and Dixon, 1983). Cellular and humoral immunity have been shown to synergize in the CIA (Seki et al., 1988).

Histologically, CIA inflammations are characterized by synovial hyperplasia, infiltrates of PMN cells and mononuclear cells (MNC), exudation of cells in the joint space, marginal erosion, periostitis and destruction of the cartilage surface (Trentham et al., 1977). Immunohistologically, T cells with the helper phenotype appear early in the infiltrates, whereas a moderate increase of T cells with the cytotoxic/suppressor phenotype, B cells and plasma cells was seen later in time. Class II positive macrophage-like cells are thought to occur as the result of lymphokine production by the activated T cells (Holmdahl et al., 1985b).

As in rats with AA also humoral as well as cellular sensitivity to collagen has been shown (Trentham, 1980), the question was raised whether the CIA and the AA model are based on the common feature of immunity to collagen type II. By induction of antigen specific suppression by cyclosporin this was shown not to be the case (Kaibara et al., 1984). Synergy between CIA and AA has been described by Taurog et al. (1985) and DeJoy et al. (1988). These investigators described that subarthritogenic doses of anti-collagen IgG and adjuvant sensitized spleen cells together show an enhanced capacity to induce arthritis.

A comparison of the histological appearance of human RA, AIA in rabbits, AA in rats and CIA in mice has been made by Doble (1987). This is schematically summarized in Table 2. Plasma cell infiltration and lymphoid aggregates were not found in AA by this author, which is in contradiction with the results described above in the AA model.

Table 2

Comparison of the histological features of human RA and four animal models

	RA (man)	AIA (rabbit)	AA (rat)	CIA (mouse)
Plasma cell infiltration	+	+	-	-
Lymphoid aggregates	+	+	-	-
PMN cells attacking hyaline cartilage	+	-	-	+
Earliest site of arthritis	joint synovium	joint synovium	tendon sheath	articular surface

After Doble, 1987.

3.5. MRL mice

MRL mice spontaneously develop arthritis, have IgG and IgM RF (Hang et al., 1982), anti-collagen type I and II antibodies (Phadke et al., 1984; Tarkowski et al., 1986), anti-DNA antibodies and circulating immune complexes (Theofilopoulos and Dixon, 1981). These mice have been developed by Murphy and Roths (1978) and were first used as a model for studying SLE by Theofilopoulos and Dixon (1981).

Histologically, initially a proliferation of synovial cells is seen, followed by infiltrates of lymphocytes, a few plasma cells, histiocytes, PMN cells and eosinophils (Pataki and Rordorf-Adam, 1985). Concerning the nature of the infiltrate the opinions differ. Some authors find that during the progression of the disease exudation and tissue destruction occur without the direct participation of inflammatory cells (O'Sullivan et al., 1985). In an immunohistochemical study only a few MHC class II expressing cells were found and only small numbers of lymphocytes (Tarkowski et al., 1987). The histopathology observed in MRL mice is rather different from that in human RA.

3.6. Arthritis by graft versus host reactions

A chronic progressive arthritis can also occur during graft-versus-host reactions in mice due to injection of parental spleen cells into F1 mice (Pals et al., 1985). Sjögren like lesions were also observed. The lesions

were found to be caused by the donor T cells. Histologically the synovial and subsynovial tissues were thickened and infiltrated by lymphocytes and plasma cells. In the articular cavities lymphocytes and plasma cells occurred together with exudates of fibrin, mononuclear cells and some PMN cells.

4. INTRODUCTION TO THE EXPERIMENTAL WORK

In human rheumatic diseases and in the arthritis models in experimental animals discussed above, often exacerbations and remissions occur, in most cases with an unknown origin.

In the AIA model in mice it has been shown that the joint inflammations can show exacerbations (flare-up reactions) after repeated administration of the antigen, either locally or systemically (iv or orally) (van Beusekom et al., 1981; van de Putte et al., 1983; Lens et al., 1983, 1984). In the induction of AIA T cells are thought to play a pivotal role (Brackertz et al., 1977a), but it is unclear whether antibodies are also involved. In view of the increasing knowledge about the cloning of T cells, it became feasible to investigate the role of T cells separately by making use of cloned T cell populations. Therefore, T cell clones were isolated from C57BL/6 (H-2^b) mice immunized with methylated bovine serum albumin (mBSA). This is an antigen that retains well to cartilage structures (van den Berg et al., 1984, 1986). The T cell clones isolated were $MT4^+$,$Lyt\text{-}2^-$ and thus of the helper phenotype.

Briefly, the aim of the study described in this thesis was to investigate whether the joint inflammations that occur in AIA can also be induced by cloned helper T cells and the antigen they recognize, and whether these joint inflammations can also show flare-up phenomena after repeated administration of the antigen. In Chapter 2 it is shown that cloned T cells with the helper phenotype can induce delayed type hypersensitivity (DTH) reactions and that these reactions can give rise to flare-up reactions after local, iv or oral administration of the antigen. We hypothesized that these DTH reactions underly the development of joint inflammations in the AIA model, and presumably in human rheumatoid diseases.

In Chapter 3 we present the model of induction of joint inflammation by similar cloned T cells. Dose response curves of the antigen and the cloned T cells injected into the joints are shown, as are the kinetics of the joint inflammations induced. Furthermore we show that it is possible

to evoke flare-up reactions of these joint inflammations, but also of joint inflammations induced by systemically administered T cells and local injection of the antigen. The induction of joint inflammation by the cloned T cells was found to be dependent on H-2 restricted interactions with recipient cells or tissues.

In Chapter 4 we show that both the joint inflammation and the flare-up reactions can be evoked in T cell deficient nude mice as well. Furthermore, this paper pays attention to the role of antigen in the retention of the cloned T cells in the joint.

In our model, the induction of an inflammatory reaction is dependent on H-2 restricted interactions between T cells and recipient cells and tissues, presumably antigen presenting cells. In Chapter 5 the characterization of a macrophage cell line, AP284, is described that is able to present antigen efficiently in vivo to the cloned helper T cells used in our studies. Injection of AP284 and syngeneic cloned helper T cells into the hind foot of allogeneic mice induces a full blown DTH reaction. This model, in which both the antigen presenting cells and the T cells are monoclonal, is attractive to investigate the cellular interactions in the induction of T cell dependent inflammations. Therefore we applied this approach in our studies on AIA to investigate whether antigen activated helper T cells and macrophages are sufficient for the induction of joint inflammation in allogeneic recipients. The data presented in Chapter 6 show that this was indeed the case. Finally in Chapter 7 we studied in detail the histological and immunohistochemical characteristics of the inflammations and flare-up reactions in our model.

The data emerging from our studies are discussed in the perspective of literature data in Chapter 8.

5. REFERENCES

Abdel-Nour, A.N., C.J. Elson and P.A. Dieppe. Proliferative responses of T-cell lines grown from joint fluids of patients with rheumatoid arthritis and other arthritides. Immunology Lett. 12:329-333, 1986.

Abrahamsen, T.G., S.S. Froland and J.B. Natvig. In vitro mitogen stimulation of synovial fluid lymphocytes from rheumatoid arthritis and juvenile rheumatoid arthritis patients: dissociation between the response to antigens and polyclonal mitogens. Scand. J. Immunol. 7:81-90, 1978.

Al-Balaghi, S., H. Ström and E. Möller. Demonstration of a helper factor(s) with T-cell-replacing activity in synovial fluid. Scand. J. Immunol. 20:493-501, 1984.

Alspaugh, M.A. and E.M. Tan. Serum antibody in rheumatoid arthritis reactive with a cell-associated antigen: demonstration by precipitation and immuno-

fluorescence. Arthritis Rheum. 19:711-719, 1976.
Bennett, J.C. The infectious etiology of rheumatoid arthritis. Arthritis Rheum. 21:531-538, 1978.
Bergroth, V., Y.T. Konttinen, P. Nykanen, R. von Essen and K. Koota. Proliferating cells in the synovial fluid in rheumatic disease. Scand. J. Immunol. 22:383-388, 1985.
Bishop, C.J., R.A. Hazelton, D.J. Moss and J.M. Ryan. T Lymphocyte lines from arthritic synovial fluid: establisment and function. Ann. Rheum. Dis. 45:120-125, 1986.
Brackertz, D., G.F. Mitchell and I.R. Mackay. Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum. 20:841-850, 1977a.
Brackertz, D., G.F. Mitchell, M.A. Vadas, I.R. Mackay and J.F.A.P. Miller. Studies on antigen-induced arthritis in mice. II. Immunologic correlates of arthritis susceptibility in mice. J. Immunol. 118:1639-1644, 1977b.
Brackertz, D., G.F. Mitchell, M.A. Vadas and I.R. Mackay. Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. J. Immunol. 118:1645-1648, 1977c.
Brahn, E. and D.E. Trentham. Effect of antithymocyte serum on collagen arthritis in rats: evidence that T cells are involved in its pathogenesis. Cell. Immunol. 86:421-428, 1984.
Brahn, E. and D.E. Trentham. Attenuation of collagen arthritis and modulation of delayed-type hypersensitivity by type II collagen reactive T-cell lines. Cell. Immunol. 109:139-147, 1987.
Buchan, G., K. Barrett, T. Fujita, T. Taniguchi, R. Maini and M. Feldmann. Detection of activated T cell products in the rheumatoid joint using cDNA probes to interleukin-2 (IL-2), IL-2 receptor and IFN-γ. Clin. exp. Immunol. 71:295-301, 1988.
Burmester, G.R., D.T.Y. Yu, A.-M. Irani, H.G. Kunkel and R.J. Winchester. Ia^+ T cells in synovial fluid and tissues of patients with rheumatoid arthritis. Arthritis Rheum. 24:1370-1376, 1981.
Burmester, G.R., A. Dimitriu-Bona, S.J. Waters and R.J. Winchester. Identification of three major synovial lining cell populations by monoclonal antibodies directed to Ia antigens and antigens associated with monocytes/macrophages and fibroblasts. Scand. J. Immunol. 17:69-82, 1983.
Burmester, G.R., J. Schneeberger, B. Jahn, M. Gramatzki, J. Zacher and J.R. Kalden. Rheumatoid non-lymphoid synovial cells and the induction of mixed leukocyte reactions. Rheumatol. Int. 4:31-37, 1984.
Carson, D.A. 1985. Rheumatoid factor. In: Textbook of Rheumatology, W. Kelley, E. Haris, S. Ruddy and C. Sledge, eds. Saunders, Philadelphia. pp. 664-679.
Cathcart, E., K. Hayes, W. Gonnerman, A. Lazzari and C. Franzblau. Experimental arthritis in a non-human primate. I. Induction by bovine type II collagen. Lab. Invest. 54:26-31, 1986.
Cesario, T.C., B.S. Andrews, D.A. Martin, M. Jason, T. Treadwell, G. Friou and J.G. Tilles. Interferon in synovial fluid and serum of patients with rheumatic disease. J. Rheumatol. 10:647-650, 1983.
Chen, J-H., D.H. Kono, Z. Yong, M.S. Park, M.M.B.A. Oldstone and D.T.Y. Yu. A Yersinia pseudotuberculosis protein which cross-reacts with HLA-B27. J. Immunol. 139:3003-3011, 1987.
Choi, E.K.K., P.A. Gatenby, N.W. McGill, J.F. Bateman, W.G. Cole and J.R. York. Autoantibodies to type II collagen: occurrence in rheumatoid arthritis, other arthritides, autoimmune connective tissue diseases, and chronic inflammatory syndromes. Ann. Rheum. Dis. 47:313-322, 1988.
Cohen, I.R., J. Holoshitz, W. van Eden and A. Frenkel. T lymphocyte clones illuminate pathogenesis and affect therapy of experimental arthritis. Arthritis Rheum. 28:841-845, 1985.

Cohen, I.R.. The self, the world of autoimmunity. Sci. Amer. 258:52-60, 1988.
Combe, B., M. Andary, B. Klein, J. Clot and J. Sany. Regulation of interleukin-2 production in rheumatoid arthritis. J. Rheumatol. 14:226-229, 1987.
Courtenay, J., M. Dallman, A. Dayan, A. Martin and B. Mosedale. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 283:666-668, 1980.
Cremer, M.A., A.D. Hernandez, A.S. Townes, J.M. Stuart and A.H. Kang. Collagen-induced arthritis in rats: antigen specific suppression of arthritis and immunity by intravenously injected native type II collagen. J. Immunol. 131:2995-3000, 1983.
Cromartie, W.J., J.G. Craddock, J.H. Schwab, S.K. Anderle and C.H. Yang. Arthritis in rats after systemic injection of streptococcal cells or cell walls. J. exp. Med. 146:1585-1602, 1977.
Dallman, M. and C.G. Fathman. Type II collagen-reactive T cell clones from mice with collagen-induced arthritis. J. Immunol. 135:1113-1118, 1985.
Danis, V.A., L.M. March, D.S. Nelson and P.M. Brooks. Interleukin-1 secretion by peripheral blood monocytes and synovial macrophages from patients with rheumatoid arthritis. J. Rheumatol. 14:33-39, 1987.
Decker, J.L., D.G. Malone, B. Haraoui, S.M. Wahl, L. Schrieber, J.H. Klippel, A.D. Steinberg and R.L. Wilder. NIH conference. Rheumatoid arthritis: evolving concepts of pathogenesis and treatment. Ann. Intern. Med. 101:810-824, 1984.
De Maria, A., M. Malnati, A. Moretta, D. Pende, C. Bottino, G. Casorati, F. Cottafava, G. Melioli, M.C. Mingari, N. Migone, S. Romagnani and L. Moretta. $CD3^{+}4^{-}WT31^{-}$ (T cell receptor) cells and other unusual phenotypes are frequently detected among spontaneously interleukin 2-responsive T lymphocytes present in the joint fluid in juvenile rheumatoid arthritis. A clonal analysis. Eur. J. Immunol. 17:1815-1819, 1987.
Degré, M., O.J. Mellbye and O. Clarke-Jenssen. Immune interferon in serum and synovial fluid in rheumatoid and related disorders. Ann. Rheum. Dis. 42:672-676, 1983.
DeJoy, S.Q., K.M. Ferguson, A.L. Oronsky and S.S. Kerwar. Studies on the synergy between collagen and adjuvant arthritis in rats. Cell. Immunol. 113:117-129, 1988.
Dijkstra, C.D., E.A. Dopp, I.M.C. Vogels and C.J.F. van Noorden. Macrophages and dendritic cells in antigen-induced arthritis: an immunohistochemical study using cryostat sections of the whole knee joint of rat. Scand. J. Immunol. 26:513-523, 1987.
Duke, O., G.S. Panayi, G. Janossy, L.W. Poulter and N. Tidman. Analysis of T cell subsets in the peripheral blood and synovial fluid of patients with rheumatoid arthritis by means of monoclonal antibodies. Ann. Rheum. Dis. 42:357-361, 1983.
Doble, A.T.A. Local cell populations in experimental arthrides. Agents Actions. 19:323-325, 1986.
Dumonde, D.C. and L.E. Glynn. The production of arthritis in rabbits by an immunological reaction to fibrin. Br. J. exp. Pathol. 43:373-383, 1962.
Editorial, Intestinal bypass syndrome. Lancet i:1419-1420, 1983.
Egeland, T., O.J. Mellbye and J.A. Pahle. Inflammatory synovial tissue mononuclear cells release leucocyte migration factor in antigen- and mitogen-free cultures. Ann. Rheum. Dis. 40:388-395, 1981.
Egeland, T. and H. Lund. Immunoregulatory lymphokines in rheumatoid joints. Scand. J. Immunol. 25:101-106, 1987.
Eisenbarth, G.S. Type I diabetes mellitus. A chronic autoimmune disease.

New Engl.J.Med. 314:1360-1368, 1986.
Emery, P., K.C. Gentry, I.R. Mackay, K.D. Muirden and M. Rowley. Deficiency of the suppressor inducer subset of T lymphocytes in rheumatoid arthritis. Arthritis Rheum. 30:849-856, 1987.
Esser, R.E., S.A. Stimpson, W.J. Cromartie and J.H. Schwab. Reactivation of streptococcal cell wall-induced arthritis by homologous and heterologous cell wall polymers. Arthritis Rheum. 28:1402-1411, 1985.
Esser, R.E., S.K. Anderle, C. Chetty, S.A. Stimpson, W.J. Cromartie and J.H. Schwab. Comparison of inflammatory reactions induced by intra-articular injection of bacterial cell wall polymers. Am. J. Pathol. 122:323-334, 1986.
Fay, A.C., A. Trudgett, J.D. McCrea, F. Kirk, J.M. Thompson, E.S. Mitchell, M.J.W. Boyd, S.D. Roberts and T.A. McNeill. Detection and partial characterization of human B cell colony stimulating activity in synovial fluids of patients with rheumatoid arthritis. Clin. exp. Immunol. 60:316-322, 1985.
Fong, S., P.P. Chen, R.I. Fox, R.D. Goldfien, G.J. Silverman, V. Radoux, F. Jirik, J.H. Vaughan and D.A. Carson. Rheumatoid factors in human autoimmune disease: their origin, development and function. Pathol. Immunopathol. Res. 5:305-316, 1986.
Fontana, A., H. Hengartner, E. Weber, K. Fehr, P.J. Grob and G. Cohen. Interleukin 1 activity in the synovial fluid of patients with rheumatoid arthritis. Rheumatol. Int. 2:49-53, 1982.
Ford, D.K., D.M. da Roza and M. Schulzer. Lymphocytes from the site of disease but not blood lymphocytes indicate the cause of arthritis. Ann. Rheum. Dis. 44:701-710, 1985.
Forre, O., T. Egeland, J. Dobloug, T. Kvien and J. Natvig. Autologous mixed lymphocyte reactions in patients with rheumatoid arthritis and juvenile rheumatoid arthritis: both non-T cells and in vivo-activated T cells can act as stimulator cells. Scand. J. Immunol. 16:173-179, 1982.
Fox, A., R.R. Brown, S.K. Anderle, C. Chetty, W.J. Cromartie, H. Gooder and J.H. Schwab. Arthropatic properties related to the molecular weight of peptidoglycan-polysaccharide polymers of streptococcal cell walls. Infect. Immun. 35:1003-1010, 1982.
Gardner, D.L. 1986. Pathology of rheumatoid arthritis. Scott, J.T. ed., Churchill Livingstone, Edinburgh. pp 604-652.
Geppert, T.D. and P.E. Lipsky. Antigen presentation by interferon-treated endothelial cells and fibroblasts: differential ability to function as antigen presenting cells despite comparable Ia expression. J. Immunol. 135:3750-3762, 1985.
Goldstein, R. and J. Karsh. In vitro synthesis of IgM rheumatoid factor in response to Staphylococcus aureus, by lymphocytes from healthy adults. Arthritis Rheum. 29:1440-1445, 1986.
Greenblatt, J.J., R.J. Boackle and J.H. Schwab. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. Infect. Immun. 19:296-303, 1978.
Greenstein, A.J., H.D. Janowitz and D.B. Sachar. The extra-intestinal complications of Crohn's disease and ulcerative colitis. A study of 700 patients. Medicine 55:401-412, 1976.
Hafez, M., F. El-Shannawy, Sh. El-Salab, Z. El-Morsi, M. El-Ziny, Y. Al-Tonbary, A. Abdalla and A. Abou-El-Enein. Studies of peripheral blood T lymphocytes in assesment of disease activity in rheumatic fever. Br. J. Rheumatol. 27:181-186, 1988.
Hahn, B.H. 1985. Antibodies to DNA, epiphenomena or pathogens? Gupta, S. and N. Talal, eds., Plenum Medical Book Company, New York and London. pp. 221-235.

Hang, L., A.N. Theofilopoulos and F.J. Dixon. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. J. exp. Med. 155:1690-1701, 1982.
Harris, E.D. Pathogenesis of rheumatoid arthritis. Am. J. Med. 80:4-10, 1986.
Holmdahl, R., L. Klareskog, K. Rubin, E. Larsson and H. Wigzell. T lymphocytes in collagen II-induced arthritis in mice. Scand. J. Immunol. 22:295-306, 1985a.
Holmdahl, R., K. Rubin, L. Klareskog, L. Dencker, G. Gustafson and E. Larsson. Appearance of different lymphoid cells in synovial tissue and in peripheral blood during the course of collagen II-induced arthritis in rats. Scand. J. Immunol. 21:197-204, 1985b.
Holmdahl, R., L. Klareskog, K. Rubin, J. Bjork, G. Smedegard, R. Jonsson and M. Andersson. Role of T lymphocytes in murine collagen induced arthritis. Agents Actions 19:295-305, 1986.
Holoshitz, J., Y. Naparstek, A. Ben-Nun and I.R. Cohen. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 219:56-58, 1983.
Holoshitz, J., A. Matitiau and I.R. Cohen. Arthritis induced in rats by cloned T lymphocytes responsive to Mycobacteria but not to collagen type II. J. Clin. Invest. 73:211-215, 1984.
Holoshitz, J., I. Drucker, A. Yaretzky, W. van Eden, A. Klajman, Z. Lapidot, A. Frenkel and I.R. Cohen. T lymphocytes of rheumatoid arthritis patients show augmented reactivity to a fraction of Mycobacteria cross-reactive with cartilage. Lancet ii:305-309, 1986.
Hom, J.T., J.M. Stuart and J.M. Chiller. Murine T cells reactive to type II collagen. I. Isolation of lines and clones and characterization of their antigen-induced proliferative responses. J. Immunol. 136:769-775, 1986a.
Hom, J.T., J.M. Stuart, J. Tovey and J.M. Chiller. Murine T cells reactive to type II collagen, II. Functional characterization. J. Immunol. 136:776-782, 1986b.
Hopkins, S.J., M. Humphreys and M.I.V. Jayson. Cytokines in synovial fluid. I. The presence of biologically active and immunoreactive IL-1. Clin. exp. Immunol. 72:422-427, 1988.
Houssiau, F.A., J.-P. de Vogelaer, J. van Damme, C. Nagant de Deux chaisnesand J van Snick. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthritis Rheum. 31:784-788, 1988.
Hunter, N., S.K. Anderle, R.R. Brown, F.G. daldorf, R.L. Clark, W.J. Cromartie and J.H. Schwab. Cell-mediated immune response during experimental arthritis induced in rats with streptococcal cell walls. Clin. exp. Immunol. 42:441-449, 1980.
Jahn, B., G.R. Burmester, M. Gramatzki, G. Weseloh, P. Stock and J.R. Kalden. Intraarticular T lymphocytes in monoarticular and oligoarticular inflammatory joint diseases. J. Rheumatol. 13:254-257, 1986.
Jahn, B., G.R. Burmester, H. Schmid, G. Weseloh, P. Rohwer and J.R. Kalden. Changes in cell surface antigen expression on human articular chondrocytes induced by gamma-interferons. Arthritis Rheum. 30:64-74, 1987.
Janossy, G., O. Duke, L.W. Poulter, G.S. Panayi, M. Bofill and G. Goldstein. Rheumatoid arthritis: a disease of T-lymphocyte/macrophage immunoregulation. Lancet ii:839-842, 1981.
Johnell, O., A. Hulth and A. Henricson. T-lymphocyte subsets and HLA-DR-expressing cells in the osteoarthritic synovialis. Scand. J. Rheumatol. 14:259-264, 1985.
Kaibara, N., T. Hotokebuchi, K. Takagishi, I. Katsuki, M. Morinaga, C. Arita and S. Jingushi. Pathogenic difference between collagen arthritis and adjuvant arthritis. J. exp. Med. 159:1388-1396, 1984.

Kakimoto, K., M. Katsuki, T. Hirofuji, H. Iwata and T. Koga. Isolation of T cell line capable of protecting mice against collagen-induced arthritis. J. Immunol. 140:78-83, 1988.

Kalden, J., O. Leitner, O. Klockel, H. Kirchner, B. Manger and B. Koch. The autologous mixed lymphocyte reaction in patients with rheumatoid arthritis. Behring Inst. Mitt. 72:193-202, 1983.

Kaplan, H.J. and J.C. Waldrep. Immunological insight into uveitis and retinitis. Ophthalmol. 91:655-665, 1984.

Keat, A. Is spondylitis caused by Klebsiella? Immunol. Today 7:144-149, 1986.

Keystone, E.C., L. Poplonski, R.G. Miller, R. Gorczynski and D. Gladman. In vivo activation of lymphocytes in rheumatoid arthritis. J. Rheumatol. 13:694-699, 1986.

Keystone, E.C., K.M. Snow, C. Bombardier, C.-H. Chang, D.L. Nelson and L.A. Rubin. Elevated suluble interleukin-2 receptor levels in the sera and synovial fluids of patients with rheumatoid arthritis. Arthritis Rheum. 31:844-849, 1988.

Kingsley, G.H., C. Pitzalis and G.S. Panayi. Abnormal lymphocyte reactivity to self-major histocompatibility antigens in rheumatoid arthritis. J. Rheumatol. 14:667-673, 1987.

Klareskog, L., U. Forsum, U. Malmns Tjernlund, D. Kabelitz and A. Wigren. Appearance of anti-HLA-DR-reactive cells in normal and rheumatoid synovial tissue. Scand. J. Immunol. 14:183-192, 1981.

Klareskog, L., U. Forsum, A. Scheynius, D. Kabelitz and H. Wigzell. Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis. Proc. Natl. Acad. Sci. USA 79:3632-3636, 1982.

Knobloch, C., M. Schlesier, R. Dräger, M. Gärtner and H.H. Peter. Quantitative absorption of interleukin-2 by peripheral blood and synovial fluid lymphocytes from patients with rheumatoid arthritis. Rheumatol. Int. 5:133-139, 1985.

Koga, T., K. Kakimoto, T. Hirofuji, S. Kotani, H. Ohkuni, K. Watanabe, N. Okada, H. Okada, A. Sumiyoshi and K. Saisho. Acute joint inflammation in mice after systemic injection of the cell wall, its peptidoglycan, and chemically defined peptidoglycan subunits from various bacteria. Infect. Immun. 50:27-34, 1985.

Konttinen, Y.T., V. Bergroth and P. Nykanen. Lymphocyte activation in rheumatoid arthritis synovial fluid in vivo. Scand. J. Immunol. 22:503-507, 1985.

Koopman, W.J. and R.E. Schrohenloher. In vitro synthesis of IgM rheumatoid factor by lymphocytes from healthy adults. J. Immunol. 125:934-939, 1980.

Korn-Lubetzki, I. and O. Abromsky. Acute and chronic demyelinating inflammatory polyradiculoneuropathy. Arch. Neurol. 43:604-608, 1986.

Kruijsen, M.W.M., W.B. van den Berg and L.B.A. van de Putte. Sequential alterations of periarticular structures in antigen-induced arthritis in mice. Histological observations on fibrous capsule, ligaments, bone and muscles, using whole joint sections. Br. J. exp. Pathol. 64:298-305, 1983.

Kurosaka, M. and M. Ziff. Immunoelectron microscopic study of the distribution of T cell subsets in rheumatoid synovium. J. exp. Med. 158:1191-1210, 1983.

Lasky, H.P., K. Bauer and R.M. Pope. Increased helper inducer and decreased suppressor inducer phenotypes in the rheumatoid joint. Arthritis Rheum. 31:52-59, 1988.

Lautenschlager, I., U. Kankaanpää and S. Kontiainen. Mononuclear cells in synovial fluid of rheumatoid arthritis patients undergoing joint surgery.

Acta Path. Microbiol. Immunol. Scand. 95:123-127, 1987.
Lehman, T.J.A., J.B. Allen, P.H. Plotz and R.L. Wilder. Polyarthritis in rats following the systemic injection of Lactobacillus casei cell walls in aqueous suspension. Arthritis Rheum. 26:1259-1265, 1983.
Lehman, T.J.A., J.B. Allen, P.H. Plotz and R.L. Wilder. Bacterial cell wall composition, lysozyme resistance, and the induction of chronic arthritis in rats. Rheumatol. Int. 5:163-167, 1985.
Lemm, G. and H. Warnatz. Evidence for enhanced interleukin 2 (IL-2) secretion and IL-2 receptor presentation by synovial fluid lymphocytes in rheumatoid arthritis. Clin. exp. Immunol. 64:71-79, 1986.
Lens, J.W., W.B. van den Berg and L.B.A. van de Putte. Flare-up of antigen-induced arthritis in mice after challenge with intravenous antigen: studies on the characteristics of and mechanisms involved in the reaction. Clin. exp. Immunol. 55:287-294, 1983.
Lens, J.W., W.B. van den Berg, L.B.A. van de Putte and L. van den Bresselaar. Flare-up of antigen-induced arthritis in mice after challenge with oral antigen. Clin. exp. Immunol. 58:364-371, 1984.
Lider, O., N. Karin, M. Shinitzky and I.R. Cohen. Therapeutic vaccination against adjuvant arthritis using autoimmune T cells treated with hydrostatic pressure. Proc. Natl. Acad. Sci. USA 84:4577-4580, 1987.
Londei, M., C.M. Savill, P. de Berardinis, F. Brennan, A. Verhoef, V. Duance, R.N. Maini and M. Feldmann. Cloning of activated T cells from rheumatoid arthritis joints: detection of collagen type II specific T cells (abstract). J. Cell. Biochem. Suppl. 12E:20, 1988.
Lotz, M., C.D. Tsoukas, C.A. Robinson, C.A. Dinarello, D.A. Carson and J.H. Vaughan. Basis for defective responses of rheumatoid arthritis synovial fluid lymphocytes to anti CD3 (T3) antibodies. J. Clin. Invest. 78:713-721, 1986.
Luyten, F., S. Suykens, E.M. Veys, J. van Lerbeirghe, C. Ackerman, H. Mielants and G. Verbruggen. Peripheral blood T lymphocyte subpopulations determined by monoclonal antibodies in active rheumatoid arthritis. J. Rheumatol. 13:864-868, 1986.
McCluskey R. and A.K. Bahn. Cell-medeated mechanisms in renal diseases. Kidney Int. 21 (suppl. 11):S6-S12, 1984.
Macdermott, R. and M. Stacey. Further characterization of the human autologous mixed leukocyte reaction (MLR). J. Immunol. 126:729-734, 1981.
MacFarlin, D.E. and H.F. McFarland. Multiple sclerosis. New Engl. J. Med. 307: 1183-1188, 1246-1251, 1982.
Malone, D.G., S.M. Wahl, M. Tsokos, H. Catell, J.L. Decker and R.L. Wilder. Immune function in severe, active rheumatoid arthritis, a relationship between peripheral blood mononuclear cell proliferation to soluble antigens and synovial tissue immunohistologic characteristics. J. Clin. Invest. 74:1173-1185, 1984.
Mikecz, K., T.T. Glant, M. Baron and A.R. Poole. Isolation of proteoglycan-specific T lymphocytes from patients with ankylosing spondylitis. Cell. Immunol. 112:55-63, 1988.
Morimoto, C., N.L. Letvin, A.W. Boyd, M. Hagan, H.M. Brown, M.M. Kornacki and S.F. Schlossman. The isolation and characterization of the human helper inducer T cell subset. J. Immunol. 134:3762-3769, 1985a.
Morimoto, C., N.L. Letvin, J.A. Distaso, W.R. Aldrich and S.F. Schlossman. The isolation and characterization of the human suppressor inducer T cell subset. J. Immunol. 134:1508-1515, 1985b.
Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin and R.L. Coffman. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348-2357, 1986.
Murphy, E.D. and J.B. Roths. 1978. Autoimmunity and lymphoproliferation:

induction by mutant gene lpr, and acceleration by a male-associated factor in strain BXSB mice, genetic control of autoimmune disease. Rose, N.R., R.E. Bigazzi and N.L. Warner, eds., Elsevier/North-Holland, New York. pp. 207-219.

Nagler-Anderson, C., L.A. Bober, M.E. Robinson, G.W. Siskind and G.J. Thorbecke. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. Proc. Natl. Acad. Sci. USA 83:7443-7446, 1986.

Nemazee, D.A. Immune complexes can trigger specific, T cell dependent, autoanti-IgG antibody production in mice. J. exp. Med.:242-255, 1985.

Nilsson, E., L.-V. von Stedingk and G. Biberfeld. Immunoregulatory function of T8 and T4 cells from synovial fluid and blood of patients with rheumatoid arthritis or other forms of chronic arthritis. Scand. J. Immunol. 24:729-737, 1986.

Nouri, A.M.E. and G.S. Panayi. Cytokines and the chronic inflammation of rheumatic disease. III. Deficient interleukin-2 production in rheumatoid arthritis is not due to suppressor mechanisms. J. Rheumatol. 14:902-906, 1987.

Nykanen, P., V. Bergroth, P. Raunio, D. Nordstrom and Y.T. Konttinen. Phenotypic characterization of 3H-thymidine incorporating cells in rheumatoid arthritis synovial membrane. Rheumatol. Int. 6:269-271, 1986.

O'Sullivan, F., H.-G. Fassbender, S. Gay and W.J. Koopman. Etiopathogenesis of the rheumatoid arthritis-like disease in MRL/l mice. I. The histomorphologic basis of joint destruction. Arthritis Rheum. 28:529-536, 1985.

Ofosu-Appiah, W.A., R.M. McKenna, R.J. Warrington and J.A. Wilkins. Characterization of synovial T lymphocytes in rheumatoid arthritis. I. Production of IL-2 dependent T cell clones from synovial fluid and peripheral blood. Clin. exp. Immunol. 64:555-562, 1986.

Ogawa, H. and T. Tsunematsu. Suppression of adjuvant arthritis in rats by transfer of cultered spleen cells. Clin. exp. Immunol. 64:88-93, 1986.

Ottenhoff, T.H.M., P. Torres, J.T. de las Aguas, R. Fernandez, W. van Eden, R.P. de Vries and J.L. Stanford. Evidence for HLA-DR4-associated immune-response gene for Mycobacterium tuberculosis. Lancet ii: 310-312, 1986.

Palacios, R. and G. Moller. HLA-DR antigens render resting T cells sensitive to interleukin-2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. Cell. Immunol. 63:143-153, 1981.

Palacios-Boix, A.A., I. Estrada-G, M.J. Colston and G.S. Panayi. HLA-DR4 restricted lymphocyte proliferation to a Mycobacterium tuberculosis extract in rheumatoid arthritis and healthy subjects. J. Immunol. 140:1-844-1850, 1988.

Pals, S.T., T. Radaskiewicz, L. Roozendaal and E. Gleichmann. Chronic progressive polyarthritis and other symptoms of collagen vascular disease induced by graft-vs-host reaction. J. Immunol. 134:1475-1482, 1985.

Panayi, G.S., E. Celinska, P. Emery, J. Griffin, K.I. Welsh, R. Grahame and T. Gibson. Seronegative and seropositive rheumatoid arthritis: similar diseases. Br. J. Rheum. 26:172-180, 1987.

Parekh, R.B., R.A. Dwek, B.J. Sutton, D.L. Fernandes, A. Leung, D. Stanworth, T.W. Rademacher, T. Mizuochi, T. Taniguchi, K. Matsuta, F. Takeuchi, Y. Nagano, T. Miyamoto and A. Kobata. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 316:452-457, 1985.

Pataki, A. and C. Rordorf-Adam. Polyarthritis in MRL lpr/lpr mice. Rheumatol. Int. 5:113-120, 1985.

Pearson, C.M. and F.D. Wood. Studies of polyarthritis and other lesions

induced in rats by injection of mycobacterial adjuvant. I. General clinical and pathologic characteristics and some modifying factors. Arthritis Rheum. 2:440-459, 1959.
Pearson, C.M. and F.D. Wood. Passive transfer of adjuvant arthritis by lymph node or spleen cells. J. exp. Med. 120:547-560, 1964.
Phadke, K., R. Fouts, J. Parrish and R.S. Baker. Autoreactivity to collagen in a murine lupus model. Arthritis Rheum. 27:313-319, 1984.
Phillips, P.E. and C.L. Christian. 1985. Infectious agents in chronic rheumatic disease. Lea and Febiger, Philadelphia. pp. 431-449.
Pitzalis, C., G.H. Kingsley, J.S.S. Lanchbury, J. Murphy and G.S. Panayi. Expression of HLA-DR, DQ and DP antigens and interleukin-2 receptor on synovial fluid T lymphocyte subsets in rheumatoid arthritis: evidence for 'frustrated' activation. J. Rheumatol. 14:662-666, 1987.
Polla, B.S. A role for heat shock proteins in inflammation? Immunol. Today 9:134-137, 1988.
Poulter, L.W., O. Duke, G.S. Panayi, S. Hobbs, M.J. Raftery and G. Janossy. Activated T lymphocytes of the synovial membane in rheumatoid arthritis and other arthropathies. Scand. J. Immunol. 22:683-690, 1985.
Raeman, F., W. Decock, T. Debeukelaar, J. Decree and H. Verhagen. Enumeration of T lymphocytes and T lymphocyte subsets in autoimmune disease using monoclonal antibodies. Clin. exp. Immunol. 45:475-479, 1981.
Res, P.C.M., C.G. Schaar, F.C. Breedveld, W. van Eden, J.D.A. van Embden, I.R. Cohen and R.R.P. de Vries. Synovial fluid T cell reactivity against 65 kD heat shock protein of Mycobacteria in early chronic arthritis. Lancet ii:478-480, 1988.
Reynolds, M.D. and N.I. Abdou. Comparative study of the in vitro proliferative responses of blood and synovial fluid leukocytes of rheumatoid arthritis patients. J. Clin. Invest. 52:1627-1631, 1973.
Ridge, S.C., J.B. Zabriskie, H. Osawa, T Diamantstein, A.L. Oronsky and S.S. Kerwar. Administration of group A Streptococcal cell walls to rats induces an interleukin 2 deficiency. J. exp. Med. 164:327-332, 1986.
Rowley, M.J., M.E. Gershwin and I.R. Mackay. Collagen antibodies in juvenile arthritis and adult rheumatoid arthritis: differences in levels and type-specificity. J. Rheumatol. 15:289-293, 1988.
Salmon, M. and P.A. Bacon. A cellular deficiency in the rheumatoid one-way mixed lymphocyte reaction. Clin. exp. Immunol. 71:79-84, 1988.
Savill, C.M., P.J. Delves, D. Kioussis, P. Walker, P.M. Lydyard, B. Colaco, M. Shipley and I.M. Roitt. A minority of patients with rheumatoid arthritis show a dominant rearrangement of T-cell receptor β chain genes in synovial lymphocytes. Scand. J. Immunol. 25:629-635, 1987.
Schlesier, M., C. Ramb-Lindhauer, M. Gartner and H.-H. Peter. Analysis of T-cell cultures and clones from a patient with classic rheumatoid arthritis-evidence for the existence of autoreactive T-cell clones in blood and synovial fluid. Rheumatol. Int. 4:1-9, 1984.
Schlesier, M., C. Ramb-Lindhauer, R. Drager, A. Urlacher, M. Robin-Winn and H.-H. Peter. Autoreactive T cells in rheumatic disease. II. Function and specificity of an autoreactive T helper cell clone established from a HLA-B27+ reactive arthritis. Submitted for publication.
Schwab, J.H., J.B. Allen, S.K. Anderele, E. Dalldorf, R. Eisenberg and W.J. Cromartie. Relationship of complement to experimental arthritis induced in rats with Streptococcal cell walls. Immunology 46:83-88, 1982.
Seki, N., Y. Sudo, T. Yoshioka, S. Sugihara, T. Fujitsu, S. Sakuma, T. Ogawa, T. Hamaoka, H. Senoh and H. Fujiwara. Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. J. Immunol.

140:1477-1484, 1988.
Severijnen, A.J., M.P. Hazenberg and J.P. van de Merwe. Induction of chronic arthritis in rats by cell wall fragments of anaerobic coccoid rods isolated from the faecal flora of patients with Crohn's disease. Digestion 39:118-125, 1988.
Severijnen, A.J., R. van Kleef, M.P. Hazenberg and J.P. van de Merwe. Cell wall fragments from major residents of the human intestinal flora induce chronic arthritis in rats. Submitted for publication.
Shinnick, T.M., M.H. Vodkin and J.C. Williams. The Mycobacterium tuberculosis 65-Kilodalton antigen is a heat shock protein which corresponds to common antigen and to the Escherichia coli GroEL protein. Infect. Immun. 56:446-451, 1988.
Simpson, R.W., L. McGinty, L. Simon, C.A. Smith, C.W. Godzeski and R.J. Boyd. Association of parvoviruses with rheumatoid arthritis of humans. Science 223:1425-1428, 1984.
Smith, J. and R. DeHoratius. Deficient autologous mixed lymphocyte reactions correlate with disease activity in systemic lupus erythematosus and rheumatoid arthritis. Clin. exp. Immunol. 48:155-162, 1982.
Smolen, J., S. Sharrow, J. Reeves, W. Boegel and A. Steinberg. The human autologous mixed lymphocyte reaction. J. Immunol. 127:1987-1993, 1981
Stamenkovic, I., M. Stegagno, K.A. Wright, S.M. Krane, E.P. Amento, R.B. Colvin, R.J. Duquesnoy and J.T. Kurnick. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc. Natl. Acad. Sci. USA 85:1179-1183, 1988.
Stanescu, R., O. Lider, W. van Eden, J. Holoshitz and I.R. Cohen. Histopathology of arthritis induced in rats by active immunization to mycobacterial antigens or by systemic transfer of T lymphocyte lines. Arthritis Rheum. 30:779-791, 1987.
Steinman, R.M. and M.C. Nussenzweig. Dendritic cells: features and functions. Immunol. Rev. 53:125-147, 1981.
Stewart-Tull, D.E.S. The immunological activities of bacterial peptidoglycans. Ann. Rev. Immunol. 34:311-340, 1980.
Stimpson, S.A., R.R. Brown, S.K. Anderle, D.G. Klapper, R.L. Clark, W.J. Cromartie and J.H. Schwab. Arthropathic properties of cell wall polymers from normal flora bacteria. Infect. Immun. 1:240-249, 1986.
Stimpson, S.A., R.A. Lerch, D.R. Cleland, D.P. Yarnall, R.L. Clark, W.J. Cromartie and J.H. Schwab. Effect of acetylation on arthropathic activity of group A streptococcal peptidoglycan-polysaccharide fragments. Infect. Immun. 55:16-23, 1987a.
Stimpson, S.A., R.E. Esser, P.B. Carter, R. Balfour Sartor, W.J. Cromartie and J.H. Schwab. Lipopolysaccharide induces recurrence of arthritis in rat joints previously injured by peptidoglycan-polysaccharide. J. exp. Med. 165:1688-1702, 1987b.
Stimpson, S.A., F.G. Dalldorf, I.G. Otterness and J.H. Schwab. Exacerbation of arthritis by IL-1 in rat joints previously injured by peptidoglycan-polysaccharide. J. Immunol. 140:2964-2969, 1988.
Stuart, J.M., M.A. Cremer, A.S. Townes and A.H. Kang. Type II collagen-induced arthritis in rats, passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. J. exp. Med. 155:1-16, 1982.
Stuart, J.M. and F.J. Dixon. Serum transfer of collagen-induced arthritis in mice. J. exp. Med. 158:378-392, 1983a.
Stuart, J.M., K. Tomoda, T.J. Yoo, A.S. Townes and A.H. Kang. Serum transfer of collagen-induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats. Arthritis Rheum. 26:1237-1244, 1983b.
Takagishi, K., N. Kaibara, T. Hotokebuchi, C. Arita, M. Morinaga and K.

Arai. Serum transfer of collagen arthritis in congenitally athymic nude rats. J. Immunol. 134:3864-3867, 1985.
Tarkowski, A., R. Holmdahl, K. Rubin, L. Klareskog, L.A. Nilsson and K. Gunnarsson. Patterns of autoreactivity to collagen type II in autoimmune MRL/l mice. Clin. exp. Immunol. 63:441-449, 1986.
Tarkowski, A., R. Jonsson, R. Holmdahl and L. Klareskog. Immunohistochemical characterization of synovial cells in arthritic MRL lpr/lpr mice. Arthritis Rheum. 30:75-81, 1987.
Taurog, J.D., G.P. Sandberg and M.L. Mahowald. The cellular basis of adjuvant arthritis. I. Enhancement of cell-mediated passive transfer by concanavalin A and by immunosuppressive pretreatment of the recipient. Cell. Immunol. 75:271-282, 1983a.
Taurog, J.D., G.P. Sandberg and M.L. Mahowald. The cellular basis of adjuvant arthritis. II. Characterization of the cells mediating passive transfer. Cell. Immunol. 80:198-204, 1983b.
Taurog, J.D., S.S. Kerwar, R.A. McReynolds, G.P. Sandberg, S.L. Leang and M.L. Mahowald. Synergy between adjuvant arthritis and collagen-induced arthritis in rats. J. exp. Med. 162:662-678, 1985.
Theofilopoulos, A.N. and F.J. Dixon. Etiopathogenesis of murine SLE. Immunol. Rev. 55:179-213, 1981.
Thoen, J., O. Forre, K. Waalen and E. Kass. Phenotypes of T lymphocytes from peripheral blood and synovial fluid of patients with rheumatoid arthritis and juvenile rheumatoid arthritis. Scand. J. Rheumatol. 16:247-256, 1987.
Thompson, H.S.G. and N.A. Staines. Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritus in rats. Clin. exp. Immunol. 64:581-586, 1985.
Tiku, M.L., S. Liu, C.W. Weaver, M. Teodorescu and J.L. Skosey. Class II histocompatibility antigen-mediated immunologic function of normal articular chondrocytes. J. Immunol. 135:2923-2928, 1985.
Toivanen, A., K. Granfors, R. Lahesmaa-Rantala, R. Leino, T. Stahlberg and R. Vuento. Pathogenesis of Yersinia-triggered reactive arthritis: immunological, microbiological and clinical aspects. Immunol. Rev. 86:47-70, 1985.
Tomana, M., R.E. Schrohenloher, W.J. Koopman, G.S. Alarcón and W.A. Paul. Abnormal glycosylation of serum IgG from patients with chronic inflammatory diseases. Arthritis Rheum. 31:333-338, 1988.
Trentham, D., A. Townes and A. Kang. Autoimmunity to type II collagen: an experimental model of arthritis. J. exp. Med. 146:857-868, 1977.
Trentham, D.E., R.A. Dynesius and J.R. David. Passive transfer by cells of type II collagen-induced arthritis in rats. J. Clin. Invest. 62:359-366, 1978.
Trentham, D.E., W.J. McCune, P. Susman and J.R. David. Autoimmunity to collagen in adjuvant arthritis of rat. J. Clin. Invest. 66:1109-1117, 1980.
Trentham, D.E. 1985. Immune response to colagen. Gupta, S. and N. Talal, eds., Plenum Medical Book Company, New York and London. pp. 301-323.
Valdimarsson, H., B.S. Baker, I. Jonsdottir and L. Fry. Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. Immunol. Today 7:256-259, 1986.
Van Beusekom, H.J., L.B.A. van de Putte, W.B. van den Berg, W.J.M. van den Broek and W.C.A.M. Buijs. Antigen handling in antigen-induced joint inflammation: kinetics of a second intra-articulary injected dose of antigen in an already established antigen-induced joint inflammation. Immunology 44:153-161, 1981.
Van de Putte, L.B.A., J.W. Lens, W.B. van den Berg and M.W.M. Kruijsen. Exacerbation of antigen-induced arthritis after challenge with intra-

venous antigen. Immunology 49:161-167, 1983.
Van den Berg, W.B., M.W.M. Kruijsen, L.B.A. van de Putte, H.J. van Beusekom, M. van der Sluis-van der Pol and W.A. Zwarts. Antigen-induced and zymosan-induced arthritis in mice: studies on in vivo cartilage proteoglycan synthesis and chondrocyte death. Br. J. exp. Pathol. 62:308-316, 1981.
Van den Berg, W.B., H.J. van Beusekom, L.B.A. van de Putte, W.A. Zwarts and M. van der Sluis. Antigen handling in antigen-induced arthritis in mice, an autoradiographic and immunofluorescence study using whole joint sections. Am. J. Pathol. 108:9-16, 1982.
Van den Berg, W.B., B.A. van de Putte, W.A. Zwarts and L.A.B. Joosten. Electrical charge of the antigen determines intraarticular antigen handling and chronicity of arthritis in mice. J. Clin. Invest. 74:1850-1859, 1984.
Van den Berg, W.B., P.L.E.M. van Lent, L.B.A. van de Putte and W.A. Zwarts. Electrical charge of hyaline articular cartilage: its role in the retention of anionic and cationic proteins. Clin. Immunol. Immunopathol. 39:187-197, 1986.
Van den Broek, M.F., W.B. van den Berg and L.B.A. van de Putte. Monoclonal anti-Ia antibodies suppress the flare up reaction of antigen induced arthritis in mice. Clin. exp. Immunol. 66:319- 330, 1986.
Van den Broek, M.F., M.C.J. van Bruggen, L.B.A. van de Putte and W.B. van den Berg. T cell responses to streptococcal antigens in rats: Relation to susceptibility to streptococcal cell wall induced arthritis. Cell. Immunol. In press a.
Van den Broek, M.F., W.B. van den Berg, L.B.A. van de Putte and A.J. Severijnen. Streptococcal cell wall induced arthritis and flare up reaction in mice induced by homologous or heterologous cell walls. Am. J. Pathol. In press b.
Van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman and I.R. Cohen. Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc. Natl. Acad. Sci. USA 82:5117-5120, 1985.
Van Eden, W., J. Holoshitz and I.R. Cohen. Antigenic mimicry between Mycobacteria and cartilage proteoglycans: the model of adjuvant arthritis. Concepts Immunopathol. 4:144-170, 1987.
Van Eden, W., J.E.R. Thole, R. van der Zee, A. Noordzij, J.D.A. van Embden, E.J. Hensen and I.R. Cohen. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331:171-173, 1988.
Van Lent, P.L.E.M., W.B. van den Berg, J. Schalkwijk, L.B.A. van de Putte and L. van den Bersselaar. Allergic arthritis induced by cationic antigens: relationship of chronicity with antigen retention and T-cell reactivity. Immunology 62:265-272, 1987.
Veys, E.M., P. Hermanns, G. Verbruggen, J. Schindler and G. Goldstein. Evaluation of T cell subsets with monoclonal antibodies in synovial fluid in rheumatoid arthritis. J. Rheumatol. 9:821-826, 1982.
Waalen, K., G.W. Duff, O. Forre, E. Dickens and G. Nuki. Interleukin I activity produced by human rheumatoid and normal dendritic cells. Scand. J. Immunol. 23:365-371, 1986.
Waalen, K., O. Forre, M. Linker-Israeli and J. Thoen. Evidence of an activated T-cell system with augmented turnover of interleukin 2 in rheumatoid arthritis. Scand. J. Immunol. 25:367-373, 1987a.
Waalen, K., O. Forre, J. Pahle, J.B. Natvig and G.R. Burmester. Characteristics of human rheumatoid synovial and normal blood dendritic cells. Scand. J. Immunol. 26:525-533, 1987b.
Wahl, S.M., R.L. Wilder, I.M. Katona, L.M. Wahl, J.B. Allen, I. Scher and J.L. Decker. Leukapheresis in rheumatoid arthritis. Association of clinical improvement with reversal of anergy. Arthritis Rheum.

26:1076-1084, 1983.
Waksman, B.H. and C. Wennersten. Passive transfer of adjuvant arthritis in rats with living lymphoid cells of sensitized donors. Int. Arch. Allergy 23:129-139, 1963.
Weisbart, R.H., D.T. Noritake, K.K. Colburn and R.E. Saxton. Human monoclonal IgG rheumatoid factor has structural homology with bacterial Fc receptor proteins. J. Immunol. 139:2925-2928, 1987.
Whitehouse, D.J., M.W. Whitehouse and C.M. Pearson. Passive transfer of adjuvant-induced arthritis and allergic encephalomyelitis in rats using thoracic duct lymphocytes. Nature 224:1322, 1969.
Wick, G., J. Möst, K. Schauenstein, H. Krmer, H. Dietrich, A. Ziemiecki, R. Fässler, S. Schwartz, N. Neu and K. Hala. Spontaneous autoimmune thyroiditis - a bird's eye view. Immunol. Today 6:359-364, 1985.
Wilder, R.L., J.B. Allen, L.M. Wahl, G.B. Calandra and S.M. Wahl. The pathogenesis of group A streptococcal cell wall-induced polyarthritis in the rat. Arthritis Rheum. 26:1442-1451, 1983.
Wooley, P.H., H.S. Luthra, J.M. Stuart and C.S. David. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. J. exp. Med. 154:688-700, 1981.
Zatarain-Rios, E. and M. Mannik. Charge-charge interactions between articular cartilage and cationic antibodies, antigens, and immune complexes. Arthritis Rheum. 30:1265-1273, 1987.
Zvaifler, N.J., R.M. Steinman, G. Kaplan, L.L. Lau and M. Rivelis. Identification of immunostimulatory dendritic cells in the synovial effusions of patients with rheumatoid arthritis. J. Clin. Invest. 76:789-800, 1985.
Zvaifler, N.J. Pathogenesis of joint disease of rhematoid arthritis. Am. J. Med. 75 (suppl.):3-8, 1988.

CHAPTER 2

FLARE-UP OF DELAYED-TYPE HYPERSENSITIVITY INITIALLY INDUCED BY MURINE CLONED HELPER T CELLS

I.S. Klasen, Th.H. van der Kwast*, I.G. Donselaar, R.M.T. Ladestein and R. Benner

Department of Cell Biology, Immunology and Genetics and *Department of Pathology, Erasmus University, The Netherlands.

This paper has been published in Cellular Immunology 108: 235-241 (1987).

Flare-up of Delayed-Type Hypersensitivity Initially Induced by Murine Cloned Helper T Cells

I. S. KLASEN, TH. H. VAN DER KWAST,* I. G. DONSELAAR, R. M. T. LADESTEIN, AND R. BENNER

*Department of Cell Biology, Immunology and Genetics, and *Department of Pathology, Erasmus University, P.O. Box 1738, 3000DR Rotterdam, The Netherlands*

Received January 6, 1987; accepted February 12, 1987

Delayed-type hypersensitivity (DTH) reactions were induced in mice by cloned helper T cells directed against methylated bovine serum albumin (mBSA). The DTH reactions were induced either by local injection of the helper T cells together with the antigen in the hind feet or by intravenous (iv) administration of the cloned T cells and local injection of the antigen. Local or systemic (oral or iv) administration of mBSA after waning of the DTH induced by the cloned helper T cells caused a flare-up reaction. This indicates that functional helper T cells persist at the inflammation site. The inflammations were quantified in a foot swelling assay and were examined histologically. The inflammation measured in the flare-up reaction was generally lower than in the acute reaction. Histologically the acute inflammation showed edema and a large proportion of granulocytes, whereas the flare-up reaction appeared more histiocytic and showed less edema.

INTRODUCTION

The role of T cells in diseases such as rheumatoid arthritis, multiple sclerosis, and autoimmune thyroiditis has become more and more clear (1). Several of these diseases in which T cells are thought to play an important role show a time course characterized by exacerbations and remissions. They show flare-up reactions in which local hyperreactivity as a result of the retention of T cells (2) and/or the retention of antigen (3) are thought to be important.

It has been shown that clones of helper T cells can induce delayed-type hypersensitivity (DTH) reactions (4). For the present study we used a helper-T-cell clone directed against methylated bovine serum albumin (mBSA). mBSA is an antigen with a net positive charge and this protein can be used to induce chronic joint inflammations in mice (5) in a so-called antigen-induced arthritis model.

The positive charge of the antigen is claimed to be imperative to induce chronicity of the joint inflammation in the antigen-induced arthritis model. Because of charge interactions, the antigen molecules persist in the cartilage (3). Flare-up reactions of these inflammations can be induced by local or systemic administration of the antigen only (6, 7). Others have demonstrated flare-up reactions at sites of immunization with complete Freund's adjuvant after systemic administration of the antigen (8).

In this study we induced DTH reactions by means of cloned helper T cells. We investigated whether it is possible to induce flare-up reactions of the inflammations

evoked by the cloned helper T cells by administration of the antigen only. These inflammations were quantified in a foot swelling assay and were examined histologically.

MATERIALS AND METHODS

Mice. Female C57BL/6J, B10.A(4R), and B10.A(5R) mice were purchased from OLAC Ltd., Bicester, United Kingdom. Female B10.MBR mice were derived from our own stock. Female C57BL/Ka *nu/nu* mice were purchased from the REP Institutes TNO, Rijswijk, The Netherlands. They were used at the ages of 2–3 months.

Cloned helper T cells. A helper-T-cell clone directed against methylated bovine serum albumin was generated according to procedures described elsewhere (8). As a control clone, an ovalbumin (OVA)-specific helper-T-cell clone was used that was described elsewhere (9). They were of C57BL/6J origin. These clones were cultured in serum-free medium (IMDM-ATL) containing 1 g/liter BSA, 241 mg/liter human transferrin ($\frac{1}{3}$ Fe-saturated) (both from Hoechst-Behringwerke, Marburg, FRG), and 50 mg/liter soybean lipids (Astec FLPV obtained from Associated Concentrates, New York) (10).

Every 5–6 weeks the clones were restimulated with the antigen (5 μg/ml mBSA for the mBSA-specific clone and 300 μg/ml OVA for the OVA-specific clone were optimal). Irradiated (3000 R) syngeneic spleen cells were used as a source of antigen-presenting cells. After 3 days, the restimulated T cells were transferred to medium containing 600 U/ml recombinant human interleukin-2 (IL-2), which was a kind gift of Dr. J. Besemer from Sandoz Ltd., Vienna, Austria. Both clones were of the $L3T4^+Lyt2^-$ phenotype.

Induction of DTH and induction of flare-up reactions. DTH was induced in two ways: first, by local injection of 3×10^5 cloned helper T cells together with 20 μg mBSA or OVA in 50 μl balanced salt solution (BSS) into the dorsa of the hind feet of the mice, and second, by intravenous (iv) injection of 1.5×10^6 helper T cells in 0.5 ml BSS followed by injections of the antigens into the hind feet. mBSA and OVA were obtained from Sigma Chemical Company, St. Louis, Missouri.

Flare-up reactions were induced by local administration of the antigen in 50 μl BSS or by systemic (oral or iv) administration of the antigen in 0.5 ml BSS. This was done 1.5–3 weeks after induction of inflammation.

Measurement of foot swelling. The thicknesses of the hind feet were measured 24 and 48 hr after induction of inflammation. In the figures 24-hr values are presented. The 48-hr responses were similar to the 24-hr responses, but generally lower. The DTH responses are expressed as the specific increase in hind foot thickness and were calculated as [(thickness right foot − thickness left foot)/thickness left foot] × 100%. The Student *t* test was used to determine significance of the differences between data from different groups of mice.

Histology. The dorsa of the hind feet of the mice were removed and fixed in 10% phosphate-buffered Formalin. Standard (5-μm) sections were prepared of paraffin wax-embedded material and stained with hematoxylin and eosin.

RESULTS

We investigated whether cloned helper T cells specific for mBSA can induce DTH reactions and whether it is possible to induce flare-up reactions of these inflammations.

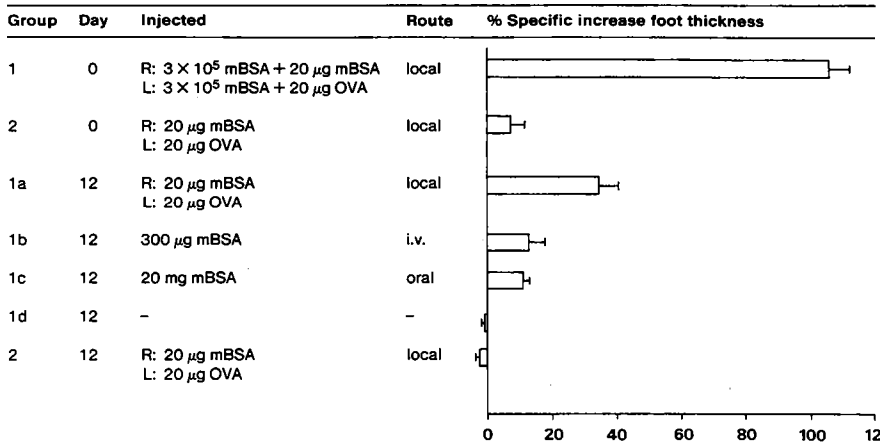


FIG. 1. DTH reaction and flare-up of DTH in mice by locally injected cloned helper T cells. Group 1 shows the DTH reactivity by cloned mBSA-specific helper T cells after direct injection into the dorsa of the hind feet, together with mBSA or, as a control, OVA. Twelve days after induction of DTH, flare-up reactions were induced by local administration of the antigen again (group 1a); iv administration of the antigen (group 1b); oral administration of the antigen (group 1c); or without readministration of the antigen (group 1d). As a control, foot swelling was measured 24 hr after injection of the antigen only (group 2). These mice also were rechallenged on Day 12. DTH responses are expressed as the specific increase in hind foot thickness (mean ± SEM; $n = 6$).

Group 1 (Fig. 1) shows the response measured 24 hr after injection of 3×10^5 mBSA-specific cloned helper T cells into the right hind foot together with 20 μg mBSA. As a control similar helper T cells were injected together with the non-cross-reacting antigen OVA into the left hind foot of the same mice.

Local reinjection of mBSA 12 days later caused a flare-up reaction (Fig. 1, group 1a). For comparison, group 1d in Fig. 1 shows the response measured 12 days after a single injection of mBSA-specific helper T cells and mBSA. These inflammations were not caused by injection of the antigen only as appears from group 2 in Fig. 1.

We also tried to induce flare-up reactions of DTH by systemic (iv and oral), instead of local, administration of the antigen. Groups 1b and 1c (Fig. 1) show that it is possible to induce a small, but significant flare-up response by systemic administration of the antigen (both $P < 0.05$ compared with the response measured after a single injection of the antigen (group 1d)).

DTH reactions could also be induced by iv injection of cloned mBSA-specific helper T cells and local injection of the antigen. Group 1 (Fig. 2) shows the specific increase in foot thickness after iv injection of 1.5×10^6 cloned helper T cells and local injection of the antigen. When the mice were challenged 3 weeks later by another local injection of the same antigen, a flare-up reaction was found (Fig. 2, group 1a).

The induction of DTH by the cloned mBSA-specific T cells was H-2 I-A^b-restricted and a DTH reaction could also be induced in thymusless C57BL/6 nude mice (Fig. 3). The I-E haplotype of the mice is not shown in Fig. 3 as I-E^b is never expressed (11).

The DTH infiltrates induced by injection of cloned mBSA-specific T cells together with mBSA were examined histologically 24 hr after induction of DTH and 24 hr after induction of flare-up reactions by local rechallenge with the antigen.

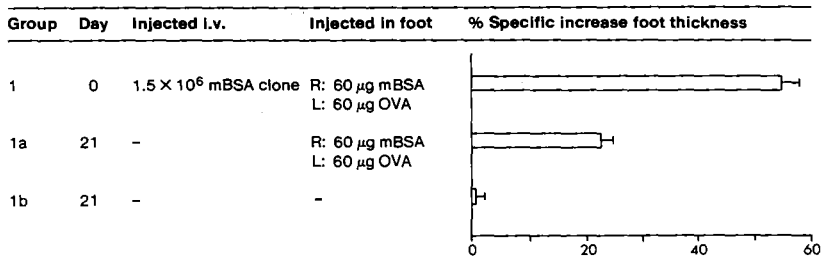


FIG. 2. DTH reaction and flare-up of DTH in mice by iv injected cloned helper T cells. DTH reactions were induced by iv injection of cloned mBSA-specific helper T cells and, immediately thereafter, a sc injection of mBSA or, as a control, OVA into the dorsa of the hind feet. Group 1 was divided into subgroups 1a and 1b; 1a was rechallenged with the same antigens 21 days later and 1b was not. DTH responses are expressed as the specific increase in hind foot thickness (mean ± SEM; $n = 6$).

Twenty-four hours after induction of DTH an infiltrate was observed that largely consisted of granulocytes. Mononuclear cells occurred scattered in the infiltrate. The papillary dermis was swollen by edema (Fig. 4a). In the left foot, into which 3×10^5 mBSA-specific helper T cells were injected together with the irrelevant antigen, OVA, almost no infiltrate was observed (Fig. 4b).

The infiltrate that was seen in the right hind foot 24 hr after induction of a flare-up reaction appeared different from the acute reaction as it was largely composed of histiocytes, while the proportion of granulocytes was considerably less compared to the acute reaction. In addition, during the flare-up reaction the edema was less prominent (Fig. 4c). The left (control) hind feet of the same mice showed a small infiltrate mainly composed of granulocytes and some edema (Fig. 4d).

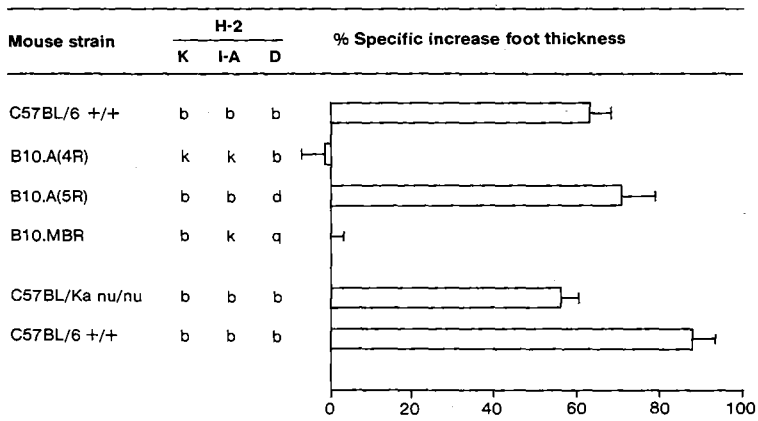


FIG. 3. H-2 I-restricted recognition of mBSA (upper part) and functional activity in athymic nude mice (lower part) by the cloned helper T cells used. The upper part shows the DTH reactivity by 3×10^5 mBSA-specific helper T cells after direct injection into the dorsum of the right hind feet of various H-2 congenic mouse strains together with 20 μg mBSA. As a control 3×10^5 OVA-specific helper T cells were injected into the left hind feet of the same mice together with 20 μg mBSA. DTH responses are expressed as the specific increase in foot thickness (mean ± SEM; $n = 6$). The lower part shows the DTH reactivity by 1.5×10^5 mBSA-specific helper T cells after direct injection into the dorsum of the right hind feet of C57BL +/+ or *nu/nu* mice together with 20 μg mBSA. As a control 1.5×10^5 OVA-specific helper T cells were injected into the left hind feet of the same mice together with 20 μg mBSA. DTH responses are expressed as the specific increase in hind foot thickness (mean ± SEM; $n = 6$).

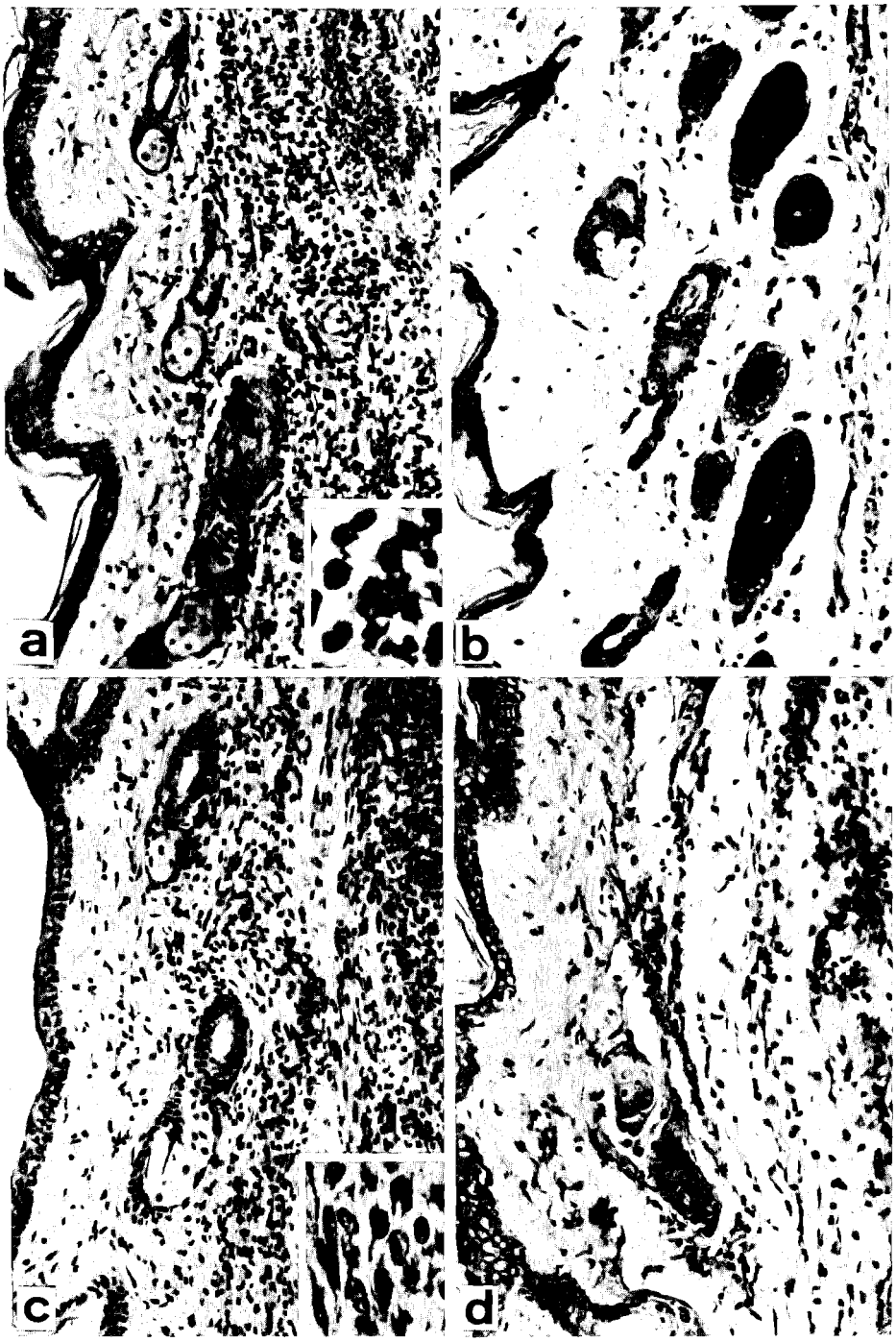


FIG. 4. (a) DTH infiltrate 24 hr after induction of the inflammation by local injection of 3×10^5 mBSA-specific helper T cells and 20 μg mBSA into the right hind foot of a mouse (HE, 60×). The inset shows detail (240×). (b) A left (control) hind foot 24 hr after injection of 3×10^5 mBSA-specific helper T cells and 20 μg OVA (HE, 60×). (c) The flared inflammation of a is shown 24 hr after local rechallenge with 20 μg mBSA (HE, 60×). The inset shows detail (240×). (d) The left (control) hind foot after local rechallenge of b with 20 μg OVA (HE, 60×).

DISCUSSION

The present study shows that it is possible to provoke a flare-up of a DTH reaction initially induced by cloned helper T cells. A flare-up reaction can be induced by a second local administration of antigen. Figure 1 shows that this flare-up was not caused by the antigen injection per se. A control group of mice that was not injected with helper T cells, but whose hind feet were injected twice with the antigens only (right: mBSA; left: OVA), did not show foot swelling. Furthermore, the data show that such flare-up reactions can also be induced by systemic (oral or iv) administration of the antigen. In the antigen-induced arthritis model, mice are immunized with the mBSA antigen in complete Freund's adjuvant. Several weeks later they are challenged with mBSA in a knee joint. This causes joint inflammation. In this model it was shown to be possible to induce flare-up reactions, after waning of the inflammation, by local injection of small amounts of mBSA or by oral or iv administration of the antigen.

Histologically, the acute inflammation measured in our model was characterized by granulocytes and edema, although mononuclear cells also occurred. The flare-up reaction, however, was more histiocytic and showed less edema.

So far we cannot induce a DTH reaction by local injection of cloned helper T cells and a single systemic administration of the antigen immediately thereafter. Maybe it is not possible to reach the high local levels of antigen necessary for the induction of DTH by systemic administration of antigen.

The induction of DTH by iv injection of the cloned helper T cells depends heavily on the migratory capacities of the cloned T cells themselves as the experiments with nude mice (Fig. 3) indicate that host T cells are not involved. Restimulation of the clone with the antigen some days before the iv administration did not enhance subsequent DTH responses. After local injection of the restimulated T cells, a weaker foot swelling was measured as compared to nonrestimulated T cells. In those cases where foot swelling did occur after iv administration of the cloned T cells together with a local injection of the antigen, less foot swelling was measured when the antigen was administered 1, 2, or 3 days after iv infusion of the cloned T cells (data not shown). Problems encountered in *in vivo* migration of cloned T cells were already described by others (12, 13). The T-cell clones used by us were negative for the monoclonal MEL-14, which is specific for the lymphocyte homing receptor (14), also after restimulation (data not shown).

The question arises whether the capacity to mediate a flare-up reaction and, thus, the persistence of the cloned T cells depend on the continued presence of the relevant antigen. In our experiments, the flare-up reactions induced by systemic administration of antigen indicate that locally injected T cells persist at the injection site and do not or hardly at all migrate to other sites. This can be deduced from the observation that systemic administration of the antigen did not cause flare-up reactions in the left hind feet of mice from which both hind feet were injected with the same clone of helper T cells, but whose right hind feet were injected with the homologous antigen and whose left hind feet were injected with a non-cross-reacting antigen. This is consistent with studies on the antigen-induced arthritis model in mice in which it was shown that the positive charge of the antigen mBSA is essential for chronicity of the joint inflammations and for the possibility to induce flare-up reactions (3). Such studies have shown that the antigen attaches to the negatively charged cartilage structures.

Because of this is thought that the antigen-specific T cells persist at the site of antigen deposition.

However, preliminary and contradictory results show that when the mBSA-specific cloned helper T cells are injected locally into a hind foot without the antigen, it was also possible to induce an inflammation by local injection of the antigen several days later. We are currently investigating whether the induction of an inflammation and the presence of the specific antigen are obligatory for the retention of cloned helper T cells.

T cells are thought to play an important role in the etiology and pathogenesis of various autoimmune diseases such as rheumatoid arthritis. Flare-up reactions by specifically sensitized helper T cells as shown in this report might play an important role in the exacerbations of such diseases. Our current studies are aimed at investigating this by using T-cell clones in an arthritis model in the mouse.

ACKNOWLEDGMENTS

The authors thank Miss R. Tees of Sandoz Ltd., Basel, for production of the T-cell clones used, Dr. M. H. Schreier of Sandoz Ltd., Basel, for stimulating discussions, Mr. J. Brandenburg for animal care, and Miss G. de Korte for assistance in the preparation of the manuscript. This investigation was supported by the Netherlands League against Rheumatism.

REFERENCES

1. Cohen, I. R., Ben-Nun, A., Holoshitz, J., Maron, R., and Zerubavel, R., *Immunol. Today* **4,** 227, 1983.
2. Scheper, R. J., Von Blomberg, M., Boerrigter, G. H., Bruynzeel, D., Van Dinther, A., and Vos, A., *Clin. Exp. Immunol.* **51,** 141, 1983.
3. Van den Berg, W. B., Van de Putte, L. B. A., Zwarts, W. A., and Joosten, L. A. B., *J. Clin. Invest.* **74,** 1850, 1984.
4. Bianchi, A. T. J., Hooijkaas, H., Benner, R., Tees, R., Nordin, A. A., and Schreier, M. H., *Nature (London)* **290,** 62, 1981.
5. Brachertz, D., Mitchell, G. F., and Mackay, I. R., *Arthritis Rheum.* **20,** 841, 1977.
6. Van de Putte, L. B. A., Lens, J. W., Van den Berg, W. B., and Kruysen, M. W. M., *Immunology* **49,** 161, 1983.
7. Lens, J. W., Van den Berg, W. B., Van de Putte, L. B. A., and Van den Bersselaar L., *Clin. Exp. Immunol.* **58,** 364, 1984.
8. Tew, J. G., Mandel, T. E., and Rice, P. L., *Immunology* **40,** 425, 1980.
9. Schreier, M. H., Tees, R., Nordin, A. A., Benner, R., Bianchi, A. T. J., and Van Zwieten, M. J., *Immunobiology* **161,** 107, 1982.
10. Iscove, N. N., *In* "Cell Culture Methods for Molecular and Cell Biology. IV. Methods for Serum-Free Culture of Neuronal and Lymphoid Cells" (D. W. Barnes, D. A. Sirbasku, and G. H. Sato, Eds.) pp. 169–185. Liss, New York, 1984.
11. Jones, P. P., Murphy, D. B., and McDevitt, H. O., *Immunogenetics* **12,** 321, 1981.
12. Dailey, M. O., Fathman, C. G., Butcher, E. C., Pillemer, E., and Weissman, I. L., *J. Immunol.* **128,** 2134, 1982.
13. Daily, M. O., Gallatin, W. M., and Weissman, I. L., *J. Mol. Cell. Immunol.* **2,** 27, 1985.
14. Gallatin, W. M., Weissman, I. L., and Butcher, E. C., *Nature (London)* **304,** 30, 1983.

CHAPTER 3

JOINT INFLAMMATION IN MICE INDUCED BY A MT4$^+$,LYT-2$^-$ T CELL CLONE: CHARACTERISTICS AND THE INDUCTION OF FLARE-UP REACTIONS

Ina S. Klasen, Renée M.T. Ladestein, Inge G. Donselaar, Wim B. van den Berg*, Reet Tees# and Robbert Benner

Department of Cell Biology, Immunology and Genetics, Erasmus University Rotterdam, *Department of Rheumatology, University Hospital St. Radboud, Nijmegen, The Netherlands and #Department for Preclinical Research, Sandoz Ltd., Basel, Switzerland.

This paper has been published in The Journal of Immunology 139: 3275-3280 (1987).

ABSTRACT

Joint inflammations were induced in mice by cloned $MT4^+$,Lyt-2^- T cells specific for methylated bovine serum albumin (mBSA). This was done either by intra-articular (ia) or by intravenous (iv) administration of the cloned T cells, together with local injection of the antigen. Local rechallenge with mBSA several weeks after waning of the joint inflammation caused a flare-up reaction. The inflammations were quantified by a ^{99m}Technetium (Tc)-uptake method and examined histologically. The arthritis induced by the cloned T cells showed aspects of a delayed type hypersensitivity reaction characterized by an intense infiltrate which resembles the inflammation in the human rheumatoid joint.

The data presented show that joint inflammations can be induced by T cells only and that after waning, reexposition to the original antigen can induce a flare-up reaction. The data suggest a central role of T cells in the induction and the exacerbations observed in rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis shares several characteristics with delayed type hypersensitivity (DTH) reactions (1). As clones of helper T cells can induce DTH reactions in mice (2) we tested whether our T cell clones were also able to induce joint inflammations. Therefore we used a T cell clone directed against methylated bovine serum albumin (mBSA).

The antigen mBSA has been used before in the antigen induced arthritis (AIA) model in mice (3,4). The AIA is a model system for human rheumatoid arthritis and was first described in 1962 by Dumonde and Glynn in rabbits (5). To induce AIA it is necessary to immunize the animals with antigen emulsified in complete Freund's adjuvant. When 2 or 3 weeks later the antigen is injected directly into the knee joint of the sensitised animal, an inflammation occurs which resembles the inflammation in the human rheumatoid joint (6,7,8).

mBSA, which has a positive net charge because of the methylation, is a suitable antigen in this model. This antigen is retained in the cartilage structures, which have a negative charge. The retention of the antigen in the joint is thought to be important for chronicity of the inflammation (9). In transfer studies it was demonstrated that the development of a chronic AIA is T cell dependent (10).

In the AIA model it has been shown that flare-up reactions can be induced after waning of the inflammation. This is achieved by reinjection of the antigen in the knee joint, but also by intravenous (iv) or oral readministration of the antigen (11,12).

We have previously shown that it is possible to induce flare-up of a waned DTH reaction that had been induced by mBSA specific cloned T cells, as was determined in a foot swelling assay (13). After having established that the cloned T cells can induce joint inflammations in mice, we investigated whether such reactions, after waning, can show flare-up phenomena after injection of the antigen only. This paper describes the characteristics of such flare-up reactions.

MATERIALS AND METHODS

Mice

C57BL/6J mice were purchased from OLAC Ltd., Bicester, UK., or from Bomholtgard, Ry, Denmark. B10.A(4R) and B10.A(5R) mice were also purchased from OLAC. C57BL/6J nu/nu mice were purchased from Bomholtgard. B10.MBR mice were bred in our own department. In all experiments female mice were used at the age of 2-3 months.

Cloned $MT4^+$,Lyt-2^- T cells

A T cell clone directed against mBSA was made. As a control clone an ovalbumin (OVA) specific T cell clone (OVA-7) was used. The procedures for the generation of T cell clones and some characteristics of the OVA specific T cell clone have been described elsewhere (14). The clones were of C57BL/6J origin and were cultured in serum-free medium (IMDM-ATL) containing 1 g/l BSA, 241 mg/l human transferrin (1/3 Fe saturated) (both from Hoechst-Behringwerke, Marburg, F.R.G.) and 50 mg/l soybean lipids (Astec FLPV obtained from Associated Concentrates, New York, U.S.A.) (15). Every 5-6 weeks the clones were restimulated with the antigens (5 μg/ml mBSA was optimal for the mBSA specific clone, 300 μg/ml OVA for the OVA specific clone). Irradiated (3000 Röntgen) syngeneic spleen cells were used as a source of antigen presenting cells. After three days the stimulated T cells were transferred to medium containing 600 U/ml recombinant human Interleukin 2 (IL-2), which was a kind gift of Dr. J. Besemer from Sandoz Ltd., Vienna, Austria. Both the clones were of the $MT4^+$,Lyt-2^- phenotype.

The cloned T cells were transferred to fresh culture medium in a one to ten dilution two times a week. Before they were injected *in vivo* they were refreshed at least three times. The irradiated antigen presenting cells died in the first week after restimulation and the adherent cells were not transferred to the fresh cultures. This means that virtually no antigen presenting cells and antigen were present in the inoculum used for *in vivo* injection.

Induction of arthritis and induction of flare-up reactions

Arthritis was induced in knee joints in several ways. (a) By injection of cloned T cells together with the antigen mBSA or OVA in 10 μl balanced salt solution (BSS) into a knee joint. (b) By injection of a dose of mBSA

or OVA in 6 μl BSS, 0.5 to 1 hour later followed by intra-articular (ia) injection of the cloned T cells into the same joint. (c) By iv injection of the cloned T cells in 0.5 ml BSS, directly followed by ia injection of the mBSA or OVA. Flare-up reactions were induced by local administration of the homologous antigen 1.5-3 weeks after the joint inflammation was induced. Primary arthritis could also be induced by injection of cloned OVA specific T cells together with the antigen OVA (data not shown). Significance of the difference between various experimental groups was determined by the Student's t test.

^{99m}Tc-uptake measurements

Measurement and quantitation of joint swelling caused by joint inflammation of the right knee was performed by the ^{99m}Tc-uptake method. This method has been described in detail elsewhere (16). Briefly 20 μCi (0.74 MBq) ^{99m}Technetium-pertechnetate was injected subcutanously (sc). Forty minutes later the uptake by the knee joints was measered by external gamma counting. The animals were sedated 10 minutes before starting the measurements. The uptake of both knee joints was measured three times with a duration of ten seconds. The severity of the inflammatory swelling in the right knee was expressed as the specific increase of ^{99m}Tc- uptake and was calculated as [(uptake right knee - uptake left knee)/uptake left knee] x 100%. 24 and 48 hrs after induction the inflammations were measured. In the figures 24 hour values, designated as specific increase of knee thickness, are presented unless indicated otherwise.

The correlation between the ^{99m}Tc-uptake and the histological joint inflammation scores is examined by Lens et al. (17). In our experiments an increased ^{99m}Tc-uptake was always caused by a perceptible joint inflammation upon histological examination.

Histology

Both hind knee joints of the same mouse were removed in toto, fixed in 10% phosphate-buffered formalin and decalcified in 5% formic acid. Standard frontal sections (6 μm) were prepared of paraffin-wax embedded knee joints and stained with hematoxylin and eosin.

RESULTS

Induction of joint inflammations by a $MT4^+$,$Lyt2^-$ T cell clone

We firstly examined the joint inflammations induced by local injection of cloned T cells and the relevant antigen. To determine the best conditions for induction of joint inflammation we defined the optimal antigen dose. As we were afraid that high doses of the cationic antigen might affect the functional activity of the cells, we compared the joint inflammations induced by injection of a mixture of cloned T cells and the antigen, and by separate injection of the cloned T cells and the antigen. Fig. 1 shows the inflammation measured 24 hours after injection of 3 x 10^5 mBSA specific cloned T cells into the right knee joint and 3 x 10^5 OVA specific cloned T cells into the left knee joint of the mice. The upper two bars show the responses measured when the T cells were injected together with the antigens, while the next four bars show a dose response curve of the antigen when the antigen was injected 0.5-1 hour before the injection of the cloned T cells. It can be concluded that an antigen dose of 60 μg mBSA injected separately from the T cells is optimal for induction of a joint inflammation by the cloned T cells used. The lowest bar shows the

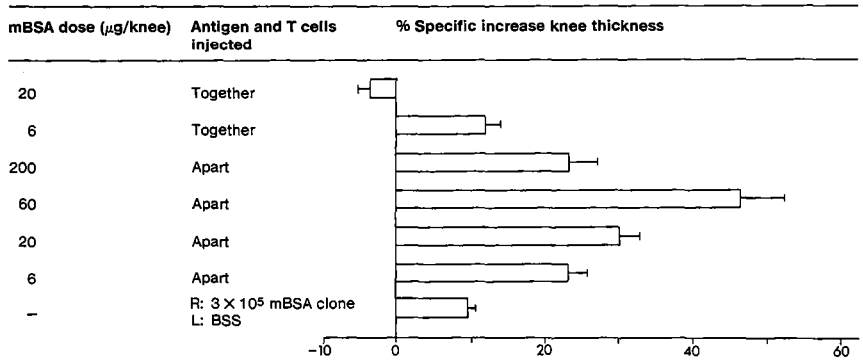


Fig. 1. Influence of mBSA dose on the induction of joint inflammation by mBSA specific cloned T cells. Joint inflammations were induced by injection of 3 x 10^5 mBSA specific cloned T cells into the right knee joint and 3 x 10^5 OVA specific T cells into the left knee joint together with mBSA, or by injection of different mBSA doses followed by injection of the cloned T cells 0.5-1 hr later. The lowest bar shows the ^{99m}Tc-uptake measured 24 hours after injection of 3 x 10^5 mBSA specific cloned helper T cells into the right knee joint and BSS into the left knee joint.

The responses are expressed as specific increase in knee thickness (mean ± SEM). Data of the upper six bars represent the mean of three experiments each involving 5 or 6 mice. The lowest bar represents one experiment involving 5 mice.

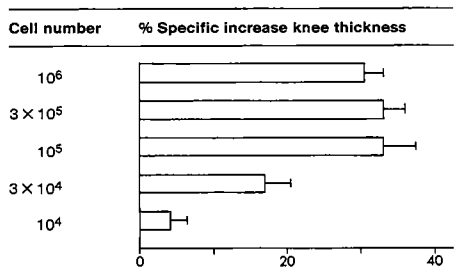


Fig. 2. Joint inflammation inducing capacity of different numbers of mBSA specific cloned T cells. A dose of 60 μg mBSA was injected into the right and the left knee joint, 0.5-1 hr later followed by different numbers of cloned T cells. mBSA specific cloned T cells were injected into the right knee joint, while OVA specific T cells were injected into the left knee joint. The responses are expressed as specific increase in knee thickness (mean ± SEM; n=6).

^{99m}Tc-uptake measured 24 hours after injection of 3 x 10^5 mBSA specific cloned helper T cells into the right knee joint and BSS into the left knee joint.

In Fig. 2 a dose-response curve is shown of the joint inflammation inducing capacity of different numbers of cloned T cells. This was measured 24 hours after injection of mBSA specific cloned T cells into the right knee joint and OVA specific cloned T cells into the left knee joint. 60 μg of mBSA was injected in both knee joints 0.5-1 hr before the cloned T cells were injected. Fig. 2 shows a plateau in severity of joint inflammation at 10^5 cloned T cells per knee joint.

The recognition of mBSA by the cloned T cells in the knee joint is subject to H-2I restriction. This was apparent from experiments involving the injection of 3 x 10^5 mBSA specific cloned T cells in the right as well as into the left hind knee joint of mice that had shortly before been injected with 60 μg mBSA into the right knee joint and with 60 μg of te non-crossreacting antigen OVA into the left knee joint. Fig. 3 shows that the joint inflammation was the most severe in C57BL and B10.A(5R) mice, which express H-2-IA^b antigens. In Fig. 3 the I-E haplotype is not mentioned as H-2I-E^b is not expressed (18). The lower line of Fig. 3 shows that part of the inflammation measured is caused by injection of the antigens only.

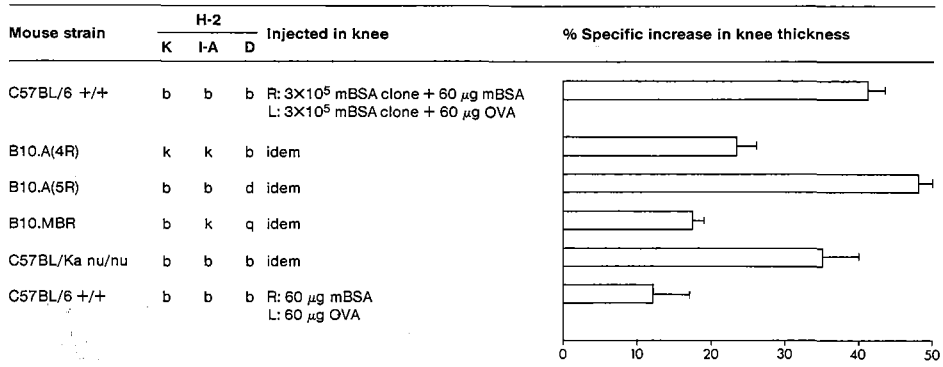


Fig. 3. H-2 restriction of the induction of joint inflammation by cloned T cells and induction of joint inflammation in nude mice. 60 μg of mBSA was injected into the right knee joint and 60 μg of OVA into the left knee joint of the mice. This was followed by local injection of 3 x 10^5 mBSA specific cloned T cells. A control group received the antigens only. The responses are expressed as specific increase in knee thickness (mean ± SEM; n=6).

Fig. 3 also shows that the cloned $MT4^+$,$Lyt2^-$ T cells can mediate a joint inflammation in H-2 compatible thymusless nude mice, indicating that T cells of the recipient mice are not necessary for induction of joint inflammation.

Induction of flare-up reactions of joint inflammation

In the antigen induced arthritis model it is possible to induce flare-up reactions of waned joint inflammations by local or systemic rechallenge with the homologous antigen (10,11). We examined whether waned joint inflammations that had been induced by cloned T cells can also flare-up. Therefore we rechallenged the mice with various doses of mBSA 13 days after induction of a joint inflammation by local injections of 60 μg mBSA and 3 x 10^5 mBSA specific cloned T cells. Fig. 4 shows that local rechallenge with 60 μg of mBSA induces the highest flare-up reaction. The lowest bar in the figure shows the response measured when no antigen was administered again. This represents the chronic, smouldering phase of the joint inflammation.

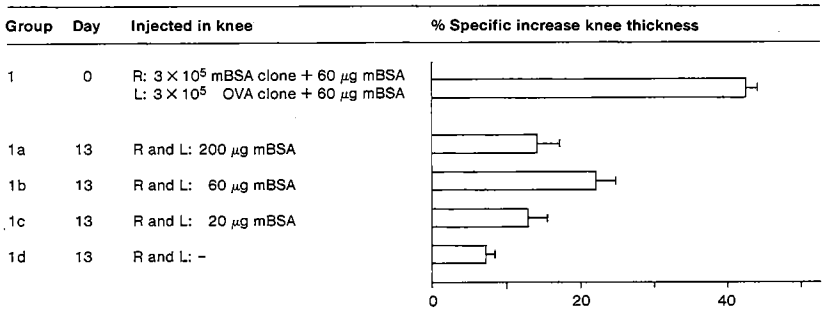


Fig. 4. Influence of the mBSA dose on the induction of a flare-up reaction. On day 0 a joint inflammation was induced in group 1 by injection of 60 μg mBSA into the right and the left knee joint, 0.5-1 hr later followed by injection of 3 x 10^5 mBSA specific cloned T cells into the right knee joint and 3 x 10^5 OVA specific T cells into the left knee joint. On day 13 group 1 was split into 4 subgroups (1a-d) of which groups 1a, 1b and 1c were rechallenged with different doses of the mBSA. Group 1d was not rechallenged. The responses are expressed as specific increase in knee thickness (mean ± SEM; n=6).

Subsequently we investigated the time course of the joint inflammation induced by mBSA specific cloned T cells and of the flare-up reaction of such a joint inflammation. We therefore injected 60 μg of mBSA into the right knee joint of the mice and 60 μg of OVA into the left knee joints, followed by injection of 3 x 10^5 mBSA specific cloned T cells into both knee joints. The time course of the induced joint inflammation is shown in Fig. 5. As a control we studied a group of mice that was injected with the antigens only, and not with the cloned T cells. On day 14 half of both groups of mice were rechallenged with the homologous antigens. As can be concluded from Fig. 5, only the mice that had received the cloned T cells on day 0 showed a flare-up reaction in their right knee joint. As the control group did not show an inflammatory reaction after local re-challenge there is no evidence for a contribution of sensibilisation of the recipient mice in the flare-up reaction measured.

Fig. 6 shows that a joint inflammation can also be induced by systemic administration of the cloned T cells. 1.5 x 10^6 mBSA specific cloned T cells were injected iv, while 60 μg mBSA was injected locally into the right knee joint and 60 μg of OVA into the left knee joint. 24 Hours later

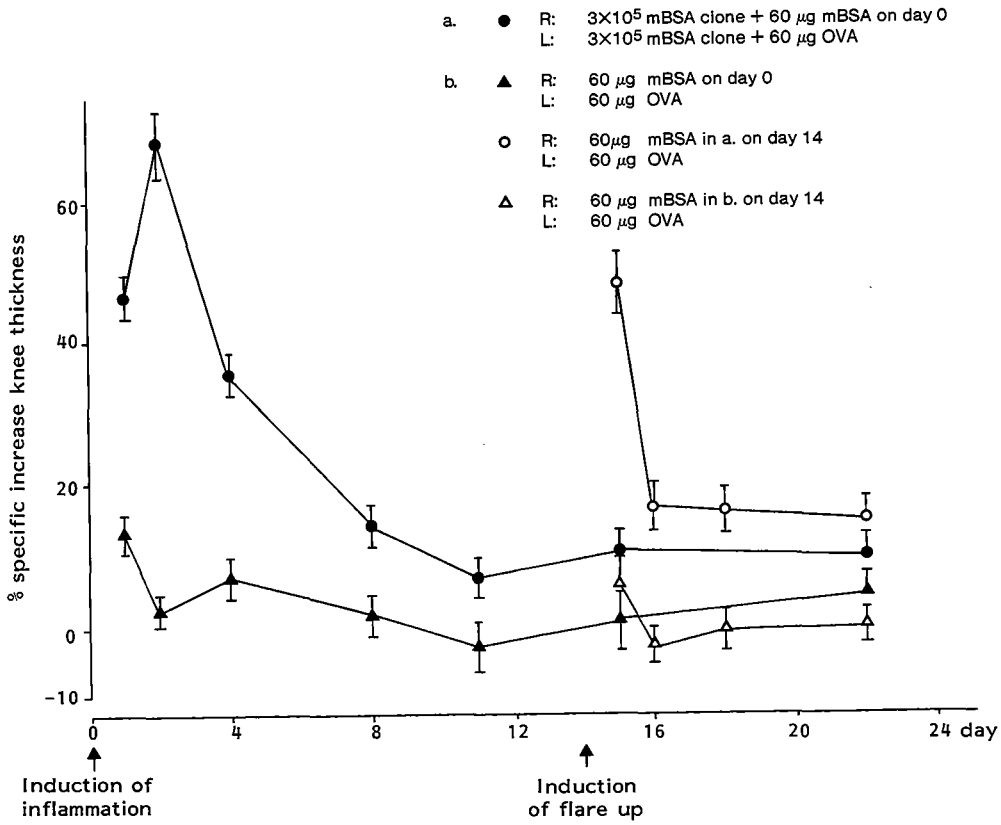


Fig. 5. Kinetics of the joint inflammation and the flare-up reaction mediated by cloned T cells. On day 0 a joint inflammation was induced as described in Fig. 3. A control group received the antigens only. On day 14 both groups were split into two subgroups of which one subgroup was rechallenged by injection of 60 µg mBSA into the right knee joint and 60 µg OVA into the left knee joint. The other group was not rechallenged. The responses are expressed as specific increase in knee thickness (mean ± SEM; n=6).

a joint inflammation was measured. Three weeks later half of this group of mice were rechallenged by local reinjection of the homologous antigens. This caused a flareup reaction. The lowest bar in Fig. 6 shows the inflammation that was measured three weeks after induction of the joint inflammation, and thus represents the chronic phase of the inflammation.

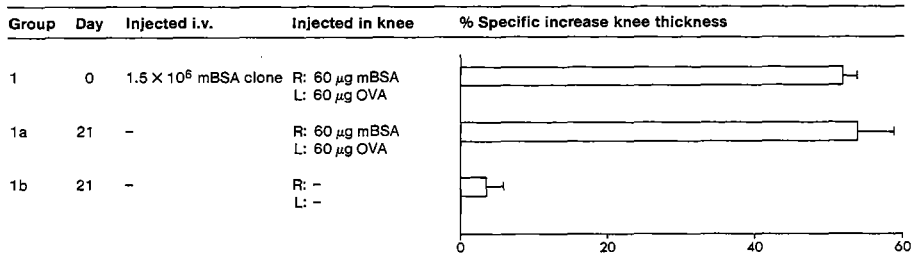


Fig. 6. Flare-up reaction of a joint inflammation induced by iv injection of cloned T cells. On day 0 a joint inflammation was induced in group 1 by iv injection of 1.5 x 10^6 mBSA specific cloned T cells and injection of 60 µg mBSA into the right knee joint and 60 µg OVA into the left knee joint. 21 Days later group 1 was split into the subgroup 1a and 1b, of which subgroup 1a was rechallenged with 60 µg of the homologous antigens (right: 60 µg mBSA, left: 60 µg OVA) and subgroup 1b was not rechallenged. The responses are expressed as specific increase in knee thickness (mean ± SEM; group 1: n=12; subgroup 1a: n=4; subgroup 1b: n=3).

Histological examination of the joint inflammations and their flare-up reactions

The joints of the mice used for Fig. 6 were examined histologically. In the right knee 24 hours after induction of the inflammation an infiltrate was observed that largely consisted of granulocytes with small numbers of lymphocytes and macrophages. In the left knee of the same mice - in which the irrelevant antigen OVA was injected - only a small infiltrate was seen with some edema and few granulocytes. The infiltrate found during the flare-up reaction was comparable to the one seen 24 hours after the induction of the inflammation (Fig. 7). However in some flare-up experiments we saw a larger percentage of mononuclear cells in combination with fibrosis, likely as a result of the smouldering inflammation which was still present before the flare-up reaction was induced. In the control group where no flare-up reaction was induced, foci of inflammation were present and a higher percentage of monocytes along with some granulocytes. Pannus formation and cartilage destruction were not generally observed.

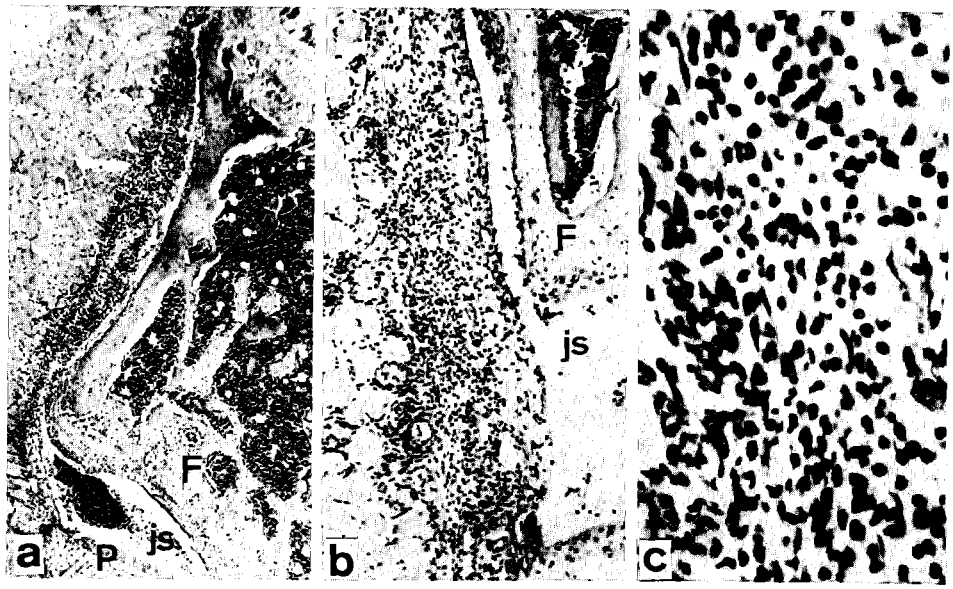


Fig. 7. (a) Histology of the arthritic inflammation 24 hrs after induction by iv injection of 1.5 x 10^6 mBSA specific cloned T cells and injection of 60 μg mBSA into the right knee joint (HE; 5.4x). (b) Flared arthritis 24 hrs after local rechallenge with 60 μg mBSA (HE; 14x).(c) Detail of (b) which shows the polymorphonuclear infiltrate (HE; 54x). P is patella, F is femur, js is joint space.

DISCUSSION

The data presented here show that a joint inflammation can be induced in mice by cloned T cells with the $MT4^+$,$Lyt\text{-}2^-$ phenotype. We were able to induce these inflammations by intra-articular injection of the T cells in joints in which the antigen had already been injected, but also by iv injection of the T cells in combination with intra-articular injection of the antigen (Fig. 6). The latter method is of course less artificial and therefore a better model for joint inflammation.

It is clear that the induction of joint inflammation by iv injection of cloned T cells heavily depends on the migratory capacities of these T cells, as cloned T cells tend to be retained in the lungs and the liver (19). Restimulation of the mBSA specific T cell clone with mBSA did not

enhance inflammatory responses after local or systemic administration, as determined in a foot swelling assay (data not shown).

The joint inflammations could also be induced in H-2 compatible athymic nude mice (Fig. 3), which indicates that T cells of the recipient mice are not necessary for the joint inflammation. The joint inflammation induced by the cloned T cells was highest in mice expressing H-2I-A^b antigens. The H-2 restriction was not absolute, since in mice with another H-2I-A haplotype also a joint inflammation was found, though weaker than in H-2I-A^b recipient mice. Part of this inflammation is caused by the injection of the antigens only, possibly because mBSA tends to cause damage when injected in high concentrations.

The inflammation induced by the cloned mBSA specific $MT4^+$,Lyt-2^- T cells is of a chronic nature. As in the AIA model, severe inflammation lasts several days (Fig. 5). Although the inflammation wanes within 7 days as was determined in ^{99m}Tc-uptake measurements, a low grade chronic infiltrate can be seen histologically at day 21 and flare-up reactions can be induced by local rechallenge with the antigen only. This flare-up reaction cannot be attributed to an immunization effect, because two intra-articular injections of 60 μg mBSA two weeks apart led to a minor specific Tc uptake, indicating a negligible inflammation. This was confirmed histologically.

The dose of mBSA required for inducing a flare-up reaction is relatively high. This is in contrast to the AIA model where intra-articular injection of nanogram amounts is sufficient to induce a flare-up of the inflammation. This discrepancy might be due to the immunization of the mice in the AIA model or to the fact that the T cell clone used needs a rather high antigen dose for optimal functional activity.

In the AIA model flare-up reactions can also be induced by administration of the antigen systemically, namely iv or orally. We are currently investigating whether this is also possible in our model.

Up to now we did not succeed in inducing a joint inflammation by local injection of the naive mice with cloned T cells and a single systemic administration of the antigen directly thereafter. Maybe it is not possible to reach local levels of antigen that are high enough for the induction of joint inflammation by systemic administration of antigen.

mBSA has been extensively used in AIA studies in which mice are severely immunized with the antigen and the inflammation is induced by local injection of the antigen into the joint. mBSA is an appropriate antigen for

such studies because it is retained in the cartilage structures because of charge interactions (9). It is thought that because of this the antigen specific T cells persist at the site of antigen deposition. However, preliminary and contradictory results show that when the mBSA specific cloned T cells are injected intra-articularly without the antigen it is also possible to induce an inflammation by local injection of the antigen several days later. Experiments are in progress to investigate whether the induction of an inflammation and the presence of the specific cationic antigen are obligatory for the retention of T cells.

Histologically a substantial inflammatory reaction was seen in the joints of mice injected with the cloned mBSA specific T cells and mBSA. This was the case after local as well as after iv injection of the cloned T cells. These infiltrates were mainly composed of granulocytes and could not be discriminated from the inflammations induced in the AIA model. The predominantly granulocytic nature of T cell dependent inflammatory reactions is characteristic for Jones-Mote type DTH reactions in mice (14). At 2-3 weeks after induction of the joint inflammation, a more monocytic infiltrate in combination with fibrosis was observed in some cases, which indicates a more chronic type of inflammation. When such mice were rechallenged, again a granulocytic infiltrate was observed, in some cases in combination with a larger percentage of monocytes and fibrosis, likely the result of the smouldering inflammation, which was still present before the flare-up reaction was induced. This was comparable with the flare-up reactions of DTH inflammations induced by cloned T cells (13).

The inflammations in the AIA model (12) and the rheumatoid joint (1) both show aspects of a DTH reaction. In the mouse AIA model, flare-up reactions can be induced analogous to the exacerbations seen in human rheumatoid arthritis. The data presented here show that similar joint inflammations and flare-up reactions can be induced by cloned T cells with the $MT4^+$,$Lyt\text{-}2^-$ phenotype. These observations support the notion (20,21) that autoimmunities like rheumatoid arthritis might well be caused by T cells only.

ACKNOWLEDGMENTS

The authors thank Miss W.A. Zwarts for preparation of the histological sections, Dr. Th.H. van der Kwast for examination of the histology, Dr. M.H Schreier of Sandoz Ltd. Basel for stimulating discussions, the team of the department of Nuclear Medicine of the Dijkzigt Hospital for pre-

paration of the ^{99m}Tc-pertechnetate, Mr. J. Brandenburg for animal care and Miss G. de Korte for assistance in the preparation of the manuscript. Mr. T van Os is gratefully acknowledged for expert photography.

REFERENCES

1. Klareskog, L., U. Forsun, A. Scheynius, D. Kabelitz, and H. Wigzell. 1982. Evidence in support of a self-perpetuating HLA-DR dependent delayed-type cell reaction in rheumatoid arthritis. Proc. Natl. Acad. Sci. U.S.A. 79: 3632.
2. Bianchi, A.T.J., H. Hooijkaas, R. Benner, R. Tees, A.A. Nordin, and M.H. Schreier. 1981. Clones of helper T cells mediate antigen-specific H-2 restricted DTH. Nature 290: 62.
3. Brackertz, D., G.F. Mitchell, and I.R. Mackay. 1977. Antigen induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum. 20: 841.
4. Van den Berg, W.B., H.J. van Beusekom, L.B.A. van de Putte, W.A. Zwarts, and M. van der Sluis. 1982. Antigen handling in antigen-induced arthritis in mice. Am. J. Pathol. 108: 9.
5. Dumonde, D.C., and L.E. Glynn. 1962. The production of arthritis in rabbits by an immunological reaction to fibrin. 1962. Brit. J. Exp. Path. 43: 373.
6. Glynn, L.E. 1968. The chronicity of inflammation and its significance in rheumatoid arthritis. Ann. Rheum. Dis. 27: 105.
7. Steinberg, M.E., C.R. McCrae, L.D. Cohen, and H.R. Schumacher. 1973. Pathogenesis of antigen induced arthritis. Clin. Orthop. Rel. Res. 97: 248.
8. Gall, E.P., and E.A. Gall. 1980. Histopathogenesis of bovine serum albumin-induced arthritis in the rabbit. J. Rheumatol. 7: 13.
9. Van den Berg, W.B., L.B.A. van de Putte, W.A. Zwarts, and L.A.B. Joosten. 1984. Electrical charge of the antigen determines intra-articular antigen handling and chronicity of arthritis in mice. J.Clin Invest. 74: 1850.
10. Brackertz, D., G.F. Mitchell, M.A. Vadas, and I.R. MacKay. 1977. Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. J. Immunol. 118: 1645.
11. Van de Putte, L.B.A., J.W. Lens, W.B. van den Berg, and M.W.H. Kruysen. 1983. Exacerbation of antigen-induced arthritis after challenge with intravenous antigen. Immunology 49: 161.
12. Lens, J.W., W.B. van den Berg, L.B.A. van de Putte, and L. van den Bresselaar. 1984. Flare-up of antigen-induced arthritis in mice after challenge with oral antigen. Clin. Exp. Immunol. 58: 364.
13. Klasen, I.S., Th.H. van der Kwast, I.G. Donselaar, R.M.T. Ladestein, and R. Benner. 1987. Flare-up of delayed type hypersensitivity initially induced by murine cloned helper T cells. Cellular Immunol. 108: 235.
14. Schreier, M.H., R. Tees, A.A. Nordin, R. Benner, A.T.J. Bianchi and M.J. van Zwieten. 1982. Functional aspects of helper T cell clones. Immunobiology 161: 107.
15. Iscove, N.N. 1984. Culture of lymphocytes and hemopoietic cells in serum-free medium. In Cell Culture Methods for Molecular and Cell Biology IV. Methods for Serum-Free Culture of Neuronal and Lymphoid Cells. (Eds D.W. Barnes, D.A. Sirbasku and G.H. Sato) Allen R. Liss, New York. Pp. 169-185.

16. Kruysen, H.W.M., W.B. van den Berg, L.B.A. van de Putte, and W.J.H. van den Broek. 1981. Inflammation revised: general topics. Detection and quantification of experimental joint inflammation in mice by measurement of ^{99m}Tc-pertechnetate uptake. Agents and Actions 11:640.
17. Lens, J.W., W.B. van den Berg and L.B.A. van de Putte. 1984. Quantification of arthritis by ^{99m}Tc-uptake measurements in the mouse knee-joint: correlation with histological joint inflammation scores. Agents and Actions 14: 723.
18. Jones, P.P., D.B. Murphy, and H.O. McDevitt. 1981. Variable synthesis and expression Eα and A_C (Eβ) Ia polypeptide chains in mice of different H-2 haplotypes. Immunogenetics 12: 321.
19. Daily, M.O., C.G. Fathman, E.C. Butcher, E. Pillemer, and I.L. Weissman. 1982. Abnormal migration of T lymphocyte clones. J. Immunol. 128: 2134.
20. Holoshitz, J., Y. Naparastek, A. Ben-Nun, and I.R. Cohen. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 219: 56.
21. Zamvil, S., P. Nelson, J. Trotter, D. Mitchell, R. Knobler, R. Fritz, and L. Steinman. 1985. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. Nature 317: 355.

CHAPTER 4

REQUIREMENTS FOR FLARE-UP REACTIONS OF JOINT INFLAMMATIONS IN MICE INDUCED BY CLONED $MT4^+$,$LYT\text{-}2^-$ T CELLS

Ina S. Klasen, Renée M.T. Ladestein, Wim B. van den Berg* and Robbert Benner

Department of Cell Biology, Immunology and Genetics, Erasmus University Rotterdam and *Department of Rheumatology, University Hospital St. Radboud, Nijmegen, The Netherlands

Arthritis and Rheumatism, in press.

ABSTRACT

Joint inflammation was induced in C57BL/6 mice by injection of cloned $MT4^+$,Lyt-2^- T cells specific for the antigen methylated bovine serum albumin (mBSA) together with mBSA. In this model flare-up reactions can be induced by a rechallenge with the specific antigen after waning of the inflammation. In this paper we show that such flare-up reactions can still be induced several weeks after waning of the joint inflammation. This was found for both normal C57BL/6 mice and athymic C57BL nude mice. The latter indicates that T cells of the recipient mice are not necessary for the elicitation of flare-up reactions. The inflammatory infiltrates in the knee joints of the nude mice were histologically examined. The infiltrates appeared to be mainly granulocytic in nature.

The cloned T cells were found to persist and to remain functionally reactive in the knee joint for at least two weeks in the absence of the antigen and thus in the absence of an inflammation. In view of the similarities between joint inflammations induced in mice by this $MT4^+$,Lyt-2^- T cell clone and human reumatoid arthritis these data may well be relevant for understanding the processes involved in the latter disease.

INTRODUCTION

In the antigen induced arthritis (AIA) model animals are immunized with a suitable soluble antigen in Complete Freund's Adjuvant and intra-articularly (ia) challenged with the same antigen several weeks later. Subsequently the animals may develop an arthritis. This model for human rheumatoid arthritis was first described in rabbits (1). When mice are immunized with positively charged antigens, e.g. methylated bovine serum albumin (mBSA), a chronic arthritis may develop. It has been shown that the electrical charge of the antigen is important for the induction of a chronic arthritis (2-5). Positively charged antigens are retained in the cartilage structures most likely as a result of charge interactions to the negatively charged cartilage structures (2). These cationic antigens can also induce good T cell responses (5). Joint inflammations induced by positively charged antigens have been shown to exhibit flare-up reactions when the animals are rechallenged with the antigen. Such flare-up reactions were found after local as well as after systemic (intravenous or oral) administration of the antigen (6,7). The general idea is that the antigen reactive T cells remain in the synovia as the antigen persists there.

The antigen induced arthritis is T cell dependent as was shown in transfer experiments (8), and by the lack of arthritis induction in athymic nu/nu mice (9). As it was already known that T cells with the helper phenotype can induce delayed type hypersensitivity (DTH) reactions (10), we investigated the role of such T cells in this T cell dependent animal model for human rheumatoid arthritis.

We have previously shown that it is possible to induce arthritis in mice by local or intravenous injection of cloned mBSA specific T cells with the $MT4^+$,$Lyt\text{-}2^-$ phenotype and local injection of mBSA (11,12). The induction of joint inflammation by these T cells was H-2I-A restricted and independent of T cells of the recipient mice as the joint inflammation could also be induced in athymic nude mice (12). This suggests a central role for T cells in the inflammations and exacerbations of human rheumatoid arthritis. In the present study we examined some prerequisites for the induction of flare-up reactions of joint inflammations evoked by injection of mBSA-specific cloned T cells with the $MT4^+$,$Lyt\text{-}2^-$ phenotype and the antigen.

MATERIALS AND METHODS

Mice

C57BL/6J mice were purchased from Olac, Bicester, U.K. and Bomholtgard, Ry, Denmark. C57BL/6J nu/nu mice were purchased from Bomholtgard. In all experiments female mice were used at the age of 2 to 3 months.

Cloned $MT4^+$,Lyt-2^-T cells

A T cell clone derived from C57BL/6J origin was used. The production and culture conditions optimal for the cloned T cells have been described previously (12,13). The mBSA specific cloned T cells were restimulated with the antigen mBSA every 5 to 6 weeks (5 μg/ml was optimal; mBSA was purchased from Sigma Chemical Company, St. Louis, Missouri, USA) using irradiated (30 Gy) syngeneic spleen cells as a source of antigen presenting cells. After 3 days the cells were transferred to medium containing 600 U/ml recombinant human interleukin 2, which was a kind gift of Dr. J. Besemer from Sandoz Ltd., Vienna, Austria. The cloned T cells were transferred to fresh culture medium in a 1 to 10 dilution twice a week. This was done at least three times after restimulation before the cloned T cells were injected in vivo. The inoculum used did contain virtually no antigen presenting cells as the irradiated cells died in the first week after restimulation and the adherent cells were not transferred to the fresh culture medium.

Induction of joint inflammations and flare up reactions

Joint inflammation was induced by local injection of mBSA into the right knee joint and ovalbumin (OVA) into the left knee joint in 6 μl balanced salt solution (BSS), 0.5-1 hr later followed by local injection of the cloned mBSA specific T cells in 10 μl BSS, unless indicated otherwise. Flare-up reactions were induced by a second administration of the antigens in 10 μl BSS into the right and the left knee joint several weeks later.

^{99m}Technetium- uptake measurements

Joint swelling of the right knee was quantified by a ^{99m}Technetium(Tc)-uptake method, described previously (14). ^{99m}Tc is an isotope with a half life of only 6 hrs. For this reason the absolute uptake values are variable between the various mice in the experiments. Therefore, the inflammatory

swelling measured was expressed as the specific increase in knee thickness which was calculated as [(uptake right knee - uptake left knee)/uptake left knee] x 100%. 24 and 48 hrs after induction the swelling was measured. In the figures 24 hour values are expressed. The correlation between the ^{99m}Tc-uptake and the histologic joint inflammation has been examined by Lens et al. (15). In the work as it is described here, knee joints with an increased Tc-uptake always showed histologic signs of inflammation. Significance of the differences measured in the various experimental groups was determined by the Student's t-test.

Histology

Hind knee joints of the mice were removed *in toto*, fixed in 10% phosphate-buffered formalin and decalcified in 5% formic acid. Standard frontal sections (6 μm) were prepared of paraffin-wax embedded knee joints and stained with hematoxylin and eosin (HE).

RESULTS

We first examined how long after induction of a joint inflammation a flare-up reaction could be induced. Therefore, on day 0 we injected MT4$^+$,Lyt-2$^-$ mBSA specific cloned T cells and mBSA into the right knee joints of C57BL/6J mice, and mBSA specific cloned T cells and the non-crossreacting antigen OVA into the left knee joint. 24 Hours later a significant joint swelling could be measured in the right knee, as was determined by ^{99m}Tc-uptake. Subgroups of these mice were rechallenged with mBSA and OVA at 14, 21, 28 and 42 days after induction of the joint inflammation. The knee swelling was measured 24 hrs later. At these different intervals after the primary joint inflammation a significant flare up reaction could be induced by ia rechallenge with the antigens (Fig. 1). The flare-up reactions, however, were weaker when induced at the later points of time. 42 Days after induction of the joint inflammation a small but significant flare-up reaction was still measurable ($P<0.05$). Two ia injections with only the antigens several weeks apart resulted in a minor joint inflammation. The values measured in these control groups injected with the antigens only were subtracted from the values measured in the groups in which also cloned T cells were ia injected on day 0. Two weeks after induction of

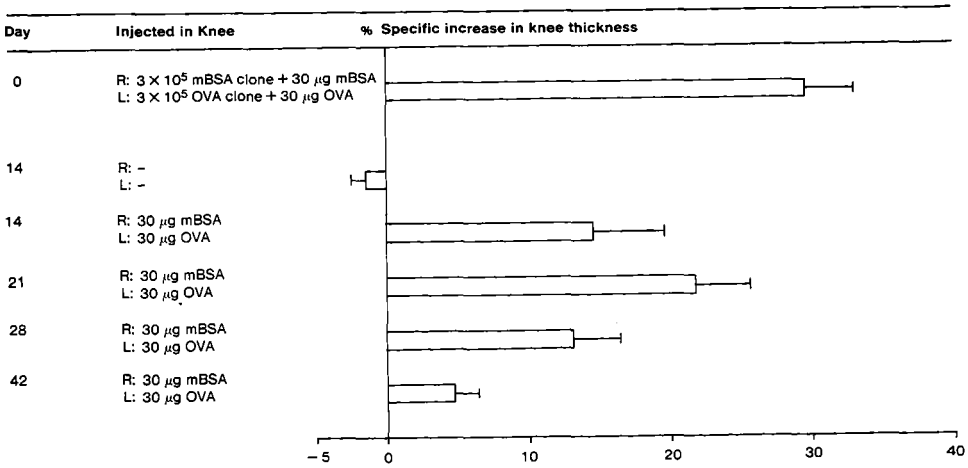


Fig. 1. Flare-up reactions evoked at various intervals after induction of a joint inflammation by cloned mBSA specific T cells and the antigen mBSA. At day 0 30 μg mBSA was injected into the right knee joint and 30 μg OVA into the left knee joint. This was 0.5 to 1 hr later followed by ia injection of 3 x 10^5 mBSA-specific cloned T cells in both knee joints. The inflammatory joint swelling of the right knee was measured 24 hrs later. On day 14, 21, 28 and 42 flare-up reactions were induced by injection of 30 μg mBSA into the right knee joint and 30 μg of OVA in 10 μl into the left knee joint, and were measured 24 hrs later. The values measured after only one (day 0) or two injections (day 0 and day 14, 21, 28 and 42) of the antigens were also measured and substracted from the values measured in the groups that also received the cloned T cells. The joint swelling of the right knee was expressed as the specific increase in knee thickness (mean ± SEM; n=5).

the primary joint inflammation by the mBSA specific T cells, the joint inflammation was no longer measurable when the mice were not rechallenged, as is shown in the second bar of Fig. 1.

Subsequently we investigated whether the cloned mBSA specific T cells retained their immunocompetence in a knee joint in the absence of the specific antigen mBSA. We therefore ia injected the mBSA specific cloned T cells, several days later followed by ia injection of mBSA in the right knee joint and OVA into the left knee joint. 24 Hrs later knee swelling was determined by ^{99m}Tc-uptake. Injection of only the T cells did not result in a measurable joint inflammation, as is shown in Fig. 2, upper bar (group 1). Mice that had been ia injected with mBSA specific cloned T cells on day 0, and were ia injected with antigen 0 - 14 days later,

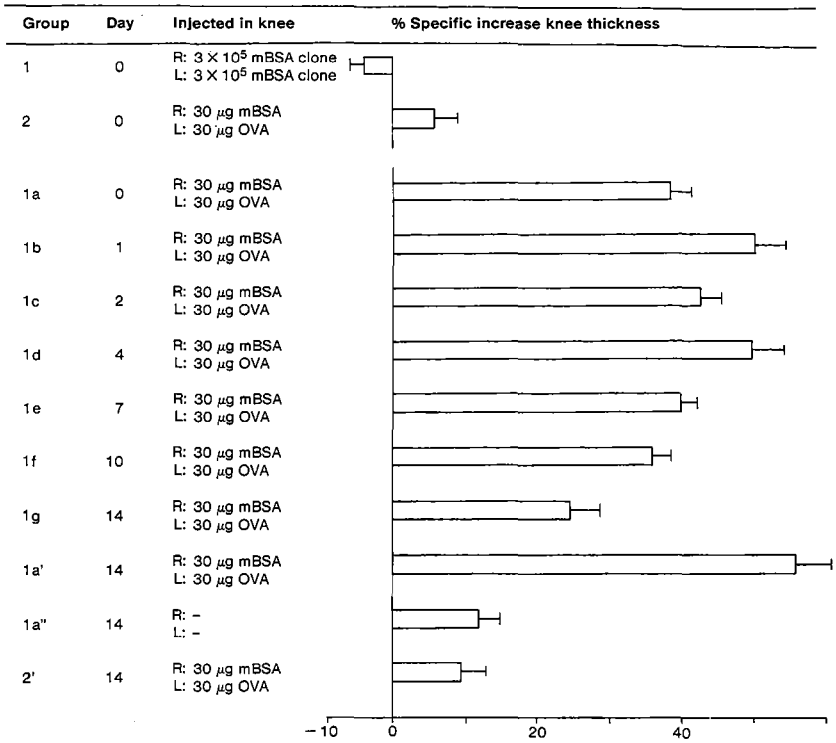


Fig. 2. Joint inflammations and flare-up reactions induced by ia injection of cloned mBSA specific T cells and ia injection of the antigen mBSA at various days thereafter. On day 0, 3 x 10^5 mBSA specific cloned T cells were injected into the right and the left knee joint. At various days thereafter 30 μg mBSA was injected into the right knee joint and 30 μg of OVA into the left knee joint. One group (1a) received the antigens on day 0 and was rechallenged on day 14 to induce a flare-up reaction (group 1a'). The response of a control group for the ia injection of the antigens only is also indicated in the figure (group 2). The joint swelling of the right knee was expressed as the specific increase in knee thickness (mean ± SEM; n=5).

developed a clear joint inflammation. This is shown in group 1a to 1g of Fig. 2. In group 1a' a flare-up reaction was induced by ia rechallenge with mBSA in the right knee joint and OVA in the left knee joint. In this group a significantly higher inflammation ($p<0.005$) was measured compared to the group that received the antigen for the first time at this time point (group 1g). Without such a rechallenge virtually no joint inflammation was measurable 14 days after the primary joint inflammation (group 1a'').

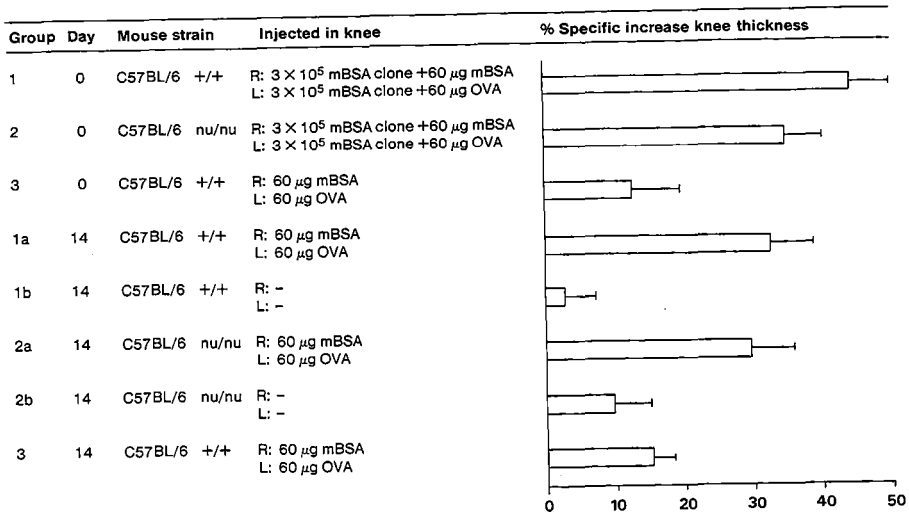


Fig. 3. Induction of flare-up reactions in nude mice. Joint inflammation was induced by injection of 60 μg mBSA into the right knee joint and 60 μg OVA into the left knee joint, 0.5 to 1 hr later followed by injection of 3 x 10^5 mBSA specific cloned T cells into both knee joints. This was done in C57BL/6J +/+ mice as well as in C57BL/6J nu/nu mice. 14 Days later the mice were rechallenged with the antigens, by ia injection of 60 μg mBSA into the right knee joint and 60 μg OVA into the left knee joint. Flare-up reactions were measured 24 hrs later (groups 1a and 2a). The values measured after one or two injections of the antigens only are also expressed (groups 3 and 3'), as are the values measured of the waned inflammation 14 days after induction of the inflammation (groups 1b and 2b). The joint swelling of the right knee was expressed as the specific increase in knee thickness (mean ± SEM). The data represent the mean of two experiments each involving 5 mice.

Finally we studied whether flare-up reactions can be evoked in the athymic nude mice. Therefore mBSA specific cloned T cells were, after injection of either mBSA or OVA ia, injected into the right and the left knee joint of C57BL/6J +/+ and C57BL/6J nu/nu mice. 24 Hrs later, both groups of mice displayed a clear joint inflammation (Fig. 3, groups 1 and 2, $P<0.01$ as compared to group3). 14 Days later in both groups the inflammation had almost waned (groups 1b and 2b). However, when these groups were rechallenged with the antigens mBSA and OVA ia, a flare-up reaction occurred in both the normal C57BL/6 mice and the C57BL/6 athymic nude mice (groups 1a and 2a; group 1a compared to group 1b and group 2a compared to group

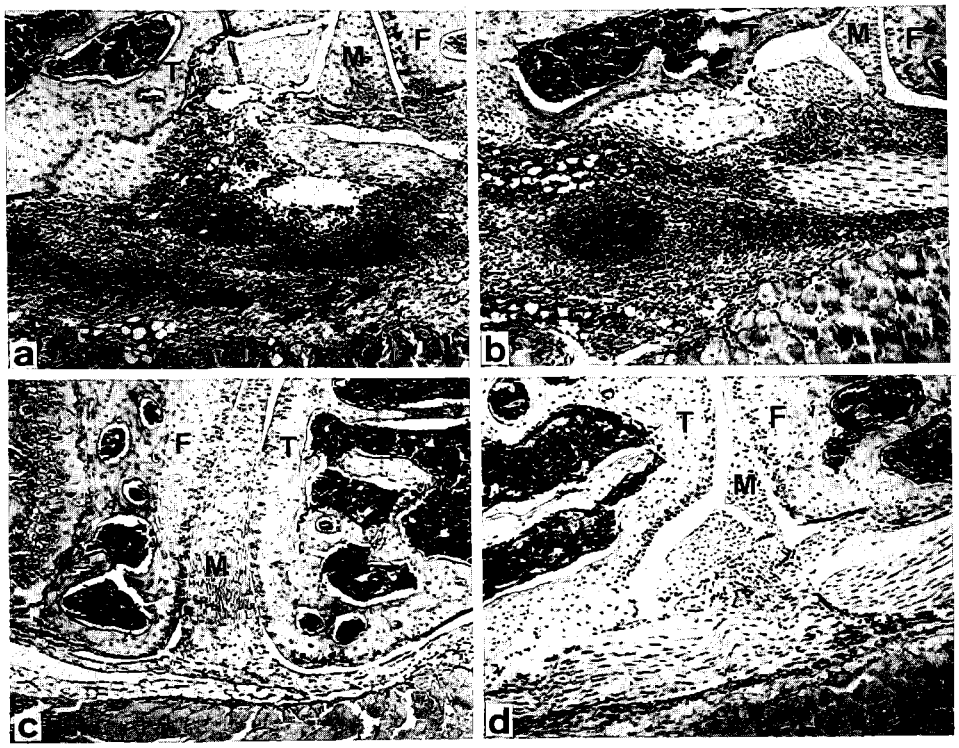


Fig. 4. Histological examination of flare-up reactions in euthymic and nude mice. (a) Flared arthritis in the right knee joint of a +/+ mouse 24 hrs after rechallenge with 60 μg mBSA (HE; 120x). The flare-up reaction was induced 14 days after induction of the joint inflammation by local injection of 3 x 10^5 mBSA specific cloned T cells and 60 μg mBSA. (b) Flared arthritis in the right knee joint of a nu/nu mouse 24 hrs after rechallenge with 60 μg mBSA (HE; 120x). (c) Right knee joint of a nu/nu mouse that was not rechallenged 14 days after induction of a joint inflammation (HE; 120x). (d) Right knee of a +/+ mouse that was injected two times with 60 μg mBSA two weeks apart (HE; 120x). F is femur, T is tibia, M is meniscus.

2b $P<0.01$). Injections of the antigens into C57BL/6 +/+ mice only led to a minor specific increase in knee thickness (group 3'). This was also the case in C57BL/6 nu/nu mice (tested in a separate experiment; data not shown).

Flare-up reactions of inflammations induced by the cloned $MT4^+$,Lyt-2^- T cells in euthymic and nude mice were also histologically examined (Fig. 4a and 4b). The flare-up reactions in the nude mice did not appear histologically different from those in normal euthymic C57BL/6 mice. The histological aspects of these reactions in the latter mice have been described previously (12). Such inflammatory infiltrates predominantly consist of granulocytes along with small numbers of monocytes and lymphocytes. Fig. 4c shows the knee joint of a mouse in which a joint inflammation had been induced 14 days before and was not rechallenged with the antigen. In such mice only foci of inlammation were found. Almost no joint inflammation was observed in normal +/+ mice that were injected two times with 60 μg mBSA 14 days apart as is shown in Fig. 4d. This indicates that the inflammations found were not caused by an immunization effect.

In all the groups mentioned here cartilage destruction and pannus formation were generally not observed.

DISCUSSION

Our previous studies showed that it is possible to induce flare-up reactions of waned inflammations that had been induced by local or iv injection of cloned $MT4^+$,Lyt-2^- T cells and local injection of the antigen (11,12). The present studies show that such flare-up reactions can still be induced 4 weeks after induction and subsequent waning of a primary joint inflammation. This indicates that at this time after induction of the joint inflammation antigen reactive T cells still occur in the knee joint. It is imaginable that by that time the number of T cells still present in the knee joint is very low as others have shown that a single T cell can mediate a measurable DTH reaction (16).

The general idea about chronicity and flare-up of joint inflammations induced in mice immunized with a positively charged antigen is that the T cells are retained in the joint as a result of the persistence in the cartilage structures of the positively charged antigen (2-5). In this study we investigated whether cloned T cells can also be retained in a

joint in the absence of the specific antigen. The persistence of T cells was determined by the ability to induce joint inflammation. The data show that the cloned T cells were still present and immunocompetent in the knee joint 14 days after their ia injection without the antigen. However, when such mice are ia injected with antigen 14 days after ia injection of the cloned T cells, the inflammation induced is weaker than at earlier points of time. This possibly indicates that the T cells are leaking out of the joint or that the T cells are losing their functional capacity. The T cells injected had been cultured for 14 days without the antigens, which implies that virtually no antigen was present in the inoculum.

The flare-up reaction evoked by rechallenge 14 days after ia injection of cloned helper T cells and the specific antigens was significantly higher than the joint inflammation measured after the first administration of antigen at day 14. This might be due to a better retention of the cloned T cells in the presence of the specific antigen, but might also be caused by the in vivo restimulation and proliferation of the injected T cells within the joint. We cannot exclude the possibility that the mBSA specific cloned T cells plus the mBSA have induced a local antibody response resulting in a better retention of the antigen and therefore of the cloned T cells. We are currently investigating these possibilities. In the AIA model the mBSA specific T cells reach the knee joint after challenge with the antigen. So it is hard to study the persistence of the antigen specific T cells in the absence of antigen in this model. It could be argued that in the work, involving the mBSA specific cloned T cells, these cloned T cells show migratory defects (12) and as a result of this may persist in the knee joints. Migratory defects of cloned T-cells have been reported previously (17,18). Anyhow, these cloned T cells can remain functionally reactive in a knee joint.

Local hyperreactivity as a result of the retention of T cells is not restricted to the joint but is thought to be also important in flare-up like phenomena in allergic skin reactions (19).

We did not use comparable points of time in Figs. 1 and 2, so we can not compare the severity of the inflammations measured in these experiments elicited by T cells that were injected with or without the antigen at the later points of time. For this reason we do not know whether inflammations measured after a first administration of the antigen could still be induced at later points of time as used in Fig. 2. More generally stated, it is

always misleading to compare different experiments using these cloned T cell populations as their functional activity can greatly differ between the experiments (data not shown).

In Fig. 3 we show that flare-up reactions of joint inflammations induced by the cloned $MT4^+$,$Lyt\text{-}2^-$ T cells could also be evoked in athymic nude mice. This indicates that T cells of the recipient mouse are not responsible and not neccessary for the flare-up reaction. We do not expect that in these T-cel deficient mice T cells might be induced as a result of factor production of the locally injected cloned T cells, or that such T cells of the recipient mouse play a role in the flare-up reactions measured. We have no indications that an immunization effect might play a role in the flare-up reactions, as two ia injections of the antigen several weeks apart did not lead to a histological or measurable joint inflammation.

Histological examination of the flared joints of the nude mice of Fig. 3 is shown in Fig. 4. The flare-up reactions appeared to be mainly granulocytic in nature, which is a normal feature for Jones-Mote type DTH reactions in rodents (13). The flare-up reactions were histologically indistinguishable from the joints 24 hours after induction of the joint inflammations (data not shown) and were also indistinguishable from the flare-up reactions in normal euthymic C57BL/6 mice. We have no indications that an immunization effect might play a pivotal role in the flare-up reactions, as two ia injections of the antigen several weeks apart lead to a minor histological (Fig. 4d) or hardly measurable minor joint inflammation (Fig. 3, group 3').

The joint inflammation caused by ia injection of the antigens mBSA and OVA only, without injection of T cells, were always determined in the experiments presented here. This is necessary as ia injection of the relatively high doses of a cationic protein like mBSA can cause a small but measurable inflammation.

In our experiments the antigens and T cells were always injected separately as ia injection of the cloned T cells and mBSA together resulted in a weaker joint inflammation (12). This is due to the deleterious effect of high doses of mBSA for the cloned T cell population used, when added to the T cells directly. Also for this reason we diminished the amount of antigen added ia from 60 μg (as used in Fig. 3 and 4) to 30 μg per joint (as used in Fig. 1 and 2).

We are currently investigating whether it is also possible to induce

flare-up reactions by systemic (oral or iv) administration of the antigens after induction of a joint inflammation by cloned $MT4^+$,$Lyt\text{-}2^-$ T cells. We have already shown that it is possible to induce a flare-up of a DTH reaction induced by cloned mBSA specific T cells and mBSA in a hind foot (20). Based on the data presented in this and previous papers we suggest, as others do (21,22), that T cells play an important role in inflammatory autoimmune diseases. In the model system in mice employing cloned T cells with the $MT4^+Lyt\text{-}2^-$ phenotype we show that the persistence of T cells can also acount for the flare-up reactions. These flare-up reactions are thought to be comparable to the exacerbations as occur in human rheumatoid arthritis. Therefore, we conclude that T cells and the specific antigen alone, without the involvement of e.g. B cells, can account for the inflammations as well as their exacerbations.

ACKNOWLEDGMENTS

The authors thank Dr. M.H. Schreier of Sandoz Ltd., Basel, Switzerland for stimulating discussions, Miss W.A. Zwarts for preparation of the histological sections, the team of the Department of Nuclear Medicine of the Dijkzigt Hospital for preparation of the ^{99m}Tc-pertechnetate and Mr. J. Brandenburg for animal care. Miss G. de Korte and Mrs. J. de Goey-van Dooren are gratefully acknowledged for their assistance in the preparation of the manuscript and Mr. T. van Os for expert photography. This work was supported by a grant from the Netherlands League against Rheumatism.

REFERENCES

1. Dumonde DC, Glynn LE: The production of arthritis in rabbits by an immunological reaction to fibrin. Brit J Exp Path 43:373-383, 1962.
2. Van den Berg WB, van de Putte LBA, Zwarts WA, Joosten LAB: Electrical charge of the antigen determines intra-articular antigen handling and chronicity of arthritis in mice. J Clin Invest 74:1850-1859, 1984.
3. Van den Berg WB, and van de Putte LBA: Deep penetration of cationic BSA in hyaline articular cartilage. Am J Pathol 121:224-234, 1985.
4. Van den Berg WB, van Lent PLEM, van de Putte LBA, Zwartz WA: Electrical charge of hyaline articular cartilage: its role in the retention of anionic and cationic proteins. Clin Immunopath 39:187-197, 1986.
5. Van Lent PLEM, van den Berg WB, Schalkwijk J, van de Putte LBA, van den Bersselaar L: Allergic arthritis induced by cationic antigens: relationship of chronicity with antigen retention and T-cell reactivity. Immunology 62:265-272, 1987
6. Van de Putte LBA, Lens JW, van den Berg WB, Kruysen MWH: Exacerbation of antigen-induced arthritis after challenge with intravenous antigen. Immunology 49:161-167, 1983.
7. Lens JW, van den Berg WB, van de Putte LBA, van den Bersselaar L:

Flare-up of antigen-induced arthritis in mice after challenge with oral antigen. Clin Exp Immunol 58:364-371, 1984.
8. Brackertz D, Mitchell GF, Vadas MA, MacKay IR: Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. J Immunol 118:1645-1648, 1977.
9. Brackertz D, Mitchell GF, Mackay IR: Antigen induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum 20:841-850, 1977.
10. Bianchi ATJ, Hooijkaas H, Benner R, Tees R, Nordin AA, Schreier MH: Clones of helper T cells mediate antigen-specific H-2 restricted DTH. Nature 290:62-63, 1981.
11. Klasen IS, Donselaar IG, Ladestein RMT, van den Berg WB, Benner R: Joint inflammations and flare-up reactions in mice induced by a helper T cell clone. Agents Actions 19:331-334, 1986.
12. Klasen IS, Ladestein RMT, Donselaar IG, van den Berg WB, Tees R, Benner R: Joint inflammation in mice induced by a $MT4^+$ $Lyt\text{-}2^-$ T cell clone. Characteristics and the induction of flare-up reactions. J Immunol 139:3275-3280, 1987.
13. Schreier MH, Tees R, Nordin AA, Benner R, Bianchi ATJ, van Zwieten MJ: Functional aspects of helper T cell clones. Immunobiology 161:107-138, 1982.
14. Kruysen HWM, van den Berg WB, van de Putte LBA, van den Broek WJH: Inflammation revisited: general topics. Detection and quantification of experimental joint inflammation in mice by measurement of ^{99m}Tc-pertechnetate uptake. Agents Actions 11:640-642, 1981.
15. Lens JW, van den Berg WB, van de Putte LBA: Quantification of arthritis by ^{99m}Tc-uptake measurements in the mouse knee-joint: correlation with histological joint inflammation scores. Agents Actions 14:723-728, 1984.
16. Marchal G, Seman M, Milon G, Truffa-Bachi P, Zilberfarb V: Local adoptive transfer of skin delayed-type hypersensitivity by a single T lymphocyte. J Immunol 129:954-958, 1982.
17. Daily MO, Fathman CG, Butcher EC, Pillemer E , Weissman IL: Abnormal migration of T lymphocyte clones. J Immunol 128:2134-2136, 1982.
18. Daily MO, Gallatin WM, Weissman I: The in vivo behaviour of T cell clones: altered migration due to loss of the lymphocyte surface homing receptor. J Mol Cell Immunol 2:27-36, 1982.
19. Scheper RJ, von Blomberg M, Boerrigter GH, Bruynzeel D, van Dinther A, Vos A : Induction of immunological memory in the skin. Role of local T cell retention. Clin Exp Immunol 51:141-148, 1983.
20. Klasen IS, van der Kwast ThH, Donselaar IG, Ladestein RMT, Benner R: Flare-up of delayed type hypersensitivity initially induced by murine cloned helper T cells. Cell Immunol 108:235-241, 1987.
21. Holoshitz J, Naparastek Y, Ben-Nun A, Cohen IR: Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 219:56-58, 1983.
22. Zamvil S, Nelson P, Trotter T, Mitchell D, Knobler R, Fritz R, Steinman, L: T- cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. Nature 317:355-358, 1985.

CHAPTER 5

MURINE MACROPHAGE CELL LINE AP284 PRESENTS ANTIGEN TO CLONED MT4$^+$,LYT-2$^-$ T CELLS IN VITRO AND IN VIVO

Ina S. Klasen, Johannes P. de Jong, Jane S.A. Voerman, Renée M.T. Ladestein, Pieter J.M. Leenen and Robbert Benner

Department of Cell Biology, Immunology and Genetics, Erasmus University Rotterdam, The Netherlands

Immunobiology, in press.

SUMMARY

A murine macrophage cell line AP284 was isolated that appeared to be mature in phenotype. After repeated cloning the cell line expressed the markers Mac-1, Mac-2, Mac-3, 2.4G2, F4/80 as well as Ia antigens. Moreover it was positive for the enzymes nonspecific esterase and acid phosphatase, negative for alkaline phosphatase and was able to phagocytise latex beads. We studied whether this cell line was able to present antigen to cloned $MT4^+$,$Lyt\text{-}2^-$ T cells specific for methylated bovine serum albumin (mBSA) or ovalbumin (OVA). The *in vitro* proliferative response of the cloned T cells specific for mBSA or OVA was found to be effectively supported by AP284. This proliferation could be blocked by monoclonal antibodies against Ia determinants. AP284 also effectively presented antigen *in vivo* as was shown in a foot swelling assay measuring delayed type hypersensitivity (DTH) to mBSA caused by specific cloned T cells with the helper phenotype. This offers a unique model system for studying the process of antigen presentation in which both the antigen presenting cells and the T cells are monoclonal.

INTRODUCTION

Helper T cells, in contrast to B cells, only respond to antigen when presented by antigen presenting cells (APC). To this end APC have to express class II (Ia) antigens syngeneic to the helper T cells (1, reviewed in 2). Particulate antigens have to be processed, which means catabolyzed by lysosomes, before they can be effectively presented to the T cells (3,4, reviewed in 5). Glutaraldehyde-fixed macrophages are unable to present such antigens unless they process the antigen before fixation (5).

Several cell types can effectively present antigen to T cells. Firstly monocytes and macrophages (Mϕ) (6,7). However, these cells do not constitutively express Ia antigens (8,9). The period of Ia expression by Mϕ after its induction is very short (12-48 hr) (8). Ia can be induced by a lymphokine present in the supernatant of stimulated T cells. The major lymphokine responsible for Ia induction is Interferon-γ (IFN-γ) (10). Furthermore Mϕ, like all other APC, need to produce IL-1 (11,12, reviewed in 13) in order to activate T cells. Recently it has been shown that only the Th2 population of the helper T cells (Th1 produces IL-2 and IFN-γ, Th2 secretes IL-4; 14) needs IL-1 as an activation signal (15).

A second category of APC is the dendritic cell (16). Dendritic cells are unable to phagocytise and exhibit constitutive Ia expression. They are very efficient in the induction of mixed lymphocyte responses (MLR), polyclonal responses and responses to several antigens (17,18). Dendritic cells are thought to be unable to present particulate antigens to T cells without previous phagocytosis and processing of the antigens by Mϕ (19). However others (20,21) have found that dendritic cells are also able to process antigens. Klein debates the necessity of antigen processing as Ia containing liposomes can present antigens to T cells (22,23). This might well be dependent on the specificity of the T cells involved (24). Also B cells (25-27) and epithelial cells (28,29) are reported to be able to present antigen to T cells.

As indicated above, the role of phagocytosis, antigen processing, Ia expression and IL-1 production in T cell activation are still under debate. In most studies on antigen presentation peritoneal Mϕ or long term bone marrow culture Mϕ are used. Some studies have been published using cultured tumor Mϕ lines. These lines can be induced to express Ia by IFN-γ (30,31).

Some other lines have been produced that constitutively express Ia (32,33). Moreover, macrophage hybridomas have been produced (34) and macrophages have been immortilized by viral infection (35,36).

We here report on a mature Mϕ tumor cell line AP284, constitutively expressing Ia antigens. This Mϕ cell line is able to present antigen to cloned T cells with the helper phenotype in vitro and in vivo and provides us with a unique model for studying antigen presentation in which both the helper T cells and the APC are monoclonal.

MATERIALS AND METHODS

Mice

C57BL/6J and BALB/c mice were purchased from OLAC LTD., Bicester, UK., or from Bomholtgard, Ry, Denmark. In all experiments female mice were used at the age of 2-3 months.

Cloned $MT4^+$,$Lyt\text{-}2^-$ T cells

The T cell clone specific for mBSA was made in collaboration with Ms. R. Tees and Dr. M.H. Schreier from Sandoz Basel, Basel, Switzerland, according to procedures described elsewhere (37). The T cell clone specific for ovalbumin (OVA) was described previously (37) as were the culture conditions optimal for both T cell clones (38-42). Briefly, the mBSA and OVA specific cloned T cells were restimulated with the antigens every 5 to 6 weeks. For the mBSA specific T cell clone and for total spleen cells 5 μg/ml mBSA was optimal, for the OVA specific T cell clone 300 μg/ml OVA was used. mBSA and OVA were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. Irradiated (30 Gy) syngeneic spleen cells were used as a source of antigen presenting cells. After 3 days the cells were transferred to medium containing 600 U/ml recombinant human interleukin 2, which was a kind gift of Dr. J. Besemer from Sandoz Ltd., Vienna, Austria. The cloned T cells were transferred to fresh IL-2 containing culture medium in a 1 to 10 dilution twice a week. This was done at least three times after restimulation before the cloned T cells were used. The cloned T cells used in the experiments did contain virtually no antigen presenting cells as the irradiated cells died in the first week after restimulation

and the adherent cells were not transferred to the fresh culture medium. Both T cell clones are of C57BL (H-2^b) origin and are H-2 I-A^b restricted in antigen recognition (37,41,42). These clones are of the Th1 phenotype, which means that they produce IL-2 and IFN-γ upon antigenic stimulation and are able to induce DTH reactions (14,41,42).

Macrophage cell line

Macrophage cell line AP284 was derived from the spleen of a male (C57BL/Rij x CBA/Rij)F1 (H-$2^{b/q}$) mouse suffering from a spontaneously arisen tumor. The spleen was processed to a single cell suspension and cultured in a humidified atmosphere of 5% CO_2 in air in alpha modification of Dulbecco's medium supplemented with 10% fetal calf serum. Out of this culture the AP284 Mϕ line was obtained. Several vials of these cells were frozen in liquid nitrogen. After the original line was lost by overgrowth of fibroblasts, the line was subcloned out of one of these frozen samples. The subcloning was performed by limiting dilution in 96-well flat bottom microtiter plates (Costar, Cambridge, U.K.). Under subcloning conditions 10% L cell conditioned medium (LCM) was added to the alpha modification of Dulbecco's medium. The subclones used here are AP284.D4 or AP284.B11. The latter is a subclone of AP284.D4. AP284.B11 appeared to be more stable in culture. The subclones were cultured in the same culture medium, without LCM, under the same coditions as described above. The Mϕ were passaged twice a week.

Chromosomal aberrations of the AP284 cell line were determined. The cell line appeared to be hypotetrapoid and showed two translocational events, t(4;18) and t(16;19) in addition to a $4p^+$, $9p^+$ (2 variations) and mosaic $13p^+$ aberration.

Phenotyping of AP284

Phenotyping of AP284.D4 line and 3 other Mϕ lines (WEHI-3 (43), Pu5-1.8 (44), P388.D1 (45)) was performed in an ELISA system described previously (46). The monoclonal antibodies M1/70, M3/38, M3/84, 2.4G2, F4/80, 59AD2.2, M5/114, ER-TR3, and MIV-113 were used. The level of antibody binding was expressed relative to the level of 30G12 antibody binding (anti-T-200; 57) and the levels of T200 expression of the 4 cell lines phenotyped here were comparable (47). The characteristics and references of the monoclonal

antibodies used (48-61) are mentioned in Table 1.

TABLE 1

PHENOTYPING OF AP284

Monoclonal antibody	Target antigen	References	WEHI-3	Pu5-1.8	P388D1	AP284.D4
M1/70	Mac-1, complement receptor type 3	48-50	8	13	22	55
M3/38	Mac-2	49,51	23	63	69	49
M3/84	Mac-3	49,52	11	9	14	19
2.4G2	Fc receptor type II	53	8	11	24	25
F4/80	F4/80	54	7	2	5	25
59AD2.2	Thy-1	55-57	125	5	1	1
30G12	T-200, common leukocyte antigen	57	100	100	100	100
M5/114	$I\text{-}A^{b/d/q}$, $I\text{-}E^{d/k}$	58	23	3	11	
ER-TR3	$I\text{-}A^{b/d/k/q/r}$	59	2	3	4	1
MIV 113	MIV 113	60,61	0	0	0	1

The Mϕ lines were phenotyped in an ELISA system as described by van Soest et al. (46) and with the monoclonal antibodies described in the Materials and Methods section. Data represent antibody binding relative to the level of 30G12 binding. Negative control values are subtracted.

Proliferation assay

Proliferation assays were performed in IMDM-ATL (38). This is the serum-free medium in which the mBSA or OVA specific cloned $MT4^+$,$Lyt\text{-}2^-$ T cells were cultured. 2×10^4 cloned T cells were cultured for 3 days in 200 µl medium in 96 well flat bottom microtiter plates (Costar, Cambridge, U.K.) together with the desired number of irradiated (30 Gy) AP284 cells or C57BL spleen cells and with or without antigen. Pulsing with antigen was done by incubation of the APC with 30 µg/ml mBSA or 1 mg/ml OVA during 1 hr at 37°C. Fixation of the APC was performed in 0.05% glutaraldehyde during 20 minutes at 4°C. IL-2 induced T cell proliferation was achieved by addition of 600 U/ml recombinant human IL-2. Proliferation was measured by ^3H-thymidine incorporation during the last 8 or 18 hrs of culture (1 µCi/well, spec. act. 50 Ci/mmol; Amersham, UK.). The cultures were harvested by means of an automatic cell harvester (Cryoson, Midden

Beemster, The Netherlands) and counted in a beta counter (Hewlet Packard, Brussels, Belgium).

Blocking of antigen presentation by monoclonal antibodies

The monoclonal antibody employed for blocking of antigen presentation (M5/114) was used as cell culture supernatant. As a control supernatant 236$\alpha\gamma$3, which is an anti-isotype monoclonal antibody, was used (62). The supernatants were dialysed overnight against 200 times the volume of fresh Dulbecco's medium. This was refreshed once.

Induction and measurement of DTH

DTH was induced by local injection of 3 x 10^5 mBSA specific cloned helper T cells together with 20 μg mBSA or OVA in 50 μl balanced salt solution (BSS) into the dorsum of the hind feet of the allogeneic BALB/c mice. Increasing numbers of AP284 cells or peritoneal exudate cells (PEC) of C57BL/KA mice were added to the inoculum. The PEC were obtained from peritoneal cavity washings of naieve mice. 24 and 48 hours after induction of inflammation, the thickness of the hind feet was measured. In the figures 24 hrs values are presented. The 48 hrs responses were much like the 24 hrs responses, but generally lower. DTH responses were expressed as the specific increase in hind foot thickness and were calculated as [(thickness right foot - thickness left foot)/thickness left foot] x 100%

Statistics

Significance of the diferences between various experimental groups was determined by Student's t-test.

RESULTS

Phenotyping of AP284

The phenotype of AP284.D4 and of 3 control lines was determined was in the ELISA system described by van Soest et al. (46). The results are shown in Table 1. The phenotypes of the control lines have been described previously (47). The level of antibody binding was expressed relative to the level of 30G12 (T-200) antibody binding. By means of membrane immunofluo-

rescence it was shown that all cells are positive for T-200 (data not shown). Table 1 shows the four Mϕ cell lines in order of maturity (47). The more mature macrophage cell line are Mac-1^+, Mac-2^+, Mac-3^+, F4/80^+ and Thy-1^-, MIV 113^-, so AP284 is concluded to be a mature Mϕ cell line, which in addition constitutively expresses Ia antigens.

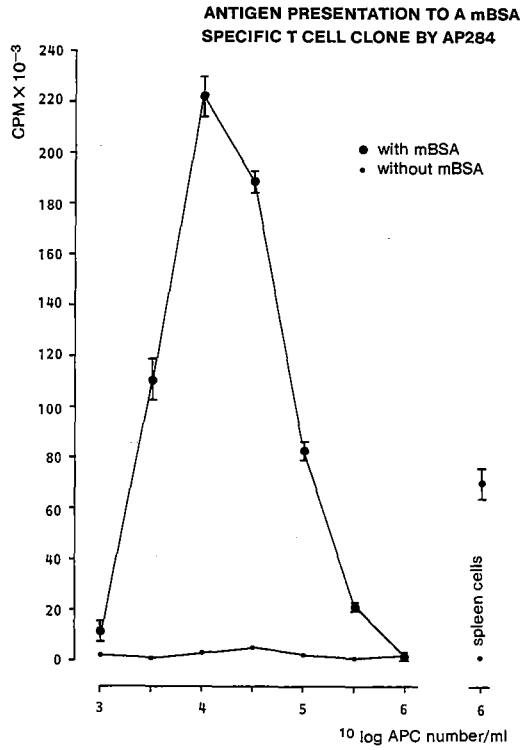


Fig. 1. Dose-response curve of the effectiveness of antigen presentation by AP284.D4 in the proliferative response of cloned mBSA specific T cells in vitro. As a source of antigen presenting cells increasing numbers of irradiated (30 Gy) AP284.D4 cells or, for comparison, 2 x 10^5 irradiated (30 Gy) C57BL/6 spleen cells were added to 2 x 10^4 cloned T cells in 200 µl. This was done in the presence or in the absence of the antigen mBSA. Data were calculated as the mean of five replicate cultures and expressed as counts per minute (cpm) ± SEM.

Antigen presentation by AP284 in vitro

The capacity of AP284 to present antigen to T cells was examined in a T cell proliferation assay in vitro. As a source of T cells we used mBSA specific cloned $MT4^+$,Lyt-2^- T cells (39,40). Fig. 1 shows that AP284.D4 can effectively present mBSA to cloned T cells. In our assay 10^4 AP284 cells per ml caused maximal proliferation of the cloned T cells. mBSA presentation by 10^6 irradiated C57BL/6 spleen cells per ml resulted in a much weaker T cell proliferation. In this respect it should be mentioned that 10^6 cells/ml is the most optimal concentration of irradiated spleen cells for presentation of mBSA to the mBSA specific cloned $MT4^+$,Lyt-2^- T cells.

Subsequently we investigated whether AP284.B11 was also able to present antigen to an OVA specific T cell clone. Moreover the role of antigen processing was studied by incubation of AP284 cells with either mBSA or together with the relevant T cell clones. The capacity of fixed AP284 cells to present antigen which is present free in culture to the cloned T cells was also examined. It is shown in Fig. 2 that AP284 was able to

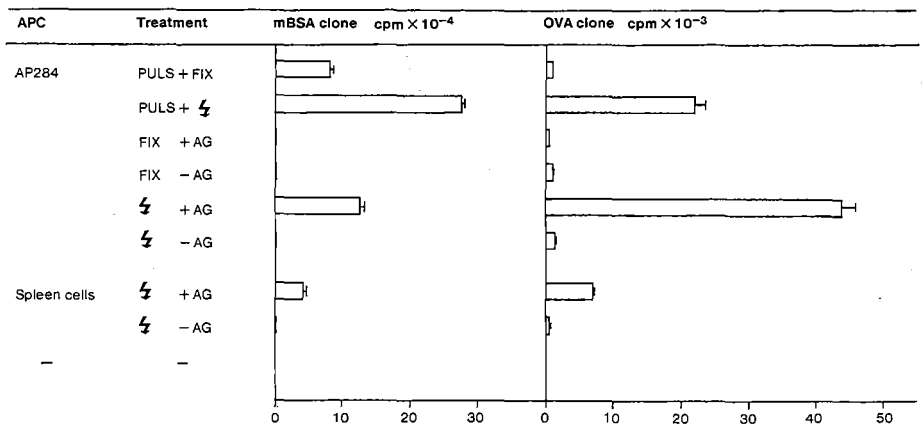


Fig. 2. Antigen presentation by pulsed and/or fixed or irradiated AP284.B11 cells to mBSA or OVA specific cloned T cells in vitro. As a source of antigen presenting cells 2 x 10^4 irradiated (30 Gy) or glutaraldehyde fixed AP284 cells or 2 x 10^5 irradiated total spleen cells were added to 2 x 10^4 mBSA or OVA specific cloned T cells in 200 μl. Pulsing of the AP284 cells with the antigen was performed by incubation with 30 μg/ml mBSA or 1 mg/ml OVA for 1 hr at 37°C. Data are calculated as the mean of four replicate cultures and expressed as counts per minute (cpm) ± SEM.

present mBSA to the mBSA specific cloned T cells after processing the antigen and fixation with glutaraldehyde. The OVA specific cloned T cells, however, did not respond to AP284 cells that were pulsed with the antigen and fixed thereafter. Fixed AP284 cells were unable to present antigen to the cloned T cells and to induce antigen specific proliferation.

Blocking of antigen presentation in vitro

The role of Ia antigen in the antigen presentation by AP284.B11 to the cloned mBSA specific $MT4^+$,$Lyt\text{-}2^-$ T cells was investigated by using monoclonal antibodies directed against Ia antigens. We therefore replaced a part of the culture medium in the proliferation assay by monoclonal anti-Ia or control antibodies. Fig. 3 shows that the monoclonal anti-Ia antibody M5/114, which can bind to AP284 (Table 1), blocks the antigen presentation effectively. The monoclonal had a minor effect on the IL-2 induced proliferation of the cloned T cells. A control supernatant ($236\alpha\gamma3$) did only block the antigen presentation and the IL-2 induced proliferation in the highest concentration used.

Antigen presentation by AP284 in vivo

The mBSA specific cloned $MT4^+$,$Lyt\text{-}2^-$ T cells are H-2I-A^b restricted in vivo with regard to their recognition of mBSA in DTH reactions (41,42). In order to investigate whether AP284 can effectively present mBSA to these cloned T cells in vivo, we injected 3×10^5 cloned T cells into either hind foot of several groups of allogeneic BALB/c mice. These mice bear the H-2^d haplotype and therefore do not express I-A^b antigens, which is the restriction element of these T cells. The cloned mBSA specific helper T cells injected into the right hind foot had been supplemented with 20 μg mBSA and those injected into the left hind foot had been supplemented with 20 μg OVA. 24 Hours later only a minor inflammation was found in the right hind foot of these mice (Fig. 4, first bar). In other groups an increasing number of AP284.D4 cells was added to the inocula to be injected in the right and the left hind feet. In the case that 10^5 AP284 cells were added to the inocula significant foot swelling was measured ($p<0.005$) compared to the controls without AP284 cells. This swelling was comparable to the foot swelling that was measured when 10^5 PEC were added to the inoculum. Thus, antigen presentation by the AP284 cells in vivo

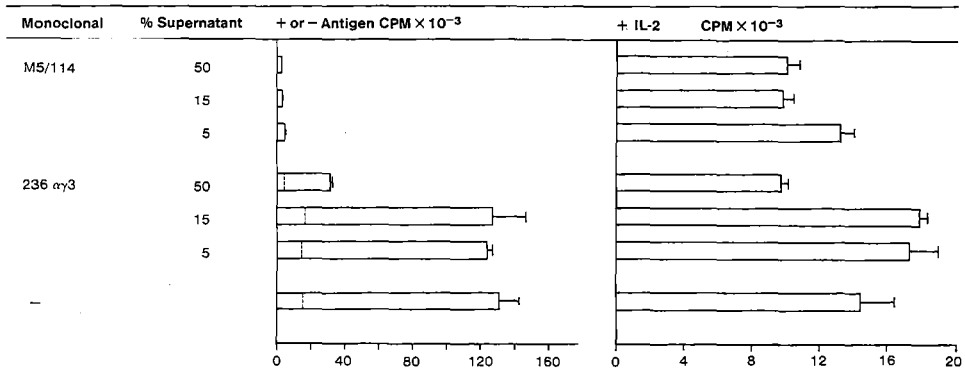


Fig. 3. Blocking of antigen presentation by AP284.B11 in vitro by a monoclonal anti-Ia antibody. The blocking of antigen presentation by 2 x 10^4 AP284 cells was assayed in a proliferation assay employing 2 x 10^4 cloned T cells. A part (50, 15 or 5%) of the culture medium was replaced by supernatant of the hybridomas M5/114 containing anti-Ia antibodies or by the control supernatant 236αγ3. This was done in the presence or absence of the antigen mBSA. The dotted lines in the bars represent the proliferation of the cloned T cells in the absence of antigen. As a control the effect of the monoclonals on the IL-2 induced proliferation (600 U/ml IL-2) was determined (right part). Data were calculated as the mean of four replicate cultures and expressed as cpm ± SEM.

was equally effective to induce an inflammatory reaction was the as antigen presentation by PEC.

DISCUSSION

This paper describes a new Mϕ cell line AP284 which is capable of antigen presentation to syngeneic T cells in vitro as well as in vivo. From the phenotyping data of this cell line it can be concluded that AP284 is a mature Mϕ cell line. This maturity is indicated by the relatively high surface expression of Mac-1, Mac-2, Mac-3 and F4/80. The expression of Mac-1, Mac-2 and F4/80 increases during Mϕ differentiation and is characteristic for mature Mϕ (49,50,53,62). The expression of Thy-1 and MIV-113, on the other hand, decreases during Mϕ differentiation (56). The latter markers indeed were undetectable on AP284. The expression of the enzymes

nonspecific esterase and acid phosphatase, the lack of alkaline phosphatase and the capacity to phagocytise latex beads also support the conclusion that AP284 is a Mϕ cell line.

The line constitutively expresses Ia as appears from the binding of the M5/114 monoclonal antibody. ER-TR3, which also detects I-A^b and I-A^q, but is a very weak monoclonal, showed no binding to AP284. Binding of M5/114 inhibited the antigen-induced proliferation of cloned mBSA specific T cells in the presence of AP284. The IL-2 induced proliferation, however, was blocked to a minor degree by M5/114.

The replacement of half of the culture medium by hybridoma-supernatants generally deminished the antigen specific proliferation as well as the IL-2 induced proliferation. This is probably caused by the addition of the high percentage of 'used' culture medium containing more demolition products.

AP284 was originally obtained from the spleen of a mouse suffering of a spontaneously arisen tumor. It was cultured without growth factors but was virtually lost after several months of culture because of the overgrowth of contaminating fibroblasts. Upon subcloning growth was only obtained in the presence of LCM. The subclones however were further expanded without LCM so this cannot account for the constitutive Ia expression. Moreover it is known that LCM is a poor inducer of Ia expression (5).

AP284 can effectively present antigen to T cells <u>in vitro</u> and is even more potent than the most optimal dose of irradiated spleen cells (Fig. 1). It is unclear why T cell proliferation was weaker when high doses of AP284 were used for antigen presentation. This reduced proliferation might be due to exhaustion of nutrients in the culture medium or Prostaglandin E_2 (PGE_2) production or by the induction of nonspecific cytotoxicity by the AP284 cells.

In Fig. 2 it is shown that the AP284 cells can present antigen not only to mBSA specific cloned T cells but also to an OVA specific T cell clone. This figure also shows that it is possible to present mBSA by AP284 cells when it is processed before fixation of the cells. We have to remind here that the optimal mBSA concentration for the mBSA specific T cell clone is much lower compared to the optimal concentration for the OVA specific cloned T cells. From the difference in antigen presentation of antigen pulsed AP284 cells after fixation or irradiation one may conclude that

antigen processing is not yet optimal for the presentation to the cloned T cells after the incubation period of 1 hour at 37°C. However, it is equally well possible that glutaraldehyde fixation decreases the antigen presenting capacities of AP284.

The dose of AP284 cells used in Figs. 2 and 3 is higher than the optimal dose determined for antigen presentation in Fig. 1. In Figs. 2 and 3, however, the AP284.B11 was used, which is a subclone of AP284.D4 and needs a higher cell concentration for optimal antigen presentation. This subclone otherwise is essentially similar to AP284.D4.

The AP284 cells can also effectively present mBSA to cloned $MT4^+$,$Lyt\text{-}2^-$ T cells in vivo, leading to the release of mediators accounting for a DTH reaction measured in a foot swelling assay (Fig. 4). This is apparent from our studies injecting the cloned T cells together with mBSA into fully allogeneic mice. Without APC syngeneic to the cloned T cells virtually no foot swelling is induced. Addition of semi-allogeneic AP284 cells to the cloned T cells, however, enabled a substantial DTH reaction in fully allogeneic BALB/c mice. These data show that AP284 not only enables antigen-specific proliferation of the cloned $MT4^+$,$Lyt\text{-}2^-$ T cells, but also the release of mediators characteristic for and underlying to their functional activity as involved in inflammatory responses. A comparably high foot swelling could be induced by PEC syngeneic to the cloned T cells. In vivo the AP284 cells and the PEC are equally effective in antigen presentation resulting in a comparable foot swelling. The inflammations measured were not based upon alloreactivity, as the left foot, in which also the cloned T cells and the Mϕ cells were injected, did not show an inflammation.

Other Mϕ cell lines have been reported that are able to present antigen to T cells (31-34). In our studies however, we have used the antigen presenting cells as well as the T cells monoclonal. Moreover we have shown that the combination of these monoclonal cell populations is functionally effective within an allogeneic environment in vivo.

In conclusion this paper describes a model system for T cell-macrophage interactions based upon the use of purely monoclonal cell populations which offers great perspectives for dissecting the processes involved in these interactions. Especially the role of soluble mediators is now open to more in-depth investigations.

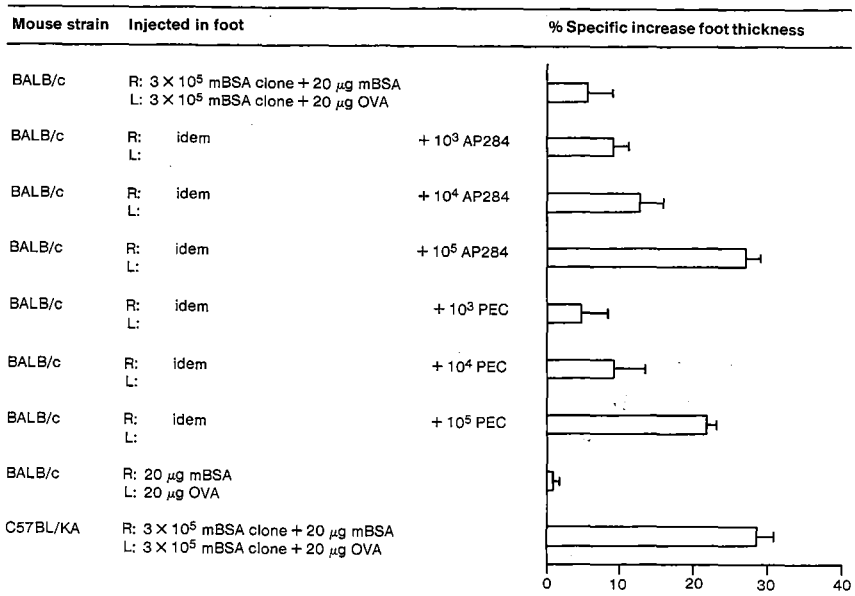


Fig. 4. Antigen presentation by AP284.D4 to mBSA specific cloned T cells with the helper phenotype in DTH. 3 x 10^5 mBSA specific cloned T cells were injected together with 20 μg mBSA into the right hind foot and together with 20 μg OVA into the left hind foot of allogeneic BALB/c mice. Increasing numbers of AP284 cells or PEC were added to the inocula injected. DTH reactions were measured 24 hrs later and expressed as the specific increase of foot thickness (mean ± SEM; n=5).

ACKNOWLEDGMENTS

The authors thank Ms R. Tees and Dr. M.H. Schreier from Sandoz Ltd. Basel, Switzerland, for assistance in the production of the mBSA specific cloned helper T cells, Mr. A. Piersma for initiating the AP284 cell line culture, Mrs. E. Franken-Postma for analysing the chromosomal aberrations, Mr. J. Brandenburg for animal care and Miss G. de Korte and Mrs. J. de Goey-van Dooren for secretarial assistance. This investigation was supported by the Netherlands League against Rheumatism and the Netherlands Asthma Foundation.

REFERENCES

1. Rosenthal, A.S., and E.M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig lymphocytes. J. Exp. Med. 138: 1194.

2. Schwartz, R.H. 1985. T-lymphocyte recognition of antigen in association with gene products of the major histocopatibility complex. Ann. Rev. Immunol. 3: 237.
3. Ziegler, H.K., and E.R. Unanue. 1981. Identification of a macrophage antigen- processing event required for I-region restricted antigen presentation to T lymphocytes. J. Immunology 127: 1689.
4. Ziegler, H.K., and E.R. Unanue. 1982. Decrease in macrophage antigen catabolism by ammonia and chloroquine is associated with the inhibition of antigen presentation to T cells. Proc. Natl. Acad. Sci. USA 79: 175.
5. Unanue, E.R. 1984. Antigen-presenting function of the macrophage. Ann. Rev. Immunol. 2: 395.
6. Unanue, E.R. 1981. The regulatory role of macrophages in antigenic stimulation. II. Symbiotic relationship betwwen lymphocytes and macrophages. Adv. Immunol. 31: 1.
7. Erb, P., A.C. Stern, S.S. Alkan, S. Studer, E. Zoumbou, and R.H. Gisler. 1980. Characterization of accessory cells required for helper T cell induction in vitro: evidence for a phagocytic, Fc receptor, and Ia bearing cell type. J. Immunol. 125: 2504.
8. Beller, D.I., and E.R. Unanue. 1981. Regulation of macrophage populations. II Synthesis and expression of Ia antigens by peritoneal exudate macrophages is a transient event. J. Immunol. 126: 263.
9. Beller, D.I. 1984. Functional significance of the regulation of macrophage Ia expression. Eur. J. Immunol. 14: 1204.
10. Steeg, P.S., R.N. Moore, H.M. Johnson, and J.J. Oppenheim. 1982. Regulation of murine Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. 156: 1780.
11. Beller, D.I., and E.R. Unanue. 1982. Reciprocal regulation of macrophage and T cell function by a way of soluble mediators. Lymphokines 6: 25.
12. Kurt-Jones, E.A., D.I. Beller, S.B. Mizel, and E.R. Unanue. 1985. Relation of macrophage Ia and membrane IL-1 expression to antigen presentation. Proc. Natl. Acad. Sci. USA. 82: 1204.
13. Durum, S.K., J.A. Schmidt, and J.J. Oppenheim. 1985. Interleukin 1: an immunological perspective. Ann. Rev. Immunol. 3: 263.
14. Cher, D.J., and T.R. Mossman. 1987. Two types of murine helper T cell clone. J. Immunol. 138: 3688.
15. Kurt-Jones, E.A., S. Hamberg, J. Ohara, W.E. Paul, and A.K. Abbas. 1987. Heterogeneity of helper /inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. J. exp. Med. 166: 1774.
16. Steinman, R.M., G. Kaplan, M.D. Witmer, and Z.A. Cohn, 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. J. Exp. Med. 149: 1.
17. Steinman, R.M., and M.G. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed lymphocyte reaction in mice. Proc. Natl. Acad. Sci. USA 75: 5132.
18. Klingeurt, W.E.F., J.H. La Baglie, and W.E. Bowers. 1982. Accessory and stimulating properties of dendritic cells and macrophages isolated from various rat tissues. J. Exp. Med. 156: 1.
19. Kapsenberg, M.L., W.B.M Teunissen, F.E.M. Stiekema, and H.G. Keizer. 1986. Antigen-presenting cell function of dendritic cells and macrophages in proliferative T cell responses to soluble and particulate antigens. Eur. J. Immunol. 16: 345.
20. Kaye, P.M., B.M. Chain, and M. Feldman. 1985. Nonphagocytic dendritic cells are effective accessory cells for anti-mycobacterial responses

in vitro. J. Immunol. 134: 1930.
21. Chain, B.M., P.M. Kay, and M. Feldmann. 1986. The cellular pathway of antigen presentation: biochemical and functional analysis of antigen processing in dendritic cells and macrophages. Immunology 58: 271.
22. Walden, P., Z.A. Nagy and J. Klein. 1985. Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and foreign antigens. Nature 315: 327.
23. Walden, P., Z.A. Nagy, and J. Klein. 1986. Major histocompatibility complex-restricted and unrestricted activation of helper T cell lines by liposome-bound antigens. J. Mol. Cell. Immunol. 2/4: 191.
24. Katz, D.R., M. Feldmann, R. Tees, and M.H. Schreier. 1986. Heterogeneity of accessory cells interacting with T-helper clones. Immunology 58: 167.
25. Chesnut, R.W., and H.M. Grey. 1981. Studies on the capacities of B cells to serve as antigen-presenting cells. J. Immunol. 126: 1075.
26. Hayglass, K.T., S.J. Naides, C.F. Scott, B. Benacerraf, and M.-S. Sy. 1986. T cell development in B cell-deficient mice. IV. The role of B cells as antigen-presenting cells in vivo. J. Immunol. 136: 823.
27. Malynn, B.A., D.T. Romeo, and H.H. Wortis. 1985. Antigen-specific B cells efficiently present low doses of antigen for induction of T cell proliferation. J. Immunol. 135: 980.
28. Nunez, G., E.J. Ball, and P.J. Stastny. 1983. Accessory cell function of human endothelial cells. A subpopulation of Ia positive cells is required for antigen presentation. J. Immunol. 131: 666.
29. Geppert, T.D., and P.E. Lipsky. 1985. Antigen presentation by Interferon-γ-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. J. Immunol. 135: 3750.
30. Walker, E.B., L.L. Lanier, and N.L. Warner. 1982. Concomitant induction of the cell surface expression of Ia determinants and accessory cell function by a murine macrophage tumor cell line. J. Exp. Med. 155: 629.
31. Birmingham, J.R., R.W. Chesnut, J.W. Kappler, P. Marrack, R. Kubo, and H.M. Grey. 1982. Antigen presentation to T cell hybridomas by a macrophage cell line: an inducible function. J. Immunol. 128: 1491.
32. Schwarzbaum, S., and B. Diamond. 1983. The J744.2 cell line presents antigen in an I region restricted manner. J. Immunol. 131: 674.
33. Schwarzbaum, S., and B. Diamond. 1985. Generation of functional I-E^d variants from an antigenpresenting macrophage cell line. J. Immunol. 135: 141.
34. Uchida, T., S.-T. Ju, A. Fay, Y.-N. Liu, and M.E. Dorf. 1985. Functional analysis of macrophage hybridomas. I. Productional Characterization. J. Immunol. 134: 772.
35. Schwartzbaum, S., R. Halpern, and B. Diamond. 1984. The generation of macrophage-like cell lines by transfection with SV40 origin defective DNA. J. Immunol. 132: 1158.
36. Blasi, E., D. Radzioch, S.K. Durum, and L. Varesio. 1987. A murine macrophage cell line, immortalized by v-raf and v-myc oncogenes, exhibits normal macrophage functions. Eur. J. Immunol. 17: 1491.
37. Schreier, M.H., R. Tees, A.A. Nordin, R. Benner, A.T.J. Bianchi, and M.J. van Zwieten. 1982. Functional aspects of helper T cell clones. Immunobiology 161: 107.
38. Iscove, N.N. 1984. Culture of lymphocytes and hemopoietic cells in serum-free medium. In: D.W. Barnes, D.A. Sirbasku, G.H. Sato (eds.)

Cell Culture Methods for Molecular and Cell Biology IV. Methods for Serum-Free Culture of Neuronal and Lymphoid Cells. Allen R. Liss, New York. Pp. 169.
39. Schreier, M.H., N.N. Iscove, R. Tees, L. Aarden, and H. von Boehmer. 1980. Clones of killer and helper T cells: growth requirements, specificity and reteention of function in long-term culture. Immunological Rev. 51, 315, 1980.
40. Klasen, I.S., I.G. Donselaar, R.M.T. Ladestein, W.B. van den Berg, and R. Benner. 1986. Joint inflammations and flare-up reactions in mice induced by a helper T cell clone. Agents and Actions 19: 331.
41. Klasen, I.S., Th.H. van der Kwast, I.G. Donselaar, R.M.T. Ladestein, and R. Benner. 1987. Flare-up of delayed-type hypersensitivity initially induced by murine cloned helper T cells. Cell. Immunol. 108: 235.
42. Klasen, I.S., R.M.T. Ladestein, I.G. Donselaar, W.B. van den Berg, R. Tees and R. Benner. 1987. Joint inflammation in mice induced by a $MT4^{+}$ $Lyt\text{-}2^{-}$ T cell clone. Characteristics and the induction of flare-up reactions J. Immunol. 139: 3275.
43. Warner, N.L., M.A.S. Moore and D. Metcalf. 1969. A transplantable myelomonocytic leukemia in BALB/c mice: cytology, karyotype and muramidase content. J. Natl. Canc. Inst. 43: 963.
44. Ralph, P., I. Nakoinz and W.C. Raschke. 1974. Lymphosarcoma cell growth is selectively inhibited by B lymphocyte mitogens: LPS, dextrane sulphate and PPD. Biochem. Biophys. Res. Commun. 61: 1268.
45. Koren, H.S., B.S. Handwerger and J.R. Wunderlich. 1975. Identification of macrophage-like characteristics in a cultured murine tumor line. J. Immunol. 114:894.
46. Van Soest, P.L., J. Josselin de Jong, P.M. Lansdorp, and W. van Ewijk. 1984. An automatic fluorescence micro-ELISA system for quantitative screening of hybridoma supernatants using a protein-A-galactosidase conjugate. Histochem. J. 16: 21.
47. Leenen, P.J.M., A.M.A.C. Jansen, and W. van Ewijk. 1986. Murine macrophage cell lines can be ordered in a linear differentiation sequence. Differentiation 32: 157.
48. Beller, D.I., T.A. Springer, and R.D. Schreiber. 1982. Anti-Mac-1 selectivity inhibits the mouse and human type three complement receptor. J. Exp. Med. 156: 1000.
49. Flotte, T.J., T.A. Springer, and G.J. Thorbecke. 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections and epidermal sheets. Am. J. Pathol. 111: 112.
50. Springer, T.A., G. Galfre, D.S. Secher, and C. Milstein. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. 9: 301.
51. Ho, M-K., and T.A. Springer. 1982. Mac-2, a novel 32,000 Mr mouse macrophage subpopulation specific antigen defined by monoclonal antibodies. J. Immunol. 128: 1221.
52. Ho, M-K., and T.A. Springer. 1983. Tissue distribution, structural characterization, and biosynthesis of Mac-3, a macrophage surface glycoprotein exhibiting molecular weight heterogeneity. J. Biol. Chem. 258: 636.
53. Unkeless, J.C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med. 150: 580.
54. Austyn, J.M., and S. Gordon. 1981. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. 11: 805.

55. Berman, J.W., and R.S. Basch. 1985. Thy-1 antigen expression by murine hemapoietic precursor cells. Exp. Hematol. 13: 1152.
56. Boswell, H.S., P.M. Wade jr., and P.J. Quesenberry. 1984. Thy-1 antigen expression by murine high-proliferative capacity hematopoietic progenitor cells. I. Relation beteen sensitivity to depletion by Thy-1 antibody and stem cell generation potential. J. Immunol. 133: 2940.
57. Ledbetter, J.A., and L.A.Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. 47: 63.
58. Bhattacharya, A., M.E. Dorf, and T.A. Springer. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. J. Immunol. 127: 2488.
59. Van Vliet, E., M. Melis, and W. van Ewijk. 1984. Monoclonal antibodies to stromal cell types of the mouse thymus. Eur. J. Immunol. 14: 524.
60. Leenen, P.J.M., U. Willmer, F.W. Falkenberg, A.M.A.C. Jansen, and W. van Ewijk. 1986. Monoclonal antibodies reactive in different stages in murine macrophage differentiation. In: Oppenheim J.J., Jacobs, D.M. (eds.) Leukocytes and host defense. Alan R. Liss, New York. Pp. 289.
61. Willmer, U., B. Boenke, and Falkenberg. 1984. Identification of differentiation antigens by the murine myeloid/macrophage cell line M1 by monoclonal antibodies. Immunobiology 167: 163.
62. Greenspan, N.S., and J.M. Davie. 1985. serologic and topographic characterization of idiotypes on murine monoclonal anti-streptococcal group A carbohydrate antibodies. J. Immunol. 134: 1065.

CHAPTER 6

JOINT INFLAMMATION INDUCED BY A MATURE MACROPHAGE CELL LINE AND CLONED $MT4^+$,$Lyt\text{-}2^-$ T CELLS IN ALLOGENEIC MICE

I.S. Klasen, R.M.T. Ladestein and R. Benner

Department of Cell Biology, Immunology and Genetics, Erasmus University
Rotterdam, The Netherlands

Agents and Actions, in press.

SUMMARY

Recently we reported the isolation of the mature macrophage cell line AP284. This cell line can efficiently present antigen to cloned helper T cells *in vitro* and *in vivo*. In this paper we show that AP284 can also present antigen to cloned helper T cells in an *in vivo* model system in which joint inflammation is induced. It appeared that injection of cloned helper T cells specific for methylated bovine serum albumin (mBSA) together with mBSA and AP284 into the joints of allogeneic mice induced a substantial joint inflammation. This system offers great prospects for studying the involvement of soluble mediators in the induction of joint inflammation as well as regulatory aspects of this inflammation.

INTRODUCTION

Helper T cells, in contrast to B cells, do not recognize antigen in its native form. These T cells recognize the antigens in a H-2 class II restricted manner. This means that the antigen has to be processed and presented by antigen presenting cells (APC), e.g. macrophages (Mϕ) or dendritic cells bearing class II (Ia) H-2 molecules which have to be syngeneic to the T cells (1).

Helper T cells are thought to play an important role in the induction of inflammatory autoimmune diseases such as rheumatoid arthritis (2). It has been shown in animal models that cloned helper T cells can induce joint inflammations resembling human rheumatoid arthritis (3). We have shown previously that cloned T cells of the helper phenotype specific for mBSA can induce joint inflammation upon intravenous or local injection of the antigen (4). mBSA is an antigen that is often used in the antigen induced arthritis (AIA) model as it is well retained to the negatively charged cartilage structures in the joint. It is thought that the antigen specific T cells remain in the joint as the antigen persists there, resulting in a chronic joint inflammation which can show flare-up phenomena after rechallenge with the antigen (5). Joint inflammations induced by cloned helper T cells can also show flare-up reactions after rechallenge with the antigen (4), suggesting a pivotal role of helper T cells in exacerbations and remissions in reumatoid arthritis.

We here present a model system of AIA in which the inflammation is induced by purely monoclonal cell populations in allogeneic mice. The antigen presenting cells we used are of the mature Mϕ cell line AP284 (6) which presents antigen efficiently *in vitro*, as well as *in vivo*. In the present experiments we show that injection of cloned T cells, AP284 and the antigen in a knee joint leads to the induction of joint inflammation.

MATERIALS AND METHODS

Mice

C57BL/6J and BALB/c mice were purchased from Bomholtgard, Ry, Denmark. Female mice were used at the age of 2-3 months.

Cloned $MT4^+$,$Lyt\text{-}2^-$T cells

The T cell clone specific for mBSA and the culture conditions used were described previously (4). This T cell clone is of C57BL ($H\text{-}2^b$) origin and $H\text{-}2I\text{-}A^b$ restricted in antigen recognition (4).

Macrophage cell line

The macrophage (Mϕ) cell line AP284 was derived from the spleen of a male (C57BL/Rij x CBA/Rij)F1 ($H\text{-}2^{b/q}$) mouse suffering from a spontaneously arisen tumor. The Mϕ cell line was subcloned repeatedly, subclone AP284.D4 was used for the experiments reported here. The cells were cultured in alpha-modification of Dulbecco's medium supplemented with 10% FCS and were passaged twice a week. The Mϕ cell line appeared to be mature in phenotype as it expressed the markers Mac-1, Mac-2, Mac-3 and F4/80 and did not express the immature Mϕ markers Thy-1 and MIV 113. Moreover it constitutively expressed Ia antigens. The Mϕ character was confirmed by the observation that AP284 can phagocytise carbon particles and is positive for the enzymes nonspecific esterase and acid phosphatase and negative for alkaline phosphatase.

Induction and measurement of joint inflammation

Joint inflammation was induced by injection of 30 μg mBSA in a volume of 6 μl balanced salt solution (BSS) into the right knee joint and 30 μg ovalbumin (OVA) in 6 μl BSS into the left knee joint of the mice (mBSA and OVA were derived from Sigma Chemical Company, St. Louis, Missouri, U.S.A.), followed by injection of 3×10^5 mBSA specific cloned T cells in 10 μl BSS into both knee joints. Increasing numbers of AP284 cells were added to this inoculum. 24 Hrs later joint swelling was measured by a ^{99m}Technetium-uptake method described previously (4). The inflammatory swelling measured in the right knee was expressed as [(Technetium-uptake right knee joint - Technetium-uptake left knee joint)/Technetium-uptake right knee joint] x 100%.

Statistics

Significance of the differences between various experimental groups was determined by Student's t-test.

RESULTS AND DISCUSSION

Joint inflammations were induced by local injection of cloned mBSA specific helper T cells of C57BL/6 origin, mBSA and H-2 semicompatible cloned Mϕ of (C57BL x CBA)F1 origin. As a control for the antigen specificity of this inflammatory reaction the irrelevant antigen OVA was injected into the left knee joint, together with helper T cells and Mϕ. In order to prevent participation of host antigen presenting cells in the inflammatory reactions to be observed, the joint inflammations were induced in allogeneic BALB/c mice.

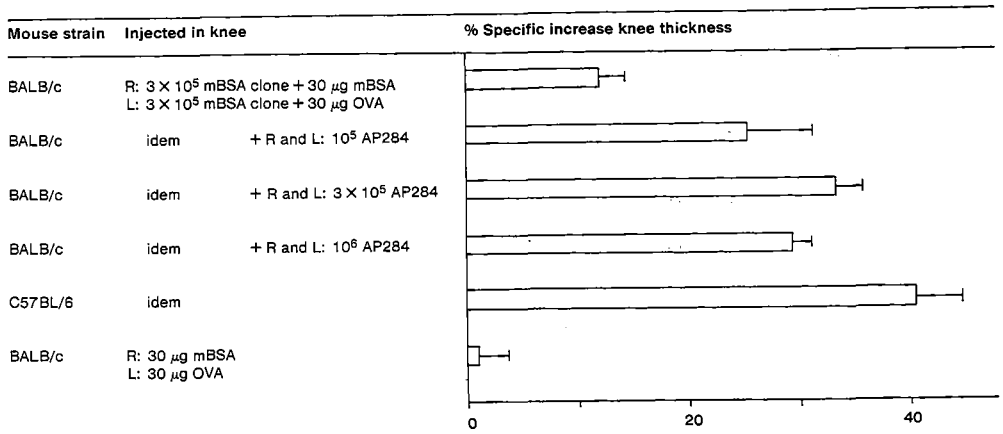


Fig. 1. Antigen presentation by AP284 to mBSA specific cloned $MT4^+$,$Lyt\text{-}2^-$ T cells in the induction of joint inflammation. 3 x 10^5 mBSA specific cloned T cells were injected 0.5-1 hr after injection of 30 μg mBSA into the right kneejoint and 30 μg OVA into the left kneejoint. This was done in syngeneic C57BL/6 or allogeneic BALB/c mice. Increasing numbers of AP284 cells were added to the T cell inocula to be injected into the BALB/c mice. Joint inflammations were measured 24 hrs later and expressed as the specific increase in knee thickness (mean ± SEM; n=5).

As is shown in Fig. 1, injection of 3 x 10^5 mBSA specific cloned helper T cells and the antigen mBSA into the joints of allogeneic BALB/c mice resulted in a minor joint inflammation. Addition of 10^5, 3 x 10^5 or 10^6 AP284 cells in the inoculum to be injected into the knee joints resulted in a significantly higher swelling of the right knee joint as compared to

the group that did not receive AP284 cells ($p<0.05$ for addition of 10^5 AP284 cells and $p<0.005$ for addition of 3×10^5 or 10^6 AP284 cells as compared to the group of BALB/c mice that did not receive AP284 cells).

No clear-cut relationship was found between the different numbers of AP284 cells added to the cloned helper T cells and the extent of joint inflammation, indicating that 10^5 antigen presenting cells were already sufficient for a sizable inflammatory reaction. Thus, in this system employing 3×10^5 cloned helper T cells and 30 μg of mBSA the number of antigen presenting cells appeared not to be the limiting factor.

Histologically the joint inflammation induced in this system, using AP284 antigen presenting cells in allogeneic mice, did not appear different from the joint inflammation induced in mice syngeneic to the cloned helper T cells. In both systems the inflammatory infiltrates mainly consisted of granulocytes and a small number of monocytes (data not shown).

The results show that the combination of a population of well-defined cloned helper T cells and cloned Mϕ together with the specific antigen can produce the mediators that cause a joint inflammation. This is highly dependent on the constitutive expression of Ia antigens by the antigen presenting Mϕ. We have shown in in vitro experiments that the antigen presentation by AP284 to the mBSA specific cloned T cells was almost completely blocked by monoclonal anti-Ia antibodies (6). Others also have reported on Mϕ lines that were able to present antigen to T cells (f.e. 7). These autors, however, did not investigate the antigen presenting capacities of these Mϕ cell line in in vivo experiments.

In the model for AIA presented here, the T cell - Mϕ interaction underlying joint inflammation can be studied in detail in vivo. In particular the soluble mediators involved in this inflammatory responses are now open for further investigations under well defined conditions.

ACKNOWLEDGMENTS

The authors thank Ms. R. Tees and Dr. M.H. Schreier from Sandoz Ltd. Basel, Switzerland, for assistance in the production of the mBSA specific cloned helper T cells, Mr. J. Brandenburg for animal care and Miss G. de Korte and Mrs. J. de Goey-van Dooren for secretarial assistance. This investigation was supported by the Netherlands League against Rheumatism.

REFERENCES

1. E.R. Unanue. Antigen-presenting function of the macrophage. Ann. Rev. Immunol. 2, 395-428 (1984).
2. L. Klareskog, U. Forsun, A. Scheynius, D. Kabelitz and H. Wigzell. Evidence in support of a self-perpetuating HLA-DR dependent delayed-type cell reaction in rheumatoid arthritis. Proc. Natl. Acad. Sci. U.S.A. 79, 3632-3636 (1982).
3. J. Holoshitz, Y. Naparastek, A. Ben-Nun and I.R. Cohen. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 219, 56-58 (1983).
4. I.S. Klasen, R.M.T. Ladestein, I.G. Donselaar, W.B. van den Berg and R. Benner. Joint inflammation in mice induced by a $MT4^+$ $Lyt\text{-}2^-$ T cell clone. Characteristics and the induction of flare-up reactions. J. Immunol. 139, 3275-3280 (1987).
5. L.B.A. van de Putte, J.W. Lens, W.B. van den Berg and M.W.H. Kruysen. Exacerbation of antigen induced arthritis after challenge with intravenous antigen. Immunology 49, 161-167 (1983).
6. I.S. Klasen, J.P. de Jong, J.S.A. Voerman, R.M.T. Ladestein, P.J.M. Leenen and R. Benner. Murine macrophage cell line AP284 presents antigen to cloned $MT4^+$,$Lyt\text{-}2^-$ T cells <u>in vitro</u> and <u>in vivo</u>. Immunobiology, in press.
7. S. Schwartzbaum and B. Diamond. The J774.2 cell line presents antigen in an I region-restricted manner. J. Immunol. 131, 674-677 (1983).

CHAPTER 7

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF JOINT INFLAMMATION AND FLARE-UP REACTIONS INDUCED BY CLONED MT4$^+$,Lyt-2$^-$ T CELLS

Ina S. Klasen*, Renée M.T. Ladestein*, Arianneke A. Grandia*, Theo H. van der Kwast# and Robbert Benner*

*Department of Cell Biology, Immunology and Genetics and #Department of Pathology, Erasmus University Rotterdam, The Netherlands

Submitted for publication.

SUMMARY

This report describes the histological and immunohistochemical characterization of joint inflammations and flare-up reactions in mice induced by cloned $MT4^+$,$Lyt\text{-}2^-$ T cells. The T cell clone used was specific for the antigen methylated bovine serum albumin (mBSA) and inoculated locally into a joint together with the antigen. The histological examination was performed in methyl-methacrylate sections and the various cell types were quantified in distinct regions of the knee joint. The infiltrates consisted predominantly of granulocytes admixed with small numbers of histiocytes. Few lymphocytes were present, while plasma cells were not found. Fibrosis was prominent in the later stages of the inflammation.

Immunohistochemical analysis of total unfixed, undecalcified sections using monoclonal antibodies revealed the presence of T cells which were predominantly of the helper phenotype, sporadic B cells and a considerable number of Ia positive cells. Macrophages were scattered throughout the infiltrate. The synovial lining was shown to express Ia antigens and to contain cells that stained with macrophage markers. Cell clusters were found including helper T (Th) cells, some B cells and Ia positive cells.

These results are in line with immunohistological examinations in other arthritis models and resemble the early events in human rheumatoid arthritis. The data indicate that activated helper T cells are required and sufficient to give rise to the inflammatory infiltrates that are characteristic for the inflammations and exacerbations in human rheumatoid arthritis.

INTRODUCTION

Several animal models for human rheumatoid arthritis have been developed to investigate the processes that are involved in the pathogenesis and the chronicity of this inflammatory disease. The cellular composition of arthritic joints is a topic of interest of many investigators in man as well as in animal models. Human arthritis has been shown to express characteristics of a delayed type hypersensitivity (DTH) reaction in which T cells are in close contact with class II positive cells (1). The antigen induced arthritis (AIA) model in the rat, mouse or rabbit is a model that is frequently used. In the murine model, the animals are immunized by a protein antigen emulsified in Complete Freund's adjuvant (CFA). Several weeks later the animals are challenged with the same antigen. In the case of a cationic antigen, such as methylated bovine serum albumin (mBSA), this treatment results in a chronic joint inflammation (2).

By transfer studies this phenomenon has been shown to be T cell mediated (3). As the cationic antigen is retained to the negatively charged cartilage structures, T cells in the inflammatory infiltrate might persist in the knee joint, resulting in a chronic inflammation (4). Flare-up phenomena in these inflammations may be provoked by a second (local, intravenous or oral) administration of the antigen (5-7).

Recently, we have shown that comparable joint inflammations could also be induced by local inoculation of cloned mBSA specific T cells of the helper phenotype and the antigen mBSA (8,9). These joint inflammations also showed flare-up reactions after a second local administration of mBSA. By making use of this model the functional importance of T cells could be clearly demonstrated during the induction and the flare-up reactions in AIA.

In the present study we investigated the histological and immunohistochemical appearance of the joint inflammations in this well defined model. Histological examination revealed a predominantly granulocytic infiltrate along with smaller numbers of histiocytes and lymphocytes. For immunohistochemical analysis of the induced joint inflammations we adopted a method of tape attached sections of total unfixed, undecalcified knee joints (10). Monoclonal antibodies against T cells (Th and Tc/s), B cells, Ia antigen and macrophages were used. During flare-up reactions the infiltrates showed T cells predominantly of the helper phenotype, few B cells, and a large number of cells that expressed Ia antigens.

MATERIALS AND METHODS

Mice

Female C57BL/Ka mice were used that were obtained from Bomholtgard, Ry, Denmark or from our own breeding stock.

Cloned T cells

The production of the cloned $MT4^+$,Lyt-2^- T cells and the culture conditions optimal for these T cells have been described previously (8,11).

Induction of arthritis and flare-up reactions

Arthritis was induced as described before (8,9). Briefly, 3 x 10^5 or 5 x 10^5 mBSA specific $MT4^+$,Lyt-2^- cloned T cells were injected intra-articularly 0.5 - 1 hour after the injection of 30 μg of the antigen mBSA into the right knee joint and the irrelevant control antigen ovalbumin (OVA) into the left knee joint. Flare-up reactions were induced by a repeated local injection of the same amount of the antigens two weeks later. 24 Hours after the induction of joint inflammation or flare-up reaction the inflammation was quantified by the ^{99m}Technetium-uptake method described by Kruysen et al. (12). The inflammatory swellings were expressed as the specific increase of knee thickness which was designated as ([Technetium uptake right joint - Technetium uptake left joint]/ Technetium uptake left joint) x 100%.

Histology

24 Hours after induction of joint inflammation or flare-up reaction the right and the left knee joints of the mice were removed in toto. Similarly the knee joints were removed from mice that had a waned joint inflammation 14 days after induction of the inflammation and that did not receive a second challenge with the antigens. The dissected joints were fixed in formaldehyde/methanol and subsequently embedded in methyl-methacrylate as described previously (13). Sections of 3 μm thickness were stained by Giemsa. The sections of representative mice were examined by two independent investigators. The numbers of the various cell types in the infiltrates were determined at a total magnification of 400-800 times using a counting grid in which a total of 100 cells was counted. This was done in 3 (first induction) or 4 (flare-up) separate levels of the joint. The arithmic

mean of the counts of these levels was calculated. Each section was examined in three different regions indicated in Fig. 1.

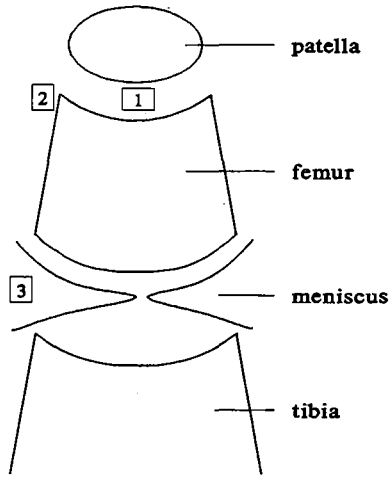


Fig. 1. Schematic presentation of a cross-section of a mouse knee joint showing the location of the regions in which the quantification of the various cell types was performed.

Table 1

Monoclonals used for the immunohistochemical characterization.

Monoclonal antibody	Antigen	Specificity	Reference
59AD2.2	Thy-1	T lymphocytes, stem cells myeloid progenitors	15
MT4	CD4	Th cells	16
Lyt-2	CD8	Tc/s cells	15
Ra3-6B2	B220	B lymphocytes	17
M5/114	$I\text{-}A^{b/d/q}$, $I\text{-}E^{d/k}$	Ia	18
F4/80	F4/80	macrophages	19

Immunohistochemistry and monoclonals

Cryostate sections (8 μm) of the total unfixed, not decalcified knee joints were made using a Bright cryostate with a tungsten carbide tipped knife. The sections were attached to adhesive tape as has been described before (10,14), attached to slides, fixed for 7 seconds in acetone (analytical grade, Merck, Darmstadt, FRG) and air dried. Subsequently the slides were incubated with one of the monoclonal antibodies listed in Table 1 during 45 min at 4°C. Undiluted cell culture supernatants were used. Negative control slides were incubated with phosphate-buffered saline (PBS) pH 7.4 with 0.2% bovine serum albumin (BSA) only. The slides were washed with the same PBS and incubated in the second step with peroxidase labelled rabbit anti-rat-Ig (Dakopatts, Copenhagen, Denmark) diluted 1:40 in PBS containing 0.2% BSA and 1% normal mouse serum. Peroxidase activity was visualised by incubation with 3,3'-diaminobenzidine-tetrahydrochloride (DAB, Sigma, St. Louis, USA). 0.5 mg/ml DAB was used in Tris-HCl buffer (pH 7.6) supplemented with 0.03% H_2O_2. The slides were counterstained with haematoxylin and embedded in glycerin/gelatin. In these sections the staining by the monoclonals used was easily distinguished from the endogenous peroxidase activity as the monoclonals showed a clear membrane or cytoplasmic staining whereas the granulocytes as a result of endogenous peroxidase activity appeared as dark brown dots. Therefore, no attempt was made to block endogenous peroxidase activity.

RESULTS

Joint inflammations in C57BL/Ka mice were induced by local injection of 3×10^5 or 5×10^5 syngeneic, mBSA specific cloned T cells. 0.5 - 1 Hour before injection of the cloned T cells 30 μg mBSA had been injected in the right knee joint and 30 μg OVA into the left knee joint. Fourteen days later in a part of these mice a flare-up reaction was provoked by injection of 30 μg of the same antigens. The specific increase in knee thickness due to the inflammatory reactions are shown in Table 2.

The joints of the mice of experiment a. were histologically examined, whereas the joints of the mice of experiment b. were used for immunohistochemical investigation.

Table 2

Specific increase of knee thickness (± SEM) of the mice used for histological and immunohistochemical examination.

Expt.	Injected	24 hours after induction	14 days after induction	24 hours after flare-up induction
a.	R: $5x10^5$ mBSA clone+30 μg mBSA L: $5x10^5$ mBSA clone+30 μg mBSA	41.0 ± 2.6	2.1 ± 2.0	21.5 ± 4.2
b.	R: $3x10^5$ mBSA clone+30 μg mBSA L: $3x10^5$ mBSA clone+30 μg mBSA	56.3 ± 4.7	11.7 ± 2.3	23.5 ± 4.0

Experiment a. was the experiment of which the histological appearance is presented in Figs. 2 and 3; experiment b. was immunohistochemically investigated and is shown in Fig. 4.

Histopathology

The mean numbers of the various cell types counted in the sections of the joints 24 hours after induction of the inflammation and 24 hours after induction of the flare-up reaction are shown in Fig. 2. It demonstrates that both the infiltrates in the initial inflammation and in the flare-up reaction were mainly granulocytic.

In the initial inflammation fibroblasts were found in a higher percentage in the region that was next to the menisci (region 3) than in the joint space between patella and femur (region 1). In region 3 infiltration of the surrounding muscles by the inflammatory cells was found. Basophils and mast cells were only observed in regions 2 and 3. Not a single mast cell was observed in region 1. Fibrin-like deposits were found in region 1. Plasma cells were not observed in these infiltrates.

During flare-up reaction relatively more fibroblasts were found in region 3 than during the initial joint inflammation. Moreover, region 1 tended to contain more histiocytes in the flare-up reaction. Fig. 3a shows the inflammatory infiltrate in the right knee at 24 hours after induction of the inflammation. Fig. 3b shows a similar site 24 hours after induction of a flare-up reaction. The flare-up infiltrate is shown in more detail in Figs. 3c and 3d.

The very limited infiltrate 14 days after induction of the joint in-

flammation precluded reliable quantification of the different cell types. This infiltrate consisted of fibroblasts, histiocytes, some lymphocytes and mast cells. This is shown in Fig. 3e.

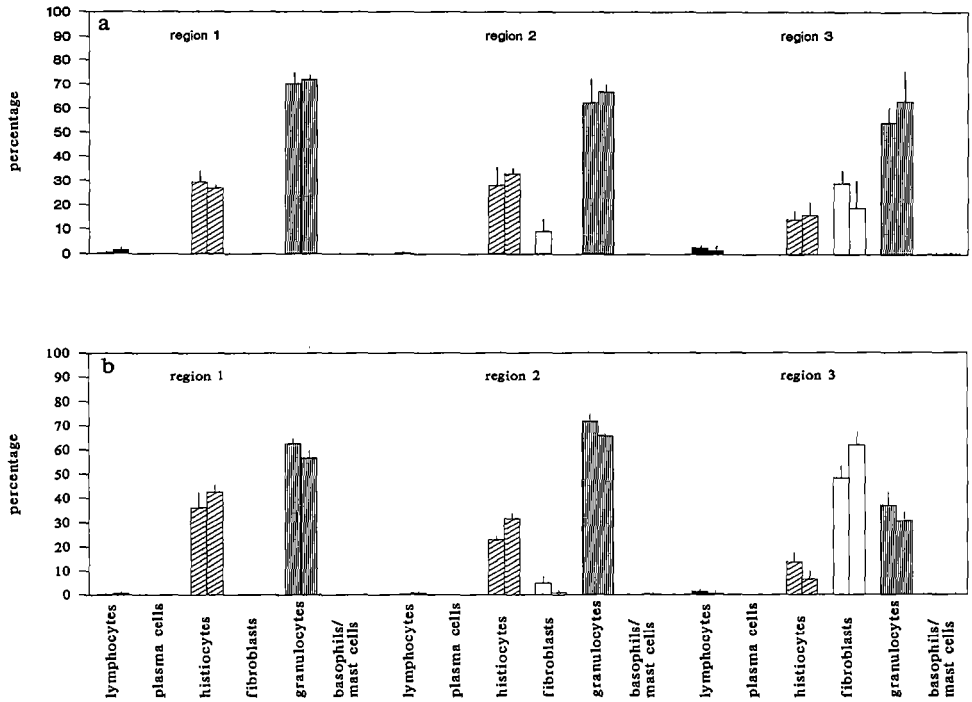


Fig. 2. Scores of the various cell types upon examination by two independent investigators of (a) the inflammatory infiltrate 24 hours after the induction of inflammation by injection of 5 x 10^5 mBSA specific cloned T cells and 30 μg of mBSA; and (b) 24 hours after the induction of a flare-up reaction after a rechallenge with 30 μg mBSA. The arithmatic mean of the counts of 3 or 4 levels ± SEM are presented.

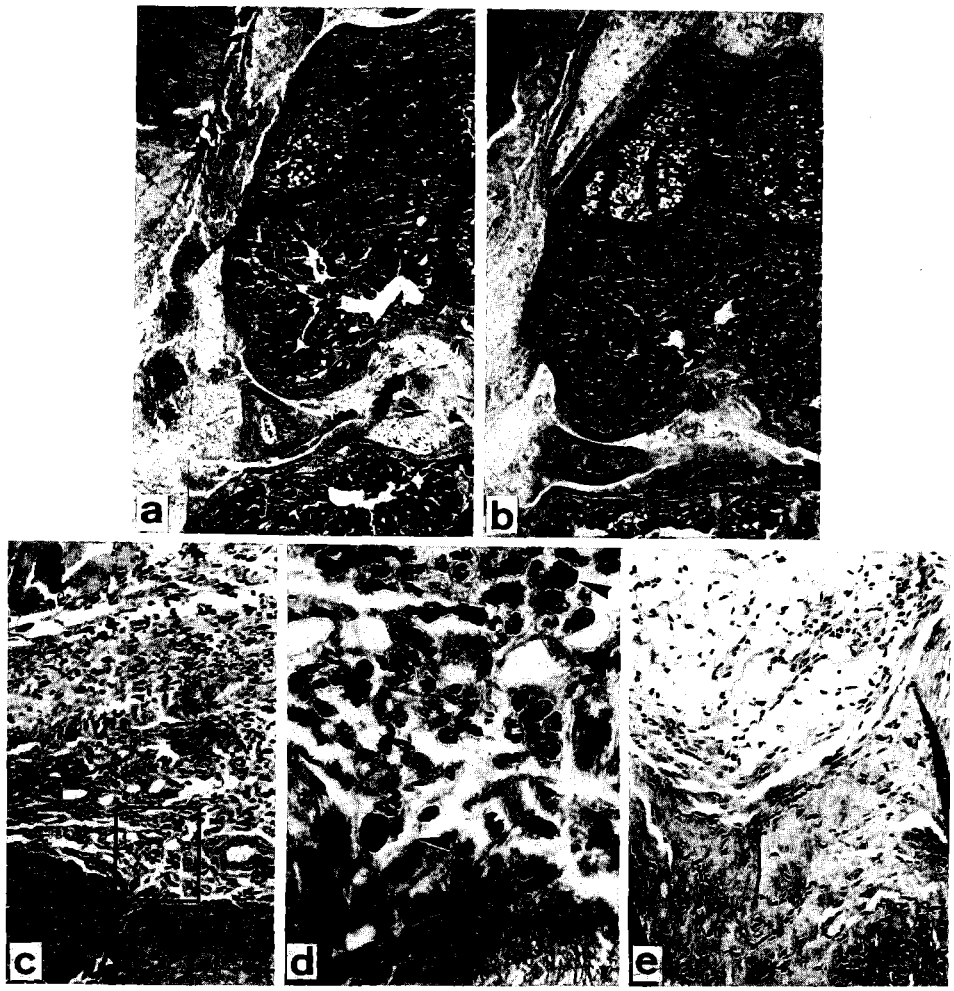


Fig. 3. Histological appearance of Giemsa stained methyl-methacrylate sections of the right knee joint of a mouse (a) 24 hours after injection of 5 x 10^5 mBSA specific cloned T cells and 30 μg of mBSA, 5 x; (b) 24 hours after induction of a flare-up reaction by rechallenge with 30 μg mBSA 5 x; (c) detail of b., 50 x; (d) detail of c., 200 x and (e) 14 days after induction of joint inflammation as described in a., 50 x; F is femur, arrows indicate mast cells.

The left knee joints of the mice served as a control as the cloned T cells were injected there together with the non-crossreacting antigen ovalbumin. No inflammatory reaction was found in these control knee joints (data not shown).

24 Hours after the initial joint inflammation no cartilage destruction or hyperplasia of the synovial lining was observed. 13 Days later some cartilage destruction was found, while the synovial lining was swollen, with focal synovial cell hyperplasia. The surrounding bone showed an increased remodeling activity.

Immunohistochemistry

The staining pattern produced by the monoclonal antibodies used (Table 1) was analysed at 24 hours and 14 days after induction of the initial joint inflammation and 24 hours after induction of the flare-up reaction. Generally, the staining patterns after the initial induction of joint inflammation and in the flare-up reaction were much alike.

During flare-up several Thy-1 positive cells were present in the joint space (Fig. 4a), mainly in region 3 as defined in Fig. 1 and in the region where the menisci were located. In the joint space between patella and femur almost no Thy-1 positive cells were observed. Upon further examination the T cells appeared to be predominantly MT4-positive (i.e. helper phenotype) (Fig. 4b), while only a few Lyt-2 positive cells (i.e. cytotoxic/suppressor cell phenotype) were found (Fig. 4c). The T cells were not observed in the synovial lining. Ia positive cells, determined by the monoclonal antibody M5/114 were found throughout the infiltrate (Fig. 4d). Occasionally cells of the synovial lining were found to be Ia positive. F4/80 positive cells were scattered throughout the infiltrate and were also present within the synovial lining membrane (Fig. 4e). Only a few B cells, positive for the monoclonal Ra3-6B2 were found (not shown), but they were hard to discriminate as this monoclonal antibody gave a considerable background staining. Clusters of T cells (mostly Th with some Tc/s) in combination with Ia positive cells and macrophages were found, with some tendency for perivascular localization. The small infiltrate at 14 days after induction of the inflammation was mainly situated in the region adjacent to the menisci. It consisted predominantly of Th lymphocytes and class II positive cells. At this time the synovial lining was almost class II negative. F4/80 positive cells were present in the infiltrate and in the synovial lining.

Fig. 4. Immunohistochemical appearance of the right knee joint of a mouse 24 hours after induction of a flare up reaction by a rechallenge with 30 μg mBSA. The initial inflammation had been induced by injection of 3 x 10^5 mBSA specific cloned T cells and 30 μg mBSA. The cryosections were stained by the monoclonals (a) 59AD2.2; (b) MT4; (c) Lyt-2; (d) M5/114; and (e) F4/80 (c.f. Table 1). Arrows indicate positive cells. SL is synovial lining, C is cartilage. Magnification 50 x.

DISCUSSION

In the first part of this paper we presented the histology of joint inflammations and flare-up reactions induced by cloned $MT4^{+}Lyt\text{-}2^{-}$ T cells and the antigen recognized by the T cell clone. The cellular composition of the infiltrate cells in these inflammations was quantified by counting cell types in various sections of the joint in different regions. The infiltrates observed 24 hours after the initial induction of the joint inflammation and 24 hours after the flare-up reaction were much alike. In both situations granulocytes predominated. Since granulocytes have a short lifetime, the infiltrate that is left and is observed 14 days after induction of the inflammation was more histiocytic and characterized by fibrosis.

Probably after a second administration of the antigen the retained T cells in the joints are stimulated again and start to produce lymphokines that attract granulocytes again. In the flare-up reaction somewhat more histiocytes and fibroblasts were found probably as the result of the longer existing inflammation. In the flare-up reaction we observed some cartilage destruction with a 'ruffled' appearance. This cartilage destruction was not described in our previous papers (8,9). Probably the cartilage destruction depends on the intensity of the inflammation during the initial induction stage.

Subsequently we investigated the immunohistochemistry of the joint inflammations induced by the cloned helper T cells and the antigen. Also in this examination the inititial inflammation and the flare-up reaction were much alike. Quite a few T cells were observed, predominantly of the helper phenotype. Experiments are in progress using Thy-1 congenic mice to investigate how many of these T cells belong to the cloned T cells that we injected into the joint or are derived from them. The number of T cells that we observed after immunohistochemical staining was higher than the number of lymphocytes that we determined histologically. So, probably we underestimated the number of lymphocytes in the latter study.

The Ia expression on various cell types was superfluous and the synovial lining contained Ia positive cells as well. This Ia expression is probably due to the release of gamma-interferon by the cloned $MT4^{+}$,$Lyt\text{-}2^{-}$ T cells (20). F4/80 positive cells, were scattered throughout the infiltrate. Cells of the synovial lining were also stained by F4/80. We were unable to discriminate between the type I, II, and III cells in the synovium as

defined by Burmester (21) and studied by Dijkstra et al. in the AIA model in the rat (10), as we did not apply monoclonal antibodies specific for these subpopulations. We assume that the F4/80 monoclonal antibody mainly stains the type I (macrophage-like) cells of the synovial lining.

Clusters of cells were found at several places in the infiltrate in which T cells (mainly Th and some Tc), and Ia positive cells were present as well as some B cells. In these clusters presentation of antigen probably takes place (10). The predominance of helper T cells is in line with the data reported in the adjuvant arthritis model (22) and the collagen type II model (23) in rats. Furthermore, in the adjuvant arthritis model it has been shown that a depletion of the Tc/s cells did not affect the disease course. In biopsy specimens or cell suspensions from synovial effusions in man a predominance of $CD8^+$ cells has been found (24,25). However, others have found predominantly $CD4^+$ cells (26,27). The contradictory results probably in part result from the different sites of investigation (28). Still the occurrence of $CD4^+$ cells is more in line with the DTH nature (1) of arthritis in experimental models as well as in human rheumatoid diseases.

In conclusion, we have shown that injection of cloned T cells of the helper phenotype and the corresponding specific antigen can result in a joint inflammation that is histologically and immunohistochemically comparable to the inflammations observed in the AIA model in immunized animals (10) and the initial stages of human rheumatoid arthritis (29). Moreover, the flare-up reactions of joint inflammations provoked by reintroduction of the specific antigen appeared histologically and immunohistochemically comparable to the infiltrates of the initial inflammations. Thus in mice only T cells and the specific antigen can account for the flare-up reactions that resemble the exacerbations occurring in human rheumatic diseases.

ACKNOWLEDGMENTS

The assistance of Mrs. Ilse Vogels and Mr. Ed Döpp in the preparation of the immunohistochemical slides is gratefully acknowledged. We also thank Prof. Dr. O. Vos and Dr. W. van Ewijk for continuous support, Mr. Tar van Os for expert photography and Ms. Geertje de Korte for secretarial assistance.

REFERENCES

1. Klareskog, L., U. Forsum, A. Scheynius, D. Kabelitz and H. Wigzell. Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis. Proc. Natl. Acad. Sci. USA 79:3632-3636, 1982.
2. Brackertz, D., G.F. Mitchell and I.R. Mackay. Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum. 20:841-850, 1977.
3. Brackertz, D., G.F. Mitchell, M.A. Vadas and I.R. Mackay. Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. J. Immunol. 118:1645-1648, 1977.
4. Van den Berg, W.B., L.B.A. van de Putte, W.A. Zwarts and L.A.B. Joosten. Electrical charge of the antigen determines intraarticular antigen handling and chronicity of arthritis in mice. J. Clin. Invest. 74:1850-1859, 1984.
5. Van Beusekom, H.J., L.B.A. van de Putte, W.B. van den Berg, W.J.M. van den Broek and W.C.A.M. Buijs. Antigen handling in antigen-induced joint inflammation: kinetics of a second intra-articularly injected dose of antigen in an already established antigen-induced joint inflammation. Immunology 44:153-161, 1981.
6. Van de Putte, L.B.A., J.W. Lens, W.B. van den Berg and M.W.M. Kruijsen. Exacerbation of antigen-induced arthritis after challenge with intravenous antigen. Immunology 49:161-167, 1983.
7. Lens, J.W., W.B. van den Berg, L.B.A. van de Putte and L. van den Bresselaar. Flare-up of antigen-induced arthritis in mice after challenge with oral antigen. Clin. exp. Immunol. 58:364-371, 1984.
8. Klasen, I.S., R.M.T. Ladestein, I.G. Donselaar, W.B. van den Berg, R. Tees and R. Benner. Joint inflammations in mice induced by a $MT4^+$,Lyt-2^- T cell clone. Characteristics and flare-up reactions. J. Immunol. 139:3275-3280, 1987.
9. Klasen, I.S., R.M.T. Ladestein, W.B. van den Berg and R. Benner. Requirements for flare-up reactions of joint inflammations in mice induced by cloned $MT4^+$,Lyt-2^+ T cells. Arthritis Rheum. in press.
10. Dijkstra, C.D., E.A. Döpp, I.M.C. Vogels and C.J.F. van Noorden. Macrophages and dendritic cells in antigen-induced arthritis: an immunohistochemical study using cryostat sections of the whole knee joint of rat. Scand. J. Immunol. 26:513-523, 1987.
11. Schreier, M.H., N.N. Iscove, R. Tees, L. Aarden and H. von Boehmer. Clones of killer and helper T cells: growth requirements, specificity and retention of function in long-term culture. Immunol. Rev. 51:315-336, 1980.
12. Kruijsen, M.W.M., W.B. van den Berg, L.B.A. van de Putte and W.J.M. van den Broek. Detection and quantification of experimental joint inflammation in mice by measurement of ^{99m}Tc-pertechnetate uptake. Agents Actions 11:640-642, 1981.
13. Te Velde, J., R. Burkhardt, K. Kleiverda, L. Leenheers-Binnendijk and W. Sommerfeld. Methyl-methacrylate as an embedding medium in histopathology. Histology 1:319-330, 1977.
14. Rijntjes, N.V.M., L.B.A. van de Putte, M. van der Pol and P.J.M. Guelen. Cryosectioning of undecalcified tissues for immunofluorescence. J. Immunol. Meth. 30:263-268, 1979.
15. Ledbetter, J.A. and L.A. Herzenberg. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. 47:63-90, 1979.

16. Pierres, A., P. Naquet, A. van Agthoven, F. Bekkoucha, F. Denizot, Z. Mishal, A.M. Schmitt-Verhulst and M. Pierres. Rat anti-mouse T4 monoclonal antibody (H129.19) inhibits the proliferation of Ia-reactive T cell clones and delineates phenotypically distinct ($T4^+$, $Lyt2,3^-$ and $T4^-$, $Lyt2,3^+$) subset among anti-Ia cytotoxic T cell clones. J. Immunol. 132:2775-2782, 1984.
17. Coffman, R.L. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. Immunol. Rev. 69:5-23, 1982.
18. Bhattacharya, A., M.E. Dorf and T.A. Springer. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region duplication. J. Immunol. 127:2488-2495, 1981.
19. Austyn, J.M. and S. Gordon. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. 11:805-815, 1981.
20. Steeg, P.S., R.N.Moore, H.M. Johnson and J.J. Oppenheim. Regulation of murine Ia antigen expression by a lymphokine with immune interferon activity. J. exp. Med. 156:1780-1793, 1982.
21. Burmester, G.R., A. Dimitriu-Bona, S.J. Waters and R.J. Winchester. Identification of three major synovial lining cell populations by monoclonal antibodies directed to Ia antigens and antigens associated with monocytes/macrophages and fibroblasts. Scand. J. Immunol. 17:69-82, 1983.
22. Holmdahl, R., K. Rubin, L. Klareskog, L. Dencker, G. Gustafson and E. Larsson. Appearance of different lymphoid cells in synovial tissue and in peripheral blood during the course of collagen II-induced arthritis in rats. Scand. J. Immunol. 21:197-204, 1985.
23. Larsson, P., R. Holmdahl, L. Dencker and L. Klareskog. In vivo treatment with W3/13 (anti-pan T) but not with 0x8 (anti-suppressor/cytotoxic T) monoclonal antibodies impedes the development of adjuvant arthritis in rats. Immunology 56:383-391, 1985.
24. Duke, O., G.S. Panayi, G. Janossy, L.W. Poulter and N. Tidman. Analysis of T cell subsets in the peripheral blood and synovial fluid of patients with rheumatoid arthritis by means of monoclonal antibodies. Ann. Rheum. Dis. 42:357-361, 1983.
25. Jahn, B., G.R. Burmester, M. Gramatzki, G. Weseloh, P. Stock and J.R. Kalden. Intraarticular T lymphocytes in monoarticular and oligoarticular inflammatory joint diseases. J. Rheumatol. 13:254-257, 1986.
26. Johnell, O., A. Hulth and A. Henricson. T-lymphocyte subsets and HLA-DR-expressing cells in the osteoarthritic synovialis. Scand. J. Rheumatol. 14:259-264, 1985.
27. Poulter, L.W., O. Duke, G.S. Panayi, S. Hobbs, M.J. Raftery and G. Janossy. Activated T lymphocytes of the synovial membane in rheumatoid arthritis and other arthropathies. Scand. J. Immunol. 22:683-690, 1985.
28. Kurosaka, M. and M. Ziff. Immunoelectron microscopic study of the distribution of T cell subsets in rheumatoid synovium. J. exp. Med. 158:1191-1210, 1983.
29. Zvaifler, N.J. Pathogenesis of the joint disease of rheumatoid arthritis. Am. J. Med. 75 (suppl.):3-8, 1983.

CHAPTER 8

GENERAL DISCUSSION

Several studies in human autoimmune diseases and the corresponding animal models point to the role of T cells in these diseases. In animal models it is shown that T cells can transfer autoimmune diseases such as experimental allergic encephalitis (Ben-Nun and Cohen, 1982), uveoretinitis (Caspi et al., 1986; Rozenszajn et al., 1986) thyreoiditis (Maron et al., 1983) and also arthritis (Holoshitz et al., 1983) to naive recipients. In view of the subject of this thesis we shall here focus on data on human and experimental arthritis. In human arthritis the inflammatory reaction resembles a delayed type hypersensitivity (DTH) reaction in which T cells co-operate with HLA-DR expressing cells (Klareskog et al., 1982). After it became feasible to develop cloned T cell populations and keep them in culture for several months or even years (Hengartner and Fathman, 1980; von Boehmer and Haas, 1981; Schreier et al., 1982; Sredni, 1982), the role that T cells play in such DTH reactions could be studied more thoroughly. It was found that T cells of the helper phenotype (in mice $MT4^+$,$Lyt\text{-}2^-$) were capable of inducing DTH reactions (Bianchi et al., 1981). Today it is known that two types of helper T cells exist. The Th1 population that produces IL-2 and γ-Ifn and can mediate DTH reactions and the Th2 population that produces IL-4 and IL-5 and thereby efficiently helps B cells to produce IgG1 and IgE (Mosmann et al., 1986; Cher and Mosmann, 1987).

1. Antigen induced arthritis mediated by T cell clones

In this thesis the role of T cells in the induction and exacerbations of rheumatic diseases was studied using mice as experimental animals. The model we used is the antigen induced arthritis (AIA) model, in which we made use of cloned Th1 cells to evoke joint inflammations. This model will be discussed in detail first.

In the AIA model in mice, the mice are immunized with positively charged antigens such as methylated bovine serum albumin (mBSA) or amidated bovine serum albumin (aBSA) (Brackertz et al., 1977b; van den Berg et al., 1984). Several weeks later they are challenged with the same antigen. Consequently

a chronic joint inflammation develops that resembles human rheumatoid arthritis. An interesting feature of this model is that these joint inflammations can show the so-called flare-up reactions that are comparable with the exacerbations in human rheumatic diseases. These flare-up reactions take place after a repeated challenge with the homologous antigen by local (van Beusekom et al., 1981) or systemic (iv or oral) (van de Putte et al., 1983; Lens et al., 1983; 1984a) administration. In the induction of joint inflammations (Brackertz et al., 1977a) as well as in the flare-up reactions (Lens et al., 1984b) T cells are thought to play a pivotal role. The idea is that the chronicity of the reaction is due to the persistence of T cells in the joint thanks to the persistence of the injected antigen. The latter is retained due to charge interactions between the antigen and the joint cartilage (van den Berg et al., 1984; 1986). In the flare-up reaction these T cells most likely respond to the reinjected antigen with the production of lymphokines causing a renewed inflammation. As these experiments were performed in immunized mice, the role of cellular and humoral responses is hard to distinguish. We reasoned that experiments with cloned T cells should descriminate between these two possibilities and should shed light on the role of T cells in the induction and flare-up of joint inflammations.

In our studies we used T cell clones of the $MT4^+$,$Lyt\text{-}2^-$ phenotype that produced IL-2 and γ-Ifn and mediated DTH (Chapter 2). According to their functional properties these clones represent Th1 cells after the terminology of Mosmann et al. (1986). In most studies we used a T cell clone specific for the cationic antigen mBSA. The clones were cultured in serum free medium and restimulated with the antigen every 5 or 6 weeks. In between the restimulations they were grown on recombinant human IL-2.

These cloned T cells appeared to be able to mediate joint inflammations that continue for several days when injected into the knee joint in combination with the relevant antigen (Chapter 3). The kinetics of these joint inflammatory reactions were examined by making use of a ^{99m}Technetium uptake method for the measurement of the joint swelling (Kruijsen et al., 1981). This method has been shown to correlate well with the inflammation as judged by histological examination (Lens et al., 1984a). These joint inflammations could also be induced by systemic administration of the cloned T cells and local administration of the antigen. In the latter case cloned T cells have to migrate to the site of antigen deposition.

These are troublesome experiments as cultured T cell populations often loose their normal migratory capacities and tend to be retained in the lungs and the liver after systemic administration (Dailey et al., 1982). The loss of receptors for high endothelial venules (HEV) might account for the poor migratory capacity of cultured T cells (Dailey et al., 1985). Such HEV can also occur in inflamed joints (Gardner, 1986), although the recognition of these HEV by T cells is probably controlled by another endothelial cell recognition system than the recognition of lymph node HEV (Jalkanen et al., 1986; Schrieber et al., 1987).

Investigators who make use of cloned T cells for in vivo experiments often restimulate their T cells with the antigen before systemic administration (Holoshitz et al., 1984). In our hands this did not improve the arthritogenicity.

In our experiments the antigen recognition by the cloned T cells of C57BL (H-2^b) origin used was H-2I-A^b resticted, i.e. they only recognized the antigen when it was presented by antigen presenting cells expressing the right (H-2I-A^b) class II antigens. In vivo this resulted in an H-2I-A^b restricted induction of inflammatory reactions (Chapters 2 and 3). In mice not expressing the right H-2 molecules, this lack of H-2I-A^b could be overcome by the addition of syngeneic macrophages to the T cell inoculum. In Chapters 5 and 6 we made use of a macrophage cell line, AP284, that constitutively expresses I-A molecules and was obtained from a (C57BL/Rij x CBA/Rij)F1 (H-$2^{b/q}$) mouse.

As we knew that (joint) inflammations could be induced by the cloned helper T cells together with the relevant antigen we investigated whether these inflammations could also develop flare-up phenomena. As we considered these joint inflammations to be essentially based on DTH reactions, the first part of this study was performed in a DTH model that has been thoroughly investigated in our laboratory. In this model DTH reactions are induced in the instep of the hind foot of a mouse. Using this model we showed that flare-up reactions could be induced by local but also by systemic (iv or oral) administration of the antigen (Chapter 2). In joint inflammations flare-up reactions occurred after local administration of the antigen (Chapters 3 and 4). Flare-up reactions could not be detected after systemic rechallenge with the antigen, which is probably caused by the lower sensitivity of the method for measuring knee swelling as compared to the method for measuring foot swelling.

Subsequently we investigated whether the cloned T cells that we injected, and not the T cells of the recipient mice, were responsible for the joint inflammations and flare-up reactions. We therefore made use of syngeneic athymic nude mice. These nude mice were shown to be indeed capable of exhibiting joint inflammation after injection of the cloned helper T cells and the antigen. Moreover, they showed flare-up reactions after local rechallenge with the antigen (Chapter 4). These results indicate that the cloned T cells that were injected into the joints of the nude mice were retained there and were still functionally reactive after several weeks. Also in studies on contact hypersensitivity it has been shown that antigen specific T cells can persist in the skin (Scheper et al., 1983).

In the AIA model using immunized mice the presence of a persisting antigen in the joint is a prerequisite for chronicity and flare-up of the inflammation. We were anxious to investigate whether this is also the case in our AIA model using cloned T cells. In this model the cloned T cells appeared to be able to persist and to keep their functional reactivity in a knee joint for several days in the absense of the relevant (Chapter 4). However, in the presence of the relevant antigen the persistence and/or functional reactivity of the T cells was better.

Histological examination of the joint inflammations (Chapters 3, 4 and 7) showed that the infiltrates were comparable to those observed in the original AIA model using immunized mice. The infiltrates were mainly granulocytic, with low numbers of macrophages and few lymphocytes, which is a common feature of DTH reactions in rodents (Schreier et al., 1982). The more chronic reactions tend to display somewhat more monocytes and fibrosis. The flare-up reactions were characterized by a renewed influx of granulocytes along with some monocytes and fibrosis. Some cartilage destruction was observed in the later stages of the joint inflammations.

Immunohistochemically the initial joint inflammation and the flare-up reaction appeared much alike using an immunoperoxidase method in unfixed undecalcified cryosections. Numerous T lymphocytes, mainly of the helper - phenotype were found. Many MHC class II expressing cells were observed amongst others in the synovial lining. Cells staining with macrophage markers were found scattered through the infiltrate and in the synovial lining.

In the model system employed an artificial antigen (mBSA) was used, but the data presented show that the model shares several characteristics

with human rheumatic diseases. In research on rheumatic diseases a central question is the nature of the antigen that triggers the immune system to cause these lesions. In recent years several interesting studies were published dealing with this aspect. We shall discuss some of these data hereafter.

2. Arthritis by bacterial cell wall products

There are many indications that bacteria might play a role in the etiology of rheumatic diseases. Firstly the reactive arthritis that occurs after infections with bacteria such as Shigella, Yersinia, Campylobacter or Salmonella (Phillips and Christian, 1985; Toivanen et al., 1985). Klebsiella is suspected to be involved in the etiology of ankylosing spondylitis (Keat, 1986). Moreover, it has been described that rather frequently joint inflammations develop after intestinal bypass surgery in case of obesitas (Lancet editorial, 1983). Crohn's disease patients, that suffer from chronic gut inflammations have a higher risk to develop joint inflammatory diseases (Greenstein et al., 1976). An important question that cannot be answered clearly, is whether in these diseases an antigen is carried to the joints. A probable source of this antigen is the gut. The role of gut bacteria in the etiology of RA has been emphasized (Bennett, 1978; J. Rheumatol. editorial, 1987). An interesting observation is that in patients suffering from reactive arthritis, ankylosing spondylitis or juvenile chronic arthritis strikingly often lesions of the gut are found (Mielants and Veys, 1984; Mielants et al., 1987; J. Rheumatol. editorial, 1987). A strong correlation between clinical articular inflammation and gut inflammation was reported (Mielants et al., 1987). In cases of joint inflammation the permeability of the gut is likely increased so that antigens can leak out, enter the bloodstream and may be deposited in the joints.

The presence of the antigen in the joint may be not an absolute necessity. This is clear from the studies of the group of Cohen. They explored an experimental model in the rat, in which arthritis is induced by complete Freund's adjuvant (CFA) which contains Mycobacterium tuberculosis (Holoshitz et al., 1983; 1984; Cohen et al., 1985). These investigators showed that the arthritis is due to the cross reactivity between an epitope on Mycobacterium tuberculosis and cartilage. A T cell clone that was isolated from an arthritic rat and was arthritogenic in irradiated naive rats appeared to recognize cartilage structures (Cohen et al., 1985). Cross reactivity

involving bacteria has also been shown at the antibody level between Klebsiella, Yersinia and HLA-B27 (an HLA type that is at risk for reactive arthritis and ankylosing spondylitis) (Keat, 1986; Chen et al., 1987). Also Streptococci are suspect in the etiology of rheumatic diseases. In rats they have been shown to be capable of inducing severe joint inflammations (Cromartie et al., 1977). Moreover, cross reactivities of Streptococci with cardiac valves and connective tissue have been shown (Kaplan and Meyeserian, 1962; Sandson et al., 1968). However, in man infections of Mycobacteria and Streptococci do not correlate with the occurrence of rheumatic diseases. The observation that anaerobic bacteria from the human endogenous intestinal flora such as Peptostreptococcus productus (Stimpson et al., 1986) and Eubacterium aerofaciens (Severijnen et al., 1988; submitted) can induce chronic joint inflammations in rats is probably of greater importance. Moreover, from the faeces of normal individuals and RA patients soluble peptidoglycan-polysaccharide (PG-PS) complexes have been isolated that are likely of bacterial origin. Under certain conditions they can also be arthritogenic in the rat (M.P. Hazenberg, personal communication). We therefore hypothesize that these PG-PS complexes may be released from the gut and induce antigen specific T cell proliferation. These T cells might react toward antigens in joints, thereby releasing lymphokines and consequently inducing inflammatory lesions. Whether this reactivity is due to accumulation of PG-PS complexes in the joints or based on cross reactivity is at present unclear.

In the streptococcal model and in the model that uses endogenous human gut bacteria it has not been feasible yet to transfer the disease to naive recipients. Maybe this indicates that the antigen has to be present in the joint. It may well be that exacerbations occur when antigen reactive T cells are already present in the joint and new antigen from the gut is deposited in the joint so that the T cells are reactivated and consequently cause an exacerbation.

There is also evidence for a relationship between bacterial products and rheumatoid factors (RF). It has been shown that RF recognize the same epitopes on the Fc part of the IgG molecule as bacterial proteins such as protein A do (Weisbart et al., 1987). The idea is that antibodies directed against this type of molecules are the RF, or that the antibodies of the anti-idiotypic response against the antibodies recognizing this sort of molecules are the RF (Weisbart et al., 1987). It has to be investigated

whether bacterial cell wall preparations as discussed above are capable of inducing RF as well.

In MRL/lpr mice, which spontaneously develop autoimmune diseases among which arthritis, spontaneous production of RF occurs (Hang et al., 1982). In these mice it has been shown that the RF are not due to polyclonal stimulation of B cells but caused by stimulation with an antigen (Schlomchik et al., 1987). This can be concluded from the fact that in MRL mice RF are oligoclonal or even monoclonal and harbour numerous somatic mutations.

Some investigators have found that a clonal dominance exists in T cell receptor (TCR) usage in human rheumatic synovium lymphocytes (Savill et al., 1987; Stamenkovic et al., 1988). This probably also indicates a recognition of an exogenous or autologous antigen in contrast to polyclonal stimulation of the T cells.

A molecule that has been studied extensively in this context is the 65 kD protein of Mycobacterium tuberculosis (van Eden et al., 1985; 1987; 1988). This protein contains the epitope that is recognized by the Mycobacterium tuberculosis specific T cell clone that is arthritogenic in rats and that also recognizes cartilage structures (van Eden et al., 1985). It is noteworthy that HLA-DR4^{+} individuals (that have a higher risk to develop rheumatoid arthritis) are high responders for this particular antigen (Ottenhof et al., 1986). Holoshitz et al. (1986) and Res et al. (1988) have found that in early chronic arthritis T cells from the synovial fluid display an increased proliferative response upon stimulation with the antigen which subsides later on and than can be shown in the PB. CD4^{-}CD8^{-} T cell clones reactive to Mycobacterium tuberculosis have been isolated from the synovial fluid of RA patients (Holoshitz et al., 1988). The 65 kD protein was shown to be a heat shock (HS) protein (Shinnick et al., 1988), a protein that is produced by many bacterial species. It is an intriguing question whether there is also a relationship between the HS proteins and the bacterial extracts that are used in the streptococcal model and the models using endogenous gut bacteria. This is even more relevant as it has been recently found that a monoclonal antibody that recognizes the epitope of the 65 kD protein of Mycobacterium tuberculosis that is seen by the arthritogenic rat clone also recognizes the PG-PS structures that can be isolated from the human faeces (M.P. Hazenberg and J. van Embden, personal communication).

3. Autoreactivity

Next to the increasing evidence for the involvement of bacterial antigens in human rheumatic diseases there is also evidence for the recognition of autoantigens. Schlesier et al. (1984; submitted) have found autoreactive T cell clones directed against the own HLA structures in a patient suffering from reactive arthritis after Yersinia infection. On the antibody level cross reactivity has been shown between HLA-B27 and Yersinia (Chen et al., 1987). There seems to be an incompatibility in the results that emphasize the importance of infectious agents such as bacteria and viruses at the one hand (Saag and Bennett, 1987) and the results that show autoreactivity at the other. Since viral infections may modify auto-antigens it can be speculated that such modified autoantigens are recognized as foreign.

An explanation can be sought in recent evidence that the MHC molecules in vivo always contain peptide molecules (Townsend and McMichael, 1987; Robertson, 1988). These are peptides from own body structures but speculatively could also consist of fragments of bacterial cell wall complexes, e.g. from bacteria of the intestinal flora that might preferentially accumulate in certain organs. That large molecules from the gut content can indeed pass the gut wall and enter the bloodstream has been shown for lipopolysaccharides (Jacob et al., 1977). The existence of organ specific polypeptides has been reported (Steinmuller, 1984). Since the induction of tolerance against self molecules is thought to take place in the thymus (Kappler et al., 1987), only self peptides that are expressed in the thymus will induce tolerance. When these organ specific peptides do not enter the thymus, tolerance for these particular peptides is not induced, leaving the possibility that under certain circumstances organ specific autoimmunities will develop, e.g. after an infection causing the entrance of large amounts of bacterial cell wall molecules into the bloodstream. In this way these molecules may trigger antigen specific T lymphocytes to produce an inflammation. Similarly, antigens from the gut may account for the exacerbations in rheumatic diseases.

4. References

Bennett, J.C. The infectious etiology of rheumatoid arthritis. Arthritis Rheum. 21:531-538, 1978.

Ben-Nun, A. and I.R. Cohen. Experimental autoimmune encephalomyelitis (EAE) mediated by cloned T cell lines: process of selection of lines

and characteristics of the cells. J. Immunol. 129:303-308, 1982.
Bianchi, A.T.J., H. Hooijkaas, R. Benner, R. Tees, A.A. Nordin and M.H. Schreier. Clones of helper T cells mediate antigen-specific, H-2-restricted DTH. Nature 290:62-63, 1981.
Brackertz, D., G.F. Mitchell and I.R. Mackay. Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum. 20:841-850, 1977a.
Brackertz, D., G.F. Mitchell, M.A. Vadas and I.R. Mackay. Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. J. Immunol. 118:1645-1648, 1977b.
Caspi, R.R., F.G. Roberge, C.G. Mcallister, M. El-Saied, T. Kuwabara, I. Gery, E. Hanna and R.B. Nussenblatt. T cell lines mediating experimental autoimmune uveoretinitis (EAU) in the rat. J. Immunol. 136:928-933, 1986.
Chen, J-H., D.H. Kono, Z. Yong, M.S. Park, M.M.B.A. Oldstone and D.T.Y. Yu. A Yersinia pseudotuberculosis protein which cross-reacts with HLA-B27. J. Immunol. 139:3003-3011, 1987.
Cher, D.J. and T.R. Mosmann. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by TH1 clones. J. Immunol. 138:3688-3694, 1987.
Cohen, I.R., J. Holoshitz, W. van Eden and A. Frenkel. T lymphocyte clones illuminate pathogenesis and affect therapy of experimental arthritis. Arthritis Rheum. 28:841-845, 1985.
Cromartie, W.J., J.G. Craddock, J.H. Schwab, S.K. Anderle and C.H. Yang. Arthritis in rats after systemic injection of streptococcal cells or cell walls. J. exp. Med. 146:1585-1602, 1977.
Dailey, M.O., C.G. Fathman, E.C. Butcher, E. Pillemer and I. Weissman. Abnormal migration of T lymphocyte clones. J. Immunol. 128:2134-2136, 1982.
Dailey, M.O., W.M. Gallatin and I. Weissman. The in vivo behavior of Tcell clones: altered migration due to loss of the lymphocyte surface homing receptor. J. Mol. Cell. Immunol. 2:27-36, 1985.
Editorial. Intestinal bypass syndrome. Lancet i:1419-1420, 1983.
Editorial. Arthritis and enteritis - an interface of protean manifestations. J. Rheumatol. 14:406-410, 1987.
Fathman, C.G. and F.W. Fitch., eds. 1982. Isolation, characterization and utilisation of T lymphocyte clones. Academic press, New York.
Gardner, D.L. 1986. Pathology of rheumatoid arthritis. Scott, J.T. ed. Churchill Livingstone. pp. 604-652.
Greenstein, A.J., H.D. Janowitz and D.B. Sachar. The extra-intestinal complications of Crohn's disease and ulcerative colitis. A study of 700 patients. Medicine 55:401-412, 1976.
Hang, L., A.N. Theofilopoulos and F.J. Dixon. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. J. exp. Med. 155:1690-1701, 1982.
Hengartner, H. and C. G. Fathman. Clones of alloreactive T cells. I. A unique homozygous MLR-stimulating determinant present on B6 stimulators. Immunogenetics 10:175-184, 1980.
Holoshitz, J., Y. Naparstek, A. Ben-Nun and I.R. Cohen. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 219:56-58, 1983.
Holoshitz, J., A. Matitiau and I.R. Cohen. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. J. Clin. Invest. 73:211-215, 1984.
Holoshitz, J., I. Drucker, A. Yaretzky, W. van Eden, A. Klajman, Z. Lapidot, A. Frenkel and I.R. Cohen. T lymphocytes of rheumatoid arthritis patients

show augmented reactivity to a fraction of Mycobacteria cross-reactive with cartilage. Lancet ii:305-309, 1986.
Holoshitz, J. and S. Strober. $CD4^{-}CD8^{-}$ T-cell clones from synovial fluid in rheumatoid arthritis (abstract). J. Cell. Biochem. Suppl. 12E:18, 1988.
Jacob, A.I., P.K. Goldberg, N. Bloom, G.A. Degenshein and P.J. Konzinn. Endotoxin and bacteria in portal blood. Gastroenterology 72:12-17, 1977.
Jalkanen, S., A.C. Steere, R.I. Fox and E.C. Butcher. A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. Science 233:556-558, 1986.
Kaplan, M.H. and M. Meyeserian. An immunologic cross-reaction between group A streptococcal cells and human heart tissue. Lancet i:706-707, 1962.
Kappler, J.W., N. Roehm and P. Marrack. T cell tolerance by clonal elimination in the thymus. Cell 49:273-280, 1987.
Keat, A. Is spondylitis caused by Klebsiella? Immunol. Today 7:144-149, 1986.
Klareskog, L., U. Forsum, A. Scheynius, D. Kabelitz and H. Wigzell. Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis. Proc. Natl. Acad. Sci. USA 79:3632-3636, 1982.
Kruijsen, M.W.M., W.B. van den Berg, L.B.A. van de Putte and W.J.M. van den Broek. Detection and quantification of experimental joint inflammation in mice by measurement of ^{99m}Tc-pertechnetate uptake. Agents Actions 11:640-642, 1981.
Lens, J.W., W.B. van den Berg and L.B.A. van de Putte. Flare-up of antigen-induced arthritis in mice after challenge with intravenous antigen: studies on the characteristics of and mechanisms involved in the reaction. Clin. exp. Immunol. 55:287-294, 1983.
Lens, J.W., W.B. van den Berg and L.B.A. van de Putte. Quantification of 99mTc-uptake measurements in the mouse knee-joint: correlation with histological joint inflammation scores. Agents Actions 14:723-728, 1984a.
Lens, J.W., W.B. van den Berg, L.B.A. van de Putte and L. van den Bresselaar. Flare-up of antigen-induced arthritis in mice after challenge with oral antigen. Clin. exp. Immunol. 58:364-371, 1984b.
Maron, R., R. Zerubavel, A. Friedmann and I.R. Cohen. T lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice. J. Immunol. 131:2316-2322, 1983.
Mielants, H. and E.M. Veys. Inflammation of the ileum in patients with B27-positive reactive arthritis. Lancet i:288, 1984.
Mielants, H., E.M. Veys, R. Joos, C. Cuvelier, M. de Vos and F. Proot. Late onset pauciarticular juvenile chronic arthritis: relation to gut inflammation. J. Rheumatol. 14:459-465, 1987.
Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin and R.L. Coffman. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348-2357, 1986.
Ottenhof, T.H.M., P. Torres, J.T. de las Aguas, R. Fernandez, W. van Eden, R.P. de Vries and J.L. Stanford. Evidence for an HLA-DR4-associated immune-response gene for Mycobacterium tuberculosis. Lancet ii:310-312, 1986.
Phillips, P.E. and C.L. Christian. 1985. Infectious agents in chronic rheumatic disease. Lea and Febiger, Philadelphia. pp. 431-449.
Res, P.C.M., C.G. Schaar, F.C. Breedveld, W. van Eden, J.D.A. van Embden, I.R. Cohen and R.R.P. de Vries. Synovial fluid T cell reactivity against

65 kD heat shock protein of Mycobacteria in early chronic arthritis. Lancet ii:478-480, 1988.
Robertson, M. Tolerance, restriction and the Mls enigma. Nature 332:18-19, 1988.
Rozenszajn, L.A., C. Muellenberg-Coulombre, I. Gery, M. El-Saied, T. Kuwabara, M. Mochizuki, Z. Lando and R.B. Nussenblatt. Induction of experimental autoimmune uveoretinitis by T-cell lines. Immunology 57:559-565, 1986.
Saag, M.S. and J.C. Bennett. The infectious etiology of chronic rheumatic diseases. Arthritis Rheum. 17:1-23, 1987.
Sandson, J., d. hamerman, R. Janis and M. Rojkind. Immunologic and chemical similarities between the Streptococcus and human connective tissue. Trans. Assoc. Am. Phys. 81:249-257, 1968.
Savill, C.M., P.J. Delves, D. Kioussis, P. Walker, P.M. Lydyard, B. Colaco, M. Shipley and I.M. Roitt. A minority of patients with rheumatoid arthritis show a dominant rearrangement of T-cell β receptor chain genes in synovial lymphocytes. Scand. J. Immunol. 25:629-635, 1987.
Scheper, R.J., M. von Blomberg, G.H. Boerrigter, D. Bruynzeel, A. van Dinther and A. Vos. Induction of immunological memory in the skin. Role of local T cell retention. Clin. exp. Immunol. 51:141-148, 1983.
Schlesier, M., C. Ramb-Lindhauer, R. Drager, A. Urlacher, M. Robin-Winn and H.H. Peter. Autoreactive T cells in rheumatic disease. II. Function and specificity of an autoreactive T helper cell clone established from a HLA-B27+ reactive arthritis. Submitted for publication.
Schlesier, M., C. Ramb-Lindhauer, M. Gartner and H.H. Peter. Analysis of T-cell cultures and clones from a patient with classic rheumatoid arthritis - evidence for the existence of autoreactive T-cell clones in blood and synovial fluid. Rheumatol. Int. 4:1-9, 1984.
Schlomchik, M.J., A. Marshak-Rothstein, B. Wolfowicz, T.L. Rothstein and M.G. Weigert. The role of clonal selection and somatic mutation in autoimmunity. Nature 328:805-811, 1987.
Schreier, M.H., N.N. Iscove, R. Tees, L. Aarden and H. von Boehmer. Clones of killer and helper T cells: growth requirements, specificity and retention of function in long-term culture. Immunol. Rev. 51:315-336, 1980.
Schreier, M.H., R. Tees, A.A. Nordin, R. Benner, A.T.J. Bianchi and M.J. van Zwieten. Functional aspects of helper T cell clones. Immunobiol. 161:107-138, 1982.
Schrieber, L., N. Manolios, S. Shun To, C.L. Geczy and S.N. Breit. Hypothesis: lymphocyte trafficking in inflammatory rheumatic disease. A role for receptor mediated homing. J. Rheumatol. 14:194-196, 1987.
Severijnen, A.J., M.P. Hazenberg and J.P. van de Merwe. Induction of chronic arthritis in rats by cell wall fragments of anaerobic coccoid rods isolated from the faecal flora of patients with Crohn's disease. Digestion 39:118-125, 1988.
Severijnen, A.J., R. van Kleef, M.P. Hazenberg and J.P. van de Merwe. Cell wall fragments of gram-positive anaerobic human intestinal bacteria induce chronic arthritis in rats. Submitted for publication.
Shinnick, T.M., M.H. Vodkin and J.C. Williams. The Mycobacterium tuberculosis 65-Kilodalton antigen is a heat shock protein which corresponds to common antigen and to the Escherichia coli GroEL protein. Infect. Immun. 56:446-451, 1988.
Sredni, B. Methods for long term growth and cloning of T cells reactive with soluble antigens. J. Immunol. Meth. 49:1-10, 1982.
Stamenkovic, I., M. Stegagno, K.A. Wright, S.M. Krane, E.P. Amento, R.B.

Colvin, R.J. Duquesnoy and J.T. Kurnick. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc. Natl. Acad. Sci. USA 85:1179-1183, 1988.
Steinmuller, D. Tissue-specific and tissue-restricted histocompatibility antigens. Immunol. Today 5:234-240, 1984.
Stimpson, S.A., R.R. Brown, S.K. Anderle, D.G. Klapper, R.L. Clark, W.J. Cromartie and J.H. Schwab. Arthropathic properties of cell wall polymers from normal flora bacteria. Infect. Immun. 51:240-249, 1986.
Toivanen, A., K. Granfors, R. Lahesmaa-Rantala, R. Leino, T. Stahlberg and R. Vuento. Pathogenesis of Yersinia-triggered reactive arthritis: immunological, microbiological and clinical aspects. Immunol. Rev. 86:47-70, 1985.
Townsend, A. and A. McMichael. MHC protein structure. Those images that yet fresh images beget. Nature 329:482-483, 1987.
Van Beusekom, H.J., L.B.A. van de Putte, W.B. van den Berg, W.J.M. van den Broek and W.C.A.M. Buijs. Antigen handling in antigen-induced joint inflammation: kinetics of a second intra-articularly injected dose of antigen in an already established antigen-induced joint inflammation. Immunology 44:153-161, 1981.
Van de Putte, L.B.A., J.W. Lens, W.B. van den Berg and M.W.M. Kruijsen. Exacerbation of antigen-induced arthritis after challenge with intravenous antigen. Immunology 49:161-167, 1983.
Van den Berg, W.B., L.B.A. van de Putte, W.A. Zwarts and L.A.B. Joosten. Electrical charge of the antigen determines intraarticular antigen handling and chronicity of arthritis in mice. J. Clin. Invest. 74:1850-1859, 1984.
Van den Berg, W.B., P.L.E.M. van Lent, L.B.A. van de Putte and W.A. Zwarts. Electrical charge of hyaline articular cartilage: its role in the retention of anionic and cationic proteins. Clin. Immunol. Immunopathol. 39:187-197, 1986.
Van Eden, W., J. Holoshitz and I.R. Cohen. Antigenic mimicry between mycobacteria and cartilage proteoglycans: the model of adjuvant arthritis. Concepts Immunopathol. 4:144-170, 1987.
Van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman and I.R. Cohen. Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc. Natl. Acad. Sci. USA 82:5117-5120, 1985.
Van Eden, W., J.E.R. Thole, R. van der Zee, A. Noordzij, J.D.A. van Embden, E.J. Hensen and I.R. Cohen. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331:171-173, 1988.
Von Boehmer, H. and W. Haas. H-2 restricted cytolytic and noncytolytic T cell clones: isolation, specificity and functional analysis. Immunol. Rev. 54:27-56, 1981.
Weisbart, R.H., D.T. Noritake, K.K. Colburn and R.E. Saxton. Human monoclonal IgG rheumatoid factor has structural homology with bacterial Fc receptor proteins. J. Immunol. 139:2925-2928, 1987.

SUMMARY

The etiology of rheumatoid arthritis (RA) and the exacerbations and remissions that occur in this disease are largely unknown. Several animal models have been developed to study the pathogenetic processes that are involved in this disease. There is ample evidence that the immune system plays an important role.

The purpose of the investigations for this thesis was to increase insight into the role of T cells in the pathogenesis of rheumatic diseases. The model used in these studies was the antigen induced arthritis (AIA) model in mice. In this model mice are immunized by protein antigens emulsified in Complete Freund's adjuvant (CFA). Several weeks later a dose of the same antigen is injected in the knee joint whereafter the mice develop a joint inflammation. When a positively charged antigen is used, the course of the joint inflammation is chronic. Such chronic joint inflammations can show flare-up reactions (exacerbations) when the antigen is administered again. This can be done locally, but also systemically (intravenously (iv) or orally).

It is assumed that in AIA the joint inflammations are chronic due to the retention of antigen specific T cells in the joint. This retention is dependent on the presence of small amounts of antigen in the joint. It is important that positively charged antigens are used as these are better retained by the negatively charged cartilage structures. These T cells mediate flare-up reactions after repeated administration of the antigen. Antigen specific antibodies are thought to be of minor importance.

Several years ago it has been shown that T cell clones of the helper phenotype can cause delayed type hypersensitivity (DTH) reactions when injected into the instep of the foot of a syngeneic mouse together with the antigen. This type of inflammatory reaction is thought to be involved in the joint inflammations in the AIA model as well as in human RA.

To study the role of T cells in joint inflammations, we produced T cell clones with the helper phenotype ($MT4^+$,$Lyt\text{-}2^-$) and specific for the positively charged antigen methylated bovine serum albumin (mBSA). The

studies that have been performed using one of these mBSA specific T cell clones are described in this thesis. Therefore the original AIA model was modified. The aim of our studies was to investigate (a) whether it is possible to induce, in naive mice, joint inflammations by cloned helper T cells together with the antigen and, if so, what the characteristics of these joint inflammations are; (b) whether it is possible to evoke flare-up reactions of chronic joint inflammations that are induced by cloned helper T cells and the antigen by repeated administration of the antigen and, if so, what the characteristics of these flare-up reactions are.

As we believe that DTH reactions play an important role in the pathogenesis of joint inflammatory diseases and their flare-up reactions, we first investigated whether DTH reactions that are evoked in the foot of a mouse by injection of cloned mBSA specific helper T cells and mBSA are capable of showing flare-up reactions. In Chapter 2 it is shown that this is indeed the case after local (sc) as well as after systemic (oral or iv) administration. In Chapter 3 we show that it is also possible to induce joint inflammations by local administration of the cloned helper T cells and the antigen. This was feasible after local (ia) administration of the T cells and the antigen, but also after systemic (iv or oral) administration of the T cells and ia administration of the antigen. These inflammatory reactions were measured by a ^{99m}Technetium uptake method and were confirmed histologically. These joint inflammations were capable of showing flare-up reactions after repeated ia injection of the antigen (Chapters 3 and 4).

Subsequently we investigated whether these joint inflammations and flare-up reactions were indeed caused by the cloned T cells that were administered and not by T cells of the recipient mice. We therefore used T cell deficient athymic nude mice. These mice also exhibited joint inflammations after administration of cloned T cells and the antigen and were capable of showing flare-up reactions as well. This indicates that T cells of the recipient mice are not required for these inflammations and their flare-up reactions. These experiments are described in Chapter 4.

In chronic joint inflammations T cells are thought to be retained in the joint when the antigen that they recognize persists there. We investigated whether this was also the case in our system in which cloned T cells were injected locally. In Chapter 4 it is shown that the cloned T cells can remain functionally reactive in a joint in the absence of the antigen. However, in the case that the antigen is present in the joint,

the T cells display a higher reactivity as judged from their capacity to evoke a joint inflammation.

The histological and immunohistochemical characteristics of the joint inflammations and flare-up reactions are described in Chapter 7. These reactions are mainly granulocytic and do not appear differently from the joint inflammations and flare-up reactions observed in mice that are immunized with antigen emulsified in CFA and that subsequently are ia challenged.

The mBSA specific cloned helper T cells that we used were obtained from a C57BL/6 mouse and are H-2I-A^b restricted in their antigen recognition. This is also clear from the smaller capacity to induce inflammations in recipient mice unless H-2I-A^b is expressed by the recipients (Chapters 2 and 3). This emphasizes the importance of the antigen presenting cell in this process. In Chapters 5 and 6 a macrophage tumor cell line (AP284) is presented that constitutively expresses H-2I-A^b and can efficiently present antigen <u>in vitro</u> to the cloned T cells used. This cell line was also capable of antigen presentation <u>in vivo</u> since local injection of the cloned helper T cells, AP284 cells and mBSA in an allogeneic mouse caused an inflammatory reaction. This was found in DTH (Chapter 5) as well as in the modified AIA model (Chapter 6). The power of this approach is that in this system both the T cell population and the antigen presenting cell population are monoclonal, so that the system is open to a more in-depth analysis of the inflammatory process.

The results presented in this thesis emphasize the importance of helper T cells in the pathogenesis of rheumatic diseases and indicate that helper T cells may play a central role in the induction of these diseases as well as their exacerbations.

SAMENVATTING

De etiologie van rheumatoide arthritis (RA) en de exacerbaties en remissies die hierbij optreden, zijn grotendeels onbekend. Een aantal proefdiermodellen zijn ontwikkeld voor de bestudering van de pathogenetische processen die hierbij gaande zijn. Er zijn er vele aanwijzingen dat het immuunsysteem hierin een grote rol speelt.

Het doel van het onderzoek voor dit proefschrift was om het inzicht te vergroten in de mogelijke rol van T cellen in de pathogenese van rheumatische ziekten. Het model dat in deze studies gebruikt werd is gebaseerd op het antigeen geïnduceerde arthritis (AIA) model in muizen. In dit model worden muizen geïmmuniseerd met eiwitantigenen geëmulgeerd in compleet Freund's adjuvant. Enige weken later wordt een dosis van hetzelfde antigeen in het kniegewricht geïnjecteerd, waarna de muizen een gewrichtsontsteking ontwikkelen. Deze gewrichtsontstekingen hebben een chronisch verloop als positief geladen eiwitantigenen worden gebruikt. Een dergelijke chronisch verlopende gewrichtsontsteking kan weer opvlammen (exacerberen) als het antigeen opnieuw wordt toegediend. Dit kan zijn na opnieuw lokaal toedienen in het gewricht, maar ook na systemische toediening (intraveneus (iv) of oraal).

In het AIA model gaat men er van uit dat deze gewrichtsontstekingen chronisch zijn omdat de antigeenspecifieke T cellen in het gewricht retineren zolang kleine hoeveelheden van het antigeen in het gewricht aanwezig zijn. Het is daarom van belang dat positief geladen antigenen worden gebruikt, aangezien deze retineren in het negatief geladen kraakbeen. De T cellen die in het gewricht retineren geven bij het opnieuw toedienen van antigeen aanleiding tot een opvlammingsreactie. Hierbij zouden antigeenspecifieke antilichamen een ondergeschikte rol spelen.

Een aantal jaren geleden is aangetoond dat gekloneerde T cellen met het helper fenotype, wanneer deze samen met het antigeen worden geïnjecteerd in de wreef van een muizepoot, aanleiding geven tot een vertraagd type overgevoeligheids (DTH) reactie. Dit soort reacties worden gedacht betrokken te zijn bij gewrichtsontstekingen in het AIA model en ook in humane RA.

Om de rol van T cellen in de gewrichtsontstekingen van AIA te kunnen

bestuderen, werden T cel klonen met het helper fenotype ($MT4^+$,$Lyt\text{-}2^-$) geproduceerd, specifiek voor het positief geladen antigeen gemethyleerd bovine serum albumine (mBSA). De studies die met één van deze mBSA specifieke T cel klonen zijn verricht worden in dit proefschrift beschreven. Daartoe werd het oorspronkelijke AIA model gemodificeerd. Het doel van onze studies was te onderzoeken of (a) het mogelijk is om met gekloneerde helper T cellen samen met het antigeen in overigens onbehandelde muizen gewrichtsontstekingen te induceren, en zo ja, wat hiervan de karakteristieken zijn; (b) of het mogelijk is om gewrichtsontstekingen die geïnduceerd zijn door toediening van gekloneerde helper T cellen en het antigeen te laten opvlammen door een herhaalde toediening van het antigeen, en zo ja, wat de karakteristieken van deze opvlammingsreacties zijn.

Omdat wij ervan uit gaan dat DTH reacties een grote rol spelen in gewrichtsontstekingen en de opvlammingsreacties daarvan, hebben wij eerst onderzocht of een DTH reactie die geïnduceerd was in een muizepoot d.m.v. mBSA specifieke gekloneerde helper T cellen en mBSA ook in staat was om weer op te vlammen. Hoofdstuk 2 laat zien dat dit inderdaad het geval is, zowel na lokale intra-articulaire (ia) als na systemische (iv of orale) toediening. In hoofdstuk 3 laten wij zien dat het ook mogelijk is om gewrichtsontstekingen te induceren door toediening van de gekloneerde helper T cellen en het antigeen. Dit was mogelijk zowel door lokale, (ia) toediening van T cellen en antigeen, als door systemische toediening van T cellen en ia injectie van het antigeen. De ontstekingsreacties werden gemeten met een ^{99m}Technetium opname methode en werden histologisch bevestigd. Deze gewrichtsontstekingen bleken in staat opvlammingsreacties te vertonen na een hernieuwde lokale injectie van het antigeen (hoofdstuk 3 en 4). Vervolgens onderzochten wij of de gewrichtsontstekingen en opvlammingsreacties inderdaad veroorzaakt werden door de toegediende gekloneerde T cellen en niet door de T cellen van de ontvangende muizen. Wij maakten daarom gebruik van T cel deficiënte, thymusloze 'naakte' muizen. Ook deze muizen bleken na toediening van gekloneerde T cellen en het antigeen gewrichtsontstekingen te ontwikkelen en opvlammingsreacties te kunnen vertonen. Dit wijst er op dat T cellen van de ontvangende muis niet noodzakelijk zijn voor deze ontstekingen. Deze experimenten worden beschreven in hoofdstuk 4.

In een chronische gewrichtsontsteking worden de T cellen gedacht te retineren in een gewricht als het antigeen dat zij herkennen daar persisteert. Wij onderzochten of dit ook het geval was in ons systeem waarin de

gekloneerde T cellen lokaal worden toegediend. In hoofdstuk 4 wordt aangetoond dat de T cellen ook enige tijd functioneel reactief kunnen blijven in een gewricht in de afwezigheid van het antigeen. In het geval dat dit antigeen wel aanwezig is in het gewricht vertonen de T cellen een groter vermogen om een gewrichtsontsteking te ontwikkelen.

De histologische en immunohistochemische karakteristieken van de gewrichtsontsteking en opvlammingsreactie worden beschreven in hoofdstuk 7. Deze reacties zijn voornamelijk granulocytair van karakter en wijken niet af van de reacties zoals deze gezien worden bij muizen die een gewrichtsontsteking ontwikkeld hebben na immunisatie met antigeen in CFA en een ia challenge.

De mBSA specifieke gekloneerde helper T cellen die wij gebruiken zijn afkomstig van een C57BL/6 muis en zijn H-2I-A^b gerestricteerd in hun antigeen herkenning. Dit komt tot uiting in het verminderde vermogen van deze gekloneerde T cellen om ontstekingsreacties op te wekken in muizen die geen H-2I-A^b tot expressie brengen (hoofdstuk 2 en 3). Dit benadrukt het belang van de antigeen presenterende cel deze processen. In hoofdstuk 5 en 6 wordt een macrofaag tumorcellijn (AP284) gepresenteerd die constitutief H-2I-A^b tot expressie brengt en die <u>in vitro</u> efficiënt antigeen kan presenteren aan de gekloneerde T cellen. Deze lijn was ook in staat tot antigeenpresentatie <u>in vivo</u>. Locale injectie van gekloneerde helper T cellen, AP284 cellen en mBSA in een allogene muis veroorzaakte een ontstekingsreactie. Dit werd gevonden zowel in DTH (hoofdstuk 5) als in het gemodificeerde AIA model (hoofdstuk 6). De kracht van deze benadering is dat in dit systeem zowel de T cel populatie als de antigeen presenterende celpopulatie monoklonaal is, zodat dit systeem open staat voor meer diepgaande studies van het ontstekingsproces.

De resultaten die in dit proefschrift worden gepresenteerd benadrukken het belang van helper T cellen in de pathogenese van rheumatische ziekten en laten zien dat helper T cellen een belangrijke rol kunnen spelen zowel in de inductie van deze ziekten als in hun exacerbaties.

CURRICULUM VITAE

13 februari 1958	Geboren te Voorburg.
juni 1976	Eindexamen Gymnasium-β O.S.G. Voorburg-Leidschendam.
september 1979	Kandidaatsexamen Biologie, B4 Rijksuniversiteit Leiden.
april 1983	Doctoraalexamen Biologie Rijksuniversiteit Leiden Hoofdvak: Biochemie, Prof. Dr. R.A. Schilperoort, Leiden. Bijvakken: Experimentele plantkunde, Prof. Dr. A. Quispel, Leiden. Tumorimmunologie, Prof. Dr. A.C. Drogendijk, Rotterdam. Lerarenopleiding, 1^{e} graads Biologie, Leiden.
april 1983	Aanvang promotieonderzoek op de afdeling Celbiologie, Immunologie en Genetica, Erasmus Universiteit Rotterdam, bij Prof. Dr. R. Benner. Tijdens dit onderzoek werd enige weken gewerkt bij Sandoz Ltd, Basel, bij Dr. M.H. Schreier.

LIST OF PUBLICATIONS

Hille, J., I.S. Klasen and R.A. Schilperoort. Construction of R prime plasmids, carrying different segments of an octopine Ti plasmid from Agrobacterium tumefaciens for complementation of vir genes. Plasmid 7:107-118, 1982.

Hille, J., J. van Kan, I.S. Klasen and R.A. Schilperoort. Site-directed mutagenesis in Escherichia coli of a stable R772::Ti conjugate from Agrobacterium tumefaciens. J. Bacteriol. 154:693-701, 1983.

Klasen, I.S., I.G. Donselaar, R.M.T. Ladestein, W.B. van den Berg and R. Benner. Joint inflammations and flare-up reactions in mice induced by a helper T cell clone. Agents Actions 19:331-334, 1986.

Klasen, I.S., Th.H. van der Kwast, I.G. Donselaar, R.M.T. Ladestein and R. Benner. Flare-up of delayed-type hypersensitivity initially induced by murine cloned helper T cells. Cell. Immunol. 108:235-241, 1987.

Klasen, I.S., R.M.T. Ladestein, I.G. Donselaar, W.B. van den Berg, R. Tees and R. Benner. Joint inflammations in mice induced by a $MT4^+$ Lyt-2^- T cell clone. Characteristics and flare-up reactions. J. Immunol. 139:3275-3280, 1987.

Klasen, I.S., R.M.T. Ladestein, W.B. van den Berg and R. Benner. Flare-up of joint inflammations induced by cloned helper T cells. Agents Actions 23:69-73, 1988.

Van den Elzen, J.H.M., M.F. van den Broek, I.S. Klasen and W.B. van den Berg. Stimulation of antigen-specific murine T cell clones in vitro with antigen-pulsed adherent cells fixed to a carrier. J. Immunol. Meth. 112:15-22, 1988.

Klasen, I.S., R.M.T. Ladestein, W.B. van den Berg and R. Benner. Requirements for flare-up reactions of joint inflammations in mice induced by cloned $MT4^+$,Lyt-2^+ T cells. Arthritis Rheum., in press.

Klasen, I.S., R.M.T. Ladestein and R. Benner. Joint inflammations induced by a mature macrophage cell line and cloned $MT4^+$,Lyt-2^- T cells in allogeneic mice. Agents Actions, in press.

Klasen, I.S., J.P. de Jong, J.S.A. Voerman, R.M.T. Ladestein, P.J.M. Leenen and R. Benner. Murine macrophage line AP284 presents antigen to cloned T cells in vitro and in vivo. Immunobiology, in press.

Klasen, I.S., R.M.T. Ladestein and R. Benner. Joint inflammation in mice by an $MT4^+$,Lyt-2^- T cell clone and an Ia-positive macrophage cell line and requirements for the induction of flare-up reactions. J. Cell. Biochem., in press.

Klasen, I.S., R.M.T. Ladestein, A.A. Grandia, Th.H. van der Kwast and R. Benner. Histological and immunohistochemical characterization of joint inflammation and flare-up reactions induced by cloned $MT4^+$,Lyt-2^- T cells. Submitted for publication.

DANKWOORD

Hoewel het eigenlijk een onmogelijke opgave is om in één à twee bladzijden duidelijk te maken hoeveel mensen een rol gespeeld hebben bij het tot stand komen van dit proefschrift, zal ik hier toch een poging wagen.

Vanaf het allervroegste begin was er al de stimulerende invloed van mijn ouders, die mij ondanks hun twijfels (over het nut hiervan voor de toekomst) lieten studeren en steeds probeerden te begrijpen waar ik mee bezig was (dikke muizeknieën ??!). Wat later kwam Ad daarbij die gelukkig begreep dat studeren en later onderzoek doen meer tijd en dus opofferingen kost dan de meeste banen en daaraan heel wat steentjes (soms keien) bijdroeg.

Heel veel mensen speelden in de afgelopen jaren op het lab een grote rol. Natuurlijk Rob Benner, die bleef vertrouwen op een goede afloop van het onderzoek, ook in tijden dat de maanstand weer eens verkeerd was, en die bereid was mijn promotor te zijn. Zijn tomeloze energie is bij ons bijna spreekwoordelijk (Benneriaans). Professor Vos zorgde voor de nodige rust en stabiliteit op de afdeling in woelige tijden. Ik stel het op prijs dat ook hij mijn promotor wilde zijn. Ook waardeer ik de kritische kanttekeningen van de overige twee leden van de commissie, Professor van der Heul en Professor Kosten, bij het concept van dit proefschrift.

Lidia Hussaarts, Inge Donselaar (Wolterink?) en Renée Ladestein verrichtten heel veel van het in dit proefschrift beschreven werk. De zin 'zonder hen was dit proefschrift niet tot stand gekomen' is al door menig ander promovendus eerder gebruikt, maar is hier zeker waar. Lidia, Inge en Renée, jullie inzet heb ik zeer gewaardeerd, alsmede het feit dat Inge en Renée mijn paranimfen wilden zijn.

Voordat de 'proeven' gedaan konden worden was er vaak al heel wat werk verzet door mensen als Rein Smid en Tom Vermetten die de bestellingen verwerkten, mevrouw Godijn, Jopie Bolman en Elly Hoffman die zorgden dat het glaswerk schoon en/of steriel werd en die ons op de been hielden met koffie en thee. Piet Hartwijk repareerde alles wat kapot was en Marry Steinvoort, Gré Stam, Saskia Kalkman en Joop Brandenburg zorgden elke keer weer voor het schoonhouden van de stallen. Joop en zijn gezin zorgden

bovendien voor gezelligheid in 'het Rhoonse'. De medewerkers van nucleaire geneeskunde leverden ons trouw het Technetium.

Als na het practische werk dan geschreven moest/kon gaan worden kwam de expertise van een andere groep mensen om de hoek kijken. Tar van Os maakte op zeer nauwgezette wijze de foto's en Cor van Dijk zorgde op zijn 'cor'date wijze dat de figuren voor elkaar kwamen. Getypt werd er vervolgens door Gea Stout, Jacqueline de Goey of Geertje de Korte als mijn eigen eenvingerig zoeksysteem te kort schoot. Geertje bleek overigens onmisbaar bij de laatste loodjes van het in elkaar zetten van dit proefschrift.

Dan wil ik hier nog een aantal collega's van andere labs noemen. First Max Schreier and Reet Tees From Sandoz Ltd., Basel. I'd like to thank you for the opportunity you offered me to work in your laboratory and for the T cell culturing rites you taught me. Dan Wim van den Berg van de afdeling Rheumatologie in Nijmegen, die een aantal van de manuscripten beoordeelde en zorgde voor stimulerende discussies.

Arianneke Grandia sneed en kleurde in haar vrije tijd heel wat coupes, die dan vervolgens door Theo van der Kwast van de afdeling Pathologie I beoordeeld werden. Theo van der Kwast speelde ook een belangrijke rol in de eindfase van dit proefschrift. Rob Ploemacher bleek in staat tussen zijn drukke werkzaamheden door in een recordtempo een tekening voor de omslag van dit proefschrift te maken. De vele vroegere en huidige labgenoten van de afdeling Celbiologie, Immunologie en Genetica waren van groot belang door hun collegialiteit en hun inbreng in de goede sfeer op het lab en bij festijnen als labdagen, zeilweekends en cabarets. Al deze hierboven genoemde mensen wil ik van harte bedanken.

ABBREVIATIONS

AA - adjuvant arthritis
AIA - antigen induced arthritis
AMLR - autologous mixed lymphocyte reaction
APC - antigen presenting cells
BCDF - B cell differentiation factor
BSF-2 - B cell stimulating factor-2
BSS - balanced salt solution
CFA - complete Freund's adjuvant
cpm - counts per minute
DC - dendritic cell
DTH - delayed type hypersensitivity
EAE - experimental allergic encephalitis
EBV - Epstein-Barr virus
FCS - fetal calf serum
GVH - graft versus host
^{3}H-TdR - tritiated thymidine
H-2 - histocompatibility-2
HE - haematoxilin and eosin
HGF - hybridoma growth factor
HLA - human leukocyte antigen
HS - heat shock
ia - intra articular
IC - immune complex
IL-n - interleukin-n
IFA - incomplete Freund's adjuvant
IFN - interferon
Ig - immunoglobulin
IMDM-ATL - Iscove's modification of Dulbecco's modified Eagles medium supplemented with albumin, transferrin and lipids instead of FCS
ip - inraperitoneal

iv - intravenous

JRA - juvenile rheumatoid arthitis

kD - kilo dalton

LN - lymph node

mAb - monoclonal antibody

mBSA - methylated bovine serum albumin

MHC - major histocompatibility complex

MIF - migration inhibition factor

MLR - mixed lymphocyte reaction

MNC - mononuclear cell

MT - Mycobacterium tuberculosis

Mϕ - macrophage

OVA - ovalbumin

PB - peripheral blood

PGE2 - prostaglandin E2

PG-PS - peptidoglycan-polysaccharide

PMNC - polymorphonuclear cell

RA - rheumatoid arthritis

ReA - reactive arthritis

RF - rheumatoid factor

SEM - standard error of the mean

SF - synovial fluid

ST - synovial tissue

^{99m}Tc - ^{99m}Technetium

Tc - cytotoxic T cell

TCR - T cell receptor

Th - helper T cell

Thi - helper inducer T cell

Ts - suppressor T cell

Tsi - suppressor inducer T cell