REGULATION OF MACROPHAGE ACTIVITY BY EICOSANOIDS

Modulatie van de activiteit van macrofagen door eicosanoiden

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
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PROF. DR. M.W. VAN HOF
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PROMOTOR:	PROF.DR. I.L. BONTA
OVERIGE LEDEN:	PROF.DR. R. BENNER
	PROF.DR. K.F. KERREBIJN
	PROF. J.H.P. WILSON

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Arjan,

χωρα μακρινησ μυριαδεσ ονειρα
στεχια δεν εχει και παντα τρεχει
χαθεναμαο δεν χυνηγα
μη μασ φωναξη δεν υσιχαξη
σταθητε λιγο πιο μακρια
για να χρατηται την χαρα.

CONTENTS

	PREFACE	7
CHAPTER 1.	MACROPHAGES	9
CHAPTER 2.	EICOSANOIDS	21
CHAPTER 3.	ADENYLATE CYCLASE	33
CHAPTER 4.	RELEASE OF EICOSANOIDS BY MACROPHAGES	39
CHAPTER 5.	EFFECTS OF EICOSANOIDS ON MACROPHAGE FUNCTIONS	51
CHAPTER 6.	INVOLVEMENT OF CYCLIC AMP IN THE REGULATION OF MACROPHAGE ACTIVITY	69
	SUMMARY AND CONCLUDING REMARKS	85
	SAMENVATTING EN CONCLUSIES	93
	REFERENCES	101
	APPENDIX; MATERIALS AND METHODS	121
	NAWOORD	127
	CURRICULUM VITAE	129
	LIST OF PUBLICATIONS	131

PREFACE

Mononuclear phagocytes increasingly appear to have a central regulatory role in governing inflammatory and immune responses. The concept of macrophage activation dates from Metchnikoff who noted that mononuclear phagocytes from animals resistant to bacterial challenge had perfected their powers of phagocytosis and microbicidal destruction. This classical view places the macrophage in the efferent part of the immune system. More recently it has been shown that macrophages are crucially involved in the regulation of lymphocyte function, thus playing an important role in the afferent part of the immune system. Chapter 1 briefly summarizes the role of macrophages in immunity, the mechanisms leading to macrophage activation and alterations in macrophage metabolism and chemistry that accompany these changes.

Multiple signals from the extracellular milieu act on macrophages to regulate activation. In turn, the numerous secretory products of macrophages act on the environment. Among these products are arachidonic acid derived metabolites (chapter 2) which play a profound role in dictating the progression of immune events. For example, various hydroxyeicosatetraenoic acids and leukotriene B_4 are extremely potent in regulating the functions of neutrophils, the hallmark of acute inflammatory mechanisms. In addition, specific prostaglandins have been shown to modulate the function of macrophages and suppress a variety of inflammatory or immune related processes. For example, earlier observations in our laboratory showed that prostaglandins of the E-series inhibited the development of the macrophage mediated proliferative component of an inflammatory reaction. They further indicated that this inhibition was realized via stimulation of macrophage cyclic AMP synthesis. In chapter 3 the mechanisms, by which alterations in cyclic AMP content affect macrophage activity are briefly summarized.

The findings described in the previous paragraph led to the experiments described in this thesis, which are aimed at unravelling the interaction of various arachidonic acid oxygenation products with regard to the regulation of macrophage activity.

CHAPTER 1. MACROPHAGES

1 INTRODUCTION

The term "mononuclear phagocytes" is generally used to refer to a very large family of phagocytic cells which are all derived from the bone marrow or blood monocytes[98], although a self-renewing population of tissue macrophages has been postulated as well[61] (figure 1). These cells are long-lived and, in contrast to another phagocyte, the neutrophil granulocyte, they have the capacity to differentiate into a variety of macrophages or macrophage-like cells in response to different activating signals. A typical macrophage does not exist. Originally characterized as phagocytic cells[172], macrophages have over the last two decades been recognized as omnipotent cells, making up the

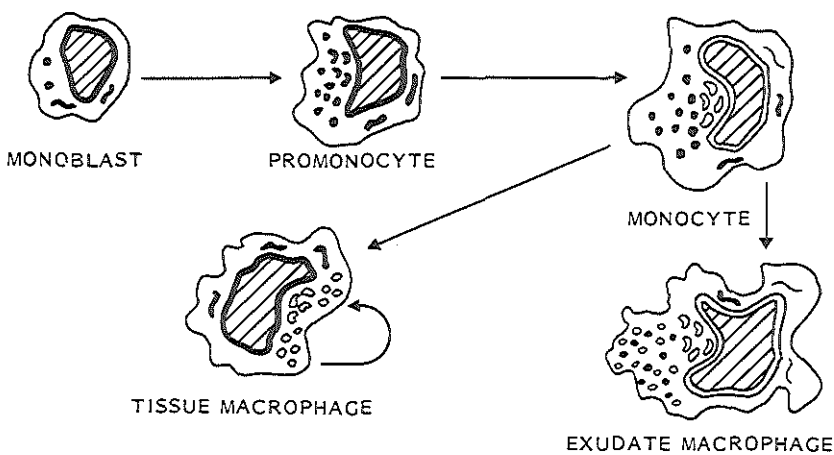


Figure 1. Origin and peroxidatic activity of macrophages. Peroxidatic activity in the nuclear envelope (monoblast, promonocyte and tissue macrophage), peroxidase positive granules (all except tissue macrophage), Golgi apparatus (promonocyte) and endoplasmic reticulum (monoblast, promonocyte and tissue macrophage).

Table 1. Secretory products of macrophages.

-hydrolytic enzymes (a.o. lysozyme)
-oxygen metabolites
-several complement components
-interferon
-interleukin-1
-fibroblast stimulating factor
-α_2-macroglobulin
-platelet-activating factor
-eicosanoids
-thymidine
-monocytopoiesis increasing factor (FIM)

non-specific branch of the immune system[180]. Not only does the macrophage ingest and kill microbes, it also plays a key role in initiating an immune reaction and subsequently modulates the immune responses. Additionally, macrophages were found to selectively and efficiently lyse tumour cells[8,124]. The efficiency of the macrophage primarily is based on the release of an enormous battery of secretory products (table 1)[77,114,134]. Most of these products are either involved in the defense against microorganisms, the destruction of damaged or neoplastic cells, the stimulation or inactivation of various enzyme systems or the modulation of immune responses.

2 FUNCTION

In spite of the danger of oversimplifying the diverse functional aspects of macrophages, in the following paragraphs the mechanisms involved in phagocytosis, cytotoxicity and immunoregulation will be discussed seperately; in fact all these functions are interrelated.

Phagocytosis and bactericidal activity.

Mononuclear phagocytes bind and efficiently destroy a variety of microorganisms. Phagocytosis is a two step process[114]. The first step involves attachment of the particle to the macrophage membrane. During the second step, the surface membrane moves over the particle until it is covered. For an efficient phagocytosis the participation of Fc- (immune complexes) and C3b- (complement cleavage products) receptors are required[77,134]. Opsonization of the antigen or particle with antibodies greatly facilitates binding as well as the actual engulfment, opsonization with complement factors only enhances binding[215]. Fc- or C3b-mediated activation of macrophages is accompanied by a rapid secretion of lysosomal enzymes[133] and various mediators including arachidonic acid oxygenation metabolites[206]. The significance of both events will be discussed in more detail in other parts of this chapter.

Phagocytosis is followed by a metabolic burst with an increase of oxygen utilization. The oxygen is used for the production of superoxide, in order to produce hydrogen peroxide, hydroxyl radicals and other oxygen radicals such as singlet oxygen[148]. Studies using cell lines which are defective for oxygen metabolism indicated that hydrogen peroxide is primarily responsible for intracellular killing of phagocytosed bacteria[63,247]. After reconstitution with a hydrogen peroxide generating system the bactericidal activity of these defective cells was restored.

A second effector mechanism of macrophages involves their capacity to secrete a variety of hydrolytic enzymes, which may enhance killing of microbes. Macrophages contain a number of enzymes with different specificities and functions.

Lysozyme (muramidase), a cationic protein with a hydrolase activity, may be considered a typical phagocytic enzyme[11,108]. However, while it is secreted by the living monocyte it is only released by the dying granulocyte[119]. Mononuclear phagocytes have a very low intracellular lysozyme content, nevertheless they continuously secrete large amounts of lysozyme, the rate of secretion being similar in resident, quiescent and activated or elicited macrophages[12]. Lysozyme secretion is a usefull parameter in assessing the viability of macrophages in culture since the rate of secretion remains very constant. The measurement of lactate dehydrogenase, a cytoplasmic enzyme, activity in supernatants can be used for the same purpose as, under normal

culture conditions, it is not released from the cells.

Lysosomal hydrolases originally were found in phagosomes where they serve the purpose of intracellular digestion[54]. Phagosomes arise from the fusion of endocytic vacuoles and lysosomes. Some fiftheen years ago[261] it was found that macrophages release large quantities of lysosomal enzymes in response to phagocytic stimuli. It was also established that the rate of stimulated release reflects the degree of activation of the cell[65]. The fact that the amounts of lysosomal hydrolases excreted, exceed the amounts which are present intracellularly, indicate that the release is based upon de novo synthesis. Indeed it could be shown that cycloheximide, a protein synthesis inhibitor, almost completely blocked the release[224].

Neutral proteinases play a major role in various inflammatory processes and may effectively destroy constituents of the connective tissues. The best-known neutral proteinase is plasminogen activator which transformes the inactive precursor plasminogen into the fibrinolytically active plasmin. Plasmin also has the capacity to activate the complement and kinin systems[149]. Only activated macrophages release neutral proteinases, which is the reason why plasminogen activator release is often used as a parameter to distinguish between resident and stimulated or elicited macrophages[225].

Cytotoxicity.

Considerable interest has been focussed on the cytotoxic activity of macrophages, a function which is believed to play a significant role in resistance to infection, tumour rejection and certain autoimmune reactions. Although the exact mechanisms which underlie cytotoxicity are beyond the scope of this thesis and are extensively reviewed elsewhere[4,166,200,251], some aspects of cytotoxicity will be discussed in the following section.

Two distinct modes of action can be distinguished by which macrophages destroy tumour cells[2]. The first of these is the rapid, highly specific lysis of antibody-coated targets in which neoplastic as well as non-neoplastic cells can be destroyed. Evidence from murine systems strongly indicates recognition of antibody-coated targets is mediated largely by Fc-receptors on the macrophages[185] and that secretion of hydrogen peroxide is a major lytic mechanism[3,144]. The second mode of action is a slow, contact-dependent cytotoxicity, which is selective for neoplastic targets and not dependent on the

presence of antibodies. The mechanisms by which activated macrophages injure neoplastic cells are incompletely defined at present. A possible factor may involve premature induction of a fatal cycle of tumor cell division. The lethal result would be the generation of an aberrant population of tumour cells with a reduced content of DNA[56]. Also, secretion of a neutral proteinase, termed cytolytic factor, that effectively lysis neoplastic cells[1,143] is considered to be one of the major events in macrophage mediated cytotoxicity. Other macrophage derived factors which have been described as cytotoxic include thymidine[242], arginase[59], cachectin[18] and a recently defined[198] soluble factor, related to lymphotoxin[73].

Immunoregulation

Recently considerable advances have been made in understanding the role of macrophages in the regulation of the immune response, whereby the attention has been focussed on the effects of secretory products, including monokines and eicosanoids. Macrophages are essential for the initiation and maintenance of a variety of lymphocyte effector cell functions (table 2). However, macrophages can act as "helper cells" during initiation of an immune response, and as "suppressor cells" at a later stage[101].

Table 2. Macrophages act as accesory cells in:

-antigen induced T-cell proliferation 211,243
-lectin induced T-cell proliferation 145,181,222
-interactions between T-and B-cells in antibody formation 199,241
-interactions with T-cells in cellular immunity reactions 107,174,250
-interactions with B-cells and polyclonal stimuli 212,270
-mixed lymphocyte reactions 57,99
-anti-tumour immunity 248

Antigen presentation by macrophages is a prerequisite for all aspects of antigen-specific T lymphocyte responses, including T cell memory[39,186,250]. The proliferative response of T cells to antigens has been one of the systems most extensively employed for studying macrophage-lymphocyte interactions. Such antigens include synthetic amino acid polymers and polypeptide hormones as well as xenogeneic antibodies. An example of the induction of antigen-specific proliferation of T cells from a study by Huber and Stingl[135] is given below. In this experiment purified T cells were sensitized to rabbit IgG pulsed human macrophages. Secondary proliferative responses were subsequently induced by restimulation of sensitized T cells with antigen-pulsed or native macrophages. A proliferative response could only be induced with autologous, antigen-pulsed macrophages. Furthermore, depleting the macrophages of the HLA-DR$^+$ cells completely abolished the observed responses. This second finding emphasizes the importance of the major histocompatibility gene complex (HLA in man, H-2 in mice)[168].

The major histocompatibility system codes for a large group of surface glycoproteins[14,238]. One type of glycoproteins corresponds serologically to the HLA-D region associated antigens in man and the I region associated antigens in rodents. These antigens, which were indentified on macrophages, B lymphocytes and on a few activated T cells[250], are identical to those structures on the surface of macrophages which control cooperation with T cells[40,50]. Thus, for effective T cell-macrophage collaboration in man, two essential features are required: (I) that the proliferating T cell and the macrophage are histocompatible, share the HLA-D/DR region; (II) that the phagocyte bears the region associated antigens (HLA-D/DR) of the major histocompatibility complex.

Secretory products. Among the secretory products of macrophages that participate in immunoregulation[109] monokines and eicosanoids[26] take the most prominent positions. As eicosanoids will be discussed in detail in chapter 2, the following paragraphs will only deal with the mode of action of monokines.

One of the most important lymphocyte stimulatory molecules is lymphocyte activating factor (LAF), first clearly demonstrated by Gery et al.[102]. Shortly after Gery's observation, Schrader[227] reported that macrophage supernatants could also stimulate the development of antibody-secreting B cells. The factor in the supernatant was termed B cell activating factor (BAF). Since studies on the chemical nature revealed that LAF and BAF were identical[152,269], it was

suggested that the term interleukin-1 (IL-1) be introduced to designate the molecule, a polypeptide with a molecular weight between 13.000-16.000 dalton.

Major effects of IL-1 are to stimulate proliferation of T lymphocytes, augment their response to mitogens, and promote secretion of a very potent lymphocyte stimulant, interleukin-2 (IL-2) (Table 3). Although from the functions enlisted in table 3 one might conclude that IL-1 is the principal immunoregulatory molecule, some caution must be taken. IL-1 indeed is capable of stimulating antigen induced T cell proliferation but only on the condition that macrophages are present in sufficient amounts to assure proper antigen presentation[194,272].

Table 3. Effects of interleukin-1

A. Immune system related effects
   -stimulation of T lymphocute proliferation 167
   -induction of IL-2 release 84
   -T lymphocyte surface alteration 15,202
   -induction of cytotoxic lymphocytes 85
   -stimulation of Natural Killer cells 66
   -enhancement of glucocorticoid resistance 175
   -enhancement of the antibody response 165
   -stimulation of antigen activated T-helper cells 147
   -induction of prostaglandin synthesis 31,68
B. Non-immunological effects
   -induction of fever 213
   -stimulation of acute phase protein synthesis 146
   -muscle breakdown 13
   -neutrophil granulocyte activation and migration 151,223
   -fibroblast, chondrocyte and synoviocyte stimulation 112,201,271

Also, the induced proliferation is not an effect of IL-1 per se, but seems to be mediated via IL-2[55,190], whereas the IL-1 induced immunoglobulin release is dependent on the presence of T cells and macrophages[165]. Finally, interactions between IL-1 and arachidonic acid oxygenation metabolites, discussed below, could provide a fine tuning mechanism by which the immune system could limit its own activity (figure 2). The discovery that IL-1 production is stimulated by metabolites of the lipoxygenase pathway[69] is of considerable interest, especially in the light of the results presented in this thesis. Cyclooxygenase derived metabolites of arachidonic acid on the other hand, have been shown to depress IL-1 release as well as the effects of IL-1 on lymphocytes[42,123].

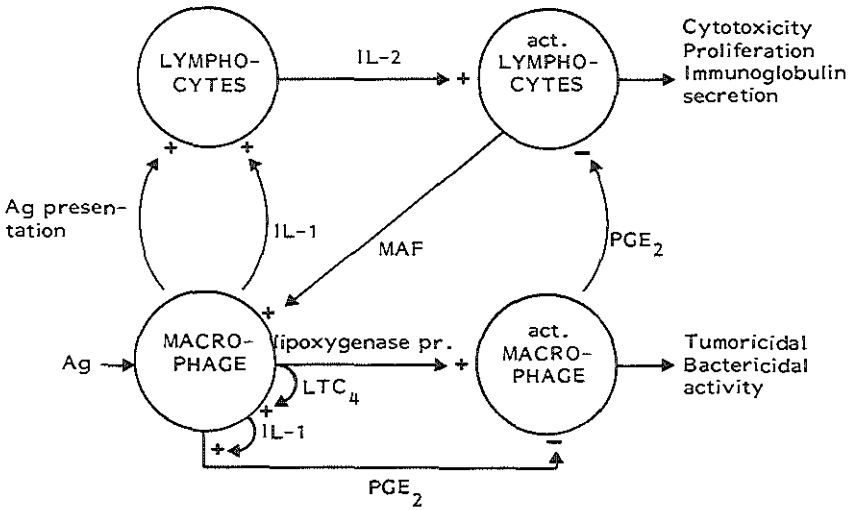


Figure 2. Interaction between eicosanoids and interleukin-1 in the regulation of macrophage activity. Abbreviations used for antigen (Ag), interleukin-1 (IL-1), interleukin-2 (IL-2), leukotriene C_4 (LTC_4), macrophage activating factor (MAF) and prostaglandin E_2 (PGE_2).

3 ACTIVATION

Macrophage activation is neither a single alteration nor a fixed set of alterations in macrophage metabolism. Rather, activation is better viewed as competence to carry out one complex function or another and, as such, depends on expression of multiple capacities, which may vary depending on the function. Thus, macrophages activated for tumoricidal activity are not necessarily activated for microbicidal function, while macrophages activated for microbicidal function are not necessarily activated for tumoricidal function[153,183]. For the sake of clearness a distinction has to be made between activation and state of activity of macrophages. Evidence has emerged that macrophages become activated by passing sequentially through a series of defined stages. These stages may be induced *in vivo* and *in vitro*, and each is operationally defined by how it is induced and how it must be further stimulated to express full activation[125,171,247]. Since thisphenomenon is excellently

Table 4. Factors used for macrophage activation

in vitro:	*in vivo*:
-latex beads/zymosan	-serum
-microorganisms	-microorganisms
-endotoxin	-endotoxin
-concanavalin A	-thioglycollate
-phorbol myristate acetate	-carrageenan
-complement factors	-protease peptone
-immune complexes	-adjuvant
-lymphokines	
-ionophore	
-arachidonic acid	

Note: the stimuli applied *in vivo* can be also used *in vitro*

reviewed elsewhere[4] and is beyond the scope of this thesis, the following paragraphs will be restricted to a brief comparison between resident and elicited/inflammatory macrophages.

Several eliciting agents have been shown in experimental animals to recruit populations of activated, inflammatory macrophages, and *in vitro* culture experiments have demonstrated that quiescent, resident tissue macrophages can be induced to differentiate in response to particulate and soluble factors (table 4). When an inflammation is induced in the peritoneal cavity, there is an increased influx of bone marrow derived monocytes from the peripheral blood into the peritoneal cavity, which differentiate into inflammatory macrophages at the site of inflammation. Inflammatory macrophages differ in numerous ways from resident tissue macrophages[61]. They are larger and more irregular in shape. In addition, their surfaces are rougher and show more prominent ridgelike processes. In inflammatory macrophages the lysosomal apparatus is stimulated, large lysosomes and an intense deposition of fat droplets in the vicinity of lysosomes can be observed[75]. Characteristic differences are found between resident and inflammatory macrophages with respect to the intracellular distribution of peroxidase activity[61]. Resident macrophages contain a peroxidase activity which is located in the rough endoplasmic reticulum and the nuclear envelope. In contrast, in inflammatory macrophages the peroxidase is located in granules which are known to fuse with phagosomes as a result of which peroxidase is released into these vesicles.

In table 5 changes in biochemical parameters of inflammatory macrophages in comparison with resident macrophages are listed. Noteworthy most alterations are increases with one major exception: the prostaglandin synthetase system and the herewith associated intracellular cAMP content. However, it must be emphasized that inflammatory macrophages respond to a second, *in vitro* stimulus with an enhanced production of prostaglandins followed by a rapid increase in intracellular cAMP content. The significance of this phenomenon will be discussed in extenso elsewhere in this thesis.

Finally, the way in which the peritoneal exudate is induced and the nature of the inducing agent itself may change the properties of the macrophage population. Support for this statement can be found in the results of studies using macrophages obtained from dialysis bags of patients on continuous ambulatory peritoneal dialysis[30]. Using this technique for the treatment of

humans with a renal disease, the population of macrophages in the peritoneal cavity is renewed every six hours. Therefore it is justified to denominate the macrophages obtained from the dialysis fluid as elicited. However with respect to functional aspects, in terms of cyclic AMP content and responsiveness to prostaglandins these macrophages did not show characteristics of inflammatory cells.

Table 5. Biochemical changes induced by stimulation of macrophages

Parameter	*Effect*	*Reference*
lysosomal enzyme release	↑	65
plasminogen activator release	↑	118
interleukin-1 release	↑	131
production of reactive O_2 metabolites	↑	197
Ia-antigen expression	↑	46
alkaline phosphodiesterase activity	↑	76
adenylate cyclase sensitivity	↑	78
lysozyme release	↑	225
c-AMP content	↓	78
prostaglandin production	↓	23
phospholipase-A_2 activity	↓	136
cyclooxygenase activity	↓	244
5'-nucleotidase activity	↓	136

CHAPTER 2, EICOSANOIDS

1 INTRODUCTION

Few areas of biological research have ever expanded as rapidly as that relating to the eicosanoids. Eicosanoids is the general term used for the metabolites which are derived via oxidative metabolism of the fatty acid arachidonic acid. They include prostaglandins, thromboxanes, leukotrienes, lipoxins and other oxygenated compounds.

Although the existence of prostaglandins and leukotrienes already was described in the 1930s by von Euler[83] and Feldberg[86], the true importance of these mediators could only be assessed after elucidation of their structure and of the pathways involved in the biosynthesis of these substances. In 1964 this was independently realized for prostaglandins by a Swedish and Netherlands group[16,71]. Platelet aggregating activity of intermediates in the prostaglandin synthetase pathway which could not be explained with the effects of the then known prostaglandins led to the discovery of thromboxanes[117]. Shortly after, in 1976, it was established that blood vessels could release a prostanoid which showed effects opposite to those of thromboxane. The newly found metabolite was termed prostacyclin[142]. In the meanwhile it was shown that non-steroidal anti-inflammatory drugs (e.g. aspirine) inhibit cyclooxygenase, the enzyme which is responsible for the conversion of arachidonic acid into prostaglandins[253]. Before being converted by cyclooxygenase, arachidonic acid has to be liberated from phospholipid pools, an event which appeared to be blocked by corticosteroids[130]. Since pronounced differences in the anti-inflammatory effects of corticosteroids and non-steroidal anti-inflammatory drugs can be observed it seemed conceivable that arachidonic acid generates an additional inflammatory mediator via a mechanism which is not dependent on cyclooxygenase activity. Indeed, it was shown that arachidonic acid can be converted by a lipoxygenase into leukotrienes[32], some of which proved to be

constituents of slow reacting substance of anaphylaxis (SRS-A)[220]. The ultimate finding in the domain of biologically active derivatives of arachidonic acid was the recent discovery of a new group of compounds, the lipoxins[234].

2 BIOSYNTHESIS AND STRUCTURE

Arachidonic acid liberation

The liberation of unesterified archidonic acid from cellular phospholipids is considered to be the prerequisite for the synthesis and secretion of eicosanoids[155]. Although there is not full agreement on which phospholipases are involved, the major route of phospholipid degradation is thought to be catalysed by phospholipase A_2[138]. Phospholipase A_2 activity is dependent on three distinct, yet related factors: (a) availability of intracellular Ca^{2+}, (b) activity of lipocortin and (c) intracellular cAMP content (figure 1). Since phospholipase A_2 activity is Ca^{2+} dependent an influx of Ca^{2+}, as can be achieved with the Ca-ionophore A23187, will enhance arachidonic acid liberation resulting in a stimulation of eicosanoid production[252]. Physiologically, the intracellular availability of Ca^{2+} is dependent on the turnover of phosphatidyl inositol[173]. Conversion of phosphatidyl inositol, catalysed by phospholipase C, yields inositol phosphates and phosphatidic acid. These products are thought to be essential for both the mobilisation of intracellular Ca^{2+} stores and the influx of Ca^{2+} from the extracellular milieu[21]. Both phospholipase A_2 and phospholipase C are inhibited by a polypeptide, termed lipocortin[93]. The name lipocortin originated from observations that the synthesis of this protein which reduces lipid metabolism, was induced by corticosteroids[92,127]. Macrophages react very rapidly to corticosteroids, and they may be unusual in that lipocortin synthesis is not induced by corticosteroids (except as a later, secondary event) but rather that the steroids induce a protein synthesis dependent release of stored lipocortin[20]. Once the lipocortin is released, the macrophages are no longer responsive to dexamethason until more protein is made;

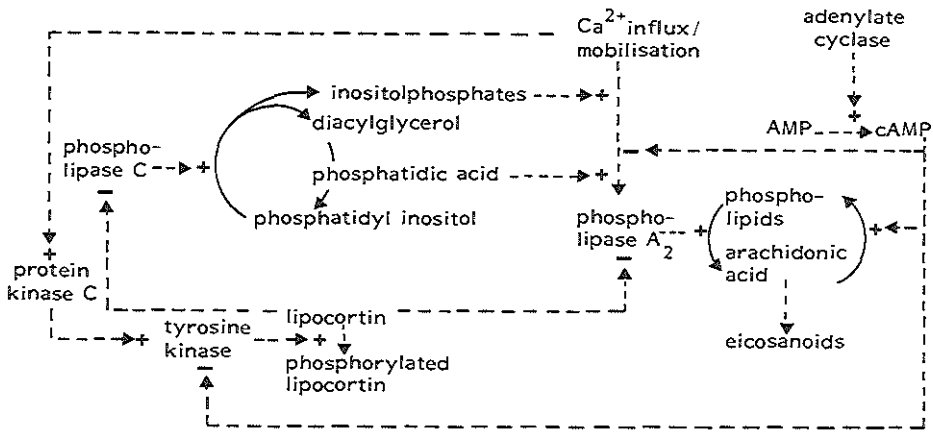


Figure 1. Interactions between lipocortin, Ca^{2+} and cyclic AMP: effects on phospholipase A_2 activity.

they are, however, responsive to added lipocortin[45]. Lipocortin is inactivated by phosphorylation, which is carried out by tyrosine kinase. Tyrosine kinase itself is subject to regulation by two serine kinases, protein kinase C and protein kinase A[128]. Whereas the Ca^{2+} dependent protein kinase C activates tyrosine kinase, the cAMP dependent protein kinase A inhibits it. Thus, increased Ca^{2+} mobilisation activates phospholipase A_2 directly and by stimulating protein kinase C activity, hence stimulating lipocortin phosphorylation, it prevents the inhibition of phospholipase A_2. cAMP has opposite effects: by stimulating Na^+/Ca^{2+} exchange it lowers the intracellular Ca^{2+} concentration[67]. Furthermore it activates protein kinase A which results in an increased availability of lipocortin.

Prostaglandins and thromboxane

Free arachidonic acid can be converted by two main pathways (cyclooxygenase and lipoxygenase) to a variety of biologically highly-active compounds. The cyclooxygenase pathway (figure 2) directly generates prostaglandin (PG) G_2 from

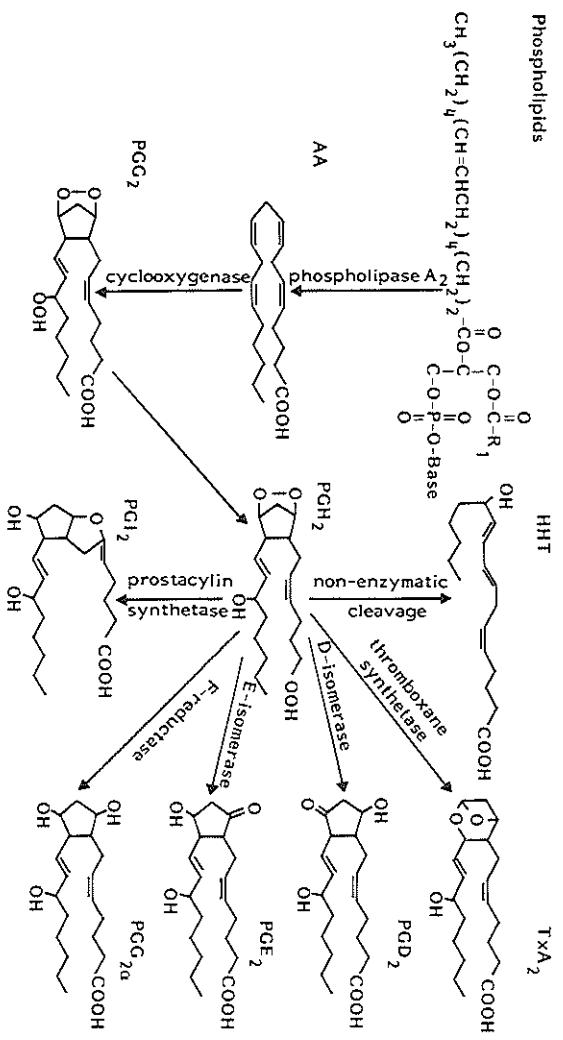


Figure 2. Cyclooxygenase pathway.

arachidonic acid by the action of an enzyme that is inhibited irreversibly by aspirin and non-competitively and reversibly by other non-steroidal anti-inflammatory compounds. The enzyme has been termed cyclo-oxygenase or PG endoperoxide synthetase and its mode of action has been proposed to be an initial lipoxygenase type of reaction with introduction of oxygen at C_{11}. Further oxygenation and cyclization yields PGG_2, an unstable endoperoxide, which is reduced in a hydroperoxidase reaction to PGH_2. PGH_2 is then subject to parallel enzymatically catalyzed reactions to yield six products. These products include four prostaglandins PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2 (prostacyclin), a 17-carbon cleavage product (HHT) and thromboxane A_2[219]. Each prostaglandin is designated by a letter, indicating the nature of the cyclopentane substituents and by a subscript which indicates the number of double bonds, being dependent upon the precursor fatty acid. Whereas archidonic acid gives rise to the prostaglandins of the dienoic "2 series", dihomo-γ-linoleic acid and eicosapentaenoic acid are converted into PG_1 and PG_3 groups of compounds respectively.

Leukotrienes

In addition to the products of the cyclooxygenase pathway, a number of non-cyclized compounds have been described as metabolites of arachidonic acid via lipoxygenase pathways that are not inhibited by the non-steroidal anti-inflammatory drugs to the same extent. These pathways include the 5-, 12- and 15-lipoxygenase routes of which the 5-lipoxygenase pathway gives rise to leukotrienes[97]. Arachidonic acid is oxidatively metabolized via the 5-lipoxygenase to 5-hydroxyperoxyeicosatetraenoic acid (5-HPETE) and then either to the related monohydroxyeicosatetraenoic acid (5-HETE) or to an epoxide intermediate known as leukotriene A_4 (LTA_4). LTA_4 can be converted by two enzymatic routes to other leukotrienes or by non-enzymatic hydrolysis to 5,12- and 5,6-di-HETE's (figure 3). The first enzymatic route of metabolism of LTA_4 involves opening of the epoxide with glutathione as a nucleophile[182] to produce the peptidolipid conjugate LTC_4. LTC_4 is sequentially metabolized to LTD_4 by removal of a glutamic acid residue from the peptide[179] and then to LTE_4 by cleavage of glycine[161]. These three leukotrienes collectively account for the

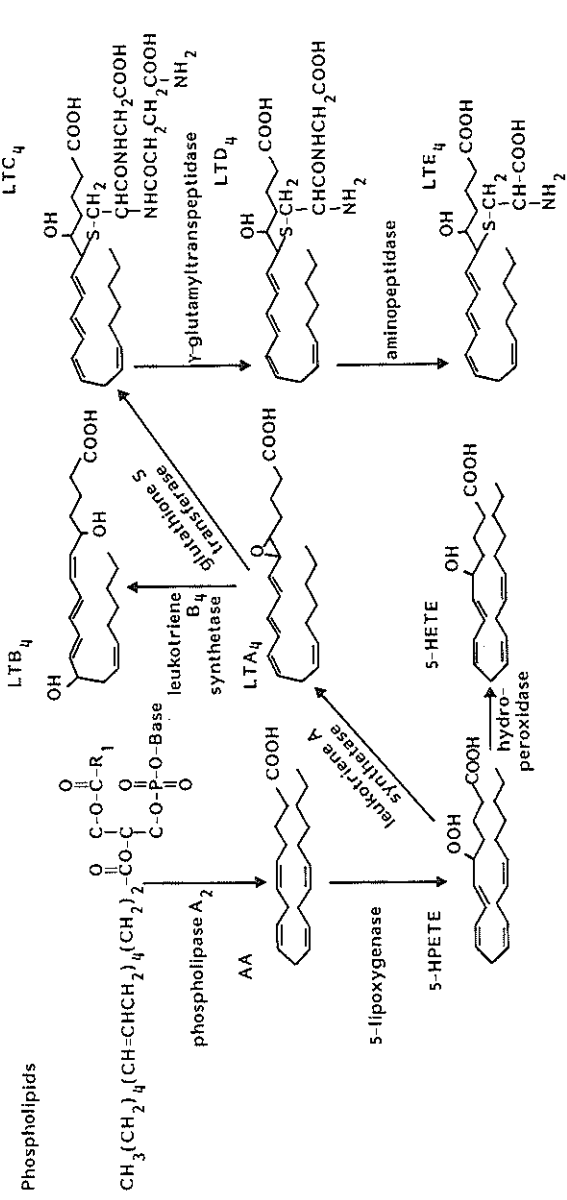


Figure 3. 5-lipoxygenase pathway.

biological activity known as slow-reacting substance of anaphylaxis (SRS-A). The second enzymatic route involves the hydration of LTA_4 to produce a 5,12-di-HETE, termed LTB_4, and was first described in polymorphonuclear leukocytes[33].

Lipoxins

In 1984 Serhan, Hamberg and Samuelsson announced the isolation of a new class of metabolites of arachidonic acid, the trihydroxyeicosatetraenoic acids or lipoxins[234]. The name lipoxin is in recognition of their being presumptive lipoxygenase products. In contrast with leukotrienes however, lipoxins are not derived via the 5-lipoxygenase but via the 15-lipoxygenase pathway. Arachidonic acid is oxidatively metabolized via this enzymatic route to 15-hydroperoxyeicosatetraenoic acid (15-HPETE). A second oxidation at C_5 yields 5,15-di-HPETE, which can undergo enzymatic dehydration to produce a 5,6 epoxide. Enzymatic hydrolysis of the epoxide at C_6 would lead to lipoxin A (LXA), while addition of water at C_{14} would produce LXB. However, in a recent publication[5] the *in vitro* synthesis of LXA and LXB was described whereby a.o. leukotriene A_4 was used as starting material. Therefore, the exact *in vivo* metabolism of lipoxins still remains to be solved.

3 FUNCTION

Eicosanoids are involved in numerous physiological or pathophysiological processes. These processes include neurotransmission, hormonal interactions and effectivity, reproductive physiology, labour and childbirth, gastrointestinal physiology, pulmonary physiology, renal physiology and diuresis, cardiovascular physiology and blood clotting and also inflammation and immunomodulation. Considering the scope of this thesis only the effects of eicosanoids on inflammation and the immune system will be discussed.

Prostaglandins

The experimental evidence which supports the view that prostaglandins influence the development and modulation of immune processes and inflammatory reactions is voluminous[26,100,105,157,250]. Among these prostaglandins, prostaglandin E_2, predominantly synthesized by macrophages[100], has gained a certain prominence as it has been shown to affect more than other cyclooxygenase products both inflammatory and immune responses[28]. Prostaglandin E_2 appears to exhibit two seemingly confusing effects, namely pro- and anti-inflammatory actions. Some of this confusion is avoided when one considers effects of prostaglandins on vascular or "acute" components of inflammation seperately from their effects on tissue components. The classical symptoms of inflammation namely redness (vasodilatation), swelling (increased capillary permeability) and pain (sensibilisation of neurones to bradykinin or histamine) which are all due to the action of prostaglandin E_2, are characterized by a rapid and early occurence during inflammation and they can impressively be inhibited by the non-steroidal anti-inflammatory drugs. However, as has been shown in the carrageenan induced granuloma model[27], in a later stage of inflammation prostaglandin E_2 exhibits anti-inflammatory properties. The anti-inflammatory and immunomodulatory effects of prostaglandin E_2 are most likely due to its capacity to stimulate the adenylate cyclase activity of a variety of cells including fibroblasts, lymphocytes, macrophages, eosinophils and neutrophils[29]. An increase in cyclic AMP predominantly represents an inhibitory signal for various functions, although some exceptions have been noted. Thus it is known that prostaglandin E_2 reduces a.o. fibroblast proliferation and collagen synthesis, lymphocyte proliferation, killing by cytolytic T cells, natural killer cell activity and lymphokine production by macrophages[250]. On the other hand, prostaglandin E_2 induces collagenase production by macrophages[257]. Also it appears that maturation of thymocytes is enhanced by cyclic AMP generating hormones such as prostaglandin E_2[105]. More information is definitively required to elucidate the role of prostaglandin E_2 as an immuno/inflammatory regulator molecule with either suppressive or stimulating properties.

Leukotrienes

The rationale for seeking pharmacotherapeutic agents to limit leukotriene biosynthesis and/or end organ effects is based upon: 1- the demonstrated *in vitro* capacities of certain cells and tissues to generate leukotrienes in response to selected agonists, 2- the potent pro-inflammatory actions of these compounds in pharmacological studies (table 1) , 3- the recovery and measurement of leukotrienes in biological fluids associated with certain disease states (table 2) and 4- the knowledge that non-steroidal anti-inflammatory drugs have substancial efficacy, but modify only one of the two major routes of arachidonic acid metabolism.

Among the cells relevant to an inflammatory response the production of leukotrienes seems to exhibit a remarkable cellular specificity. Whereas polymorphonuclear leukocytes generate leukotriene B_4 in a 10-fold excess relative to leukotriene C_4, this ratio is reversed with eosinophils[262]. Human peripheral blood monocytes release both leukotriene B_4 and leukotriene C_4[266] and human alveolar macrophages are reported to respond to stimulation with the Ca-ionophore A23187 with a preferential leukotriene B_4 generation[87]. However some caution has to be taken with respect to the interpretation of these data. Thus it is shown that stimulation of human alveolar macrophages with arachidonic acid resulted in the production of leukotriene D_4[64], whereas human peritoneal macrophages responded to A23187 with a release of both leukotriene B_4 and leukotriene C_4[74]. Therefore depending on the site in the inflammatory focus, the cell type involved and the stimulus applied, the leukotriene production can predominantly be leukotriene B_4 or leukotrienes C_4/D_4 with distinct biological activities.

A number of biological activities have been described for the 5-lipoxygenase products. Leukotriene B_4 has been shown to be one of the most powerful agents that stimulate chemokinesis of leukocytes and to act as a chemotactic compound with a potency comparable to other important chemotactic agents such as the complement-derived peptide C5a and the synthetic peptide formylmethionylleucylphenylalanine. In addition, leukotriene B_4 seems to augment leukocyte activity as could be observed with respect to aggregation, superoxide production, lysosomal enzyme release, glucose metabolism and expression of C3b receptor sites. *In vivo* studies showed that leukotriene B_4 mediated inflammatory reactions by recruiting leukocytes, by mediating vascular

Table 1. Biological effects, induced by 5-lipoxygenase products.

in vitro effects

target	metabolite	effect	reference
PMN/eo/M//Mo	LTB_4	chemokinesis	36,94,237
PMN/eo	"	chemotaxis	195
"		C3b receptor site expression	184
"		complement dependent cytotoxic reaction	178
PMN	"	adherence	95
		Ca^{2+} and Na^+ influx	177
		intracellular cAMP level	48
		superoxide production	233
		lysosomal enzyme release	204
PMN	LTC_4	adherence	104
M/	"	chemiluminescence	121
"	"	release of cyclooxygenase metabolites	89
"	5-HETE	Ia antigen production	240
T lymphocyte	LTB_4	T suppressor cell induction	208
NK cell		natural cytotoxic cell activity	209
lung parenchymal tissue	LTC_4/D_4	contraction	9
		TxA_2 release	273

in vivo effects

	LTB_4	leukocyte accumulation	34
	"	increased vascular permeability	35
	"	hyperalgesia	203
	LTC_4	vasodilatation	19
	"	increased vascular permeability	96
	"	stimulation of mucus production	141
	"	bronchoconstriction	260

Table 2. Involvement of 5-lipoxygenase products in pathological conditions

Disease	metabolite	reference
asthma	LTC_4	62
psoriasis	LTB_4/C_4	72,113
gout	LTB_4	205
rheumatoid arthritis	LTB_4	205
ulcerative colitis	LTB_4	235
pleurisy	LTC_4/D_4	249

permeability changes and by modulating pain responses. Furthermore, suppression of human T lymphocyte function, possibly through induction of human suppressor T lymphocytes, and activation of natural cytotoxic cells are aspects of the immunomodulatory role of leukotriene B_4.

Already before the leukotriene structures were elucidated, slow reacting substance of anaphylaxis (SRS-A) was known to be a constrictor of human airway smooth muscle[37]. More recently, these effects of SRS-A have been confirmed and extended with synthetic leukotriene C_4 and leukotriene D_4 both *in vivo* and *in vitro*. Other interesting features in the action of the peptido-leukotrienes on the lung are their ability to stimulate mucus production and to induce vascular permeability changes. Contraction, mucus production and edema are major aspects of asthma and indeed it has been shown that antigen challenge of human asthmatic lung leads to the release of peptido-leukotrienes[62]. These findings and the fact that macrophages from asthmatic individuals synthesize more leukotriene D_4 than macrophages from normal subjects[64] lead to the hypothesis that peptido-leukotrienes are major mediators involved in human allergic asthma. In addition these leukotrienes have other properties that could be important in terms of mediation of inflammatory reactions. They are potent vasodilators, enhance neutrophil adherence and modulate the release of mediators from macrophages which is the subject of this thesis.

Lipoxins

Since the structure of lipoxin A and lipoxin B has only been elucidated very recently, the relevance of lipoxins on inflammatory or immune processes still is open to question. Of potential interest are the biological actions of these lipoxins, which involve: regulation of natural killer cell activity, stimulation of superoxide and lysosomal enzyme release from polymorphonuclear leukocytes and contraction of the guinea pig lung parenchymal strip[221]. However, whereas Samuelsson reported that both lipoxin A and lipoxin B inhibited natural killer cell activity[221], a recent paper showed that lipoxin A in low concentrations actually enhanced whereas higher concentrations inhibited this activity[160]. It is evident that the availability of synthetic lipoxins, as was recently reported[5], will go far towards the elucidation of the relevance of lipoxins in pathophysiological processes.

This short review of effects of eicosanoids should emphasize one important aspect. Metabolism of arachidonic acid yields a variety of mediators which differ tremendously in their respective activity. A complicated, but balanced network of interactions appears to evolve that may markedly influence inflammation or immunoregulation at an unspecific level. It probably will turn out that this immunologically unspecific system is intricately involved in the regulation of the more sophisticated and immunologically specific networks of the immune response.

CHAPTER 3. ADENYLATE CYCLASE

1 INTRODUCTION

Many hormonally responsive systems are regulated through changes in intracellular cylic nucleotide levels, and it is well documented that macrophages respond to hormones such as prostaglandins[30,79] and drugs such as β-adrenergic agents[115,263] by an increased production of 3'5'-cyclic adenosylmonophosphate (cyclic AMP). Increased levels of this nucleotide are known to modify cell metabolism and to influence such parameters as reactive oxygen production[164], lysosomal enzyme release[169], prostaglandin production[163], aggregation[216], phagocytosis[268], cytotoxicity[228] and Ia antigen expression[240]. To better understand how such macrophage functions are regulated, it is important to describe the underlying mechanisms of cAMP production and the proposed mechanism of action.

2 METABOLISM

Currently, activation of adenylate cyclase by hormones is considered to involve a ternary complex. This complex consists of: 1- a discriminator, a hormone specific receptor, 2- a transducer, transmitting the signal from the receptor to the effector system and 3- an amplifier, which is formed of the effector system (adenylate cyclase)[207]. Studies whereby adenylate cyclase deficient membranes were reconstituted with extracts from cell membranes of normal cells demonstrated that the transducer consists of a regulatory protein, which is guanosyltriphosphate (GTP) dependent[214], the G_s or guanine nucleotide

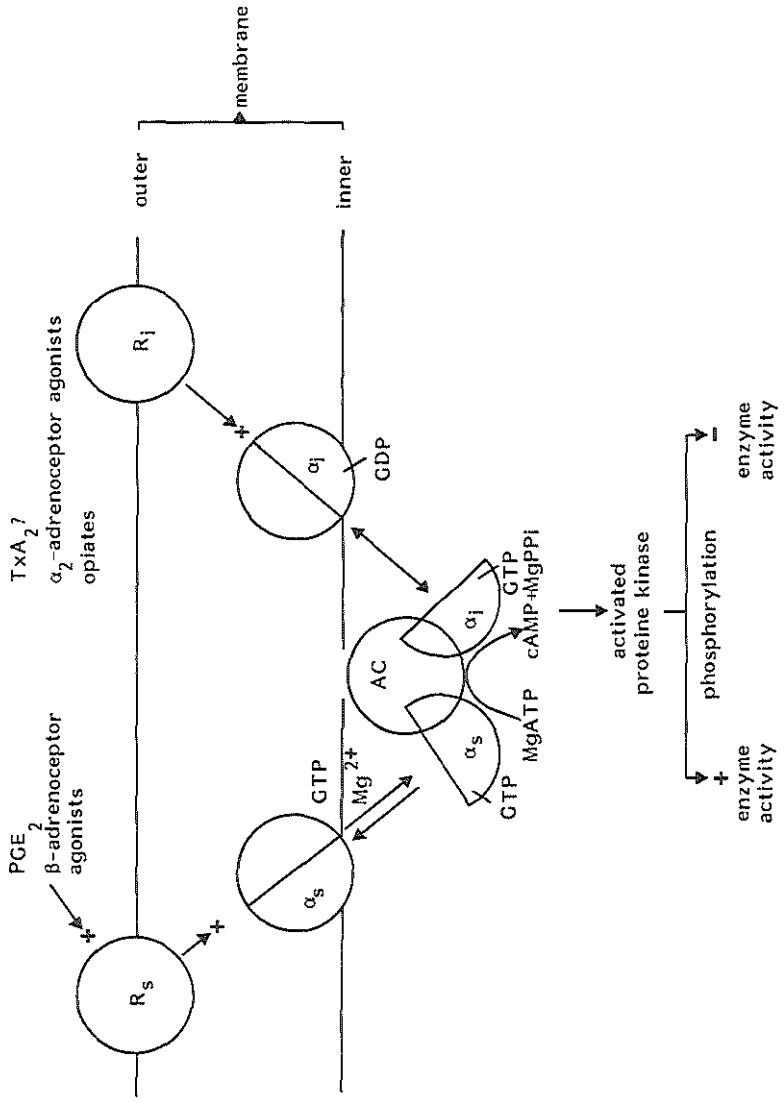


Figure 1. Regulation of adenylate cyclase activity: involvement of regulatory proteins.

regulatory protein. Furthermore it was shown that the rate limiting step in the process that leads to enhanced cyclic AMP formation is a change at the level of the G_s protein[47]. G_s protein contains two types of subunits, α and β, whereby the α subunits have been assessed to bind GTP[188]. Since it appeared that G_s protein activation is dependent on Mg^{2+}[139] and is followed by a reduction in its size[120], it has been postulated that G_s activation by GTP and Mg^{2+} is associated with a subunit dissociation event followed by combination of the GTP-loaded α subunit with the catalytic unit of adenylate cyclase[236] (figure 1).

Although many hormones act by increasing cellular levels of cyclic AMP, it is also well known that application of certain hormones lowers this level. Among these are α_2-adrenergic agonists, muscarinic agonists and opiates[140]. As was the case with stimulation, also with hormonal inhibition the presence of a guanine nucleotide regulatory protein, G_i, could be demonstrated[126]. Although the subunits of G_i are different from those of G_s, the general build-up of the protein is comparable to G_s with one difference: the inactive form of G_i contains GDP bound to the α-subunit which is exchanged for GTP upon activation[51] (figure 1).

3 FUNCTION

Although the exact mode of action by which cyclic AMP modulates cellular activity is not known yet, two mechanisms are known by which cyclic AMP affects cellular metabolism: protein phosphorylation[53] and interference with a second intracellular messenger, calcium[17]. Originally discovered as an activator of phosphorylase kinase[267], it soon became evident that the cyclic AMP dependent proteine kinase A must have a much wider role in mediating the effects of cyclic AMP as it was known that cyclic AMP affects other processes than glycogenolysis (table 1). The specificity of the response is determined by the presence of different substrate enzymes in different cells. With respect to arachidonate metabolism, one of the substrates for proteine kinase A is tyrosine kinase. As

was described in chapter 2, part 2 inactivation of tyrosine kinase by protein kinase A will lead to a decreased metabolism of arachidonic acid. These effects are counteracted by intracellular calcium which activates tyrosine kinase.

Next to the interaction between cyclic AMP and calcium described above, cyclic AMP is known to modulate the intracellular calcium concentration by two distinct mechanisms. Firstly, it has been shown that cyclic AMP stimulated Na^+/Ca^{2+} exchange, thus lowering the intracellular calcium concentrations[67]. Secondly, cyclic AMP causes the phosphorylation of the protein phospholamban, resulting in an increased uptake of calcium by the sarcoplasmic reticulum and therewith a reduced availability of calcium[154]. Whereas these mechanisms both result in a decreased concentration of calcium, one major interaction between cyclic AMP and calcium results in an increased effectivity of the latter. Phosphorylase kinase A not only is a target for cyclic AMP dependent proteine kinase A but is also dependent on calcium[122]. Phosphorylation of this enzyme

Table 1. Protein kinase affected enzymes (modified after Cohen,1982).

activated	inhibited
calmodulin	glycogen synthase
phosphorylase kinase	glycerol phosphate acyl transferase
triglyceride lipase	acetyl Co-A carboxylase
cholesterol esterase	6-phosphofructo 2-kinase
fructose 1-6 biphosphatase	pyruvate kinase
phenylalanine hydroxylase	tyrosine kinase
tyrosine hydroxylase	myosin light chain kinase
phospholamban	

by protein kinase A allowed activation by calcium to occur at lower concentrations[52]. Phosphorylase kinase has a subunit structure, whereby one of the subunits was found to be identical to calmodulin[259]. It has recently become clear that many of the biological actions of calcium are regulated by calmodulin in a manner that closely resembles the actions of cyclic AMP as a regulator of enzyme activities[150]. Whereas cyclic AMP stimulates the activation of calmodulin, calmodulin stimulates both adenylate cyclase and phosphodiesterase, the enzymes involved in the metabolism and breakdown of cyclic AMP[103]. In conclusion, it becomes increasingly apparent that cellular processes are controlled by an intricate network of intracellular signals of which the cyclic nucleotides and calcium are key components.

CHAPTER 4. RELEASE OF EICOSANOIDS FROM MACROPHAGES

1 INTRODUCTION

Of all the leukocytes macrophages regularly impress as the most efficient eicosanoid producers[101]. They are known to release both cyclooxygenase- and lipoxygenase-derived products[206,239]. Several investigators have shown a concomitant activation of the cyclooxygenase and lipoxygenase pathways, following exposure of macrophages *in vitro* to different stimuli as ionophore A23187[74], zymosan[217] and immune complexes[231]. However, it has been shown that soluble, membrane mediated inflammatory stimuli such as phorbol myristate acetate and lipopolysaccharide stimulated the cyclooxygenase pathway without affecting lipoxygenase activity[137]. On the other hand a recent paper describes the converse. It was shown that γ-hexachlorocyclohexane stimulated the formation of lipoxygenase products, having only a minor effect on prostaglandin production[170]. The results of these two groups could be explained by the existence of two different, independent sources of substrate arachidonic acid, as has been suggested previously[132]. The implication of this concept would be that prostaglandin and leukotriene production are independently mediated.

However, one should bear in mind that comparing eicosanoid release from cells using different stimuli can be quite confusing. Properties of macrophages vary according to their "state of activation". As discussed in chapter 1, part 3 the typical, activated macrophage does not exist[183]. Nevertheless some general alterations appear to be valid for all situations. Whereas *in vitro* stimulation of macrophages leads to enhanced eicosanoid release, activated macrophages, elicited *in vivo* with different inflammatory stimuli, have a reduced capacity to convert arachidonic acid to cyclooxygenase- and lipoxygenase-derived products[136,230]. Another well-known aspect of activated macrophages is their ability to release substantial amounts of lysosomal hydrolases[65]. This secretion generally is considered to be an aspecific but

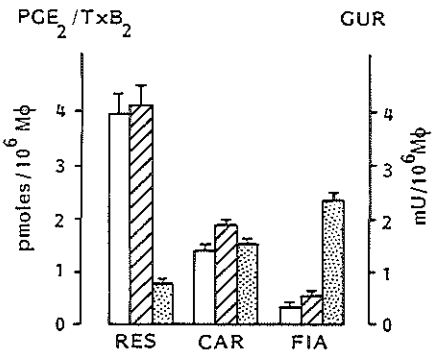


Figure 1. Basal secretory levels of resident peritoneal macrophages (RES), carrageenan (2 mg i.p.) elicited macrophages (CAR) and Freund's incomplete adjuvant (2 ml i.p.) elicited cells (FIA). Prostaglandin E_2 (PGE_2, open bars), thromboxane B_2 (TxB_2, hatched bars) and β-glucuronidase (GUR, dotted bars) were measured. An inverse relation between the production of the cyclooxygenase metabolites PGE_2 and TxB_2 and the release of the lysosomal enzyme β-glucuronidase could be observed. 2.5×10^6 cells were incubated during 1.5 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. Means ± SEM (n=5).

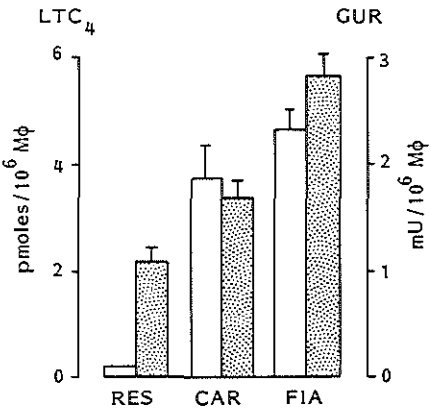


Figure 2. Basal secretory release of leukotriene C_4 (LTC_4, open bars) and β-glucuronidase (GUR, dotted bars) from resident (RES) and carrageenan (CAR) or Freund's incomplete adjuvant (FIA) elicited peritoneal macrophages. Note the positive relation between LTC_4 and GUR release. 10×10^6 cells/10 ml were incubated for 15 minutes at 37°C in the presence of gluthatione (2 mM), arachidonic acid (10 μg) and serine (0.25 mM). Means ± SEM (n=4).

important indicator of cell activity[225].

With the experiments described in the following sections of this chapter an attempt was made to correlate eicosanoid release with macrophage activity, for which lysosomal enzyme release was taken as a criterion.

2 INDEPENDENT REGULATION OF CYCLOOXYGENASE AND LIPOXYGENASE ACTIVITY

Three different populations of rat peritoneal macrophages were obtained by peritoneal lavage: resident cells without prior intraperitoneal stimulation and elicited cells on the fourth day after an intraperitoneal injection of carrageenan or Freund's incomplete adjuvant (vide Appendix, Materials and Methods for a detailed description of the experimental conditions). As can be seen in figure 1 an inverse relation could be observed between the release of the lysosomal enzyme β-glucuronidase and the production of the cyclooxygenase products prostaglandin E_2 and thromboxane A_2, measured as thromboxane B_2. Whilst enzyme activity (i.e. cell activity) was more pronounced in elicited macrophages, the reverse was observed with prostaglandin E_2 and thromboxane A_2. Leukotriene release on the other hand was found to be positively correlated with enzyme release (figure 2). No detectable levels of leukotriene C_4 were measured in the supernatant of resident cells, whereas carrageenan and Freund's incomplete adjuvant elicited macrophages released 3.85 and 4.55 pmoles leukotriene C_4 respectively. Leukotriene B_4, D_4 or E_4 could not be detected.

It has been suggested that a decreased availability of substrate arachidonic acid is the factor responsible for the reduced capacity of elicited macrophages to secrete eicosanoids[231], due, for example, to a decrease in the activity of phospholipase A_2[78]. However, the results obtained in the present experiments are in contradiction with these findings. Whereas the release of cyclooxygenase-derived products indeed was decreased, leukotriene C_4 release was enhanced. A possible explanation for this difference could be the postulated

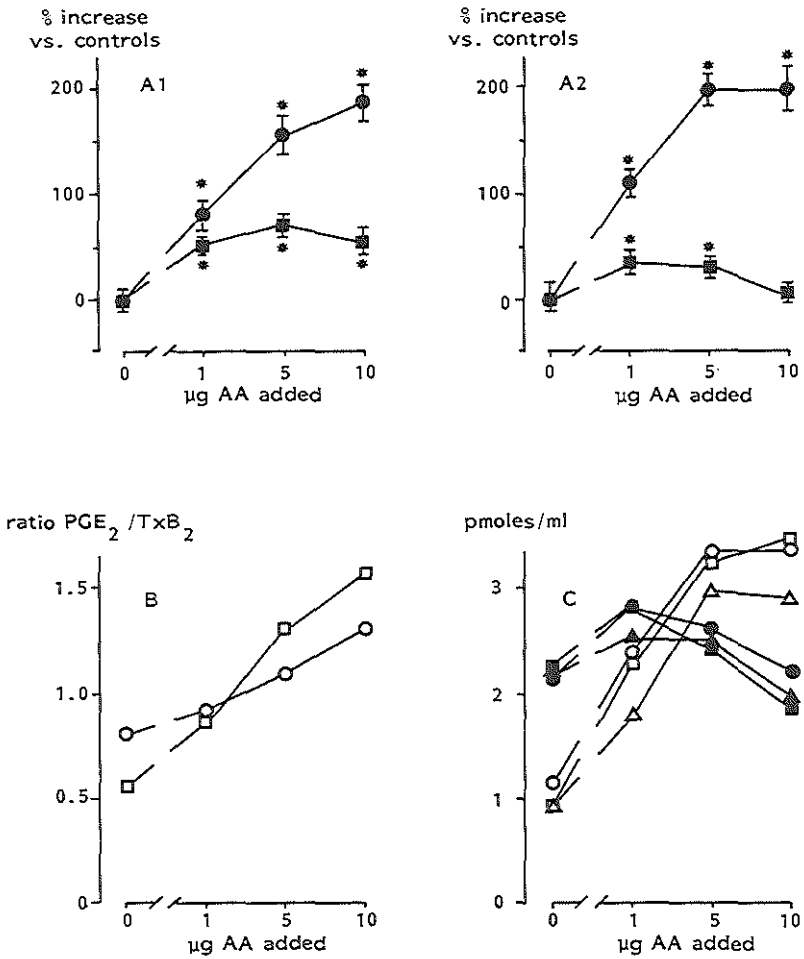


Figure 3. Effects of arachidonic acid (AA) on the production of prostaglandin E_2 (PGE_2, ●) and thromboxane B_2 (TxB_2, ■) from carrageenan elicited macrophages (panel A1) or Freund's incomplete adjuvant elicited macrophages (panel A2). TxB_2 production was saturated at much lower concentrations of AA than was PGE_2

existence of at least two phospholipase A_2 pools in macrophages[265]. Since 5-lipoxygenase, the initial enzyme in the lipoxygenase pathway, is a soluble enzyme and thus presumably resides in the cytosol, whereas cyclooxygenase is membrane bound, it is very likely that these enzymes use arachidonic acid from different phospholipid sources[210]. These findings add further support to the hypothesis that prostaglandin and leukotriene synthesis are independently regulated.

3 PREFERENTIAL PROSTAGLANDIN E_2 SYNTHESIS IN RESPONSE TO ARACEIDONIC ACID STIMULATION

As could be seen in figure 1, elicited macrophages have a reduced capacity to produce cyclooxygenase products. In addition to this decreased activity, also a shift within cyclooxygenase activity could also be observed. Whereas resident macrophages produced as much prostaglandin E_2 as thromboxane A_2, elicited macrophages produced less. In general the lower the production of prostaglandin E_2, the more active the cell, as measured by lysosomal enzyme release, and the greater, relative to prostaglandin E_2, the formation of thromboxane A_2. However, exposing the macrophages to the precursor arachidonic acid completely reversed this situation. As can be seen in figure 3, panel A, a dose dependent increase of prostaglandin E_2 production could be observed in the presence of archidonic acid, whereas thromboxane A_2 production was saturated at

production. Panel B: ratio between PGE_2 and TxB_2 as was found with carrageenan (○) or with Freund's incomplete adjuvant (□) elicited cells. Panel C: production of PGE_2 (open symbols) and TxB_2 (closed symbols) by Freund's incomplete adjuvant elicited cells; macrophages were incubated with a cell number of 2.5x (○), 5x (□) or $10x10^6$ (△) cells/ml. Note that the production of both metabolites was independent on the number of cells. Experimental conditions as described in the legend to figure 1. Means ± SEM, n=5. * $P<0.01$ (Student's t-test, two-tailed).

low concentrations of the fatty acid. Furthermore, calculating the ratio between the two cyclooxygenase products after exposure to archidonate indicated that prostaglandin E_2 was the major metabolite (figure 3, panel B). Some slight differences were observed between the two populations of elicited macrophages which were used in this study. Firstly, in carrageenan elicited macrophages (figure 3, panel A1) prostaglandin E_2 production could not be saturated, in Freund's incomplete adjuvant elicited cells it could (figure 3, panel A2). Secondly, whereas thromboxane A_2 production in carrageenan elicited macrophages was maximal with 1 μg arachidonic acid and could not be increased further, in Freund's incomplete adjuvant elicited cells thromboxane A_2 release actually decreased. Thirdly, the shift from thromboxane A_2 to prostaglandin E_2 was more pronounced in Freund's incomplete adjuvant elicited macrophages. These data were extended by recent observations in our laboratory, showing that resident macrophages responded to arachidonic acid in a comparable way[82]. Both prostaglandin E_2 and thromboxane B_2 were increased whereby thromboxane A_2 release was saturated with 5 μg arachidonate. However, the amounts of prostaglandin E_2 released never exceeded those of thromboxane B_2.

In conclusion, these results indicate that during *in vivo* activation cyclooxygenase activity is changed, whereby prostaglandin E_2 formation is preferentially inhibited. The more active the cell, the lower the production of prostaglandin E_2, both abolute and relative to other cyclooxygenase metabolites. Restoring the decreased availability of substrate by exposing the cells *in vitro* to arachidonic acid yields the converse pattern. Both elicited and resident macrophages react with a preferential increase of prostaglandin E_2 formation. Interestingly this increase was found to be more pronounced in active macrophages, indicating that prostaglandin E_2 is the major cyclooxygenase metabolite upon activation of these cells. As will be discussed later in this thesis prostaglandin E_2 plays a dominant role in the regulation of macrophage functions.

An interesting aspect of archidonate induced prostaglandin release was observed with cell suspensions containing different cell numbers. In contrast to what was expected, the production of cyclooxygenase metabolites was completely independent of the number of macrophages present in the suspension. In figure 3, panel C a graphical presentation is offered of the results obtained

with Freund's incomplete adjuvant elicited macrophages. Although absolute values differed (Freund's incomplete adjuvant elicited cells consistently produced significantly less cyclooxygenase metabolites) similar changes were observed with carrageenan elicited macrophages (data not shown). Evidently the amount of the metabolites produced was dependent on two factors: 1- the concentration of the precursor fatty acid and 2- the concentration of the metabolite produced. At a given concentration of 5 μg arachidonic acid/ml, 2.5, 5 and 10.10^6 cells/ml all released prostaglandin E_2 up to a final concentration of ± 3 pmoles/ml and thromboxane to a concentration of ± 2.5 pmoles/ml. These values were found to be dependent on cell activity, since the less active carrageenan elicited macrophages released substantially more (± 11 pmoles/ml and ± 9 pmoles/ml respectively).

It is known that elicited macrophages display, in terms of adenylate cyclase stimulation and subsequent rise of cyclic AMP, a high responsiveness to prostaglandin E_2. Resident cells with an optimally functioning cyclooxygenase system were rather unresponsive[30]. It was also reported that the reduced capacity of the cyclooxygenase pathway in elicited macrophages was accompanied by low intracellular cyclic AMP levels[78]. Furthermore it has been shown that prostaglandin E_2 inhibited the release of other cyclooxygenase metabolites from elicited macrophages[80]. In view of these facts and because prostaglandin E_2 is the major cyclooxygenase metabolite of elicited macrophages, it is possible that, via activation of adenylate cyclase, prostaglandin E_2 was responsible for the effects described in the previous paragraph.

The results discussed in the previous paragraph were obtained with rat peritoneal macrophages. It should be noted that although prostaglandin E_2 is the major cyclooxygenase metabolite in rat macrophages, due to species differences this finding cannot be extrapolated to human cells. In fact, previous investigations in our laboratory revealed that human peritoneal macrophages are more sensitive to prostacyclin than to prostaglandin E_2[30]. These macrophages had been collected from the dialysis fluid of renal patients on continuous ambulatory dialysis at a time when no complications were present. However, during an intercurrent infectious peritoneal inflammation the cells displayed a marked increase in reactivity towards prostaglandin stimulation with was more pronounced with prostaglandin E_2[7]. The original difference[30] between the responsiveness of the cells to the prostaglandins was abolished. Whereas

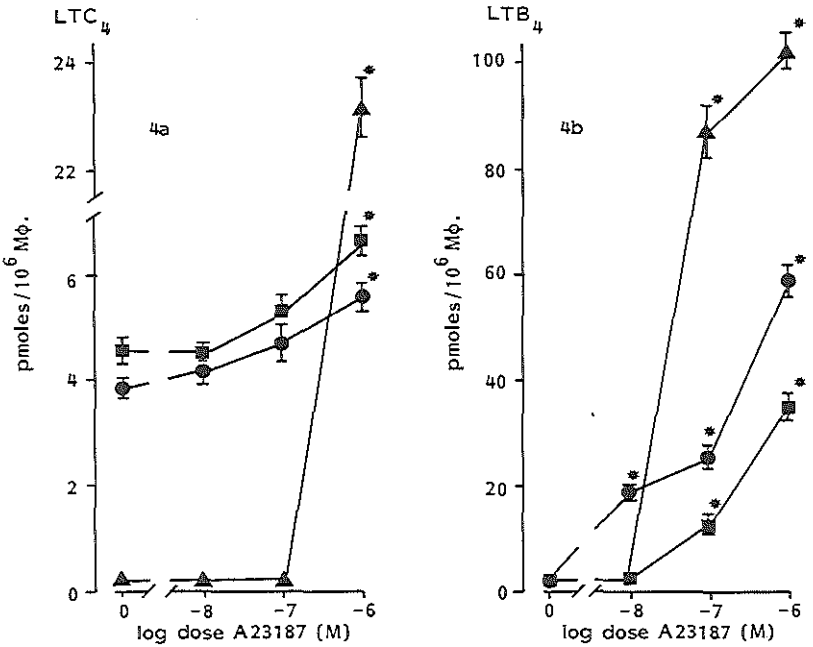


Figure 4. Release of leukotriene C_4 (LTC_4, panel A) or leukotriene B_4 (LTB_4, panel B) from resident (▲), carrageenan elicited (●) or Freund's incomplete adjuvant elicited (■) macrophages after exposure of the cells to the Ca-ionophore A23187. Although the basal leukotriene production was enhanced in elicited macrophages, resident cells responded far better to stimulation with A23187. In all cell populations leukotriene B_4 production could only be measured in the presence of the ionophore. Experimental procedure as described in the legend to figure 2. Means ± SEM (n=4). * $P<0.01$ (Student's t-test, two tailed).

these results indicate that also in human cells during inflammation prostaglandin E_2 is a major cyclooxygenase metabolite, the reason for the discrepancy between the responsiveness of inflammatory versus non-inflammatory cells to the different prostaglandins remains puzzling.

4 LEUKOTRIENE C_4 IS THE MAJOR LIPOXYGENASE METABOLITE AFTER *IN VIVO* STIMULATION, LEUKOTRIENE B_4 AFTER *IN VITRO* STIMULATION

Figure 4 depicts the release of leukotriene C_4 (panel A) and leukotriene B_4 (panel B) after stimulation of the three cell populations studied with the Ca-ionophore A23187. In general, all populations responded to the ionophore with an enhanced release of both leukotriene C_4 and leukotriene B_4. More specifically several points are of interest. Resident macrophages do not produce leukotriene C_4 unless stimulated with 10^{-6} M A23187. Elicited macrophages did release leukotriene C_4 under basal conditions whereby a positive correlation with cell activity was established. However, compared with resident macrophages these cells responded poorly to stimulation. Increases, due to A23187, were only significant at 10^{-6} M ionophore, and in this respect these cells resembled resident macrophages, but at this concentration of ionophore resident cells released about four times as much leukotriene C_4. Although leukotriene B_4 was not produced under basal conditions, this leukotriene is evidently the major metabolite after stimulation. Leukotriene B_4 levels in the presence of 10^{-6} M A23187 exceeded those of leukotriene C_4 4.4-10.6 fold. Again elicited macrophages showed a reduced capacity to secrete leukotriene B_4 as compared to resident cells.

The conclusions which can be drawn from these experiments are twofold: 1- leukotriene B_4 is the major lipoxygenase metabolite released from macrophages and 2- as with cyclooxygenase activity, the lipoxygenase pathway appears to be depressed in elicited cells. Although these conclusions seem evident, caution must be taken for the following reasons. Most other workers, who measured

macrophage leukotriene production, used mouse or human cells. These works reported a concomittant release of leukotriene B_4 and leukotriene C_4 in response to the Ca-ionophore[74,193]. When different stimuli were used, e.g. γ-hexachlorocyclohexane or IgE, a preferential synthesis of leukotriene C_4 and leukotriene B_4 respectively was observed[170,266]. Again, the existence of different arachidonic acid pools might offer a valid explanation for these variations. As yet, the simultaneous release of leukotriene C_4 and leukotriene B_4 from resident rat peritoneal macrophages has not been reported. Earlier studies showed that resident rat macrophages released leukotriene B_4, whereas elicited cells released leukotriene C_4, both in response to ionophore stimulation[10,70]. More recently the release of leukotriene C_4 from resident peritoneal macrophages was reported[206]. However, in this study the cells were harvested after an intraperitoneal lavage with saline. As was shown in our laboratory saline instantly activates macrophages, possibly via modification of the Na^+/K^+ ATPase[81]. A second study from our laboratory reported that saline elicited macrophages responded to A23187 stimulation with the release of both peptidoleukotrienes and leukotriene B_4[267]. Neither in human, mouse nor in rat macrophages basal release of leukotrienes has been studied. The present study establishes, for the first time, basal leukotriene C_4 release. Furthermore these levels were positively correlated with cell activity, indicating that leukotriene C_4 is the major lipoxygenase metabolite produced after *in vivo* stimulation. In the light of other results, discussed in different parts of this thesis, the enhanced basal release of leukotriene C_4 from elicited macrophages is of particular interest. As will be discussed in chapter 5 this peptidoleukotriene is considered to be an important mediator in cellular activation.

The general reduction in lipoxygenase activity observed in elicited macrophages, is possibly due to an effect of A23187 on cyclic AMP. It has been shown that elicited rat peritoneal macrophages respond to A23187 with an increase of the intracellular cylic AMP level, which was prostaglandin mediated[162]. Taken together with the high responsiveness of elicited macrophages to prostaglandin E_2[30], this interaction may represent a mechanism by which macrophage activity, in terms of of lipoxygenase metabolite production is regulated.

5 SUMMARY AND CONCLUSIONS

The respective capacities of rat peritoneal macrophages to metabolise arachidonic acid into prostaglandins and leukotrienes were compared. For this purpose three different cell populations were used, resident cells and carrageenan or Freund's incomplete adjuvant elicited macrophages. An inverse relation between lysosomal enzyme release, a non-specific indicator of cell activity, and cyclooxygenase derived products could be observed. Whereas Freund's incomplete adjuvant elicited cells released more β-glucuronidase than carrageenan elicited and resident cells, the reverse was observed with respect to prostaglandin E_2 and thromboxane A_2 production. Furthermore, it was shown that upon *in vivo* activation prostaglandin E_2 was preferentially inhibited. On the other hand, resident cells did not release leukotriene C_4 but elicited macrophages did. Thus leukotriene C_4 release was positively correlated to cell activity, being indicative of an important role of leukotriene C_4 in cell activation. In conclusion: ***in vivo* activation of macrophages is accompanied by increased lysosomal enzyme and leukotriene C_4 release and decreased production of cyclooxygenase metabolites, whereby prostaglandin E_2 is preferentially inhibited.**

Restoration of the decreased precursor availability by exposing elicited macrophages to various concentrations of arachidonic acid resulted in an increased release of cyclooxygenase metabolites. Varying the number of cells in the suspension had no effect on the maximal release. Apparently prostaglandins were produced up to a fixed concentration, which was found to be dependent on cell activity. The less active carrageenan elicited macrophages produced three times as much cyclooxygenase metabolites compared with the more active Freund's incomplete adjuvant elicited cells. Furthermore, exposure of the cells to arachidonate resulted in a preferential synthesis of prostaglandin E_2, which was more pronounced in the more active cells. In conclusion: ***in vitro* stimulation of macrophages with arachidonic acid leads to a preferential production of prostaglandin E_2 to a fixed maximal concentration which is dependent on cell activity.**

Finally, it was shown that elicited macrophages, despite high basal

secretory levels of leukotriene C_4, have a reduced lipoxygenase activity. Compared to resident macrophages these cells poorly responded to the Ca-ionophore A23187. The amount of leukotriene C_4 produced by resident macrophages upon maximal stimulation was at least 3.5 times as much as that produced by elicited cells. Furthermore, in response to A23187 leukotriene B_4 was the lipoxygenase metabolite which was predominantly produced, both in resident and elicited cells. No leukotriene D_4 or leukotriene E_4 could be detected. In conclusion: *in vitro* stimulation of macrophages with A23187 leads to production of leukotriene B_4 and leukotriene C_4, leukotriene B_4 being the major metabolite; despite high basal leukotriene C_4 production elicited macrophages have a reduced lipoxygenase activity.

CHAPTER 5. EFFECTS OF EICOSANOIDS ON MACROPHAGE FUNCTIONS.

1 INTRODUCTION

Macrophages are not only a source of eicosanoids, but also a target for their actions. It is well known that two cyclooxygenase products, prostaglandin E_2 and prostacyclin, are powerful elevators of cyclic AMP in rat peritoneal macrophages. Of these two metabolites mentioned, prostaglandin E_2 was shown to be the major metabolite, at least in rat macrophages[30] (vide chapter 6.1). Elevation of cyclic AMP is associated with an inhibition of several functions of macrophages. Thus, inhibitory actions of prostaglandin E_2 have been observed on chemiluminescence[197], phagocytosis and cell motility[191], Ia-antigen expression[156,240], interleukin-1 production[42], cytotoxicity[176,246], plasminogen activator release[226] and complement production[159]. In contrast, prostaglandin E_2 induces collagenase production by macrophages[257]. Although a substantial number of reports has been published on the effects of lipoxygenase products on a variety of tissues and cells (vide chapter 2, table 1), there is a scarcity of data on the actions of these metabolites on macrophages. It has been reported that leukotriene C_4 induces a respiratory burst[121] and that 5-hydroxyeicosatetraenoic acid enhances Ia-antigen expression[240]. Both factors are markers of enhanced cell activity. It was further demonstrated that leukotriene C_4 and leukotriene D_4 augmented the release of cyclooxygenase metabolites from macrophages[88,89]. These latter observations are of particular interest since the possibility was raised that macrophage activity is regulated via interaction between lipoxygenase and cyclooxygenase derived products.

With the experiments described in this chapter the interaction between leukotriene C_4 and prostaglandin E_2 in modulating macrophage activity was investigated, using lysosomal enzyme release as a marker of cell activity.

Table 1. Effects of leukotriene C_4 on resident macrophages.

LTC_4 (M)	0	10^{-9}	10^{-8}	10^{-7}	10^{-6}
PGE_2 (pmoles/ 10^6 cells)	4.19 ± 0.42	n.d.	4.73 ± 1.10	3.38 ± 1.08	25.38* ± 2.28
TxB_2 (pmoles/ 10^6 cells)	4.35 ± 0.38	3.95 ± 0.21	4.83 ± 0.21	6.52* ± 0.38	9.48* ± 0.51
GUR (mU/ 10^6 cells)	0.86 ± 0.03	1.00 ± 0.05	1.32* ± 0.05	1.63* ± 0.07	1.92* ± 0.09

Table 1. Effects of leukotriene C_4 (LTC_4) on the release of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2) and β-glucuronidase from resident macrophages. 2.5×10^6 cells were incubated during 1.5 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. Macrophages in suspension were exposed to LTC_4, the concentration varying from 10^{-9} to 10^{-6} M. Note that GUR release was augmented by a lower concentration of LTC_4 than that needed to stimulate PGE_2 and TxB_2 production. Means ± SEM (n=5). * P<0.01 vs. controls (Student's t-test, two tailed).

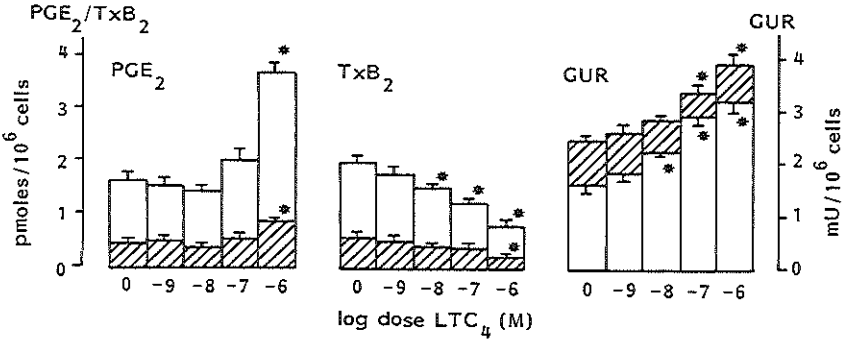


Figure 1. Effects of leukotriene C_4 (LTC_4) on the release of prostaglandin E_2, (PGE_2), thromboxane B_2 (TxB_2) and β-glucuronidase (GUR) from macrophages elicited with carrageenan (2 mg i.p., open bars) or with Freund's incomplete adjuvant (2 ml i.p., hatched bars). Whereas GUR release and PGE_2 production could be augmented by LTC_4, TxB_2 production was decreased. Experimental conditions as described in the legend to table 1. Means ± SEM (n=5). * P<0.01 (Student's t-test, two tailed).

2 LEUKOTRIENE C_4 INDUCES LYSOSOMAL ENZYME RELEASE AND PROSTAGLANDIN E_2 PRODUCTION

The exposure of resident macrophages to leukotriene C_4 resulted in the following alterations (table 1). At low ($<10^{-7}$ M) concentrations of leukotriene C_4 there were no changes in prostaglandin E_2 levels but in the presence of 10^{-6} M leukotriene C_4 a 6.1-fold increase was observed. In common with the effects of arachidonic acid on the release of cyclooxygenase metabolites a preferential increase of prostaglandin E_2 production compared to thromboxane A_2 was found in response to leukotriene C_4. Whereas basal levels of both metabolites were broadly the same, 10^{-6} M leukotriene C_4 only induced a 2.2-fold increase of thromboxane A_2 production. Lysosomal enzyme (β-glucuronidase) release was dose dependently enhanced. It is of particular interest that the observed β-glucuronidase release was augmented by a lower concentration of leukotriene C_4 than that needed to stimulate prostaglandin E_2 release.

Elicited macrophages showed a somewhat different response to leukotriene C_4 (figure 1). As was found with resident macrophages, prostaglandin E_2 and β-glucuronidase release could be augmented by leukotriene C_4. Again changes in lysosomal enzyme release were observed at lower concentrations of leukotriene C_4 than necessary to enhance prostaglandin E_2 production. However, in contrast to the increased prostaglandin E_2 production, thromboxane A_2 release markedly decreased. Although absolute values differed between the two elicited cell populations (carrageenan elicited macrophages consistently produced more cyclooxygenase metabolites and less β-glucuronidase) similar directional changes in response to leukotriene C_4 were observed.

A preferential increase in prostaglandin E_2 production from elicited macrophages under the influence of leukotriene C_4 has been shown earlier by Feuerstein et al.[89]. In contrast to this report, the present study showed that the increased prostaglandin E_2 production was associated with a decreased production of thromboxane A_2. The observed directional changes between the two cyclooxygenase products remains a question to be solved. This event was highly

reproducible, in addition a similar effect was described with respect to the leukotriene induced release of cyclooxygenase products from the guinea pig trachea[60]. A differential effect of leukotriene C_4 on thromboxane synthetase and prostaglandin E isomerase cannot be excluded. Also, the existence of at least two different pools of arachidonic acid, which can be activated by leukotriene C_4 as was shown in our laboratory could be responsible for the observed difference[274]. Re-evaluating the data from Feuerstein's report disclosed some remarkable differences between this study and the results presented here. Firstly, the concentration of leukotriene C_4 used in Feuerstein's experiments to induce thromboxane A_2 synthesis from macrophages in suspension was higher than 10^{-6} M. At 10^{-6} M or less, no effect or an actual inhibition ot thromboxane A_2 release was observed. Secondly, the effects of leukotriene C_4 on the production of cyclooxygenase metabolites were found to be most prominent in macrophages in monolayer. The macrophages were allowed to adhere for 24 hours, without addition of the *in vivo* eliciting agent. It has been shown that removal of the stimulus unblocks the cyclooxygenase and allows prostaglandin E_2 synthesis, hence deactivation occurs and a cell with properties of a resident macrophage remains[192]. The results obtained by Feuerstein and coworkers with macrophages in monolayer are in agreement with the results obtained with resident macrophages in the present study.

In the present experiments it was shown that leukotriene C_4 enhances lysosomal enzyme release, which supports the idea that lipoxygenase products of arachidonic acid are crucially involved in macrophage activation. Circumstancial evidence for a role of lipoxygenase products can be found in the effects of lipoxygenase inhibitors on macrophage activity. It has been shown that lipoxygenase inhibitors reduce: Ia-antigen expression and macrophage mediated granuloma formation[156], chemiluminescence[164,197], interleukin-1 release[69] and endotoxin induced thromboplastin production[58]. The fact that the concentration of leukotriene C_4 necessary to increase prostaglandin E_2 production by macrophages was higher than the one necessary to augment lysosomal enzyme release, combined with the observations that prostaglandin E_2 depresses macrophage activity, raised the possibility that macrophage activity is regulated via an interaction between lipoxygenase and cyclooxygenase derived products. Leukotriene C_4 being stimulatory and prostaglandin E_2 suppressive, the balance between these two products would determine the degree of macrophage

activity and represent a mechanism by which arachidonic acid oxygenation products can control inflammatory/immune reactions. The following sections of this chapter present the results of experiments which were aimed at unravelling this interaction. In these experiments the effects of cyclooxygenase inhibitors and prostaglandin E_2 on the leukotriene C_4 induced β-glucuronidase release were investigated.

3 CYCLOOXYGENASE INHIBITORS MODULATE THE LEUKOTRIENE C_4 INDUCED LYSOSOMAL ENZYME RELEASE

The effects of four cyclooxygenase inhibitors, indomethacin, aspirin, piroxicam and Org 7258 on the release response of carrageenan elicited macrophages were studied. In the absence of leukotriene C_4 all cyclooxygenase inhibitors effectively blocked the release of cyclooxygenase metabolites without affecting lysosomal enzyme release (table 2). The relative order of potency was: Org 7258 > indomethacin > piroxicam > aspirin. Differential effects of the four cyclooxygenase inhibitors could be observed in the presence of leukotriene C_4.

Indomethacin

Figure 2a shows that exposure of the cells to 10^{-8} M leukotriene C_4 resulted in enhanced prostaglandin E_2 production, decreased thromboxane A_2 production and increased release of β-glucuronidase. These effects were more pronounced in the presence of 10^{-6} M leukotriene C_4 (figure 2b). In both cases the leukotriene C_4 induced β-glucuronidase release was dose dependently augmented by the addition of indomethacin whereas the enhanced prostaglandin E_2 production was blocked and the already diminished thromboxane A_2 production was further declined.

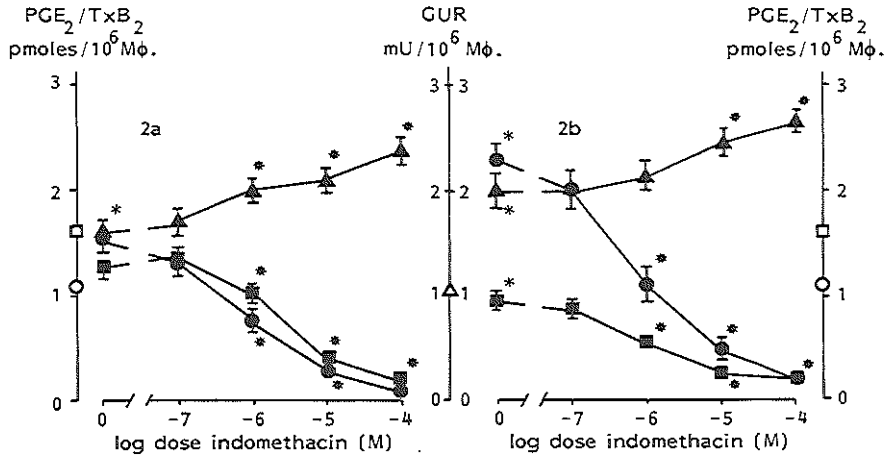
Aspirin

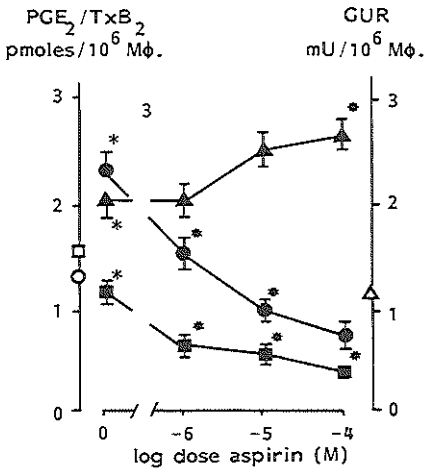
Aspirin was found to be slightly less potent than indomethacin in augmenting the leukotriene C_4 induced lysosomal enzyme release and in blocking the biosynthesis of endogenous prostaglandins (figure 3).

Table 2. Effects of cyclooxygenase inhibitors on the release of PGE_2, TxB_2 and GUR from carrageenan elicited macrophages.

treatment	PGE_2 pmoles/10^6 Mo.	TxB_2 pmoles/10^6 Mo.	GUR mU/10^6 Mo.
controls	1.10 ± 0.10	1.58 ± 0.04	1.06 ± 0.09
+ 10^{-7} M indom.	1.16 ± 0.08	1.09 ± 0.09	0.94 ± 0.07
+ 10^{-6} M indom.	0.62 ± 0.10	0.79 ± 0.05	1.16 ± 0.05
+ 10^{-5} M indom.	0.22 ± 0.02	0.29 ± 0.01	1.09 ± 0.11
+ 10^{-4} M indom.	0.14 ± 0.02	0.25 ± 0.02	1.27 ± 0.06
controls	1.31 ± 0.06	1.52 ± 0.10	1.11 ± 0.08
+ 10^{-6} M aspir.	1.32 ± 0.09	1.52 ± 0.10	1.39 ± 0.16
+ 10^{-5} M aspir.	1.03 ± 0.03	0.64 ± 0.03	1.29 ± 0.13
+ 10^{-4} M aspir.	0.71 ± 0.06	0.32 ± 0.04	1.43 ± 0.09
controls	1.93 ± 0.17	3.57 ± 0.12	0.74 ± 0.03
+ 10^{-8} M pirox.	2.08 ± 0.19	3.20 ± 0.10	0.61 ± 0.06
+ 10^{-7} M pirox.	2.29 ± 0.04	2.44 ± 0.22	0.71 ± 0.04
+ 10^{-6} M pirox.	1.05 ± 0.04	1.95 ± 0.05	0.68 ± 0.05
controls	1.53 ± 0.13	3.51 ± 0.12	0.64 ± 0.04
+ 10^{-8} M Org 7258	1.12 ± 0.09	1.65 ± 0.17	0.64 ± 0.06
+ 10^{-7} M Org 7258	0.41 ± 0.02	0.42 ± 0.02	0.76 ± 0.06
+ 10^{-6} M Org 7258	0.17 ± 0.04	0.39 ± 0.03	0.59 ± 0.04

Table 2. Effects of the cyclooxygenase inhibitors indomethacin, aspirin, piroxicam and Org 7258 on the release of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2) and β-glucuronidase from carrageenan elicited peritoneal macrophages. All cyclooxygenase inhibitors effectively reduced the production of PGE_2 and TxB_2, whereby no effects on GUR release could be observed. Experimental conditions as described in the legend to table 1. Means ± SEM (n=5).





Figures 2/3. Effects of indomethacin (figure 2) and aspirin (figure 3) on the leukotriene C_4 (LTC_4) induced release response. Figure 2A represents values found in the presence of 10^{-8} M LTC_4, figure 2B and figure 3 in the presence of 10^{-6} M LTC_4. Open symbols on the axes represent basal secretory levels (no indomethacin, aspirin or LTC_4 added) of prostaglandin E (PGE_2, ●), thromboxane B_2 (TxB_2, ■) and β-glucuronidase (GUR, ▲). Note that both indomethacin and aspirine augmented the LTC_4 induced release of GUR. Experimental conditions as described in the legend to table 1. n=5. * $P<0.01$ vs. basal secretory levels, * $P<0.01$ vs. LTC_4 induced secretory levels (Student's t-test, two tailed).

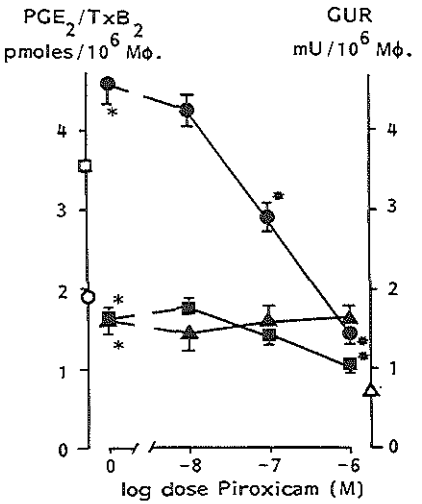


Figure 4. Effects of piroxicam on the leukotriene C_4 (10^{-6} M) induced release of prostaglandin E_2 (PGE_2, ●), thromboxane B_2 (TxB_2, ■), and β-glucuronidase (GUR, ▲) from carrageenan elicited macrophages. Open symbols on the axes represent basal secretory levels (no LTC_4 added). In contrast with indomethacin or aspirin piroxicam did not affect GUR release. Experimental conditions as described in the legend to table 1. Means ± SEM (n=5). * P<0.01 vs. basal secretory levels, * P<0.01 vs. LTC_4 induced secretory levels (Student's t-test, two tailed).

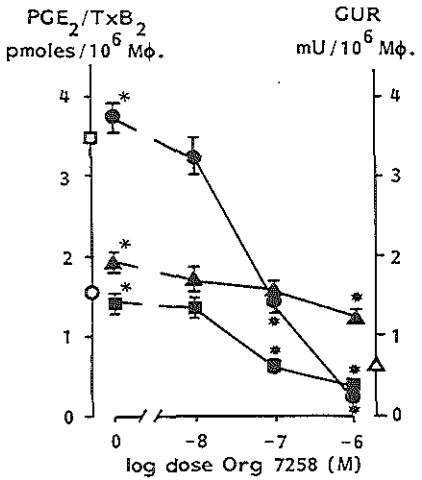


Figure 5. Effects of Org 7258 on the leukotriene C_4 (10^{-6} M) induced release of prostaglandin E_2 (PGE_2, ●), thromboxane B_2 (TxB_2, ■) and β-glucuronidase (GUR, ▲) from carrageenan elicited macrophages. Open symbols on the axes represent levels in the absence of LTC_4 or Org 7258. Note that in contrast to indomethacin, aspirin and piroxicam (figures 2, 3 and 4) Org 7258 inhibits the LTC_4 induced GUR release. * P<0.01 vs. basal secretory levels, * P<0.01 vs. LTC_4 induced release (Student's t-test, two tailed). Means ± SEM (n=5).

Piroxicam

Concerning prostaglandin E_2 and thromboxane A_2 production similar results as those found with indomethacin and aspirin were observed with piroxicam. With respect to β-glucuronidase release, in contrast to indomethacin or aspirin, piroxicam did not augment the leukotriene C_4 induced release (figure 4). Whether the observed difference is due to a different mechanism of action could not be established. Increasing the dose beyond 10^{-6} M caused damage to the membranes of the macrophages, as could be observed by an enhanced release of the cytoplasmic enzyme lactate dehydrogenase.

Org 7258

A marked difference between the actions of indomethacin or aspirin on the one side and Org 7258 on the other hand could be observed with respect to lysosomal enzyme release. Whereas indomethacin and aspirin augmented the leukotriene C_4 induced release, it was inhibited by Org 7258. Not only the leukotriene C_4 induced β-glucuronidase release could be reversed by Org 7258 but it also restored the increased prostaglandin E_2 production (figure 5).

The fact that indomethacin and aspirin enhanced the leukotriene C_4 induced lysosomal enzyme release provides further proof for the hypothesis that endogenous prostaglandins may function as inhibitory feed-back regulators of macrophage activation. With respect to the effects of cyclooxygenase inhibitors on macrophage activity several reports have been published. Two earlier reports showed that indomethacin did not affect or decrease the zymosan induced release of lysosomal enzymes[22,169]. In these studies however, concentrations of indomethacin were used of 10^{-3}-10^{-4} M. Next to inhibiting cyclooxygenase these concentrations are now known to have two other effects: inhibition of lipoxygenase and inhibition of phosphodiesterase. The stimulus used in these studies (zymosan) is known to induce leukotriene release from macrophages and it has been suggested that the zymosan induced lysosomal enzyme release was leukotriene mediated[25]. Therefore inhibition of lipoxygenase activity by high doses of indomethacin will lead to reduced lysosomal enzyme release. Also inhibition of phosphodiesterase has the same effect (vide chapter 6.4). In an elegant study performed by Schnyder and coworkers, the effect of indomethacin on plasminogen activator release was studied[226]. It was found that indomethacin enhanced the degree of activation obtained after zymosan phagocytosis. Addition

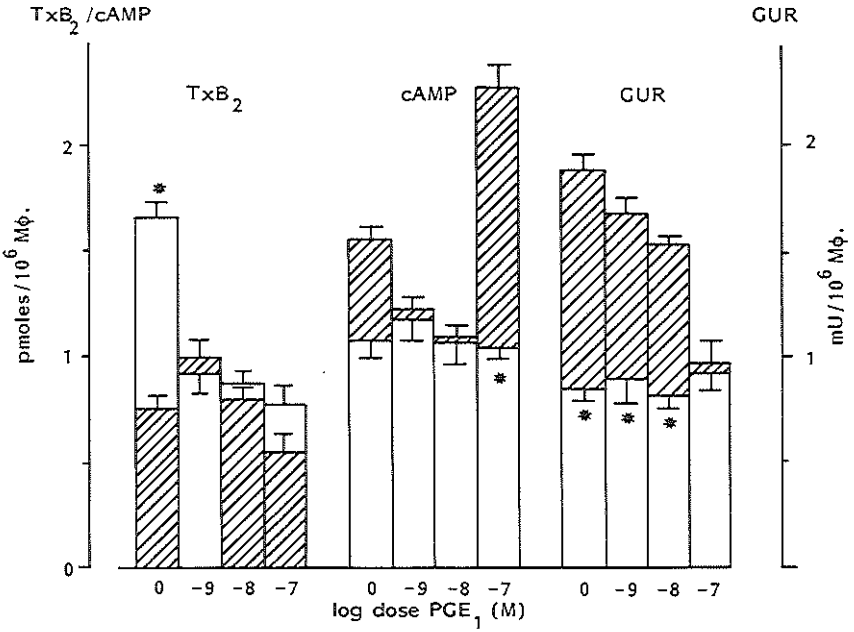


Figure 6. Modulation of the release of thromoboxane B_2 (TxB_2) and β-glucuronidase (GUR) and of intracellular cyclic AMP (cAMP) content of carrageenan elicited macrophages by leukotriene C_4 and prostaglandin E_1 (PGE_1). Open bars represent values found in the presence of prostaglandin E_1 alone, hatched bars in the presence of 10^{-6} M leukotriene C_4 and 10^{-9}-10^{-6} M prostaglandin E_1. Whereas, in the absence of leukotriene C_4, PGE_1 had no effects, in the presence of leukotriene C_4 the level of intracellular cAMP was enhanced and the leukotriene C_4 induced GUR release could be reversed. Both PGE_1 and LTC_4 caused a diminished TxB_2 production. Experimental conditions as described in the legend to table 1. * $P<0.01$ vs. values observed in the presence of leukotriene C_4 (Student's t-test, two tailed). Means ± SEM (n=5).

of exogenous prostaglandin E_2 on the other hand, had the opposite effect, it suppressed the enhanced plasminogen activator release induced either by indomethacin, zymosan or a combination of both. Futher evidence in favour of a regulatory role of endogenous prostaglandins was obtained from the following results. Cyclooxygenase inhibitors have been shown to enhance macrophage activity resulting in augmentation of: Ia-antigen expression[156], bactericidal activity and interleukin-1 secretion[42] and tumoricidal activity[43].

The results obtained in the present experiments underline a distinctive nature of Org 7258 relative to other non-steroidal anti-inflammatory drugs. Org 7258 blocked the leukotriene C_4 induced lysosomal enzyme release. Since this property was not shared by the other cyclooxygenase inhibitors studied, it is concluded that the blockade of lysosomal enzyme release is independent of the inhibition of cyclooxygenase. It has previously been reported that Org 7258 suppressed the zymosan induced secretion of the lysosomal enzyme β-glucuronidase from mouse peritoneal macrophages. This activity was paralleled by dinitrophenol, a well-known uncoupler of oxidative phosphorylation, indicating that this mechanism may be implicated in the effect of Org 7258 on lysosomal enzyme release. Although a number of studies have indicated that Org 7258 is a potent anti-inflammatory agent in several animal models, including the granuloma-thymus model, the carrageenan induced paw oedema model and the calcium-pyrophosphate induced pleurisy, the exact mechanism of action of the drug is not clear yet (Den Hollander, personal communication).

4 PROSTAGLANDIN E_1 AND PROSTAGLANDIN E_2 SUPPRESS THE LEUKOTRIENE C_4 INDUCED LYSOSOMAL ENZYME RELEASE

In a seperate set of experiments the effects of prostaglandin E_1 and prostaglandin E_2 on macrophage activity were tested. Prostaglandin E_1 was shown to have the following effects (figure 6). It should be noted that due to the addition of prostaglandin E_1, the measurement of prostaglandin E_2 was unreliable

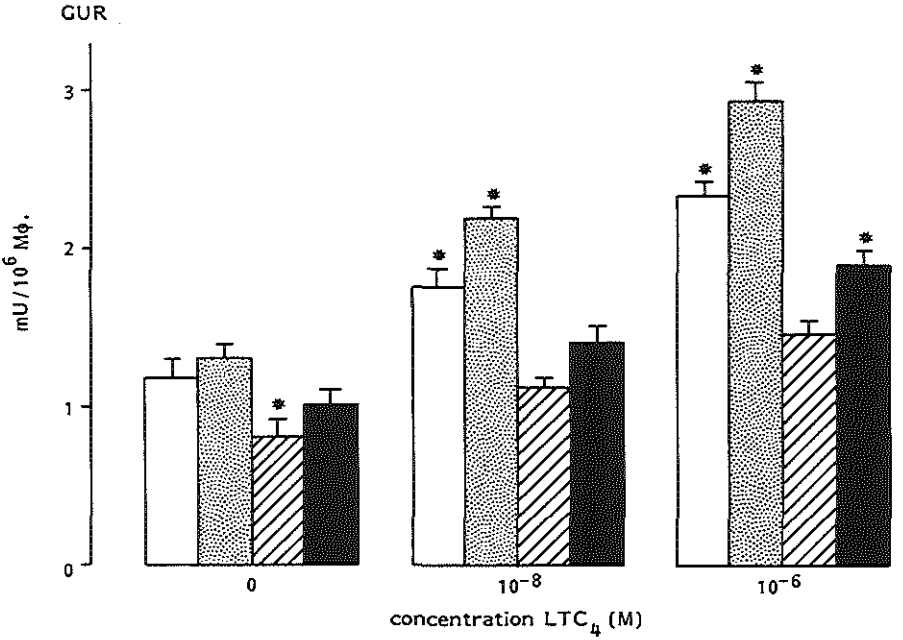


Figure 7. Effects of leukotriene C_4 (LTC_4), indomethacin and prostaglandin E_2 (PGE_2) on β-glucuronidase (GUR) release from carrageenan elicited macrophages. Open bars represent the values which were found in the absence (first panel) or presence of 10^{-8} M (second panel) or 10^{-6} M LTC_4 (third panel). Pretreatment of the cells with indomethacin (dotted bars) (10^{-5} M) resulted in an augmented GUR release. Addition of 10^{-7} M PGE_2 (hatched bars) prior to LTC_4 prevented the LTC_4 induced GUR release (second and third panel) or, in the absence of LTC_4 (first panel) diminished the release. This effect was blocked when the cells were pretreated with indomethacin (closed bars) before LTC_4 or PGE_2 were added. Experimental conditions as described in the legend to table 1. * $P<0.01$ vs. basal levels (no drugs added) (Student's t-test, two tailed). Means ± SEM (n=5).

because of cross-reactivity with the antibody used in the radioimmunoassay. Therefore no data on prostaglandin E_2 production are supplied with respect to this experiment. In the absence of leukotriene C_4 no effects of prostaglandin E_1 could be observed, except on thromboxane A_2 production. Already at the lowest concentration of prostaglandin E_1 tested, thromboxane A_2 production was inhibited, whereby no further decrease could be established by increasing the concentration of prostaglandin E_1. In the presence of 10^{-6} M leukotriene C_4, variations in all three parameters were observed. The leukotriene C_4 induced β-glucuronidase release was dose dependently reversed whereby the normal basal secretory level was restored. Thromboxane A_2 production, already inhibited by leukotriene C_4, was further depressed. Whereas prostaglandin E_1 by itself had no effect on intracellular cyclic AMP levels, in the presence of leukotriene C_4 this level was enhanced by addition of 10^{-7} M prostaglandin E_1.

Prostaglandin E_2 was tested in a single dose, which was known to elevate intracellular cyclic AMP in this system. The results of these experiments are shown in figure 7. In contrast with prostaglandin E_1, prostaglandin E_2 (10^{-7} M) reduced basal release of β-glucuronidase, an effect which was prevented when indomethacin (10^{-5} M) was added prior to prostaglandin E_2. A dose dependent increase of β-glucuronidase release was observed following addition of leukotriene C_4. Again this increase could be markedly enhanced by pretreating the cells with indomethacin. Prostaglandin E_2 completely blocked the enhanced release caused by leukotriene C_4, however in the presence of 10^{-6} M leukotriene C_4 and indomethacin this blockade was only partial.

The results of the present work demonstrate that prostaglandin E_1 suppressed both thromboxane A_2 synthesis and leukotriene C_4 induced lysosomal enzyme release. It was also shown that the latter effect was shared by prostaglandin E_2. Depressed thromboxane A_2 production, induced by prostaglandin E_2 has been described previously[78,90]. In these studies the effects of prostaglandin E_2 have been attributed to activation of adenylate cyclase activity. Since prostaglandin E_1 was shown to induce cyclic AMP generation, the present experiments seem to confirm this idea. However, as will be discussed in chapter 6, enhanced cylic AMP levels, induced either by β_2-adrenoceptor stimulation or by deactivation of phosphodiesterase have

different effects on thromboxane A_2 production. Stimulation of β_2-adrenoceptors was followed by a reversal of the inhibition of thromboxane A_2 production due to leukotriene C_4 whilst inactivation of phosphodiesterase resulted in only a modest inhibition of thromboxane A_2 production. In contrast, a decreased lysosomal enzyme release was induced by both treatments and was related to an increase in cyclic AMP levels. Whether the effects of prostaglandins E on thromboxane A_2 metabolism are due to a direct effect on thromboxane synthetase, to interference at a specific site rendering the cell unresponsive in terms of thromboxane A_2 synthesis or to activation of adenylate cyclase is not clear yet. Elucidation of the interactions between prostaglandin E_2, thromboxane A_2 and cyclic AMP will provide insight into the normal regulation of the release and effects of these metabolites and the mechanisms by which they modulate macrophage activity.

It has been postulated that endogenous prostaglandins do not play a major role in regulating lysosomal enzyme release from macrophages[169]. However, the fact that exogenous prostaglandin E_2 reduced the basal release, abolished the leukotriene C_4 induced release and counteracted the augmentation by indomethacin of the leukotriene C_4 induced release, strongly indicate that prostaglandins are involved in the regulation of lysosomal enzyme release. Furthermore they support the hypothesis that lipoxygenase- and cyclooxygenase-derived products interact in modulating macrophage activity. The fact that prostaglandin E_2 could not completely reverse the leukotriene C_4 induced β-glucuronidase release in the presence of indomethacin can be explained by the inability of leukotriene C_4 to induce prostaglandin E_2 production in this situation. In the absence of indomethacin leukotriene C_4 causes macrophages to produce prostaglandin E_2 which will act synergistic with the exogenously added prostaglandin E_2, resulting in a full reduction of leukotriene C_4 induced β-glucuronidase release.

5 PROSTAGLANDIN E_2 FAILS TO AFFECT LEUKOTRIENE SYNTHESIS

Recently, inhibition by prostaglandin E_2 of leukotriene B_4 release from activated neutrophils was reported[116]. In order to establish whether a similar relation exists in macrophages, the effect of prostaglandin E_2 on Ca-ionophore induced leukotriene production was studied. As can be seen in table 3, prostaglandin E_2 in a concentration of 10^{-7} M did not affect leukotriene B_4 nor leukotriene C_4 synthesis from Freund's incomplete adjuvant or carrageenan elicited cells with one exception. The A23187 (10^{-6} M) induced release of leukotriene C_4 from Freund's incomplete adjuvant elicited macrophages could be reduced by prostaglandin E_2. Whether this difference really is valid (the standard deviation of this point is extraordinarly small) is not clear yet. In

Table 3. Effects of prostaglandin E_2 on leukotriene production.

	A23187 (M)	0	10^{-8}	10^{-7}	10^{-6}
LTC_4	CAR.Mo.	3.85 ± 0.20	4.08 ± 0.37	4.62 ± 0.46	5.51 ± 0.42
	+ 10^{-7} M PGE_2	3.51 ± 0.31	4.32 ± 0.41	4.89 ± 0.52	5.39 ± 0.37
	FIA.Mo.	4.55 ± 0.33	4.43 ± 0.63	5.28 ± 0.43	6.55 ± 0.38
	+ 10^{-7} M PGE_2	4.35 ± 0.27	4.78 ± 0.29	5.43 ± 0.41	5.63 ± 0.09
LTB_4	CAR.Mo.	---	18.51 ± 1.47	24.80 ± 1.93	58.04 ± 4.17
	+ 10^{-7} M PGE_2	---	19.53 ± 2.01	22.84 ± 2.17	51.62 ± 6.38
	FIA.Mo.	---	---	13.04 ± 1.08	34.66 ± 2.75
	+ 10^{-7} M PGE_2	---	---	16.08 ± 2.16	32.50 ± 3.14

Table 3. Effects of prostaglandin E_2 on the (A23187-stimulated) release of leukotriene C_4 and B_4 from carrageenan (CAR) or Freund's incomplete adjuvant (FIA) elicited macrophages. No effects of prostaglandin E_2 on leukotriene release could be observed. $10x10^6$ cells in 10 ml were incubated for 15 minutes at 37° C in a humidified atmosphere containing 5% CO_2 in air, in the presence of 10 μg arachidonic acid and 2 mM glutathione. Means ± SEM (n=4). Leukotriene production is expressed as pmoles/10^6 cells.

order to clarify this matter further experiments will have to be carried out, including a dose response curve of prostaglandin E_2 in the absence or presence of cyclooxygenase inhibitors. Furthermore these experiments will have to be extended to resident macrophages. Possibly, the reduced lipoxygenase activity in elicited macrophages is to be held responsible for the lack of effect of prostaglandin E_2. However, we could observe no effect of prostaglandin E_2 on leukotriene production in a preliminary study using resident macrophages.

6 SUMMARY AND CONCLUSIONS

The effects of leukotriene C_4 and prostaglandin E_2 on macrophage activity were studied, for which lysosomal enzyme release was taken as a criterion. It was shown that leukotriene C_4 induces a release response consisting of enhanced prostaglandin E_2 and β-glucuronidase release in elicited and resident cells. Thromboxane A_2 production was increased in resident cells but inhibited in elicited macrophages. Furthermore it was shown that the concentration of leukotriene C_4 necessary to increase prostaglandin E_2 production was higher than the one necessary to augment lysosomal enzyme release. This observation, combined with observations that prostaglandin E_2 depresses diverse aspects of macrophage activity, raised the possibility that macrophage activity is regulated via interactions between lipoxygenase- and cyclooxygenase-derived products. In conclusion: *in vitro* activation of macrophages with a low dose of leukotriene C_4 leads to enhanced lysosomal enzyme release, higher doses result in a preferential production of prostaglandin E_2 as compared to thromboxane A_2, whereby thromboxane A_2 production was inhibited in elicited macrophages.

Inhibiting the synthesis of endogenous prostaglandins by preincubating the macrophages in the presence of cyclooxygenase inhibitors resulted in modulation of the leukotriene C_4 induced β-glucuronidase release. The relative order of potency, in terms of inhibiting cyclooxygenase activity was: Org 7258 > indomethacin > piroxicam > aspirin. Indomethacin and aspirin were shown to

augment leukotriene C_4 induced lysosomal enzyme release, piroxicam was ineffective and Org 7258 inhibited β-glucuronidase release. The observation that indomethacin and aspirin enhanced leukotriene C_4 induced lysosomal enzyme release provides further proof for the hypothesis that endogenous prostaglandins may function as inhibitory feedback regulators of macrophage activity. Since piroxicam was found to be toxic beyond a concentration of 10^{-6} M, it could not be established whether the ineffectivity of piroxicam was due to a different mode of action or to too low a concentration. In contrast with aspirin and indomethacin, the experimental drug Org 7258 inhibited lysosomal enzyme release induced by leukotriene C_4. The possibility was raised that this effect of Org 7258 was due to uncoupling of oxidative phosphorylation. In conclusion: **the cyclooxygenase inhibitors aspirin and indomethacin augment leukotriene C_4 induced lysosomal enzyme release, probably by blocking endogenous prostaglandin production; the experimental drug Org 7258 has the opposite effect.**

Exogenously added prostaglandin E_1 or E_2 could reverse the leukotriene C_4 induced β-glucuronidase release. Furthermore it was shown that prostaglandin E_2 reduced basal β-glucuronidase release and counteracted the augmentation by indomethacin of the leukotriene C_4 induced lysosomal enzyme release. These facts are strong indicators that prostaglandins are involved in regulating macrophage activity. Leukotriene C_4 being stimulatory and prostaglandin E_2 suppressive, the balance between these two products would determine the degree of macrophage activity and represent a mechanism by which eicosanoids can control inflammatory/immune reactions. In conclusion: **exogenous prostaglandin E_2 reverses lysosomal enzyme release induced by leukotriene C_4 and/or indomethacin.**

In view of the proposed interaction between leukotriene C_4 and prostaglandin E_2 in modulating macrophage activity, the effect of prostaglandin E_2 on the release of leukotrienes from macrophages was investigated. Prostaglandin E_2 did not affect leukotriene B_4 or leukotriene C_4 production by elicited macrophages. In conclusion: **prostaglandin E_2, in the dosage studied, has no effect on leukotriene synthesis by elicited macrophages.**

CHAPTER 6. INVOLVEMENT OF CYCLIC AMP IN THE REGULATION OF MACROPHAGE ACTIVITY.

1 INTRODUCTION

Extensive studies of leukocytes have documented the inhibitory role of cyclic AMP as an intracellular "second messenger", relaying a stimulus from the extracellular environment to biochemical machinery within the cell. The "first messenger" may be a chemical change in the cell's environment but is represented in more complex situations by neurotransmitters or hormones which activate adenylate cyclase[28,100]. With respect to macrophages, of these hormones prostaglandins are of particular interest. Elevation of intracellular cyclic AMP levels in response to prostaglandin E_2, associated with an inhibition of several macrophage functions, has been observed in a number of studies (vide chapter 3.1). Prostacyclin also stimulates the production of cyclic AMP in elicited peritoneal macrophages[24]. However, in contrast to prostaglandin E_2, prostacyclin failed to reduce the macrophage mediated component of a carrageenan induced granuloma[196]. At the time of the above studies, the reasons for the observed differences between prostaglandin E_2 and prostacyclin were poorly understood. More recent experiments performed in our laboratory revealed that in terms of adenylate cyclase activation rat peritoneal macrophages responded better to prostaglandin E_2 than to a stable synthetic analogue of prostacyclin[30]. It was also shown that low concentrations of prostaglandin E_2 inhibited the prostacyclin induced elevation of cyclic AMP[6], an effect which was mediated via displacement of prostacyclin from its receptor by prostaglandin E_2[189]. These findings suggested that the observed low responsiveness of rat granuloma or peritoneal macrophages to prostacyclin was due to permanent exposure of these cells to endogenous prostaglandin E_2. They also suggested that prostaglandin E_2 has a dominant role in the control of cyclic AMP in rat macrophages.

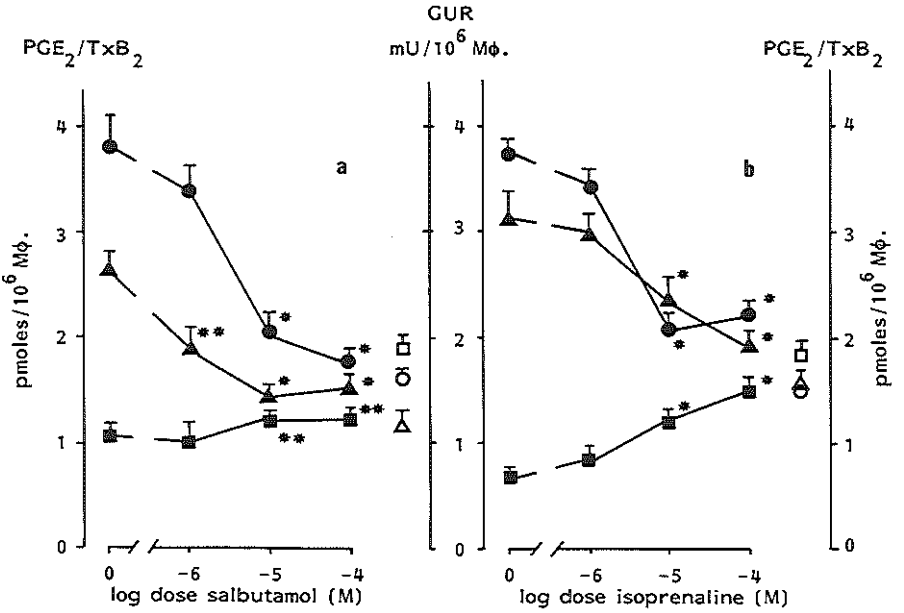


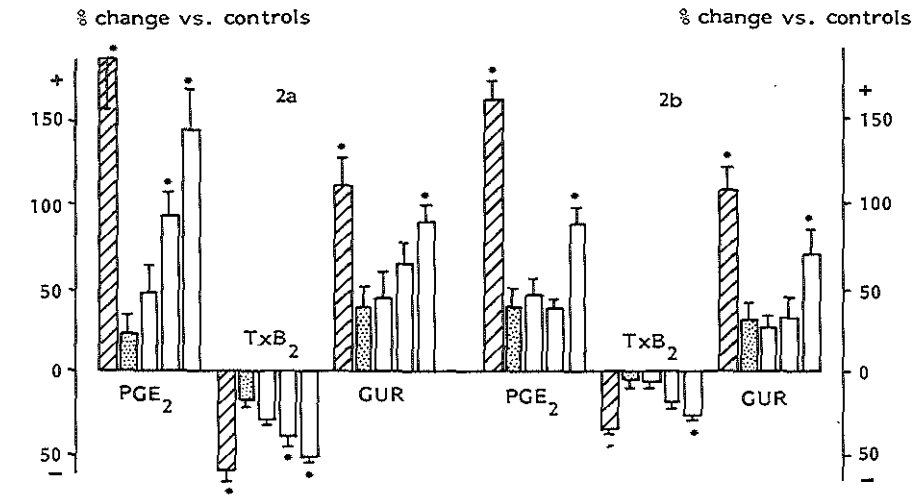
Figure 1. Beta-adrenoceptor agonists reverse the leukotriene C_4 induced release of prostaglandin E_2 (PGE_2, ●), thromboxane B_2 (TxB_2, ■) and β-glucuronidase (GUR, ▲) from carrageenan elicited macrophages. 2.5×10^6 cells/ml were incubated for 1.5 h at 37° C in a humidified atmosphere containing 5% CO_2 in air, in the presence of 10^{-6} M leukotriene C_4 (closed symbols). Open symbols represent the values as were found when the cells were not exposed to any drug. Addition of the β_2-selective adrenoceptor agonist salbutamol (panel 1^a) or the non-selective β-adrenoceptor agonist isoprenalin (panel 1^b) within the range of 10^{-6}-10^{-4} M effectively reversed the leukotriene C_4 induced release response. ** $P<0.02$, * $P<0.01$ (Student's t-test, two tailed). Means ± SEM (n=5).

The experiments described in this chapter were performed with carrageenan elicited macrophages. With respect to adenylate cyclase activity, marked differences exist between resident and elicited macrophages. Elicited macrophages have a depressed prostaglandin E_2 synthesising capacity and lowered intracellular cyclic AMP levels but an enhanced responsiveness of adenylate cyclase to exogenously added or endogenously produced prostaglandin E_2[30,80]. In light of the fact that leukotriene C_4 induces prostaglandin E_2 production, which was shown to counteract the effects of leukotriene C_4 on macrophage activity, combined with the knowledge that prostaglandin E_2 exerts its effects via stimulation of adenylate cyclase, the experiments described in this chapter were performed to investigate the involvement of cyclic AMP in the leukotriene C_4 induced release response.

2 β_2-ADRENOCEPTOR STIMULATION REVERSES THE LEUKOTRIENE C_4 INDUCED RELEASE RESPONSE

Activation of adenylate cyclase may be achieved through stimulation of a variety of adenylate cyclase-coupled receptors. Since β-adrenoceptor agonists are known to exert their effects through stimulation of adenylate cyclase, the present study was initiated to investigate the effects of the β-adrenoceptor agonists salbutamol and isoprenalin on the leukotriene C_4 induced release response. In the study the β-adrenoceptor antagonists sotalol, H3525 and practolol were also included.

Neither salbutamol, isoprenalin, practolol, H3525 or sotalol had any effect on the basal secretory parameters measured (data not shown). Addition of leukotriene C_4 (10^{-6} M) to the macrophages resulted in increased β-glucuronidase release, enhanced prostaglandin E_2 production and inhibition of thromboxane A_2 production. Both the selective β_2-adrenoceptor agonist salbutamol (figure 1B) and the non-selective β-adrenoceptor agonist isoprenalin (figure 1A), within the concentration range of 10^{-6}-10^{-4} M, dose-dependently reversed the leukotriene C_4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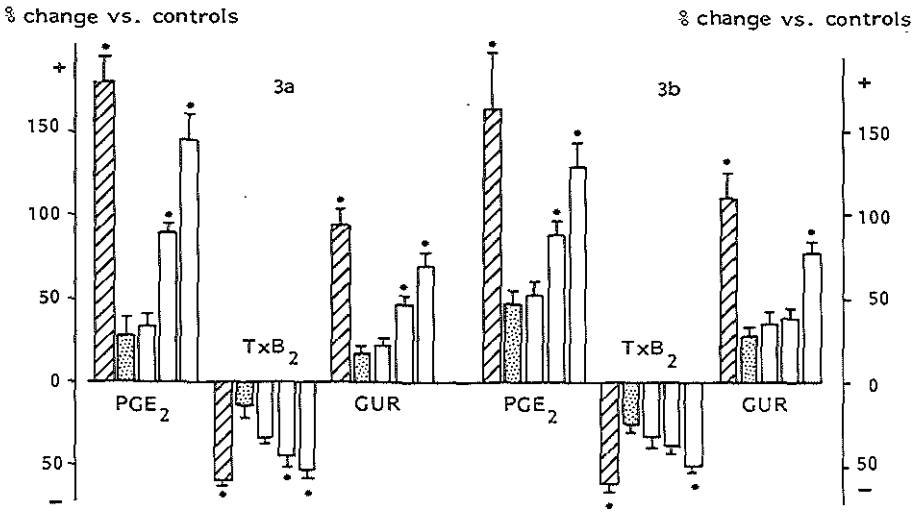
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Table 1. Effects of isoprenaline and practolol on the leukotriene C_4 induced release response from carrageenan elicited macrophages.

	PGE_2 pmoles/10^6 Mo.	TxB_2 pmoles/10^6 Mo.	GUR mU/10^6 Mo.
1. controls	1.54 ± 0.07	1.72 ± 0.13	1.32 ± 0.13
2. + LTC_4 (10^{-6} M)	3.54 ± 0.29	0.84 ± 0.07	2.92 ± 0.30
3. as 2 + isoprenaline (10^{-5} M)	1.89 ± 0.06	1.39 ± 0.09	1.81 ± 0.16
4. as 3 + practolol (10^{-6} M)	2.11 ± 0.11	1.31 ± 0.09	1.73 ± 0.12
5. as 3 + practolol (10^{-5} M)	2.03 ± 0.06	1.43 ± 0.10	1.69 ± 0.15
6. as 3 + practolol (10^{-4} M)	2.08 ± 0.09	1.46 ± 0.12	1.80 ± 0.16

Table 1. Effects of the β_1-selective adrenoceptor agonist practolol on the interaction between leukotriene C_4 (LTC_4) and the non-selective β-adrenoceptor agonist isoprenalin. The LTC_4 induced release response could be reversed by isopenalin (10^{-5} M). Addition of practolol within a concentration range of 10^{-6}-10^{-4} M was without any effect on the interaction between LTC_4 and isoprenalin. Experimental conditions as described in the legend to figure 1. Means ± SEM (n=5).

Figures 2/3. Effects of the non-selective β-adrenoceptor antagonist sotalol (figure 2) and the β_2-selective antagonist H3525 (figure 3) on the interactions between β-adrenoceptor agonists and leukotriene C_4 (LTC_4). Responses are expressed as percentages change vs. controls (no drugs added, =0%). The release response, induced by LTC_4 (10^{-6} M, hatched bars) of prostaglandin E_2, thromboxane B_2 and β-glucuronidase was reversed by the β-adrenoceptor agonists (dotted bars) salbutamol (10^{-5} M, panel $2^a/3^a$) or isoprenalin (10^{-5} M, $2^b/3^b$). Addition of sotalol or H3525 (open bars) within the concentration range of 10^{-6}-10^{-4} M dose dependently restored the LTC_4 induced release response. Experimental conditions as described in the legend to figure 1. * $P<0.01$ (Student's t-test, two tailed). Means ± SEM (n=5).

induced release response. Minimal differences were observed between the effects of salbutamol and isoprenalin. Whereas salbutamol was more effective in blocking the effects of leukotriene C_4 on prostaglandin E_2 and β-glucuronidase release, isoprenalin was more effective on thromboxane A_2 production. The actions of salbutamol and isoprenalin could be blocked by different agents known to have β-adrenoceptor blocking properties, such as sotalol (figure 2), a non-selective antagonist, and the experimental drug H3525 (figure 3) which was reported to be β_2-selective[44]. Practolol, a selective β_1-adrenoceptor antagonist failed to reverse or to reduce the inhibition of the leukotriene C_4 induced release response caused by isoprenalin (table 1).

Though these results support the view that β_2-adrenoceptors are present on macrophage membranes and that activation of these receptors can down-regulate macrophage activity, the final evidence that these receptors are present has not been furnished yet. For this purpose binding studies with radioactive ligands will have to be carried out. However modulation of macrophage activity, as was shown in the present study, may represent a mechanism by which β-adrenoceptor agonists can control inflammation. Contradictory reports on the role of β-adrenoceptors in macrophages have been published. Schultz and coworkers[229] tested a number of agents known to increase intracellular cyclic AMP levels. The β-adrenoceptor agonist, isoprenalin, was without any effect on activated macrophage functions at all concentrations tested. This observation was similar to that of Kurland and associates[158], who showed that the proliferation of macrophage progenitor cells was not inhibited by β-adrenoceptor agonists and suggested that these cells lacked β-adrenoceptors. More recently however, it was found that L-isoprenalin inhibited the phagocytosis of *Trypanosoma cruzi* by mouse peritoneal macrophages[268] and activated adenylate cyclase in membrane preparations or intact macrophages[254]. It was also found that L-isoprenalin decreased the lymphokine induced aggregation of macrophages; D-isoprenalin had no effect[216]. Possibly a difference between D- and L-isoprenalin could explain the observed variation. Schultz and Kurland do not indicate whether they used D- or L-isoprenalin, whereas a racemic mixture was used in the present experiments.

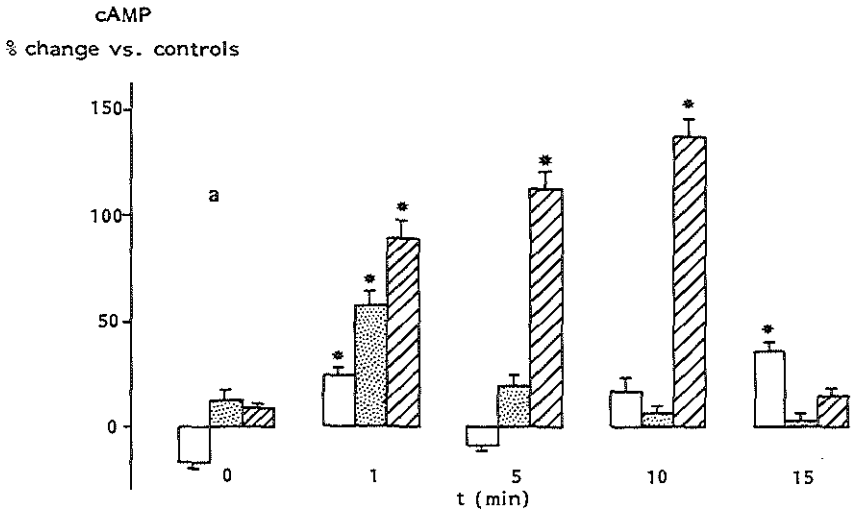
Another factor which is of importance in determining the reaction of macrophages to β-adrenergic stimulation is their "state of activity" or their

pathophysiological state. Whereas the present study indicates that β-adrenoceptor agonists do not inhibit macrophage basal secretions, but do abolish the effects of a stimulatory agent, the converse was shown in a paper by Vogel and coworkers[255]. They showed that a defective, Fc receptor mediated, phagocytosis of opsonized sheep erythrocytes could be corrected by agents known to elevate intracellular cyclic AMP. Among these was isoprenalin. On the other hand, Fc receptor mediated phagocytosis has been reported to induce cyclic AMP synthesis. Although isoprenalin enhanced cyclic AMP levels in the absence of phagocytosis, the already increased levels induced by phagocytosis could not be further increased by the β-adrenergic agonist[187]. In short, the responses controlled by the interaction of catecholamines with β-adrenergic receptors are modified through a variety of physiological factors and pharmacological perturbations and are most difficult to standardize[264].

3 LEUKOTRIENE C_4 AND SALBUTAMOL SYNERGIZE IN ELEVATING CYCLIC AMP LEVELS

Carrageenan elicited macrophages were preincubated for 15 minutes before addition of vehicle, leukotriene C_4 (10^{-6} M), salbutamol (10^{-5} M) or salbutamol and leukotriene C_4. After 1, 5, 10 and 15 minutes the cells were spun down, the supernatants were assayed for the presence of prostaglandin E_2 and the content of intracellular cyclic AMP of the macrophages in the pellet was determined (vide Appendix: Materials and Methods for a detailed description of the experimental procedures). Changes in cyclic AMP levels are presented in figure 4A, of prostaglandin E_2 production in figure 4B. Leukotriene C_4 was found to induce a rapid transient increase of cyclic AMP after 1 minute. The same effect, though more pronounced, was observed with salbutamol. However when the macrophages were exposed to a combination of leukotriene C_4 and salbutamol, cyclic AMP levels remained enhanced for as long as 10 minutes.

15 minutes after addition, leukotriene C_4 caused a second rise. If compared to control levels at each moment, significant changes in



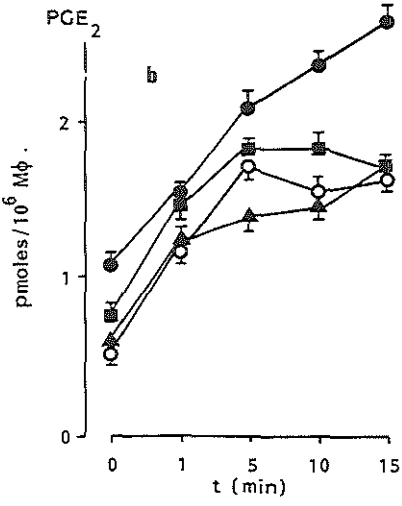


Figure 4. Kinetic changes of intracellular cyclic AMP levels (figure 4[a]) and prostaglandin E_2 production (figure 4[b]) of carrageenan elicited macrophages after exposure of the cells to LTC_4 (open bars, ●) (10^{-6} M), salbutamol (dotted bars, ■) (10^{-5} M) or to the combination of these drugs (hatched bars, ▲). The cells were preincubated for 15 minutes prior to addition of the drugs. cAMP and PGE_2 levels were assessed 0, 1, 5, 10 and 15 minutes after addition of LTC_4 or salbutamol. cAMP values are expressed as percentages vs. controls (○) (=0%), PGE_2 as pmoles/10^6 cells. Means ± SEM (n=6).

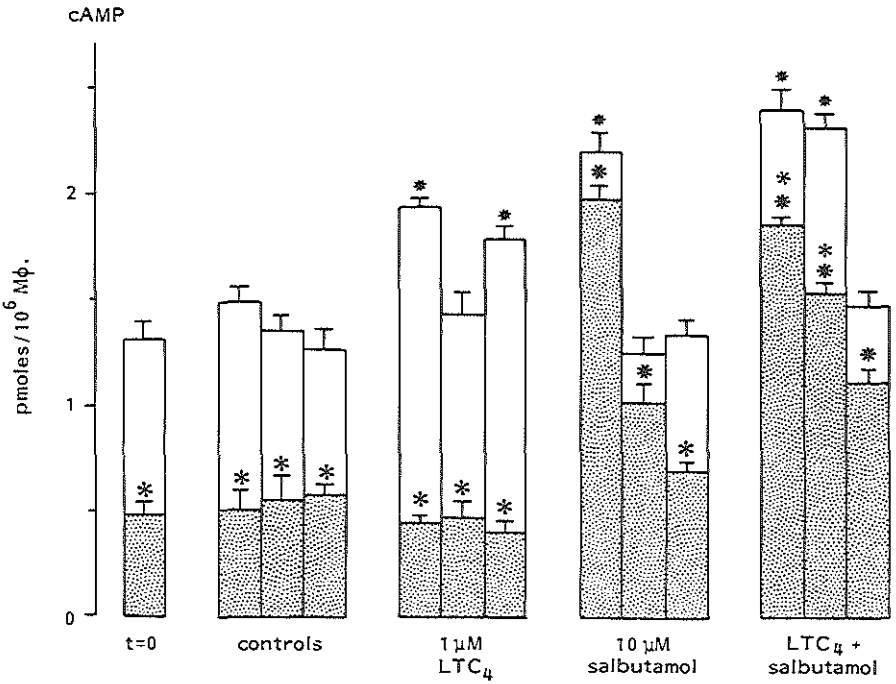


Figure 5. Kinetic changes of intracellular cyclic AMP levels of carrageenan elicited macrophages. Changes were measured 1, 10 and 15 minutes (left, middle and right bar of a block of three respectively) after exposure of the cells to vehicle (controls), LTC_4 (10^{-6} M), salbutamol (10^{-5} M) or to a combination of these two drugs. Furthermore the values were measured in the absence (open bars) and presence of 10^{-5} M indomethacin (hatched bars). The cAMP generation, induced by LTC_4 after 1 and 15 minutes, could be blocked by indomethacin. In contrast, salbutamol-mediated cAMP generation was prolonged after pretreatment with indomethacin, an effect which was even more pronounced when salbutamol and LTC_4 were both added to the cells. Experimental conditions as described in the legend to figure 4. ✱ $P<0.01$ vs. controls, ✱ $P<0.01$ vs. indomethacin treated controls, ✱ $P<0.01$, indomethacin treated vs. non-treated within each group (Student's t-test, two tailed). Means ± SEM (n=4).

prostaglandin E_2 production were only observed in the presence of leukotriene C_4, 10 and 15 minutes after addition. In order to investigate whether endogenous prostaglandins were involved in the leukotriene C_4 induced cyclic AMP generation, the experiment was repeated in the presence c.q. absence of indomethacin in a concentration (10^{-5} M) which was shown to effectively inhibit prostaglandin E_2 production. As can be seen in figure 5 absolute values differed somewhat between the two experiments, directional changes were the same. Indomethacin reduced control levels, indicating that endogenous prostaglandin production indeed is involved in determining intracellular cyclic AMP content. Again leukotriene C_4 induced a transient increase after 1 minute and a second increase after 15 minutes. Both events did not take place in the presence of indomethacin, actually leukotriene C_4 slightly reduced cyclic AMP levels (not significant) as compared to control macrophages. The effect of indomethacin pretreatment on salbutamol and salbutamol/leukotriene C_4 generated cyclic AMP production essentially was the same. In both situations a prolonged increase could be observed.

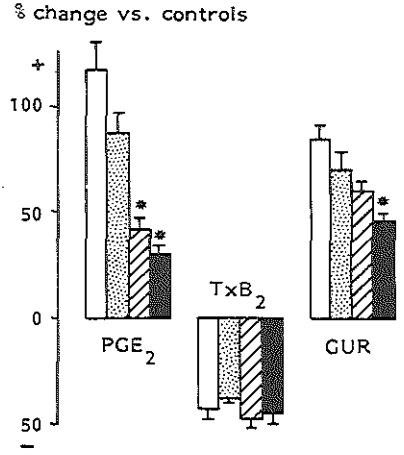


Figure 6. Effects of isobuthylmethylxanthine (IBMX) on the LTC_4 induced release of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2) and β-glucuronidase (GUR) from carrageenan elicited macrophages. Open bars represent values found when no IBMX was added, closed bars the values found in the presence of 10^{-5}–10^{-3} M IBMX. IBMX dose-dependently reversed the LTC_4 induced PGE_2 and GUR release. No effects were observed on TxB_2 production. Experimental conditions as described in the legend to figure 1. * $P<0.01$ vs. LTC_4 induced levels (Student's t-test, two tailed). Means ± SEM (n=5).

A rise in intracellular cyclic AMP can also be achieved by application of a phosphodiesterase inhibitor. Isobutylmethylxanthine (IBMX) in the absence of leukotriene C_4, inhibited prostaglandin E_2, thromboxane A_2 and β-glucuronidase release. In the presence of leukotriene C_4, IBMX counteracted the enhanced prostaglandin E_2 and β-glucuronidase release. No effects were observed on thromboxane A_2 production (figure 6). The latter phenomenon and the observed inhibition of thromboxane A_2 production in the absence of leukotriene C_4 are in contradiction with the results presented in the previous paragraph of this chapter.

The fact that indomethacin inhibited the leukotriene C_4 induced cyclic AMP generation clearly indicates that this increase was due to formation of endogenous prostaglandins. Therefore the conclusion that leukotriene C_4 itself does not activate adenylate cyclase seems justified. Also the prolonged increase in response to salbutamol after indomethacin pretreatment is not difficult to explain. Firstly, as was discussed previously, effects of β-adrenoceptor agonists are more pronounced with low intracellular cyclic AMP levels[187]. Secondly, as a consequence of indomethacin pretreatment thromboxane A_2 synthesis is inhibited. It is known that thromboxane A_2 decreases the activity of adenylate cyclase[110]. Although this phenomenon has not been shown in macrophages but in platelets, the decreased thromboxane A_2 production may account for the observed hypersensitivity to β-adrenoceptor stimulation. Thirdly but not so likely, indomethacin is known to inhibit phosphodiesterase activity. To obtain such an effect a concentration in the order of 10^{-3} M is required, which is a hundred times as much as the concentration presently used. Furthermore, if indomethacin was inhibiting phosphodiesterase activity in the concentration used, it would have reduced leukotriene C_4 induced lysosomal enzyme release as could be observed when using IBMX. In chapter 5.3 it has been shown that the same dose of indomethacin used in the experiments described above augmented the effects of leukotriene C_4. Nevertheless, since in the previously described experiments no adenylate cyclase activating stimulus was added, we cannot rule out the possibility that indomethacin inhibited phosphodiesterase.

None of the aforementioned explanations for the observed prolonged effect of salbutamol can satisfactorily account for the effects found with the

combination of leukotriene C_4 and salbutamol. Neither cyclic AMP, nor thromboxane A_2 levels were drastically reduced after application of leukotriene C_4 per se. A reduction of phosphodiesterase activity by indomethacin, thus augmenting the effect of salbutamol, cannot explain the observed prolongation of enhanced cyclic AMP levels in the presence of leukotriene C_4. Prostaglandin E_2 levels were not significantly different between the various groups treated with indomethacin. Therefore enhancement of endogenous prostaglandin production, as was found in the absence of indomethacin, is not likely to contribute to the observed increase of cyclic AMP levels. Since indomethacin abolished the leukotriene C_4 induced cyclic AMP generation in the macrophages treated with leukotriene C_4 alone, it was concluded that this effect was due to production of endogenous prostaglandins. It was also concluded that leukotriene C_4 itself does not activate adenylate cyclase. However, recently it has been reported that leukotrienes induce cyclic AMP generation, albeit in different systems.

Gorman and coworkers reported that acetylglyceryl phosphorylcholine (AGEPC) induced neutrophil aggregation which was coincident with a transient rise in cyclic AMP. Pretreatment of the cells with indomethacin potentiated the cyclic AMP response. In contrast, a 5-lipoxygenase inhibitor blocked both cyclic AMP accumulation and aggregation. These data suggested the involvement of a product of the 5-lipoxygenase pathway. In a second experiment it was found that leukotriene B_4 elevated cyclic AMP levels in intact cells and stimulated adenylate cyclase activity in neutrophil membrane preparations. Testing an adenylate cyclase inhibitor these authors found that this drug inhibited AGEPC-stimulated cyclic AMP accumulation, whereby neutrophil aggregation was actually enhanced. From these experiments they concluded that leukotriene B_4 and AGEPC (indirectly through leukotriene formation) stimulate neutrophil cyclic AMP levels but that the increase in cyclic AMP is not responsible for subsequent neutrophil aggregation. Instead, the elevation in cyclic AMP appeared to be part of a normal homeostatic mechanism that limits the aggregation response[113].

Another paper, presented by Claeson and Feinmark, reports that leukotriene B_4 stimulated the formation of cyclic AMP, the release of lysosomal enzymes and the generation of superoxide anions by neutrophils. Preincubation of the cells with (5S,12S)-diHETE or leukotriene B_4 resulted in a dose dependent inhibition of leukotriene B_4 induced degranulation, without causing parallel

changes in the levels of cyclic AMP[49]. The authors suggested that multiple leukotriene B_4 receptors may exist, whereby the leukotriene B_4 induced degranulation and superoxide anion responses are linked to receptors that are not associated with the cyclic AMP system.

The fact that leukotriene B_4 induces cyclic AMP synthesis in neutrophils does not necessarily mean that leukotriene C_4 has the same effect in macrophages. However some recent findings, listed below, allow speculation on the possibility that peptidoleukotrienes indeed affect cyclic AMP metabolism. Firstly, there is evidence for multiple receptors for peptidoleukotrienes[91]. Moreover, it has been postulated that leukotrienes act as Ca-ionophores[232]. Furthermore it is known that leukotriene C_4 induces a respiratory burst in macrophages[121]. In the light of these findings, the observation that stimulants of the oxidative burst activate macrophage adenylate cyclase is of particular interest. The elevation of cyclic AMP levels in response to the Ca-ionophore A23187 could not be blocked by indomethacin, demonstrating that prostaglandin synthesis was not involved[38].

The results described in the previous paragraphs are indicative for the existence of different cyclic AMP pools, of which one can be activated by leukotrienes via a specific receptor. However, they still do not offer an explanation for the results presently described. For, leukotriene C_4 per se did not enhance cyclic AMP levels, merely in the presence of salbutamol increased levels of cyclic AMP which were possibly due to leukotriene C_4 could be oberved. Recently Burka and associates have shown that stimulants of adenylate cyclase inhibited A23187 induced contractions of normal guinea-pig trachea[41]. This effect of A23187 is known to be leukotriene C_4 mediated[218]. Sensitized trachea responded differently. Low concentrations of adenylate cyclase stimulants, such as isoprenalin and forskolin all enhanced A23187 induced contractions, higher concentrations inhibited the response. The authors explained their results by activation of different pools of cyclic AMP that partake in either the release/contraction processes or in negative feedback, as has been described earlier[129] and concluded that sensitization has altered the modulatory mechanisms in the trachea.

As was shown in chapter 6.2 β-adrenoceptor agonists did not inhibit macrophage basal secretion, despite the fact that they do enhance cyclic AMP levels. It was also shown that β-adrenoceptor stimulation abolishes the effect

of a stimulating agent such as leukotriene C_4, an effect which was converse, though comparable to the effects of adenylate cyclase stimulants described in the previous paragraph. In light of the facts discussed above, the involvement of different cyclic AMP pools in the interaction between leukotriene C_4 and salbutamol is proposed. This proposal gains strength by the differences observed between the effects of salbutamol and IBMX. Both salbutamol and IBMX counteracted the leukotriene C_4 induced prostaglandin E_2 and β-glucuronidase release. Marked differences were observed in their respective effects on thromboxane A_2 synthesis. Salbutamol reversed the inhibition induced by leukotriene C_4, IBMX had no effect. However, IBMX inhibited basal thromboxane A_2 production, a property shared by prostaglandin E_2 (vide chapter 5). Two agents, both enhancing cyclic AMP levels, yet different in their effects. Whether salbutamol permits the expression of a specific leukotriene C_4 receptor and subsequent activation by leukotriene C_4 of a specific cyclic AMP pool or whether leukotriene C_4 permits activation of a separate cyclic AMP pool by salbutamol is by no means clear yet.

4 SUMMARY AND CONCLUSIONS

The involvement of cyclic AMP in the regulation of macrophage activity was studied. For that purpose the effects of β-adrenoceptor agonists and a phosphodiesterase inhibitor on the leukotriene C_4 induced release response were established. Also, kinetic changes in intracellular cyclic AMP levels after exposure of the macrophages to salbutamol and/or leukotriene C_4 were studied. Finally it was investigated to what extent endogenous prostaglandins were involved in the modulation of intracellular cyclic AMP levels.

Both the non-selective β-adrenoceptor agonist isoprenalin and the selective β_2-adrenoceptor agonist salbutamol reversed the leukotriene C_4 induced release response. Addition of the non-selective β-adrenoceptor antagonist sotalol or the β_2-selective antagonist H3525 abolished these effects. Practolol, a selective β_1-adrenoceptor antagonist was without any effect on the

isoprenalin-leukotriene C_4 interaction. In conclusion: **activation of β_2-adrenoceptors on macrophages abolishes the leukotriene C_4 induced release response which consists of enhanced prostaglandin E_2 production and β-glucuronidase release and inhibition of thromboxane A_2 synthesis.**

Studies on kinetic changes of intracellular cyclic AMP levels revealed that leukotriene C_4 induced a transient increase after 1 minute, followed by a second increment after 15 minutes. Both events disappeared after pretreatment of the macrophages with indomethacin, indicating that they were prostaglandin mediated. Salbutamol, like leukotriene C_4, induced a rapid transient cyclic AMP generation. However, this event was not inhibited by pretreatment with indomethacin, in fact a prolonged increase could be observed. The combination of leukotriene C_4 and salbutamol was found to have the most prominent effects on cyclic AMP levels. In contrast to the transient increments induced by leukotriene C_4 or salbutamol seperately, these substances enhanced cyclic AMP levels for as long as 10 minutes if added together. Whereas this interaction was likely to be due to a leukotriene C_4 induced prostaglandin E_2 production, surprisingly it could not be inhibited by indomethacin. Differences were observed in the effects on thromboxane A_2 production between β-adrenoceptor agonists on the one hand and the phosphodiesterase inhibitor IBMX and prostaglandin E_1 on the other side. Leukotriene C_4 induced inhibition of thromboxane A_2 synthesis could be reversed by β-adrenoceptor stimulation, whereas IBMX and prostaglandin E_1 reduced thromboxane A_2 production. These results are discussed in the light of a proposed existence of different cyclic AMP pools which can be activated either by leukotriene C_4 or by salbutamol. In conclusion: **leukotriene C_4 induces a rapid transient cyclic AMP generation which is prostaglandin mediated. In contrast, the interaction between leukotriene C_4 and salbutamol in modulating cyclic AMP metabolism appears to be independent on endogenous prostaglandin production and probably takes place via activation of separate cyclic AMP pools.**

SUMMARY AND CONCLUDING REMARKS

Over the last two decades considerable advances have been made in understanding the role of macrophages within the body's defence system. Originally these cells were characterized as simple scavengers[172], cells which adequately phagocytosed and killed bacteria or removed cell debris. A simple cell which lacked the sophisticated recognition and control mechanisms of the cellular elite: lymphocytes. The term "angry" macrophage, previously used to denominate activated macrophages, fits the macrophage perfectly if it could have realized the position it was relegated to: second-class. Thanks to the recognition of its ability to secrete an enormous battery of products[77,114,134] and its ability to present antigens to lymphocytes[39,186,250], the macrophage now is placed on the position it deserves: first-class. As was described in chapter 1, the macrophage does not only ingest and kill microbes, it selectively lysis tumour cells[4,251] and plays a key role in initiating and modulating immune reactions[101,180]. Because of the fact that the macrophage has such a central role within the body's defence system, the mechanisms by which the activity of this cell is modulated are of great importance.

Among the products secreted by macrophages are eicosanoids. Chapter 2 briefly outlines the biosynthesis, structure and general properties of eicosanoids. In chapter 4, more specifically, the production of eicosanoids by macrophages is discussed. Macrophages are known to release both cyclooxygenase- and lipoxygenase-derived arachidonic acid oxygenation products[206,239]. In the experiments described in chapter 4 eicosanoid release was correlated with macrophage activity. Comparing three different cell populations it was found that the production of cyclooxygenase metabolites was inversely correlated to the release of β-glucuronidase, a lysosomal enzyme. Lysosomal enzyme release is a well-established aspecific indicator of cell activity[65,225]. Thus, active cells showed a reduced capacity to produce cyclooxygenase metabolites. It could also be observed that after *in vivo* stimulation within the cyclooxygenase pathway a shift occurred. In comparison to thromboxane A_2, prostaglandin E_2 production was preferentially inhibited. The release of lipoxygenase

metabolites on the other hand was positively correlated to cell activity. Resident macrophages, the least active cells, did not release leukotriene C_4 whereas elicited macrophages did. Furthermore it was observed that carrageenan elicited macrophages produced less leukotriene C_4 than Freund's incomplete adjuvant elicited macrophages. A similar pattern was found with respect to β-glucuronidase release. The facts that prostaglandin E_2 production was preferentially inhibited and leukotriene C_4 production enhanced after *in vivo* activation provide further proof for the hypothesis that prostaglandin and leukotriene synthesis are independently mediated and are indications that these metabolites may be crucially involved in regulation of macrophage activity. Independent modulation of cyclooxygenase and lipoxygenase activity can be explained by the existence of two independent sources of substrate arachidonic acid[210] or the existence of two independently mediated phospholipase A_2 pools[265]. Products of the lipoxygenase pathway can have actions on macrophages which are opposite to those of the cyclooxygenase pathway. The concept that there are multiple, specific sources of arachidonic acid, giving rise to lipoxygenase or cyclooxygenase metabolites, is in accordance with the proposal that these compounds play an important role in regulating cell activities.

Table 1. Effects of arachidonic acid oxygenation metabolites/ metabolism on macrophage functions.

macrophage function	PGE_2	LTC_4	cyclooxyg. inhibitors	lipoxyg. inhibitors
Ia-antigen expression	−		+	−
chemiluminescence	−	+	ø +	−
lysosomal enzyme release	−	+	ø +	
IL-1 secretion	−		+	−
bactericidal activity	ø		+	
tumoricidal activity	−	+	+	
proliferation	−		ø	−
thromboplastin production			ø	−
plasminogen activator rel.	−		+	

The idea that leukotriene C_4 and prostaglandin E_2 modulate the activity of macrophages was sustained by the results of the experiments described in chapter 5. Leukotriene C_4 was shown to enhance macrophage activity, expressed as lysosomal enzyme release, and to induce the production of prostaglandin E_2. Prostaglandin E_2 in turn was found to inhibit macrophage activity. Further proof for the proposed interaction between these two eicosanoids could be found in results obtained by other groups. It is well documented that prostaglandin E_2 reduces macrophage activity. Prostaglandin E_2 inhibits a.o.: chemiluminescence[197], release of cyclooxygenase products[90], lysosomal enzyme release, interleukin I secretion[42] and tumoricidal activity[176]. A more complete list of the actions of prostaglandin E_2 on diverse aspects of macrophage activity is provided in table 1. Less is known about the effects of leukotriene C_4 on macrophages. Leukotriene C_4 induces a respiratory burst[121], enhances prostaglandin E_2 production, promotes the release of lysosomal enzymes, induces interleukin I secretion and tumoricidal activity (Bonta, personal communication). Interestingly, prostaglandin E_2 and leukotriene C_4 have opposite effects on all aspects mentioned. Circumstancial evidence for an interaction between cyclooxygenase and lipoxygenase derived products can be found in the opposing effects of inhibition of the respective enzymes on macrophage actvity. Ia antigen expression and interleukin I secretion, for example are promoted[42,156] by cyclooxygenase but inhibited[69,156] by lipoxygenase inhibitors. These two functions are of special importance since they represent the major mechanisms by which macrophages regulate lymphocyte activity. Thus, by influencing macrophages eicosanoids exert profound effects on the entire immune system.

Chapter 3 describes what is currently known about adenylate cyclase, how it is activated and how cyclic AMP effects are mediated. With Ca^{2+} cyclic AMP is considered as a major second messenger, relaying a stimulus from the extracellular environment to biochemical machinery within the cell[17]. Of all prostaglandins, prostaglandin E_2 was found to be the most important one in modulating cyclic AMP levels of macrophages, at least in rats[6]. Human macrophages are more sensitive to prostacyclin[30]. Since in the experiments described in this dissertation rat macrophages were used and because leukotriene C_4 promoted prostaglandin E_2 production from these macrophages, the involvement of cyclic AMP in the leukotriene C_4 induced release response was

investigated.

In order to study cyclic AMP involvement several experiments (described in chapter 6) were carried out. Firstly, since adenylate cyclase is not only activated by prostaglandin E_2 but also by β-adrenoceptor agonists, the effects of these agents on the leukotriene C_4 induced release response were studied. Secondly, inactivation of phosphodiesterase activity leads to accumulation of cyclic AMP. Therefore the effects of the phosphodiesterase inhibitor IBMX were assessed. Thirdly, studies on the kinetic changes of intracellular cyclic AMP levels were performed in the absence c.q. presence of the cyclooxygenase inhibitor indomethacin in order to investigate the contribution of endogenous prostaglandins to intracellular cyclic AMP production.

β-adrenoceptor agonists were found to reverse the leukotriene C_4 induced release response. Furthermore, studies whereby diverse agonists and antagonists were used, indicated that the effects of β-adrenoceptor agonists were mediated via activation of β_2-adrenoceptors. IBMX also effectively reversed the leukotriene C_4 induced prostaglandin E_2 and β-glucuronidase release. However, in contrast with the β-adrenoceptor agonists, but, like prostaglandins of the E-series, IBMX inhibited thromboxane A_2 production. These findings already were puzzling but the results obtained from the studies on kinetic cyclic AMP changes were even more surprising. Leukotriene C_4 as well as salbutamol induced a transient increase of cyclic AMP, whereby the leukotriene C_4 induced increment was prostaglandin mediated. However, the combination of the two drugs induced a cyclic AMP increase which was prolonged. Furthermore, this effect was not due to leukotriene C_4 induced prostaglandin production since it could not be blocked by indomethacin pretreatment. In contrast, after indomethacin pretreatment the effect was more pronounced. These results can be explained by the existence of several, separate cyclic AMP pools as has been proposed earlier[129]. Whether such a pool is activated by leukotriene C_4[49] or whether activation of the cell by leukotriene C_4 permits stimulation by salbutamol of a specific pool[41] is far from clear. Both situations have been reported earlier, albeit in different systems.

leukotrienes is reduced after activation. Still the basal release of leukotriene C_4, the activating metabolite, is higher in more active cells. The reason for this discrepancy is not known yet, but this situation might merely represent an optimally functioning peptido-leukotriene synthase sytem despite a decreased availability of precursor fatty acid. With respect to decreased arachidonic acid availability a specific interaction between T lymphocytes and macrophages is particularly interesting. T lymphocytes are not capable of producing eicosanoids but do provide macrophages with arachidonic acid, which can serve as a substrate for the synthesis of eicosanoids[106]. This interaction may serve as a unique pathway by which the T lymphocyte can modulate the activity of the macrophage and by which the macrophage may overcome its impaired capacity to produce arachidonic acid oxygenation products.

The styling of figure 1 was inspired by Francis Picabia's painting: "Petite solitude au milieu des soleils". Picabia gave his painting a subtitle which superbly fits the aim of figure 1: "Tableau peint pour raconter non pour prouver". Indeed, the figure is descriptive, not decisive and only represents an oversimplified model. Oversimplified since Ca^{2+} is not included, the postulated interaction of leukotriene C_4 with cyclic AMP is deleted and the interactions between the different cyclooxygenase metabolites are not incorporated. However, according to Picabia "there are no theories about the unknown".

In conclusion, the presently described experiments indicate that:

- a high degree of macrophage activation is associated with decreased production of prostaglandin E_2 and increased production of leukotriene C_4.
- leukotriene C_4 promotes macrophage activation.
- leukotriene C_4 promotes prostaglandin E_2 production from macrophages.
- prostaglandin E_2 cannot suppress leukotriene production.
- prostaglandin E_2 suppresses macrophage activity.
- effects of prostaglandin E_2 are mediated via activation of adenylate cyclase.
- β_2-adrenoceptor stimulation reverses the effects of leukotriene C_4.
- effects of β_2-adrenoceptor agonists are cyclic AMP mediated, but that
- leukotriene C_4 and salbutamol interact in enhancing intracellular cyclic AMP levels.

Finally, it has to be emphasized that lysosomal enzyme release, albeit important, is only one aspect of macrophage activity. To check the true importance of the proposed interaction between leukotriene C_4 and prostaglandin E_2 other aspects of macrophage activity will have to be studied, whereby special attention should be given to those aspects which are involved in the cooperation between macrophages and other cells, e.g. lymphocytes. Furthermore, as was already mentioned, in human macrophages prostacyclin, instead of prostaglandin E_2, is the major suppressive metabolite. Studies which are currently carried out in our laboratory indicate that leukotriene D_4 is the major peptido-leukotriene released by human alveolar macrophages. Obviously, much is still to be learned.

The concept however that macrophage activity is modulated by eicosanoids opens broad perspectives in terms of the development of new therapeutical agents. Application of drugs, inhibiting the lipoxygenase or the cyclooxygenase pathway could result in immunosuppression or enhancement of the immune response respectively. Although this approach is quite conventional, combined with specific alterations in/on the drugs which renders them tissue selective, such agents would be very usefull tools. Possibly, a more specific way to block the effects of leukotrienes and prostaglandins can be accomplished by the development of selective receptor antagonists. Finally, an approach which has attracted little attention consists of reinforcing physiological processes by the administration of synthetic analogues. Whereas little doubt exists that a succesfull modulation of the immune system would be beneficial for diseases as rheumatoid arthritis, AIDS and glomerulonefritis, and although the crucial role of macrophages within the immune system nowadays is recognized, the development of drugs interfering in macrophage activity still is in its infancy.

SAMENVATTING EN CONCLUSIES

Twintig jaar geleden werd de macrofaag nog op eenzelfde manier omschreven als aan het begin van deze eeuw door Metchnikoff: een cel die in staat is microörganismen te fagocyteren en vervolgens te doden. Een nuttige eigenschap weliswaar, maar, vergeleken met de lymfocyt, het neusje van de zalm onder de cellen van het afweer- of immuunsysteem, was de macrofaag een simpele cel. Zoals opgesomd in hoofdstuk 1 is deze "simpele" cel echter tot veel meer in staat dan alleen de fagocytose van microörganismen. Zij is betrokken bij de vernietiging van kankercellen en speelt een sleutelrol bij het op gang brengen van verschillende reacties van het immuunsysteem. De macrofaag is hiertoe in staat door twee eigenschappen en wel: (a) haar vermogen diverse, biologisch actieve substanties uit te scheiden en (b) het vermogen antigeen aan lymfocyten aan te bieden. Omdat de macrofaag zo'n prominente positie inneemt in de diverse verdedigingsmechanismen van het lichaam, is de activiteit van deze cel en de manier waarop deze activiteit geregeld wordt van groot belang.

Onder de door macrofagen afgegeven producten, bevinden zich eicosanoiden, metabolieten van het meervoudig onverzadigde vetzuur arachidonzuur. Het doel van de in de hoofdstukken 4, 5 en 6 van dit proefschrift beschreven studies, was het onderzoeken van de afgifte van eicosanoiden door macrofagen en van de effecten van eicosanoiden op macrofaag activiteit. Als parameter voor macrofaag activiteit werd gekozen voor de afgifte van het lysosomal enzym β-glucuronidase. Een toegenomen afgifte van lysosomale enzymen wordt algemeen geinterpreteerd als een toegenomen activiteit van de cel. Zoals behandeld in hoofdstuk 2 kan arachidonzuur omgezet worden in een groot aantal producten, ieder met een verschillende biologische werking. Bij de omzetting van arachidonzuur zijn verschillende enzymen betrokken, waarvan het cyclooxygenase en het lipoxygenase van speciaal belang zijn. Via de cyclooxygenase route worden prostaglandinen en thromboxanen gevormd; via de lipoxygenase route ontstaan onder andere de leukotrienen. Macrofagen produceren zowel cyclooxygenase als lipoxygenase metabolieten. De in hoofdstuk 4 beschreven experimenten hadden tot doel een verband te leggen tussen de activiteit van macrofagen en de afgifte van

eicosanoiden. Wanneer een vergelijking wordt gemaakt tussen drie verschillende celpopulaties, blijkt dat de productie van cyclooxygenase metabolieten omgekeerd evenredig is met de afgifte van het lysosomale enzym β-glucuronidase. Met andere woorden, actieve macrofagen produceren minder cyclooxygenase metabolieten. Voorts bleek dat tussen de verschillende cyclooxygenase metabolieten een verschuiving optrad. Bij actieve cellen was de productie van zowel thromboxaan A_2 als prostaglandine E_2 verminderd, waarbij de afgifte van de laatste naar verhouding het meest geremd was. Wat de lipoxygenase metabolieten betreft was de situatie juist omgekeerd; de minst actieve cellen produceerden geen leukotrieen C_4, terwijl de actieve, opgeroepen macrofagen dit wel deden. Deze observaties geven steun aan de al eerder gepostuleerde hypothese dat de productie van leukotrienen of van prostaglandinen, hoewel afkomstig van hetzelfde vetzuur, onafhankelijk van elkaar geregeld is. Een verklaring voor dit verschijnsel kan gevonden worden in het bestaan van meerdere, van elkaar onafhankelijke bronnen arachidonzuur of in het bestaan van diverse, op verschillende manieren geactiveerde phospholipases A_2. Zoals moge blijken uit de volgende alinea hebben lipoxygenase en cyclooxygenase metabolieten tegenovergestelde effecten op macrofagen. De hypothese dat er meerdere,

Tabel 1. Effecten van eicosanoiden/arachidonzuur metabolisme op diverse functies van macrofagen.

macrofaag functie	PGE_2	LTC_4	cyclooxyg. remmers	lipoxyg. remmers
expressie van Ia-antigeen	−	−	+	−
productie van O_2 radicalen	−	+	ø +	−
afgifte van lysosomale enz.	−	+	ø +	
afgifte van IL-1	−		+	
bactericide werking	ø		+	
tumoricide werking	−	+	+	
proliferatie	−		ø	−
afgifte v. thromboplastine			ø	−
afgifte v. plasminogeen act.	−		+	

specifieke bronnen arachidonzuur bestaan is in overeenstemming met de in deze dissertatie geopperde idee dat eicosanoiden een belangrijke rol spelen in de regulatie van macrofaag activiteit.

De resultaten van de experimenten behandeld in hoofdstuk 5, ondersteunen de gedachte dat door een interactie tussen leukotrieen C_4 en prostaglandine E_2 de activiteit van de macrofaag bepaald kan worden. Uit deze experimenten bleek dat leukotrieen C_4, behalve een toename van de cel activiteit, ook de productie van prostaglandine E_2 door macrofagen kan bewerkstelligen. Prostaglandine E_2 daarentegen remde de activiteit van de cel. Terwijl de in dit proefschrift beschreven studies de enige zijn waarbij gekeken werd naar de effecten van zowel prostaglandine E_2 als leukotrieen C_4 in een systeem, kan ondersteunend bewijs voor de voorgestelde interactie gevonden worden in resultaten, gepubliceerd door andere groepen. Deze resultaten hebben vaak betrekking op andere aspecten van macrofaag activiteit zoals: zuurstof radicaal productie, interleukin-1 afgifte, expressie van Ia-antigeen en tumoricide werking (tabel 1). Zowel uit de studies waarbij de metabolieten zelf gebruikt werden, als uit die waarbij de productie van de metabolieten geremd werd, kwam naar voren dat cyclooxygenase en lipoxygenase metabolieten tegenovergestelde effecten hebben. Bijvoorbeeld: interleukin-1 afgifte en Ia-antigeen expressie worden geremd door prostaglandine E_2, echter gestimuleerd na blokkade van de vorming van prostaglandine E_2 door cyclooxygenase remmers. Een omgekeerde situatie was waar te nemen met betrekking tot leukotrieen C_4: stimulatie van beide functies door leukotrieen C_4 zelf, remming na blokkade van de productie van leukotrieen C_4. De bovengenoemde twee functies zijn van bijzonder belang, omdat met deze functies de belangrijkste manieren genoemd zijn waarmee de macrofaag de activiteit van de lymfocyt, en daarmee van het hele immuunsysteem, kan beinvloeden. Concluderend mag dan ook gesteld worden dat eicosanoiden, door macrofaag activiteit te beinvloeden, diepgaande effecten op de werking van het immuunsysteem hebben.

Een beknopte beschrijving van het enzym adenylaat cyclase, activatie van dit enzym en effecten die daardoor teweeggebracht worden, wordt gegeven in hoofdstuk 3. Cyclisch AMP wordt, samen met Ca^{2+}, beschouwd als de belangrijkste intracellulaire "boodschapper", de stof die er voor zorgt dat een signaal van buiten de cel omgezet wordt in een chemische reactie binnen de cel. Omdat leukotrieen C_4 de afgifte van prostaglandine E_2 door macrofagen bevordert en

omdat bekend was dat de effecten van prostaglandine E_2 tot stand komen via verhoging van de intracellulaire cyclisch AMP concentratie, werd de rol van cyclisch AMP in de interactie tussen beide eicosanoiden onderzocht. Om dit te kunnen doen werd een aantal experimenten uitgevoerd (hoofdstuk 6). Daar adenylaat cyclase niet alleen door prostaglandine E_2, maar ook door β-adrenoceptor stimulatie geactiveerd kan worden, werden als eerste de effecten van diverse β-adrenoceptor agonisten op de werking van leukotrieen C_4 bestudeerd. Vervolgens werd onderzocht wat het resultaat was van ophoping van intracellulair cyclisch AMP na inactivatie van het enzym phosphodiesterase door IBMX. Tenslotte werd gekeken naar de veranderingen in de tijd van het cyclisch AMP gehalte in af- respectievelijk aanwezigheid van de cyclooxygenase remmer indomethacine. Deze laatste experimenten werden uitgevoerd om de betrokkenheid van de endogeen gevormde prostaglandinen bij de cyclisch AMP generatie te bepalen.

Stimulatie van β-adrenoceptoren leidde tot remming van alle door leukotrieen C_4 veroorzaakte effecten. Uit studies met selectieve antagonisten bleek dat de effecten van de agonisten tot stand kwamen via stimulatie van β_2-adrenoceptoren. Ook IBMX remde de door leukotrieen C_4 veroorzaakte afgifte van prostaglandine E_2 en β-glucuronidase. In tegenstelling tot de β-adrenoceptor agonisten, maar evenals prostaglandine E_1, bleek IBMX de thromboxaan A_2 afgifte te remmen. Een verwarrend resultaat dat er niet duidelijker op werd door de gegevens van de studies naar cyclisch AMP kinetiek. Hieruit bleek dat zowel leukotrieen C_4 als de β_2-adrenoceptor agonist salbutamol een tijdelijke toename van de hoeveelheid intracellulair cyclisch AMP veroorzaakten. De toename na leukotrieen C_4 kon geremd worden met indomethacine, een gegeven waardoor de conclusie gerechtvaardigd is dat deze toename tot stand kwam via prostaglandine productie. Werden leukotrieen C_4 en salbutamol gezamenlijk gegeven, dan was de cyclisch AMP toename groter en bovendien langer aanhoudend. Endogene prostaglandine vorming speelde hierbij geen rol; na voorbehandeling met indomethacine was het effect niet verdwenen maar juist versterkt. Een verklaring voor dit verschijnsel kan gevonden worden in het bestaan van meerdere, gescheiden bronnen cyclisch AMP. Of een van deze bronnen gestimuleerd wordt door leukotrieen C_4, of dat de activatie van de cel door leukotrieen C_4 aan salbutamol de gelegenheid biedt een speciale bron aan te boren, is geenszins duidelijk. Beide mogelijkheden zijn in de literatuur

geactiveerde macrofaag verliest echter, om vooralsnog duistere redenen, haar vermogen voldoende prostaglandinen te produceren om een hoog cyclisch AMP gehalte in stand te houden. Na activatie daalt dit gehalte razendsnel. Verdere activatie van de cel wordt echter voorkomen door de vergrote gevoeligheid van het adenylaat cyclase voor prostaglandine E_2, waardoor zelfs kleine hoeveelheden in staat zijn om de negatieve terugkoppeling in stand te houden. Behalve de synthese van prostaglandinen is ook de capaciteit tot productie van leukotrienen, na activatie verminderd. Toch is de basale afgifte van leukotrieen C_4 hoger in actieve cellen. Hoewel de exacte oorzaak voor deze discrepantie niet bekend is, is het heel wel mogelijk dat dit gegeven slechts een weerspiegeling is van het feit, dat ondanks verminderde beschikbaarheid van arachidonzuur, de moedersubstantie, het leukotrieen genererend enzymsysteem optimaal functioneert. Een manier waarop de macrofaag de verminderde beschikbaarheid van arachidonzuur op kan vangen ligt besloten in een unieke samenwerkingsvorm tussen macrofagen en lymfocyten. Zelf is een lymfocyt niet in staat eicosanoiden te produceren; wel kan deze cel arachidonzuur afstaan aan de macrofaag, die het op haar beurt als substraat voor de eicosanoid productie kan gebruiken. Of deze interactie *in vivo* echter ook optreedt is geenszins duidelijk.

Figuur 1 is gestyleerd naar analogie van Francis Picabia's schilderij: Petite solitude au milieu des soleils. Het onderschrift bij dit schilderij luidt: "Tableau peint pour raconter, non pour prouver", een onderschrift wat uitstekend de bedoeling van de figuur weergeeft; niets meer dan de presentatie van een simpel model, geen absolute waarheid. Het model is simpel, immers Ca^{2+} is weggelaten, de interactie tussen leukotrieen C_4 en cyclisch AMP is er niet in opgenomen en wisselwerkingen tussen verschillende cyclooxygenase metabolieten zijn niet meegeteld. Volgens Picabia is dit echter gerechtvaardigd, immers: "Het onbekende kent geen theorieën".

Samenvattend leiden de beschreven resultaten tot de volgende conclusies:
-verhoogde activiteit van een macrofaag is gecorreleerd aan verminderde productie van prostaglandine E_2 en toegenomen synthese van leukotrieen C_4,
-leukotrieen C_4 bevordert de macrofaag activiteit,
-leukotrieen C_4 bevordert de afgifte van prostaglandine E_2 door macrofagen,
-prostaglandine E_2 heeft geen effect op de afgifte van leukotrienen door

macrofagen.

-prostaglandine E_2 remt macrofaag activiteit.

-stimulatie van β_2-adrenoceptoren doet het effect van leukotrieen C_4 op macrofagen teniet.

-effecten van $\beta_2$adrenoceptor agonisten komen tot stand via activatie van adenylaat cyclase en

-er bestaat een wisselwerking tussen leukotrieen C_4 en salbutamol, leidend tot een verhoogd gehalte aan intracellulair cyclisch AMP.

Ter afsluiting nog enkele kanttekeningen. Hoewel de afgifte van lysosomale enzymen een belangrijke, aspecifieke parameter is van macrofaag activiteit, is het maar een facet hiervan. Om de werkelijke betekenis van de vastgestelde interactie tussen leukotrieen C_4 en prostaglandine E_2 te kennen, moeten andere aspecten van macrofaag activiteit bestudeerd worden, speciaal die aspecten die van belang zijn voor de samenwerking tussen macrofagen en andere cellen. Bij de hier beschreven studies werd gebruik gemaakt van ratte-macrofagen. Hoewel we nog niet in staat zijn hierover een eenduidig oordeel te geven, verschaft het nu lopende onderzoek op onze afdeling ons aanwijzingen dat bij de mens niet prostaglandine E_2, maar prostacycline de belangrijkste cyclooxygenase metaboliet is. Voorts is gebleken dat menselijke longmacrofagen voornamelijk leukotrieen D_4 produceren. Voordat de hier geopperde hypothese practische toepassingen oplevert moet er nog veel onderzocht worden. Echter, het concept dat macrofaag activiteit gemoduleerd wordt door eicosanoiden opent een scala aan mogelijkheden wat betreft de ontwikkeling van nieuwe geneesmiddelen. Toepassing van farmaca, die of de lipoxygenase of de cyclooxygenase route remmen, zou respectievelijk een onderdrukking of een verhoging van de immuunrespons tot gevolg kunnen hebben. Deze aanpak is tamelijk conventioneel. Gecombineerd echter met veranderingen aan de chemische structuur, zodanig dat de geneesmiddelen aangrijpen op specifieke cellen, zou hij leiden tot het ontstaan van bijzonder geschikte, selectief werkzame farmaca. Een andere manier om de werking van leukotrienen en prostaglandinen te remmen houdt de toediening van specifieke receptor antagonisten in. Tenslotte, versterking van fysiologische processen door toediening van synthetische analoga is een aanpak die, onverdiend, nauwelijks aandacht geniet. Het staat buiten kijf dat succesvol ingrijpen in het immuunsysteem gunstig is bij ziekten zoals rheumatoide

arthritis, AIDS en glomerulonefritis. Ook staat vast dat de macrofaag een prominente positie inneemt binnen het immuunsysteem. Echter, zoals reeds eerder gesteld, de ontwikkeling van geneesmiddelen die effectief de macrofaag activiteit beinvloeden staat pas in de kinderschoenen.

REFERENCES

1 Adams, D.O., K.J.Kao, R.Farb and S.V.Pizzo, 1980, Effector mechanisms of cytolytically activated macrophages: II. Secretion of a cytolytic factor by activated macrophages and its relationship to secreted neutral proteases, J. Immunol., 124, 293.

2 Adams, D.O., 1982, Macrophage activation and secretion, Fed. Proc., 41, 2193.

3 Adams, D.O., M.Cohen and H.S.Koren, 1983, Activation of mononuclear phagocytes for cytolysis: Parallels and contrast between activation for tumour cytotoxicity and for ADCC. In: Macrophage Mediated Antibody Dependent Cellular Cytotoxicity, (ed. H.S.Koren), Marcel Dekker, New York.

4 Adams, D.O. and P.A.Marino, 1984, Activation of mononuclear phagocytes for destruction of tumour cells as a model for study of macrophage development. In: Contempory Hematology/Oncology, (eds. A.S.Gordon, J.LoBue and R.Silver), Plenum Press, New York London, p.69.

5 Adams,J., B.J.Fiksimmons, Y.Girard, Y.Leblanc, J.F.Evans and J.Rokach, 1985, Enantiospecific and stereospecific synthesis of lipoxin A. Stereochemical assignment of the natural lipoxin A and its possible biosynthesis, J. Am. Chem. Soc., 107, 464.

6 Adolfs, M.J.P. and I.L.Bonta, 1982, Low concentrations of prostaglandin E_2 inhibit the prostacyclin induced elevation of cyclic adenosine 3',5'-monophosphate in elicited populations of rat peritoneal macrophages, Br. J. Pharmacol., 75, 373.

7 Adolfs, M.J.P, I.L.Bonta and M.W.J.A.Fieren, 1985, Infectious inflammatory changes in cyclic AMP levels and in their regulation by prostaglandins in human peritoneal macrophages, Prostagl. Leukotr. Med., 18, 217.

8 Alexander, P. and R.Evans, 1971, Endotoxin and double stranded RNA render macrophages cytotoxic, Nature, 232, 76.

9 Austen, K.F., E.J.Corey, J.M.Drassen and A.G.Leith, 1983, The effect of indomethacin on the contractile response of the guinea-pig lung parenchymal strip to leukotriene B_4, leukotriene C_4, leukotriene D_4 and leukotriene E_4, Br. J. Pharmacol., 80, 47.

10 Bach, M.K., J.R.Brashler, S.Hammarström and B.Samuelsson, 1980, Identification of leukotriene C_1 as a major component of slow reacting substance from rat mononuclear cells, J. Immunol., 125, 115.

11 Baggiolini, M., 1980, The neutrophil. In: The Cell Biology of Inflammation, Handbook of Inflammation, vol 2, (ed. G.Weissmann), Elsevier/North-Holland Biomedical Press, p.163.

12 Baggiolini, M., 1984, Lysosomal hydrolases. In: The Reticuloendothelial System, a comprehensive treatise, (eds. M.Escobar, S.Reichard and H.Friedman), Plenum Press, New York

13 Baracos, V., H.P.Rodemann, C.A.Dinarello and A.L.Goldberg, 1983, Stimulation

of muscle protein degradation and prostaglandin E_2 release by leukocytic pyrogen (interleukin-1), N. Engl. J. Med., 308, 553.

14 Barnstable, C.S. and E.A.Jones, 1978, Isolation, structure and genetics of HLA-A, -B, -C and -DRw (Ia) antigens, Br. Med. Bull., 34, 241.

15 Beller, D.S. and L.R.Unanue, 1977, Thymic maturation in vitro by a secretory product from macrophages, J. Immunol., 118, 1780.

16 Bergström, S., H.Danielsson and B.Samuelsson, 1964, The enzymatic formation of prostaglandin E_2 from arachidonic acid: prostaglandins and related factors 32, Biochim. Biophys. Acta, 90, 207.

17 Berridge, J., 1984, Cellular control through interactions between cyclic nucleotides and calcium, In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 17, (eds. P.Greengard, G.A.Robinson, R.Paoletti and S.Nicosia) Raven Press, New York, p.329.

18 Beutler, B., D.Greenwald, J.D.Humes, M.Chang, Y.-C.E.Pan, J.Mathison, R.Ulevitch and A.Cerami, 1985, Identity of tumour necrosis factor and the macrophage-secreted factor cachectin, Nature, 316, 552.

19 Bisgaard, H., J.Kristensen and J.Sondergaard, 1982, The effect of leukotriene C_4 and D_4 on cutaneous blood flow in humans, Prostaglandins, 23, 797.

20 Blackwell, G.J., R.Carnuccio, M.DiRosa, R.J.Flower, L.Parente and P.Persico, 1980, Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids, Nature, 287, 147.

21 Blaesdale, J.E., J.Eichberg and G.Hansen, 1984, Simple molecules, complex functions, TIPS, 5, 449.

22 Bonney, R.J., P.D.Wightman, P.Davies, S.J.Sadowski, F.A.Kuehl Jr. and J.L.Humes, 1978, Regulation of prostaglandin synthesis and of the selective release of lysosomal hydrolases by mouse peritoneal macrophages, Biochem. J., 176, 433.

23 Bonney, R.J., P.Davies, F.A.Kuehl and J.L.Humes, 1980, Arachidonic acid oxygenation products produced by mouse peritoneal macrophages responding to inflammatory stimuli, J. Reticuloendothel. Soc., 28S, 113S.

24 Bonney, R.J., S.Burger, P.Davis, F.A.Kuehl Jr. and J.L.Humes, 1982, Prostaglandin E_2 and prostacyclin elevate cyclic AMP levels in elicited populations of mouse peritoneal macrophages, In: Advances in Prostaglandin and Thromboxane Research, vol. 8 (eds. B. Samuelsson, P.W. Ramwell and R. Paoletti) Raven Press, New Yprk, p.1691.

25 Bonney, R.J., P.D.Wightman, M.E.Dahlgren, S.J.Sadowski, P.Davies, N.Jensen, T.Lanza and J.L.Humes, 1983, Inhibition of the release of prostaglandins, leukotrienes and lysosomal acid hydrolases from macrophages by selective inhibitors of lecithin biosynthesis, Biochem. Pharmacol., 32, 361.

26 Bonney, R.J. and P.Davies, 1984, Possible autoregulatory functions of the secretory products of mononuclear phagocytes, In: Contemporary Topics in Immunobiology, vol.13, Macrophage Activation (eds. D.O.Adams and M.G.Hanna Jr.), Plenum Press, New York and London, p.199.

27 Bonta, I.L. and M.J.Parnham, 1979, Time-dependent stimulatory and

inhibitory effects of prostaglandin E_1 on exudative and tissue components of granulomatous inflammation in rats. Br. J. Pharmacol., 65, 465.

28 Bonta, I.L. and M.J.Parnham, 1982. Immunomodulatory anti-inflammatory functions of E-type prostaglandins. Mini review with emphasis on macrophage-mediated effects. Int. J. Immunopharmacol., 4, 103.

29 Bonta, I.L., 1984, Macrophage regulation by arachidonic acid metabolites: Can this physiological control process serve as a model for novel pharmacotherapy of immuno-inflammatory conditions? In: Agents and Actions, Suppl. vol.14, Pathophysiology and Treatment of Asthma and Arthritis (eds. P.R.Saxena and G.R.Elliott), p.5.

30 Bonta, I.L., M.J.P.Adolfs and M.W.J.A.Fieren, 1984, Cyclic AMP levels and their regulation by prostaglandins in peritoneal macrophages of rats and humans, Int. J. Immunopharmacol., 6, 547.

31 Boraschi, D., S.Censini, M.Bartalini, P.Ghiara and A.Tagliabre, 1985. Arachidonic acid metabolism in macrophages: Regulation by interferons and interleukin-1. Int. J. Immunopharmacol., 7, 359.

32 Borgeat, P. and B.Samuelsson, 1979, Arachidonic acid metabolism in polymorphonuclear leucocytes: Unstable intermediates in formation of dihydroxy acids, Proc. Natl. Acad. Sci. USA, 76, 3213.

33 Borgeat, P. and B.Samuelsson, 1979, Transformations of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxy eicosatetraenoic acid, J. Biol. Chem., 254, 2643.

34 Bray, M.A., F.A.Cunningham, A.W.Ford-Hutchinson and M.J.H.Smith, 1981, Leukotriene B_4: An inflammatory mediator in vivo, Prostaglandins, 22, 213.

35 Bray, M.A., F.A.Cunningham, A.W.Ford-Hutchinson and M.J.H.Smith, 1981, Leukotriene B_4: A mediator of vascular permeability, Br. J. Pharmacol., 72, 483.

36 Bray, M.A., A.W.Ford-Hutchinson and M.J.H.Smith, 1981, Leukotriene B_4: Biosynthesis and biological activities, In: SRS-A and Leukotrienes (ed. P.J.Piper), Wiley, Chichester, p.253.

37 Brocklehurst, W.E., 1960, The release of histamine and formation of a slow-reacting substance of anaphylaxis (SRS-A) during anaphylactic shock, J. Physiol., 151, 416.

38 Bromberg, Y. and E.Pick, 1981, Activation of macrophage adenylate cyclase by stimulants of the oxidative burst and by arachidonic acid - two distinct mechanisms. Cell Immunol., 61, 90.

39 Burckhardt, J.J., 1979, Rat memory thymus-derived lymphocytes. II. Differences in macrophage dependent activation shown by *Actinomyces viscosus* antigens and by mitogens using silica in vitro, Scand. J. Immunol., 10, 229.

40 Burger, R. and E.M.Shevach, 1980, Monoclonal antibodies to guinea pig Ia antigens. II. Effect on alloantigen, antigen and mitogen induced T lymphocyte proliferation in vitro, J. Exp. Med., 152, 1011.

41 Burka, J.F., 1983, Effects of selected bronchodilators on antigen- and A23187-induced contraction of guinea pig trachea. J. Pharmacol. Exp.

Ther., 225, 427.

42 Cahill, J. and K.E.Hopper, 1984, Immunoregulation by macrophages III. Prostaglandin E suppresses lymphocyte activation but not macrophage effector function during *Salmonella enteritidis* infection, Int. J. Immunopharmacol., 6, 9.

43 Cameron, D.J. and P.O'Brian, 1982, Relationship of the suppression of macrophage mediated tumor cytotoxicity in conjunction with secretion of prostaglandin from the macrophages of breast cancer patients, Int. J. Immunopharmacol., 4, 445.

44 Carlson, E. and B.Öblad, 1976, Are there two types of β-receptors mediating the same adrenergic response ? In: Beta-Adrenoceptor Blocking Agents (eds. P.R. Saxena and R.P. Forsyth) North-Holland, Amsterdam, p.305.

45 Carnuccio, R., M.DiRosa, R.J.Flower and A.Pinto, 1981, The inhibition by hydrocortisone of prostaglandin biosynthesis in rat peritoneal leukcocytes is correlated with intracellular macrocortin levels, Br. J. Pharmacol., 74, 322.

46 Chensue, S.W., S.L.Kunkel, G.S.Higashi, P.E.Ward and D.L.Boros, 1983, Production of superoxide anion, prostaglandins and hydroxy eicosatetraenoic acids by macrophages from hypersensitivity-type (*Schistosoma mansoni* egg) and foreign body-type granulomas, Int. J. Immunol., 42, 1116.

47 Citri, Y. and M.Schramm, 1982, Probing of the coupling site of the β-adrenergic receptor. Competition between different forms of the guanyl nucleotide binding protein for interaction with the receptor, J. Biol. Chem., 257, 13257.

48 Claesson, H., 1982, Leukotrienes A_4 and B_4 stimulate the formation of cyclic AMP in human leukocytes, FEBS Lett., 139, 305.

49 Claesson, H.E. and E.J.Feinmark, 1984, Relationship of cyclic-AMP levels in leukotriene B_4-stimulated leukocytes to lysosomal enzyme release and the generation of superoxide anions, Biochim. Biophys. Acta, 804, 52.

50 Clark, R.B., J.Chiba, S.E.Zweig and E.M.Shevach, 1982, T cell colonies recognize antigen in association with specific epitopes on Ia molecules, Nature, 294, 412.

51 Codina, J., J.Hildebrandt, T.Sunyer, R.D.Sekura, C.R.Manclark, R.Syengan and L.Birnbaumer, 1984, Mechanisms in the vectorial receptor-adenylate cyclase signal transduction, In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 17, (eds. P.Greengard, G.A.Robinson, R.Paoletti and S.Nicosia) Raven Press, New York, p.111.

52 Cohen, P., C.B.Klee, C.Picton and S.Shenolikar, 1980, Calcium control of muscle phosphorylase kinase through the combined action of calmodulin and troponin, Ann. N.Y. Acad. Sci., 356, 151.

53 Cohen, P., 1982, The role of protein phosphorylation in neural and hormonal control of cellular activity, Nature, 296, 613.

54 Cohn, Z.A. and E.Wiener, 1963, The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion, J. Exp.

Med., 118, 1009.

55 Conlon, P.J., C.S.Henney and S.Gillis, 1982, Cytokine-dependent thymocyte response: Characterization of IL-1 and IL-2 target subpopulations and mechanisms of action, J. Immunol., 128, 797.

56 Connolly, K.M. and A.M.Kaplan, 1981, In vivo reduction of tumour cell DNA content by pyran, *C. parvum* and 5-fluor-uracil, Fed. Proc. 40, 761.

57 Connolly, K.M., K.D.Elgert, J.M.Conroy, S.N.Vogel and R.B.Rutherford, 1983, Immunoregulation of the mixed lymphocyte reaction by macrophage-derived factors: Functional and biochemical separation of enhancing and inhibitory factors, Immunobiol., 164, 144.

58 Crutchley, D.J., 1984, Effects of inhibitors of arachidonic acid metabolism on thromboplastin production in human monocytes, Biochem. Biophys. Res. Commun., 119, 179.

59 Currie, G.A., 1978, Activated macrophages kill tumour cells by releasing arginase, Nature, 273, 758.

60 Cuthbert, N.J. and P.J.Gardiner, 1983, Leukotriene induced release of cyclooxygenase products from the guinea-pig trachea, In: Leukotrienes and Other Lipoxygenase Products (ed. P.J.Piper), Wiley, Chichester, p.290.

61 Daems, W.T., 1980, Peritoneal macrophages, In: The Reticuloendothelial System, Vol. 1, (eds. I.Carr and W.T.Daems), Plenum Publishing Corporation, p.57.

62 Dahlen, S., G.Hansson, P.Hedqvist, T.Bj/rck, E.Granström and B.Dahlen, 1983, Allergen challenge of lung tissue from asthmatics elicits a bronchial contraction that correlates with the release of leukotrienes C_4, D_4 and E_4. Proc. Natl. Acad. Sci. USA, 80, 1712.

63 Damiani, G., C.Kyotaki, W.Soella, M.Sasada, J.Peiach and B.R.Bloom, 1980, Macrophage variants in oxygen metabolism, J. Exp. Med., 152, 808.

64 Damon, M., C.Chavis, Ph.Godard, F.B.Michel and A.Crastes de Paulet, 1983, Purification and mass spectrometry identification of leukotriene D_4 synthesized by human alveolar macrophages, Biochem. Biophys. Res. Commun., 111, 518.

65 Davies, P. and R.J.Bonney, 1980, The secretion of hydrolytic enzymes of mononuclear phagocytes, In: The Cell Biology of Inflammation (ed. G.Weismann), Elsevier/North Holland Biomedical Press, Amsterdam, p.479.

66 Dempsey, R.A., C.A.Dinarello, J.W.Mier, L.J.Rosenwasser, M.A.Uegretta, T.E.Brown and D.R.Parkinson, 1982, The differential effects of human leukocytic pyrogen/lymphocyte-activating factor, T cell growth factor and interferon on human natural killer activity, J. Immunol., 129, 2504.

67 Diez, J., P.Braquet, C.Nazaret, P.Hannaert, R.Vema and R.Garay, 1984, The effects of cyclic nucleotides and eicosanoids on Na^+ and K^+ transports systems in human red cells and mouse macrophages, In: Advances in Cyclic Nucleotide and Protein Phophorylation Research, vol.17 (eds. P.Greengard, G.A.Robinson, R.Paoletti and S.Nicosia), Raven Press, New York, p.621.

68 Dinarello, C.A., S.O.Marnoy and L.J.Rosenwasser, 1983, Role of arachidonate metabolism in the immunoregulatory function of human leukocytic

release using resident an elicited macrophages, submitted to Br. J. Pharmacol.

83 Euler, U.S. von, 1934, Zur Kenntnis der pharmakologischen Wirkungen von Nativesekreten und Extrakten männlicher aksessorischer Geslechtsdrüsen, Naunyn Schmiedb. Arch. Exp. Path. Pharmak., 175, 78.

84 Farrar, J.J., J.Mizel, S.B.J.Fuller-Bonar, M.L.Hilfiker and W.L.Farrar, 1980, Lipopolysaccharide mediated adjuvanticity: Effect of lipopolysaccharide on the production of thymus derived growth factor (interleukin-2). In: Microbiology (ed. D.Schlesinger), American Society of Microbiology, Washington D.C., p.36.

85 Farrar, W.L., H.M.Johnson and J.J.Farrar, 1981, Regulation of the production of immune interferon and cytotoxic T lymphocyte by interleukin-2, J.Immunol. 126, 1125.

86 Feldberg, W. and C.H.Kellaway, 1938, The formation of lysolecithin and of a muscle-stimulating substance by snake venoms, J. Physiol., 94, 232.

87 Fels, A.O.S., N.A.Pawlowski, E.B.Cramer, T.K.C.King, Z.A.Cohn and W.A.Scott, 1982, Human alveolar macrophages produce leukotriene B_4, Proc. Natl. Acad. Sci. USA, 79, 7866.

88 Feuerstein, N., J.A.Bash, J.N.Woody and P.W.Ramwell, 1981, Leukotriene C stimulates prostaglandin release from rat peritoneal macrophages, Biochem. Biophys. Res. Commun., 100, 1085.

89 Feuerstein, N., M.Foegh and P.W.Ramwell, 1981, Leukotrienes C_4 and D_4 induce prostaglandin and thromboxane release from rat peritoneal macrophages, Br. J. Pharmacol., 72, 389.

90 Feuerstein, N. and P.W.Ramwell, 1984, Differential effects of prostaglandin E_2 and cyclic AMP on release of arachidonic acid metabolites from resting and lipoplysaccharide-stimulated macrophages, Br. J. Pharmacol., 83, 759.

91 Fleisch, J.H., L.E.Rinkema and S.R.Baku, 1982, Evidence for multiple leukotriene D_4 receptors in smooth muscle. Life Sci., 31, 377.

92 Flower, R.J. and G.J.Blackwell, 1979, Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation, Nature, 278, 456.

93 Flower, R.J., 1984, Macrocortin and the antiphospholipase proteins, In: Advances in Inflammation Research, vol.8 (ed. J.Weissmann), Raven Press, New York, p.

94 Ford-Hutchinson, A.W., M.A.Bray, M.V.Doig, M.E.Shipley and M.J.H.Smith, 1980, Leukotriene B: A potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes, Nature, 286, 264.

95 Ford-Hutchinson, A.W., 1983, The role of leukotriene B_4 as a mediator of leukocyte function, Agents and Actions, suppl.12, 154.

96 Ford-Hutchinson, A.W. and A.Rackam, 1983, Leukotrienes as mediators of skin inflammation, Br. J. Dermatol., 109, 26.

97 Ford-Hutchinson, A.W., 1985, Leukotrienes: Their formation and role as inflammatory mediators, Fed. Proc., 44, 25.

98 Furth, R. van, 1981, Current view of the mononuclear phagocyte system. In: Haematology and Blood Transfusion, vol.27, (eds. F.Schmalz, D.Huhn and H.E.Schaefer), Springer Verlag, Berlin, Heidelberg, New York, p.3.

99 Geier, S.S. and P.Cresswell, 1980, Monocytes as acessory cells in the human mixed lymphocyte response: Lack of requirement of high cell densities, Cell.Immunol., 49, 190.

100 Gemsa, D., H.-G.Leser, M.Seitz, W.Deimann and E.Bärlin, 1982, Membrane perturbation and stimulation of arachidonic acid metabolism, Molec. Immunol., 19, 1287.

101 Gemsa, D., H.-G.Leser, M.Seitz, M.Debatin, E.Bärlin, W.Deimann and W.Kramer, 1982, The role of macrophages in inflammation. In: Cells in Inflammation (eds. M.J.Parnham and J.Winkelman), Agents and Actions, Suppl. vol.11, Birkhäuser Verlag, Basel Boston Stuttgart, p.93.

102 Gery, I. and B.H.Waksman, 1972, Potentiation of the T lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s), J. Exp. Med., 136, 143.

103 Godfraind, T., 1984, Calcium modulators: Tools for cell biology, In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 17 (eds. P.Greengard, G.A.Robinson, R.Paoletti and S.Nicosia) Raven Press, New York, p.551.

104 Goetzl, E.J. and L.I.Brindley, 1985, Enhancement of human neutrophil adherence by synthetic leukotrienes constituents of the slow-reacting substance of anaphylaxis, Immunol.

105 Goldyne, M.E. and J.D.Stobo, 1981, Immunoregulatory role of prostaglandins and related lipids, CRC Crit. Rev. Immun., 2, 189.

106 Goldyne, M.E. and J.D.Stobo, 1983, T-cell derived arachidonic acid and eicosanoid synthesis by macrophages. In: Advances in Prostaglandin, Thromboxane and Leukotriene Research (eds. B.Samuelsson, R.Paoletti and P.W.Ramwell), Raven Press, New York, p.39.

107 Gomard, E., T.Wybier-Franqui and T.P.Levy, 1981, Accessory cells in the in vitro generation of type C virus specific T killer lymphocytes. I. Role of macrophages in primary anti-FMR reaction, J. Immunol., 126, 891.

108 Gordon, S., J.Todd and Z.A.Cohn, 1974, In vitro synthesis and secretion of lysozyme by mononuclear phagocytes, J. Exp. Med., 139, 1228.

109 Gordon, S., 1980, Lysozyme and plasminogen activator: Constitutive and induced secretory products of mononuclear phagocytes. In: Mononuclear Phagocytes. Functional Aspects, (ed. R.van Furth), Nyhoff, The Hague, p.1273.

110 Gorman, R.R., W.Wieringa and D.V.Miller, 1979, Independence of the cyclic AMP-lowering activity of thromboxane A_2 from the platelet-release reaction. Biochim. Biophys. Acta, 572, 95.

111 Gorman, R.R., A.L.Lim and N.K.Hopkins, 1984, Acetylglycerylether phosphorylcholine-(AGEPC) and leukotriene B_4 stimulated cyclic AMP levels in human polymorphonuclear leukocytes. In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 17 (eds. P.Greengard,

G.A.Robinson, R.Paoletti and S.Nicosia), Raven Press, New York, p.631.

112 Cowen, M., D.D.Wood, E.J.Shrie, J.E.Meats and R.G.G.Russell, 1985, Stimulation by human interleukin-1 of cartilage breakdown and production of collagenase and proteoglycanase by human chondrocytes but not by human osteoblasts in vitro, Biochim. Biophys. Acta, .

113 Greaves, M.W., 1980, Neutrophil polymorphonuclears, mediators and the pathogenesis of psoriasis, Br. J. Dermatol., 109,115.

114 Griffin, F.M., J.A.Griffin and S.C.Silverstein, 1976, Studies of the mechanism of phagocytosis. II. The interaction of macrophages with anti-immunoglobulin SgG-coated bone marrow-derived lymphocytes, J. Exp. Med., 144, 788.

115 Hagmann, J. and P.H.Fishman, 1980, Modulation of adenylate cyclase in intact macrophages by microtubules, J. Biol. Chem., 225, 2659.

116 Ham, E.A., D.D.Soderman, M.E.Zanetti, H.W.Dougherty, E.McCauley and F.A.Kuehl Jr., 1983, Inhibition of prostaglandins of leukotriene B_4 release from activated neutrophils, Proc. Natl. Acad. Sci. USA, 80, 4349.

117 Hamberg, M., J.Svensson and B.Samuelsson, 1975, Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides, Proc. Natl. Acad. Sci. USA, 72, 2994.

118 Hamilton, J.A., 1981, Regulation of prostaglandin and plasminogen activator production by mouse peritoneal macrophages, J. Reticuloendothel. Soc., 30, 115.

119 Hansen, N.E., 1974, Plasma lysozyme. A measure of neutrophil turn-over. An analytical review, Ser. Haematol., 7, 1.

120 Hanski, E., P.C.Sternweis, J.K.Northup, A.W.Dromerick and A.G.Gilman, 1981, The regulatory component of adenylate cyclase. Purification and properties of the turkey erythrocyte protein, J. Biol. Chem., 256, 12911.

121 Hartung, H.P., 1983, Leukotriene C_4 (LTC_4) elicits a respiratory burst in peritoneal macrophages. Eur. J. Pharmacol., 91, 159.

122 Heilmeyer, L.M.G., F.Meyer, R.H.Haschk and E.H.Fischer, 1970, Control of phosphorylase activity in a muscle glycogen particle. 2. Activation by calcium, J. Biol. Chem., 245, 6649.

123 Herman, J. and A.R.Rabson, 1984, Prostaglandin E_2 depresses natural cytotoxicity by inhibiting interleukin-1 production by large granular lymphocytes, Clin. Exp. Immunol., 57, 380.

124 Hibbs, J.B., L.H.Lambert and J.S.Remington, 1972, Possible role of macrophage mediated nonspecific cytotoxicity in tumour resistance, Nature, 235, 48.

125 Hibbs, J.B., R.R.Tanitor, H.A.Chagman and J.B.Weinberg, 1977, Macrophage tumour killing: Influence of the local environment, Science, 197, 279.

126 Hildebrandt, J.D., R.D.Sekura, J.Codina, R.Syengar, C.R.Manclark and L.Birnbaumer, 1983, Stimulation and inhibition of adenylyl cyclases mediated by distinct regulatory proteins, Nature, 302, 706.

127 Hirata, F., E.Schiffmann, K.Venkatasubramanian, D.Solomon and J.Axelrod, 1980, A phospholipase A_2 inhibitor protein in rabbit neutrophils induced by

glucocorticoids, Proc. Natl. Acad. Sci. USA, 77, 2533.

128 Hirata, F., K.Matsuda, Y.Notsu, T.Hattori and R.DelCarmine, 1984, Phosphorylation at a tyrosine residue of lipomodulin in nitrogen-stimulated murine thymocytes, Proc. Natl. Acad. Sci. USA, 81, 4717.

129 Holgate, S.T., R.A.Lewis and K.F.Austen, 1980, Role of adenylate cyclase in immunological release of mediators from rat mast cells. Agonist and antagonist effects of purine- and ribose-modified adenosine analogs. Proc. Natl. Acad. Sci. USA. 77, 6800.

130 Hong, S.C.L. and L.Levine, 1976, Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. Proc. Natl. Acad. Sci. USA, 73, 1730.

131 Hopper, K.E. and J.M.Cahill, 1983, Immunoregulation by macrophages. II. Separation of mouse peritoneal macrophages having tumoricidal and bactericidal activities and those recruting PGE and interleukin-1, J. Reticuloendothel. Soc., 33, 443.

132 Hsueh, W., U.Desai, F.Gonzalez-Crussi, R.Lamb and A.Chu, 1981, Two phospholipase pools for prostaglandin synthesis in macrophages, Nature, 290, 710.

133 Hsueh, W., R.L.Jordan, H.H.Harrison and M.A.Cobb, 1983, Serum and plasma stimulate prostaglandin production by alveolar macrophages, Prostaglandins, 55, 793.

134 Huber, H. and G.Holm, 1975, Surface receptors of mononuclear phagocytes: Effect of immune complexes on in vitro function in human monocytes. In: Mononuclear Phagocytes in Immunity, Infection and Pathology, Blackwell, Oxford London Edinburgh Melbourne, p.291.

135 Huber, C. and G.Stingl, 1981, Macrophages in the regulation of immunity. In: Haematology and Blood Transfusion, vol.27, (eds. F.Schmalz, D.Huhn and H.E.Schaefer), Springer Verlag, Berlin Heidelberg New York, p.31.

136 Humes, J.L., S.Burger, M.Galavage, F.A.Kuehl Jr., P.D.Wightman, M.E.Dahlgren, P.Davies and R.J.Bonney, 1980, The diminished production of arachidonic acid oxygenation products by elicited mouse peritoneal macrophages: possible mechanisms, J. Immunol., 124, 2110.

137 Humes, J.L., S.Sadowski, M.Galavage, M.Goldenberg, E.Subers, R.J.Bonney and F.A.Kuehl Jr., 1982, Evidence for two sources of arachidonic acid for oxidative metabolism by mouse peritoneal macrophages, J. Biol. Chem., 275, 1591.

138 Irvine, R.F., 1982, How is the level of free arachidonic acid controlled in mammalian cells, Biochem. J., 204, 3.

139 Iyengar, R., 1981, Hysteretic activation of adenylyl cyclase. II. Mg ion regulation of the activation of the regulatory component as analyzed by reconstitution, J. Biol. Chem., 256, 11042.

140 Jakobs, K.H. and G.Schultz, 1980, Actions of hormones and neurotransmitters at the plasma membrane inhibition of adenylate cyclase, TIPS, 1, 313.

141 Johnson, H.G., M.L.McNee, M.A.Johnson and M.D.Miller, 1983, Leukotriene C_4 and dimethylpiperazium-induced response in canine airway tracheal muscle

contraction and fluid secretion, Int. Arch. Allergy Appl. Immunol., 71, 214.

142 Johnson, R.A., D.R.Morton, J.H.Kinner, R.R.Gorman, J.C.McGuire, F.F.Sun, N.Whittaker, S.Bunting, J.Salmon, S.Moncada and J.R.Vane, 1976, The chemical structure of prostaglandin X (Prostacyclin), Prostaglandins, 12, 915.

143 Johnson, W.J., P.A.Marino, R.D.Schrieber and D.O.Adams, 1983, Sequential activation of murine mononuclear phagocytes for tumor cytolysis: Differential expression of markers by macrophages in the several stages of development, J. Immunol., 13, 1038.

144 Johnston, R.B.Jr., 1981, Enhancement of phagocytosis-associated oxidative metabolism as a manifestation of macrophage activation, Lymphokines, 3, 33.

145 Kammer, G.M. and E.R.Unanue, 1980, Accessory cell requirement in the proliferation response of T lymphocytes to hemocyanin, Clin. Immunol. Immunopathol., 15, 434.

146 Kampschmidt, R.F., 1982, Leukocytic endogenous mediator/endogenous pyrogen. In: Infection: The Physiologic and Metabolic Responses of the Host, (eds. M.C.Powanda and P.G.Canonics), Elsevier, New York, p.505.

147 Katz, D.R., G.H.Sunshine, M.Feldman and P.Erb, 1984, Role of the reticuloendothelial system in T-helper cell induction. In: The Reticuloendothelial System, a comprehensive treatise, vol.6 (eds. J.A.Bellanti and H.B. Herscowitz), p.201.

148 Klebanoff, S.J. and C.B.Hamon, 1975, Antimicrobial systems of mononuclear pnagocytes. In: Mononuclear Phagocytes in Immunity, Infection and Pathology, Blackwell, Oxford London Edingburgh Melbourne, p.507.

149 Klebanoff, S.J. and R.A.Clark, 1978, The neutrophil: Function and clinical disorders, Elsevier/North-Holland Biomedical Press, Amsterdam New York Oxford.

150 Klee, C.B., T.H.Crouch and P.G.Richman, 1980, Calmodulin, Ann. Rev. Biochem., 49, 489.

151 Klempner, M.S., C.A.Dinarello, W.R.Henderson and J.S.Gallin, 1979, Stimulation of neutrophil oxygen-dependent metabolism by human leukocytic pyrogen, J. Clin. Invest., 64, 99.

152 Koopman, W.J., J.J.Farrar and J.Fuller-Bonar, 1978, Evidence for the identification of lymphocyte activating factor as the adherent cell-derived mediator responsible for enhanced antibody synthesis by nude mouse spleen cells, Cell. Immunol., 35, 92.

153 Krahenbuhl, J.L., J.S.Remington and R.McLeod, 1980, Cytotoxic and microbicidal properties of macrophages. In: Mononuclear Phagocytes: Functional aspects, (ed. R.van Furth), Martinus Nijhoff, The Hague, p.1631.

154 Kranias, E.G. and R.J.Solaro, 1982, Phosphorylation of troponin-1 and phospholamban during catecholamine stimulation of rabbit heart, Nature, 298, 182.

155 Kuehl, F.A.Jr. and R.W.Egan, 1980, Prostaglandins, arachidonic acid and

inflammation, Science, 210, 978.

156 Kunkel, S.L., S.W.Chensue, M.Plewa and G.I.Higashi, 1984, Macrophage function in the *Schistosomo mansoni* egg-induced pulmonary granuloma. Role of arachidonic acid metabolites in macrophage Ia antigen expression, Am. J. Pathol., 114, 240.

157 Kunkel, S.L., S.W.Chensue, J.C.Fantone and P.A.Ward, 1985, Role of prostaglandins in macrophage cell function, In: The Reticuloendothelial System, vol.6 (eds. J.A.Bellanti and H.B.Herscowitz), p.289.

158 Kurland, J.I., J.W. Hadden and M.A.S. Moore, 1977, Role of cyclic nucleotides in the proliferation of committed granulocyte-macrophage progenitor cells. Cancer Res., 37, 4534.

159 Lappin, D.F. and K.Whaley, 1982, Prostaglandins and prostaglandin synthetase inhibitors regulate the synthesis of complement components by human monocytes, Clin. Exp. Immunol., 49, 623.

160 Leblanc, Y., B.J.Fiksimmons, J.Adams and J.Rokach, 1985, Total synthesis of lipoxin B. Assignment of stereochemistry, Tetrahedron Lett., 26, 1399.

161 Lewis, R.A., J.M.Drazen, K.F.Austen, D.A.Clark and E.J.Corey, 1980, Identification of the C(6)-S-conjugate of leukotriene A with cysteine as a naturally occuring slow-reacting substance of anaphylaxis (SRS-A). Importance of the 11-cis geometry for biological activity, Biochem. Biophys. Res. Commun., 96, 271.

162 Lim, L.M., N.H.Hunt, T.Evans and M.J.Weidemann, 1981, Rapid changes in the activities of the enzymes of cyclic AMP metabolism after addition of A23187 to macrophages, Biochem. Biophys. Res. Commun., 103, 745.

163 Lim, L.K., N.H.Hunt, R.D.Eichner and M.J.Weidemann, 1983, Cyclic AMP and the regulation of prostaglandin production by macrophages, Biochem. Biophys. Res. Commun., 114, 248.

164 Lim, L.K., N.H.Hunt and M.J.Weidemann, 1983, Reactive oxygen production, arachidonate metabolism and cyclic AMP in macrophages, Biochem. Biophys. Res. Commun., 114, 549.

165 Lipsky, P.E., P.A.Thompson, L.J.Rosenwasser and C.A.Dinarello, 1983, The role of interleukin-1 in B-cell activation: Inhibition of B-cell proliferation and the generation of immunoglobulin-secreting cells by an antibody against human leukocytic pyrogen, J. Immunol., 130, 2708.

166 Lohmann-Matthess, M.-L., 1981, The macrophage as a cytotoxic effector cell. In: Haematology and Blood Transfusion, Disorders of the monocyte macrophage system, vol.27, (eds. F.Schmalz, D.Huhn and H.E.Schaefer), Springer Verlag, Berlin Heidelberg New York, p.23.

167 Maizel, A.L., S.R.Mehta, S.Hauft, D.Franzini, L.B.Lachman and R.J.Ford, 1981, Human T lymphocyte/monocyte interaction in response to lectin: Kinetics of entry into the S phase, J. Immunol., 127, 1058.

168 McDevitt, H.O., B.D.Deak, D.C.Streffer, J.Klein, J.H.Strimpling and G.D.Snell, 1972, J. Exp. Med., 135, 1259.

169 McMillan, R., D.E.MacIntyre, J.E.Beesly and J.L.Gordon, 1980, Regulation of macrophage lysosomal enzyme secretion: Role of arachidonate metabolites,

divalent cations and cyclic AMP, J. Cell. Sci., 44, 299.

170 Meade, C.J., J.Harvey, J.R.Boot, G.A.Turner, P.E.Bateman and D.J.Osborne, 1984, γ-hexachlorocyclohexane stimulation of macrophage phospholipid hydrolysis and leukotriene production, Biochem. Pharmacol., 33, 289.

171 Meltzer, M.S., 1981, Tumoricidal cytotoxicity by lymphokine-activated macrophages: Development of macrophage tumoricidal activity requires a sequence of reactions, Lymphokines, 3, 319.

172 Metchnikoff, E., 1905, Immunity to Infectious Diseases, Cambridge University Press, New York.

173 Michell, R.H. and C.J.Kirk, 1981, Why is phosphatidylinositol degraded in response to stimulation of certain receptors, TIPS, 2, 87.

174 Mishell, R.I., J.M.Shiigi, B.B.Mishell, K.H.Grabstein and S.M.Shiigi, 1980, Prevention of the immuno-suppressive effects of glucocorticosteroids by cell-free factors from adjuvant activated accessory cells, Immunopharmacol., 2, 233.

175 Mishell, R.I., D.A.Lee, K.H.Grabstein and L.B.Lachman, 1982, Prevention of the in vitro immuno-suppressive effects of glucocorticosteroids by interleukin-1 (IL-1), J. Immunol., 128, 1614.

176 Mochizuki, M., J.S.Siegler, P.Russel and I.Gery, 1984, Cytostatic and cytolytic activities of macrophages, regulation by prostaglandins, Cell. Immunol., 83, 34.

177 Molski, T.F.P., P.H.Naccache, P.Borgeat and R.I.Sha'afi, 1981, Similarities in the mechanisms by which formyl-methionyl-leucyl-phenylalanine, arachidonic acid and leukotriene B_4 increase calcium and sodium influxes in rabbit neutrophils, Biochem. Biophys. Res. Commun., 103, 227.

178 Moqbel, R., S.P.Sass-Kuhn, E.J.Goetzl and A.B.Kay, 1983, Enhancement of neutrophil- and eosinophil-mediated complement-dependent killing of schistosomula of *Schistosoma mansoni* in vitro by leukotriene B_4, Clin. Exp. Immunol., 52, 519.

179 Morris, H.R., G.W.Taylor, P.J.Piper and J.R.Tippins, 1980, Structure of slow-reacting substance of anaphylaxis from guinea pig lung, Nature, 285, 104.

180 Mullen, P.W., 1980, Recent developments in immunopharmacology, Trends Pharmacol. Sci., 1, 352.

181 Munthe-Kars, A.C., 1980, The accessory cell effects of liver macrophages in concanavalin A stimulation of rat spleen lymphocytes, Scand. J. Immunol. 11, 523.

182 Murphy, R.C., S.Hammarström and B.Samuelsson, 1979, Leukotriene C: A slow reacting substance from murine mastocytoma cells, Proc. Natl. Acad. Sci. USA, 76, 4275.

183 Nacy, C.A., C.N.Oster, S.L.James and M.S.Meltzer, 1984, Activation of macrophages to kill *Rickettsia* and *Leishmania*: Dissociation of intracellular micrbicidal activities and extracellular destruction of neoplastic and helminth targets, In: Contemporary Topics in Immunobiology (eds. D.O.Adams and M.G.Hanna Jr.), Plenum Press, New York and London,

p.147.

184 Nagy, L., T.H.Lee, E.J.Goetzl, W.E.Pickett and A.B.Kay, 1982, Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products, Clin. Exp. Immunol., 47, 541.

185 Nathan, C.F., L.H.Brukner, G.Kaplan, J.Unkeless and Z.S.Cohn, 1980, Role of activated macrophages in antibody-dependent lysis of tumour cells, J. Exp. Med., 152, 183.

186 Nelson, D.S., 1976, Immunobiology of the macrophage, Academic Press, New York.

187 Nitta, T. and T.Suzuki, 1982, Biochemical signals transmitted by Fc_b receptors: Triggering mechanisms of the increased synthesis of adenosine-3',5'-cyclic monophosphate mediated by $Fc_{\gamma 2a}$-and $Fc_{\gamma 2b}$-receptors of a murine macrophage-like cell ($P388D_1$). J. Immunol., 129, 2708.

188 Northup, J.K., M.D.Smigel and A.G.Gilman, 1982, The guanine nucleotide activating site of the regulatory component of adenylate cyclase, J. Biol. Chem., 257, 11416.

189 Opmeer, F.A., M.J.P.Adolfs and I.L.Bonta, 1983, Prostaglandin E_2 competes for adenyl cyclase coupled binding sites of (^3H) prostacyclin in rat peritoneal macrophages. Prostaglandins, 26, 467.

190 Oppenheim, J.J. and I.Gery, 1982, Interleukin-1 is more than an interleukin, Immunol. Today, 3, 113.

191 Oropeza-Rendon, R.L., V.Speth, G.Hiller, K.Webber and H.Fischer, 1979, Prostaglandin E_1 reversibly induces morphological changes in macrophages and inhibits phagocytosis, Exptl. Cell. Res., 119, 365.

192 Oropeza-Rendon, R.L., M.Ernst, U.Schade, E.T.Rietschel and H.Fischer, 1981, Activation of bone marrow-derived macrophages by repeated zymosan phagocytosis leads to enhanced prostaglandin synthesis, Immunobiol., 60, 208.

193 Ouwendijk, R.J.Th., F.J.Zijlstra, A.M.W.C.. van den Broek, A.Brouwer, J.H.P.Wilson and J.E.Vincent, Comparison of the production of eicosanoids by human and rat peritoneal macrophages and rat Kupffer cells, Prostaglandins, submitted for publication.

194 Palazios, R. and G.Moller, 1981, 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.

195 Palmer, R.M.J., R.J.Stepney, G.A.Higgs and K.E.Eakins, 1980, Chemotactic activity of arachidonic acid lipoxygenase products on leukocytes of different species, Prostaglandins, 20, 411.

196 Parnham, M.J., I.L.Bonta and M.J.P.Adolfs, 1979, Distinction between prostaglandin E_2 and prostacyclin as inhibitors of granulomatous inflammation, J. Pharm. Pharmacol., 31, 565.

197 Parnham, M.J., C.Bittner and J.Winkelmann, 1983, Chemiluminescence from mouse resident macrophages: Characterization and modulation by arachidonate metabolites, Immunopharmacology, 5, 277.

198 Pennica, D., G.E.Nedwin, J.S.Hayflick, P.H.Seeburg, R.Derynck, M.A.Palladino, W.J.Kohr, B.B.Aggarwal and D.V.Goeddel, 1984, Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature, 312, 724.

199 Pierce, C.W., 1984, Requirements for macrophages in primary and secondary humoral responses. In: The Reticuloendothelial System, a comprehensive treatise, vol.6 (eds. J.A.Bellanti and H.B.Herscowitz), p.157.

200 Poplack, D.G., D.E.Cole and U.Srinivasan, 1984, Monocyte-mediated antibody-dependent cellular cytotoxicity. In: The Reticuloendothelial System, a comprehensive treatise, vol.6 (eds. J.A.Bellanti and H.B.Herscowitz), p.303.

201 Postlethwaite, A.E., L.B.Lachman, C.L.Mainardi and A.H.Kang, 1983, Interleukin-1 stimulation of collagenase production by cultured fibroblasts, J. Exp. Med., 157, 801.

202 Puri, J., M.Shinitzky and P.Lonai, 1980, Concomitant increase in antigen binding and in T cell membrane lipid viscosity induced by the lymphocyte-activating factor, LAF, J. Immunol., 124, 1937.

203 Rackam, A. and A.W.Ford-Hutchinson, 1983, Inflammation and pain sensitivity: Effects of leukotrienes D_4, B_4 and prostaglandin E in the rat paw, Prostaglandins, 25, 193.

204 Rae, S.A. and M.J.H.Smith, 1981, The stimulation of lysosomal enzyme secretion from human polymorphonuclear leukocytes by leukotriene B_4, Br. J. Pharmacol., 33, 616.

205 Rae, S.A., E.M.Davidson and M.J.H.Smith, 1982, Leukotriene B_4, an inflammatory mediator in gout, The Lancet, Nov. 20, 1122.

206 Rankin, J.A., M.Hitchcock, W.W.Merrill, S.S.Huang, J.R.Brashler, M.K.Bach and P.W.Askenase, 1984, IgE immune complexes induce immediate and prolonged release of leukotriene C_4 (LTC_4) from rat alveolar macrophages, J. Immunol., 132, 1999.

207 Rodbell, M., L.Birnbaumer, S.L.Pohl and H.M.J.Krans, 1971, Glucagon sensitive adenyl cyclase system in plasma membranes of rat liver. 5. Obligatory role of guanyl nucleotides in glucagon action, J. Biol. Chem., 246, 1877.

208 Rola-Pleszczynski, M., L.Gagnon and P.Sirois, 1983, Leukotriene B_4 induces human suppressor lymphocytes, Biochem. Biophys. Res. Commun., 108, 15.

209 Rola-Pleszczynski, M., L.Gagnon and P.Sirois, 1983, Leukotriene B_4 augments human natural cytotoxic cell activity, Biochem. Biophys. Res. Commun., 113, 531.

210 Rollins, T.E. and W.L.Smith, 1979, Subcellular localization of prostaglandin-forming cyclooxygenase in Swiss mouse 3T3 fibroblasts by electron microscopic immunocytochemistry, J. Biol. Chem., 255, 4872.

211 Rosenberg, S.A., F.S.Ligler, V.Ugolini and P.E.Lipsky, 1981, A monoclonal antibody that identified human peripheral blood monocytes recognizes the accessory cells required for mitogen-induced T lymphocyte proliferation, J. Immunol., 126, 1473.

212 Rosenberg, S.A. and P.E.Lipsky, 1981, The role of monocytes in pokeweed mitogen-stimulated B cell activation: Separate requirements for intact monocytes and a soluble monocyte factor, J. Immunol., 126, 1341.

213 Rosenwasser, L.J. and C.A.Dinarello, 1981, Ability of human leukocytic pyrogen to enhance phytohemagglutinin induced murine thymocyte proliferation, Cell. Immunol., 63, 134.

214 Ross, E.M. and A.G.Gilman, 1977, Resolution of some components of adenylate cyclase necessary for catalytic activity, J. Biol. Chem., 252, 6966.

215 Ross, G.D. and S.L.Newman, 1984, Regulation of macrophage functions by complement, complement receptors and IgG-Fc receptors. In: The Reticuloendothelial System, a comprehensive treatise, vol.6 (eds. J.A.Bellanti and H.B.Herscowitz), p.173.

216 Rouveix, B., P.Badenoch-Jones, S.Larno and J.L.Turk, 1980, Lymphokine-induced macrophage aggregation: The possible role of cyclic nucleotides, Immunopharmacology, 2, 319.

217 Rouzer, C.A., W.A.Scott, A.L.Hamill and Z.A.Cohn, 1982, Synthesis of leukotriene C and other arachidonic acid metabolites by mouse pulmonary macrophages, J. Exp. Med., 155, 720.

218 Saad, M.H., M.A.Wilson and K.F.Burka, 1983, Antigen and ionophore induced release of leukotriene C_4 from guinea pig trachea. In: Leukotrienes and Other Lipoxygenase Products (ed. P.J. Piper) Wiley, Chichester, p.283.

219 Samuelsson, B., M.Goldyne, E.Granström, M.Hamberg, S.Hammarström and C.Malmsten, 1978, Prostaglandins and thromboxanes, Ann. Rev. Biochem., 47, 997.

220 Samuelsson, B., P.Borgeat, S.Hammarström and R.C.Murphy, 1980, Leukotrienes: A new group of biologically active compounds, In: Advances in Prostaglandin and Thromboxane Research, vol. 6, (eds. B.Samuelsson, P.W.Ramwell and R.Paoletti) Raven Press, New York, p.1.

221 Samuelsson, B., 1985, Leuktrienes and related compounds, In: Advances in Prostaglandin, Thromboxane and Leukotriene Research, in press.

222 Satoh, J., H.Rikiishi, M.Nagahashi, E.Ohuchi and E.Kumagai, 1980, Mitogen responsiveness of various immune tissues: Heterogenity of accessory cells and susceptibility to suppression by macrophages, Cell. Immunol., 56, 1.

223 Saunder, D.N., N.L.Mounessa, S.I.Katz, C.A.Dinarello and J.S.Gallin, 1983, Chemotaxic properties of human ETAF/LP/IL-1, Fed. Proc., 42, 449.

224 Schnyder, J. and M.Baggiolini, 1978, Secretion of lysosomal hydrolases by stimulated and non-stimulated macrophages, J. Exp. Med., 148, 435.

225 Schnyder, J., 1981, Biochemical properties of human and murine mononuclear phagocytes and their changes on activation. In: Haematology and Blood Transfusion, vol.27, Disorders of the Monocyte Macrophage System (eds. F.Schmalz, D.Huhn and H.E.Schaefer), Springer Verlag, Berlin Heidelberg New York, p.23.

226 Schnyder, J., B.Dewald and M.Baggiolini, 1981, Effects of cyclooxygenase inhibitors and prostaglandin E_2 on macrophage activation in vitro, Prostaglandins, 22, 411.

227 Schrader, J.W., 1973, Mechanism of activation of the bone marrow-derived lymphocyte. III. A distinction between a macrophage-produced triggering signal and the amplifying effect on triggered B lymphocytes of allogeneic interaction, J. Exp. Med., 138, 1411.

228 Schultz, R.M., N.A.Pavlidis, W.A.Stylos and M.A.Chirigos, 1978, Regulation of macrophage tumoricidal function: A role for prostaglandins of the E series, Science, 202, 320.

229 Schultz, R.M., N.A.Pavlidis, J.N.Stoychkov and M.A.Chirigos, 1979, Prevention of macrophage tumoricidal activity by agents known to increase cellular cyclic AMP. Cell Immunol., 42, 71.

230 Scott, W.A., N.A.Pawlowski, H.W.Murray, M.Andreach, J.Zrike and Z.A.Cohn, 1982, Regulation of arachidonic acid metabolism by macrophage activation, J. Exp. Med., 155, 1148.

231 Scott, W.A., C.A.Rouzer and Z.A.Cohn, 1983, Leukotriene C release by macrophages, Federation Proc., 42, 129.

232 Serhan, C.-N., J.Fridovich, E.J.Goetzl, P.B.Dunham and G.Weissmann, 1982, Leukotriene B_4 and phosphatidic acid are calcium ionophores. Studies employing arsenazo-III in liposomes. J. Biol. Chem. 257, 4746.

233 Serhan, C.-N., A.Radin, J.E.Smolen, H.Korchak, B.Samuelsson and G.Weissman, 1982, Leukotriene B_4 is a complete secretagogue in human neutrophils: a kinetic analysis, Biochem. Biophys. Res. Commun., 107, 1006.

234 Serhan, C.-N., M.Hamberg and B.Samuelsson, 1984, Trihydroxytetraenes- a novel series of compounds formed from arachidonic acid in human leukocytes, Biochem. Biophys. Res. Commun., 118, 943.

235 Sharon, P. and W.F.Stenson, 1983, Production of leukotrienes by colonic mucosa from patients with inflammatory bowel disease, Gastroenterology, 84, 1306.

236 Smigel, M., T.Katada, J.K.Northup, G.M.Bokoch, M.Ui and A.G.Gilman, 1984, Mechanisms of guanine nucleotide-mediated regulation of adenylate cyclase activity, In: Cyclic Nucleotide and Protein Phosphorylation Research, vol. 17, (eds. P.Greengard, G.A.Robinson, R.Paoletti and S.Nicosia) Raven Press, New York, p.111.

237 Smith, M.J.H., A.W.Ford-Hutchinson and M.A.Bray, 1980, Leukotriene B_4: A potential mediator of inflammation, J. Pharm. Pharmacol., 32, 517.

238 Snell, G.D., 1978, T cells, T cell recognition structures and the major histocompatibility complex, Immunol. Rev., 38, 3.

239 Snider, M.E., R.H.Fertel and B.S.Zwilling, 1983, Production of arachidonic acid metabolites by operationally defined macrophage subsets, Prostaglandins, 25, 491.

240 Snyder, D.S., D.J.Beller and E.R.Unanue, 1982, Prostaglandins modulate macrophage Ia antigen expression, Nature, 299, 163.

241 Souavannavong, V., L.Rimsky and A.Adams, In vitro response to sheep erythrocytes in macrophage depleted cultures. Restoration with interleukin-1 or a monokine from resident macrophages and stimulation by N-acetyl-muramyl-L-Alanyl-D-Isoglutamine (MDP), Biochem. Biophys. Res.

Commun., 114, 721.

242 Staedecker, M.J., J.Calderon, M.L.Karnovsky and E.R.Unanue, 1977, Synthesis and release of thymidine by macrophages, J. Immunol., 119, 1738.

243 Stevenson, H.C., P.J.Miller, M.J.Waxdal, B.F.Haynes, C.A.Thomas and A.S.Fanci, 1983, Interaction of pokeweed mitogen with monocytes in the initiation of the human lymphocyte activation process, Immunol., 49, 633.

244 Stringfellow, D.A., F.A.Fitzpatrick, F.F.Sun and J.C.McGuire, 1978, Prostacycline biosynthesis in activated, stimulated and normal mouse peritoneal cell populations, Prostaglandins, 16, 901.

245 Taffet, S.M., J.L.Pace and S.W.Russell, 1981, Lymphokine maintains macrophage activation for tumour cell killing by interfering with the negative regulatory effect of prostaglandin E_2, J. Immunol., 127, 121.

246 Taffet, S.M., T.E.Eurell and S.W.Russel, 1982, Regulation of macrophage mediated tumour cell killing by prostaglandins: comparison of the effects of PGE_2 and PGI_2, Prostaglandins, 22, 411.

247 Tanaka, Y., C.Kigotaki, H.Tanowitz and B.R.Bloom, 1981, Mechanism of killing *T. cruzi* by macrophage-like cell lines, Fed. Proc., 40, 760.

248 Ting, C.-C. and M.E.Hargrove, 1983, Activation of natural killer-derived cytotoxic T lymphocytes. I. Regulation by macrophages and prostaglandins, J. Immunol., 131, 1734.

249 Ueno, A., K.Tanaka and M.Katori, 1983, Detection of leukotriene C_4 and leukotriene D_4 in the exudate of rat carragenin-induced pleurisy, Prostaglandins, 26, 493.

250 Unanue, E.R., 1981, The regulatory role of macrophages in antigenic stimulation. Part two. Symbiotic relationship between lymphocytes and macrophages, Adv. Immunol., 31, 1.

251 Urban, J. and H.Schreiber, 1984, The surveillance role of various leukocytes in preventing the outgrowth of potentially malignant cells. In: Contemporary Topics in Immunobiology, vol.13 (eds. D.O.Adams and M.G.Hanna, Jr.), Plenum Press, New York London, p.225.

252 Van den Bosch, H., 1980, Intracellular phospholipases A, Biochim. Biophys. Acta, 604, 191.

253 Vane, J.R., 1971, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nature (New Biol.), 231, 232.

254 Verghese M.W. and R.Snyderman, 1983, Hormonal activation of adenylate cyclase in macrophage membranes is regulated by guanine nucleotides. J. Immunol., 130, 869.

255 Vogel, S.N., L.L.Weedon, J.J.Oppenheim and D.L.Rosenstreich, 1981, Defective Fc-mediated phagocytosis in C3H/HeJ macrophages.II.Correction by cAMP agonists, J. Immunol., 126, 441.

256 Waarde, D. van, 1976, Humoral regulation of monocytopoiesis, academic thesis. 257 Wahl, L.M., C.E.Olsen, A.L.Sandberg and S.E.Mergenhagen, 1977, Prostaglandin regulation of macrophage collagenase production, Proc. Natl. Acad. Sci. USA, 74, 4955.

258 Walsh, D.A., J.P.Perkins and E.G.Krebs, 1968, An adenosine

3',5'-monophosphate-dependent protein kinase from rabbit skeletal muscle. J. Biol. Chem., 243, 3763.

259 Watterson, D.M., F.Sharuf and T.C.Vanaman, 1980, Complete amino-acid sequence of the Ca^{2+}-dependent modulator protein (calmodulin) of bovine brain, J. Biol. Chem., 255, 962.

260 Weiss, J.W., J.H.Drazen, E.R.McFadden Jr., P.Weller, E.J.Corey, R.A.Lewis and K.F.Austen, 1983, Airway constriction in normal humans produced by inhalation of leukotriene D. Potency, time course, and effect of aspirin therapy, J. Am. Med. Assoc., 249, 2814.

261 Weissmann, G., P.Dukor and R.B.Zurier, 1971, Effects of cyclic AMP on release of lysosomal enzymes from phagocytes, Nature, 321, 131.

262 Weller, P.F., C.W.Lee, D.W.Foster, E.J.Corey, K.F.Austen and R.A.Lewis, 1983, Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: Predominant production of leukotriene C_4, Proc. Natl. Acad. Sci. USA, 80, 7626.

263 Welscher, H.D. and A.Cruchaud, 1978, Conditions for maximal synthesis of cyclic AMP by mouse macrophages in response to β-adrenergic stimulation, Eur. J. Pharmacol., 8, 180.

264 Wess, B., L.H.Greenberg and M.Blair Clark, 1984, Physiological and pharmaological modulation of the β-adrenergic receptor-linked adenylate cyclase system: supersensitivity and subsensitivity. In: Dynamics of Neurotransmitter Function (ed. I. Hanin) Raven Press, New York, p.319.

265 Wightman, P.D., J.L.Humes, P.Davies and R.J.Bonney, 1981, Identification and characterization of 2 phospholipase A_2 activities in resident mouse peritoneal macrophages, Biochem. J., 195, 427.

266 Williams, J.D., J.K.Czop and K.F.Austen, 1984, Release of leukotrienes by human monocytes on stimulation of their phagocytic receptor for particulate activators, J. Immunol., 132, 3034.

267 Wilson, J.H.P. and I.L.Bonta, 1985, Human peritoneal macrophages: clinical models of inflammation and potential targets of antiinflammatory drugs, Agents and Actions, in press.

268 Wirth, J.J. and F.Kierszenbaum, 1983, Inhibitory action of elevated levels of adenosine-3'5' cyclic monophosphate on phagocytosis: Effects on macrophage *Trypanosoma cruzi* interaction, J. Immunol., 129. 2759.

269 Wood, D.D., 1979, Mechanism of action of human B cell activating factor. I. Comparison of the plaque stimulating activity with thymocyte stimulating activity, J. Immunol., 123, 2400.

270 Wood, D.D. and M.J.Stamch, 1981, Control of the mitogeneicity of muramyl dipeptide, J. Immunopharmacol., 3, 31.

271 Wood, D.D., D.Hamerman, E.J.Shrie and L.Papovitch, 1983, Stimulation of synoviocyte glucosamino glycan production by synoviocyte-derived interleukin-1, Fed. Proc., 42, 1200.

272 Wood, D.D., 1984, Antigen-nonspecific factors elaborated by macrophages which stimulate lymphocytes, interleukin-1. In: The Reticuloendothelial System, a comprehensive treatise, vol.6 (eds. J.A.Bellanti and

H.B.Herscowitz), p.201.

273 Zijlstra, F.J., J.E.Vincent and I.L.Bonta, 1983. The effects of chloroquine and IBMX on the leukotriene D_4 induced contraction and thromboxane release of the guinea-pig lung parenchymal strip; comparison with the effect of phospholipase A_2. In: Leukotrienes and Other Lipoxygenase Products (ed. P.J.Piper), Wiley, Chichester, p.327.

274 Zijlstra, F.J. and J.E.Vincent, 1985, Leukotriene D_4 and phospholipase A_2 release thromboxane A_2 of different origin in the guinea pig lung parenchymal strip, Prostagl. Leukotr. Med., 17, 337.

APPENDIX: MATERIALS AND METHODS

Cell isolation and incubation.

All cells were isolated from male Wistar rats(175-200 g). Resident peritoneal cells were harvested by peritoneal lavage with 40 ml Gey's Balanced Salt Solution (GBSS). Elicited cells were harvested on the fourth day after an intraperitoneal injection with 2 mg carrageenan (1 mg/ml) (Marine Colloids Inc., USA) or 2 ml Freund's incomplete adjuvant (Difco Lab., USA). A cell population of more than 90% macrophages, as judged by differential counting of smears stained with May Grünwald Giemsa solution, was obtained by density gradient centrifugation (Lymphoprep, Nyegaard and Co., Norway). After determination of cell viability by Trypan Blue exclusion, the cells were suspended in Minimal Essential Medium, Delbucco's modification (DMEM), supplemented with penicillin (100 IE/ml), streptomycin (0.1 mg/ml) and glutamin (0.292 mg/ml). Trypan Blue solution, DMEM, penicillin, streptomycin and glutamin all were purchased from Flow Lab., USA. Regularly, 1.5 ml of the suspension, containing 2.5×10^6 viable cells/ml, was incubated for 1.5 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. In case of the measurements of leukotriene production and cyclic AMP content a somewhat different experimental procedure was followed which is described below in the respective paragraphs.

During the incubation period the cells were exposed to the following drugs or to combinations of these drugs: **arachidonic acid** (Supelco Inc., USA), **A23187** (Calbiochem Behring Inc., USA), **leukotriene C_4** (a gift from Dr.J.Rokach, Merck Frosst Lab., Canada), **indomethacin** (pharmacie AZR Dijkzigt, The Netherlands), **aspirin** (pharmacie AZR Dijkzigt, The Netherlands), **Org 7258)** (Organon NV, The Netherlands), **piroxicam** (Pfizer Inc., USA), **salbutamol** (Glaxo, UK), **isoprenalin** (Brocacef, The Netherlands), **practolol** (ICI, UK), **sotalol** (Lappe, FRG), **H3525** (dl-erythro-4'-methyl-α(1-isopropylaminoethyl)-benzylalcohol HLL) (a gift from Dr.G.Johnsson, AB Hässle, Sweden) and **isobuthylmethylxanthine** (IBMX) (Janssen Chimica, Belgium). Control studies were performed under the same experimental conditions and are referred to as basal secretory levels. At the end of the

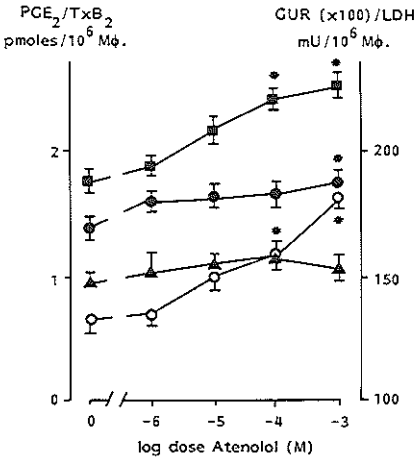


Figure 1. Effects of atenolol on the release of prostaglandin E_2 (●), thromboxane B_2 (■), β-glucuronidase (▲) and lactate dehydrogenase (○) from carrageenan elicited macrophages. Note that atenolol was toxic to macrophages as could be assessed by the release of lactate dehydrogenase. * $P<0.01$ vs. controls (Student's t-test, two tailed). Means ± SEM (n=5).

incubation period the cells were spun down at 12000 *g* for 1 minute, the supernatant was decanted and frozen at -70°C for assay of lactate dehydrogenase and β-glucuronidase activity and prostaglandin E_2, thromboxane A_2 and leukotriene content.

Enzyme measurements.

Lactate dehydrogenase activity was measured by the method of Wroblesky and LaDue[2]. In this method lactate dehydrogenase catalyses the conversion of pyruvate (Boehringer, FRG) to lactate, whereby NADH (Boehringer, FRG) is used as cofactor. The reduction of NADH concentration can be measured as a declining extinction at 340 nm and hence is a parameter for lactate dehydrogenase activity. Lactate dehydrogenase release was used as a control parameter. If any appreciable changes were observed in the activity of lactate dehydrogenase in the incubation medium, suggesting that the membranes of the cells were damaged during the test period due to a specific treatment, the effects of this treatment were considered to be toxic. An illustration of such an effect is provided in figure 1 showing that the β_1-selective adrenoceptor agonist atenolol (ICI, UK) was toxic to macrophages.

β-glucuronidase activity was quantified using the conversion of phenolphthalein glucuronide (Sigma, USA)to phenolpthalein which was then measured spectrophotometrically (λ=555 nm). Phenolphthalein used for the standard curve was obtained from Merck, FRG.

Measurements of cyclooxygenase products

Prostaglandin E_2 and thromboxane A_2 were assayed by a direct radioimmunoassay (RIA), whereby thromboxane A_2 was measured as the stable breakdown product thromboxane B_2. Antibodies were obtained from the Institute Pasteur (Paris), standard prostaglandin E_2 and thromboxane B_2 from Sigma Chem. Comp. (USA) and tritiated compounds from the Radiochemical Centre of Amersham (UK).

Measurements of 5-lipoxygenase products

In all experiments whereby leukotriene production was assessed a protocol was used as follows. 10×10^6 macrophages were suspended in 10 ml DMEM and incubated for 15 minutes at 37 °C in a humidified atmosphere containing 5% CO_2 in air. DMEM was supplemented with glutamin, penicillin and streptomycin as described earlier. Additionally gluthathion (2 mM) (Merck, FRG) and arachidonic acid (10 μg) were added. At the end of the 15 minutes incubation serine (0.25 mM) (Merck, FRG) was added to prevent breakdown of the peptidoleukotrienes and the cells were spun down (10 minutes, 1400 g). The pellets were washed once with 10 ml GBSS and the combined supernatants were stored at -70 °C. Thereafter, prostaglandin B_2 (200 μg) was added as internal standard. The supernatants were applied to a couple of Seppak C_{18} and silica cartridges (Waters/Millipore, The Netherlands) which were prewashed with 10 ml ethanol and 10 ml distilled water. The samples were eluted with 4 ml ethanol and evaporated to dryness in a vacuüm centrifuge. Dried samples were dissolved in 0.5 ml of HPLC-solvent A, centrifuged and purified by a Millex filter (Waters/Millipore, The Netherlands) and kept in a HPLC micro vial (Weichmann, Switzerland). Using this concentration technique a recovery of 60-70% regularly could be obtained. Reversed phase-HPLC of leukotrienes was carried out on a Nucleosil 5 C_{18} column (Chrompack, The Netherlands) with a solvent system (A) as follows: tetrahydrofuran/methanol/0.1% (w/v) EDTA solution in water/acetic acid (25/30/45/0.1), adjusted to pH 5.5 with ammoniumhydroxide[3]. A representative chromatogram of a leukotriene and prostaglandin B_2 standard mixture is shown in figure 2A, of a sample in figure 2B (leukotrienes B_4, C_4, D_4 and E_4 were gifts

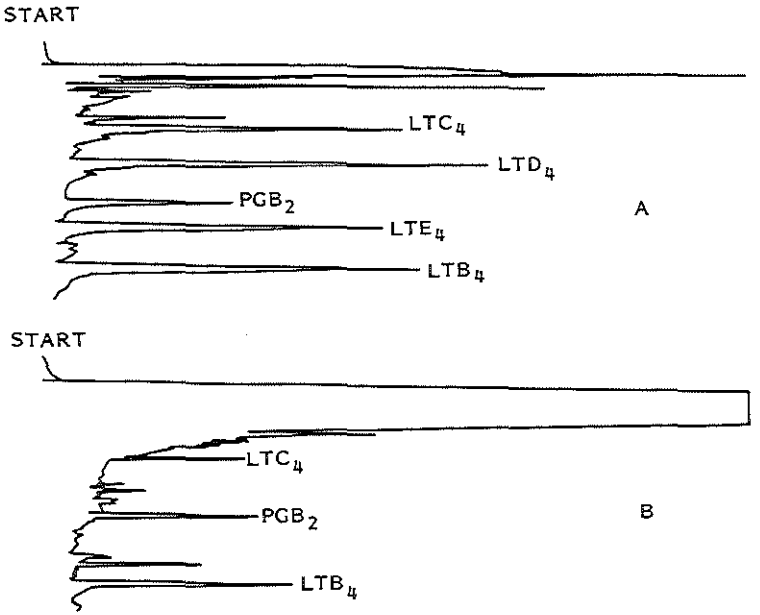


Figure 2. HPLC-chromatogram of a mixture of standards (2^a) and of a sample (2^b) containing leukotrienes C_4 and B_4. The standard mixture contained 100 ng leukotrienes C_4, D_4, E_4 and B_4 each and 40 ng PGB_2.

of Dr.J.Rokach, Merck Frosst, Canada).

Cyclic AMP measurements.

In order to study kinetic changes of intracellular cyclic AMP contents the macrophages were preincubated for 15 minutes at 37°C. Preincubation was necessary to prevent a "heating effect", since the cells were kept on ice during the isolation and purification procedure. Heating the cells to 37°C causes a rise in intracellular cyclic AMP. Following the addition of the different drugs the macrophages were allowed to remain in suspension (2.5×10^6 cells/ml, 1 ml) for 1, 5, 10 or 15 minutes. IBMX was not added to the incubation medium. After the incubation the cells were spun down at 12000 *g* for 30 seconds and the supernatant was discarded. Following the addition of tris disodium adetate (EDTA) buffer (pH 7.4) to the pellet, 5 minutes boiling in a water bath and centrifugation at 12000 *g* for 1 minute, the supernatant was stored at -20°C for subsequent cyclic AMP assay, which was carried out by the protein binding method[1].

Statistical evaluation

Student's t-test (two-tailed) was applied for statistical evaluation of the results.

REFERENCES

1 Gilman, A.G., 1970, Protein binding assay for adenosine 3'5' cyclic monophosphate, Proc. Natl. Acad. Sci. USA, 67, 305.

2 Wroblewsky, F. and J.S.LaDue, 1955, Lactic dehydrogenase activity in blood, Proc. Soc. Exptl. Biol. Med., 20, 210.

3 Zijlstra, F.J. and J.E.Vincent, 1984, Determinatrion of leukotrienes and prostaglandins in (^{14}C) arachidonic acid labelled human lung tissue by high performance liquid chromatography and radioimmunoassay, J. Chromatogr., 311, 39.

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CURRICULUM VITAE

Evert-Jan Schenkelaars
Geboren te Veldhoven op 13 januari 1955.

Na het behalen van het einddiploma Gymnasium β aan het Stedelijk Gymnasium te Schiedam in 1974, ben ik begonnen met de studie Medicijnen aan de Erasmus Universiteit te Rotterdam. Het doctoraalexamen werd behaald in 1980.
Vanaf november 1980 tot december 1984 was ik als promovendus werkzaam aan de afdeling Farmacologie van de Erasmus Universiteit Rotterdam waar onder leiding van Prof. Dr. I.L. Bonta het hier beschreven onderzoek werd verricht.
Sinds december 1984 ben ik als wetenschappelijk assistent in dienst van het Nederlands Astmafonds verbonden aan de afdeling Farmacologie van de Erasmus Universiteit Rotterdam.

LIST OF PUBLICATIONS.

-Bragt, P.C., E.J.P.M.Schenkelaars and I.L.Bonta, 1979, Dissociation between malondialdehyde formation in exudate and increased levels of malondialdehyde in plasma and liver during granulomatous inflammation in the rat, Prostagl. Med., 2, 51.

-Schenkelaars, E.-J. and I.L.Bonta, 1983, Leukotriene C_4 induced release from rat peritoneal macrophages and its inhibition by isoprenaline, In: Leukotrienes and Other Lipoxygenase Products (ed. P.J.Piper), Wiley, Chichester, p.257.

-Schenkelaars, E.-J. and I.L.Bonta, 1983, Effect of leukotriene C_4 on the release of secretory products by elicited populations of rat peritoneal macrophages, Eur. J. Pharmacol., 86, 477.

-Schenkelaars, E.-J. and I.L.Bonta, 1985, β_2-adrenoceptor agonists reverse the leukotriene C_4 induced release of macrophages, Eur. J. Pharmacol., 107, 65.

-Schenkelaars, E.-J. and I.L.Bonta, 1985, Cyclooxygenase inhibitors promote the leukotriene C_4 induced release of β-glucuronidase from rat peritoneal macrophages; prostaglandin E_2 suppresses, Int. J. Immunopharmacol., in press.

-Bonta, I.L., G.R.Elliott and E.-J.Schenkelaars, Prostaglandins and leukotrienes as regulators of macrophage functions, In: Proceedings of the Fourth Congress of the Hungarian Pharmacological Society with International Participation, Hungarian Acad. Sci., in press.

-Schenkelaars, E.-J., M.J.P.Adolfs and I.L.Bonta, Leukotriene C_4 induced cyclic AMP generation in rat macrophages; involvement of endogenous prostanoids and interactions with the β_2-adrenoceptor agonist salbutamol, submitted to Prostaglandins.

-Schenkelaars, E.-J., F.J.Zijlstra and I.L.Bonta, Release of leukotrienes C_4 and B_4 from rat peritoneal macrophages; a comparison between resident and elicited cell populations, submitted to Biochim. Biophys. Res. Commun.

-Elliott, G.R. and E.-J.Schenkelaars, Preferential synthesis of prostaglandin E_2: a comparison of arachidonic acid stimulated eicosanoid release using resident and elicited macrophages, submitted to Br. J. Pharmacol.

-Schenkelaars, E.-J., J.M.E. van Groningen and I.L.Bonta, Comparison between the effects of the two cyclooxygenase inhibitors Piroxicam and Org 7258 on the leukotriene C_4 induced release from rat peritoneal macrophages, to be submitted to Eur. J. Pharmacol.

-Schenkelaars, E.-J. and I.L.Bonta, 1984, Leukotriene C_4 and prostaglandin E_2 interact in modulating macrophage activity (abstract), Pharmaceutisch Weekblad Scientific Edition, 6, 228.

-Schenkelaars, E.-J. and I.L.Bonta, Involvement of PGE_2 in the LTC_4 induced secretory response and cyclic AMP production of rat macrophages (abstract), In: Proceedings of the Kyoto Conference on Prostaglandins 1984, in press.