Human Anti-Idiotypic T Lymphocyte Clones are Activated by Autologous Anti-Rabies Virus Antibodies Presented in Association with HLA-DQ Molecules

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Précis

The regulatory function of antigen-specific T cells in human antibody responses to protein and carbohydrate determinants of many viral and bacterial antigens has extensively been studied in systems involving *in vitro* triggering of B cells by antigens or polyclonal activators. Although amply documented in experimental murine models, the existence of T helper cells with receptor specificity for idiotypic determinants of B cell immunoglobulins has not been demonstrated in a human system. We are interested in T helper cell recognition of idiotypic determinants of virus-specific antibody, secreted by human B cells in response to viral antigens, and in the role which such idiotype-specific T helper cells play, alone or in concert with virus-specific T helper cells, to regulate the antibody response. Understanding of the function of different T helper cell subsets in an anti-viral antibody response and especially of the mechanisms of idiotype recognition by T cells is important for the development, and future application in man of idiotype vaccines, the potential of which has been indicated for different pathogens in several animal species.

It was realized that for the efficient characterization of each of the T helper cell subsets, the availability of cloned populations of T cells would be inevitable. Furthermore, we argued that if, as predicted by Jerne, idiotype recognizing T helper cells are involved in physiological idiotype regulation in the course of an immune response—e.g., following encounter with virus—cloned populations of T cells should best be obtained by immunization protocols closely mimicking the physiological situation. In a previous report we described the induction of a secondary antibody response in human peripheral blood mononuclear cells (PBMC) *in vitro* by rabies virus antigen. This response was shown to be T helper cell dependent, and rabies virus-specific T helper cell clones have recently been obtained in our laboratory.

The present study describes the generation of cloned lines of anti-idiotypic $T4^+$ cells from rabies virus immune PBMC restimulated with rabies virus antigen *in vitro*. The cloned T cell lines were found to respond to circulating autologous antibody exhibiting specificity to rabies virus, but not to rabies virus antigen. The clonal proliferation, induced by this "auto-anticlonotypic" antibody, was found to be preceded by modulation of the T3/Ti molecular complex and required presentation of the antibodies by antigen presenting cells in association with HLA-DQ molecules. This observation of MHC-restricted idiotype recognition by human T cells, may have important consequences for the understanding of the regulation of the human immune response by T cells. In addition, as regards strategies for the development of idiotype vaccines, our findings may implicate that selection of idiotypes by serological assays alone, may basically be incorrect and at a minimum should include a survey for idiotypic structures defined by T helper cells.

Introduction

Administration of antigen elicits antigen-specific B cell responses which for most antigens need the as-

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sistance of T helper cells. The functions of antigen-specific T helper cells (AgTh) which act in a MHC restricted manner by cognate recognition of their B cell targets has been extensively characterized in both mouse and man [1–10]. Observations in several different murine systems have revealed evidence for the existence of another type of T helper cells recognizing idiotypic determinants of antibodies [11–31] and in fact many experiments have allowed to distinguish two subsets of idiotype-specific T helper cells termed ThId and anti-IdTh [see 32 for review]. Thid are Thyl$^+$, Lyt1$^+$, Lyt2$^-$ idiotype binding cells specific for a certain foreign antigen. These Thid, which by itself show no recognition of MHC gene products, synergize upon reexposure to antigen with MHC restricted AgTh to generate antigen-specific B cell response [11–16, 25–27]. In contrast, Thy1$^+$, Lyt1$^+$, Lyt2$^-$, anti-IdTh, which fail to bind native idiotype, may recognize idiotype in association with MHC gene products [17–24]. Earlier reports showing that anti-IdTh could help idiotype bearing B cells in the absence of antigen [17] have recently been challenged by Kawahara et al. [18] who demonstrated the necessity for antigen exposure of anti-IdTh for its activity.

So far in man, data on the regulatory potential of idiotype specific T cells in an antigen driven antibody response are restricted to the demonstration of Id$^+$ T8$^+$ T cells with xenogeneic rabbit anti-idiotypic antisera raised against idiotypes of tetanus toxoid (TT) specific antibodies [33]. In his study, Geha showed that interaction of Id$^+$ T8$^+$ T cells with anti-Id induced antigen-specific suppression of TT driven T cell proliferation and AgTh dependent antibody response of human B cells *in vitro*. However the precise target cells of Id$^+$ T8$^+$ T cells have not been identified.

The experiments reported in this paper were designed to determine whether in addition to AgTh, T cells with the characteristics of ThId or anti-IdTh are activated in man by an autologous process during a secondary antibody response against a biological relevant infectious pathogen.

In a previous paper [34], we described an antigen driven and AgTh dependent secondary antibody response of human PBMC *in vitro* against rabies virus and rabies virus-specific T helper cell activity has recently been analyzed with cloned AgTh [Bunschoten et al., manuscript in preparation]. Here we demonstrate the induction of human cloned T cells during an *in vitro* antibody response of human PMNC against rabies virus, which have shown properties resembling those of anti-IdTh described in mice.

Table 1. Induction of proliferation by autologous serum Ig

Stimulus	T cell clones			
	TO-229	TO-227	TO-216	TK-102
Experiment 1				
medium	1.567	1.940	1.086	1.262
rabies virus (1 μg/well)	1.891	2.240	1.112	12.632
serum donor U	1.345	1.443	n.d.	n.d.
serum donor B	1.413	1.755	n.d.	n.d.
serum donor O	54.092	41.243	2.364	1.663
prot.A-Seph.eluate	35.914	29.203	n.d.	n.d.
prot.A-Seph.effluent	5.751	4.781	n.d.	n.d.
Experiment 2				
medium	1.724			
prot.A.Seph.eluate	14.444			
anti-human IgG-Seph.eluate	8.700			
anti-human IgG-Seph.effluent	2.106			
Experiment 3				
medium	4.047	5.772		
pre-immune serum donor O	4.019	5.588		
immune serum donor O	24.423	9.344		

Induction of proliferation of clones TO-299 and TO-277 by autologous rabies virus immune serum (donor O), protein A-sepharose isolated serum Ig and affinity purified serum IgG but not by pre-immune serum of donor O or heterologous rabies virus immune sera (donor U, donor B) or rabies virus. Clone TK-102 is a rabies virus-specific clone obtained from another rabies virus immune individual and clone TO-216 is a clone of unknown specificity which was derived from donor O; nd, not determined. The results are expressed as the mean cpm of triplicate cultures.

Results and Discussion

The generation of Ig-specific T cells

Interleukin-2 (Il-2) dependent T4$^+$ T cell clones were generated *in vitro* by stimulation with rabies virus vaccine of peripheral blood mononuclear cells (PBMC) from a healthy individual (donor O) who had experienced a post-exposure vaccination against rabies virus seven years earlier. Because the titer of rabies virus neutralizing antibodies in the serum of donor O has remained at a high level ever since, we speculated that T cells specific for idiotypes of anti-rabies virus antibodies may have a regulatory function in the response to rabies virus. It was observed that six out of thirty T cell clones, none of them exhibiting specificity for rabies virus antigen, proliferated to autologous (donor O) serum. The results obtained with a selection of clones T0-227, T0-229, T0-216 generated from PBMC of donor O and with clone TK-102 which was obtained from another rabies virus immune individual are shown in Table 1. Clones T0-227 and T0-229 were induced to proliferate to autologous (donor O) but not to heterologous serum of rabies virus immune individuals, while clones T0-216 and TK-102 did not respond. In addition none of a set of *Mycobacterium leprae* reactive T cell lines and T cell clones [35] tested for the proliferation to the serum from donor O did respond (data not shown). However, clone TK-102 was shown to proliferate by stimulation with rabies virus. The induction to

proliferation of clones T0-227 and T0-229 was induced by the immunoglobulin (Ig) fraction of the serum from donor O (Table 1). Both the protein A-sepharose binding-serum fraction (eluate), and the fraction binding to anti-human IgG antibody cross-linked to CnBr-activated Sepharose (eluate) in which a high titer of rabies virus-specific antibodies could be demonstrated, induced proliferation of cloned T cells. In contrast effluent fractions of both columns contained a low amount of anti-rabies virus antibodies and showed a highly reduced capacity to induce proliferation of the clones. These results indicate that the T0-227 and T0-229 cloned T cells are not specific for isotype or allotype Ig determinants.

Specificity of Ig inducing T cell proliferation

An indication that rabies virus antibodies are responsible for the proliferative signal delivered by the serum from donor O to the T cell clones *in vitro* came from the observation that in contrast to rabies virus immune sera of donor O, its pre-immune serum failed to induce proliferation of clones T0-227 and T0-229 (Table 1). Furthermore experiments showing that rabies virus antigen inhibited the proliferation of clones T0-227 and T0-229 mediated by the serum from donor O also suggested a role for rabies virus-specific antibody in inducing T cell proliferation. Both clones were cultured in the presence of serum from donor O and either rabies virus antigen (DKCV), vesicular stomatitis virus antigen (VSV-DKC) or tetanus toxoid (TT) was added in different amounts at the start of the cultures. Rabies virus and vesicular stomatitis virus—two antigenically unrelated members of the Rhabdoviridae family—were propagated in dog kidney cells. As shown in Figure 1, the induction of proliferation of clones T0-227 and T0-229 by autologous serum was inhibited by rabies virus antigen but not by vesicular stomatitis virus antigen or tetanus toxoid, suggesting that the T cells recognize V-region determinants of autologous Ig.

Ig-induced proliferation of T4$^+$ cells is mediated through triggering of the T cell receptor

Thus far the results of our experiments indicate that the serum from donor O contains rabies virus-specific antibodies which by themselves are not mitogenic but rather, initiate proliferation of clones T0-227 and T0-229 in a manner analogous to activation of T cells by antigen plus MHC determinant. If this is the case, one would expect the stimulation of

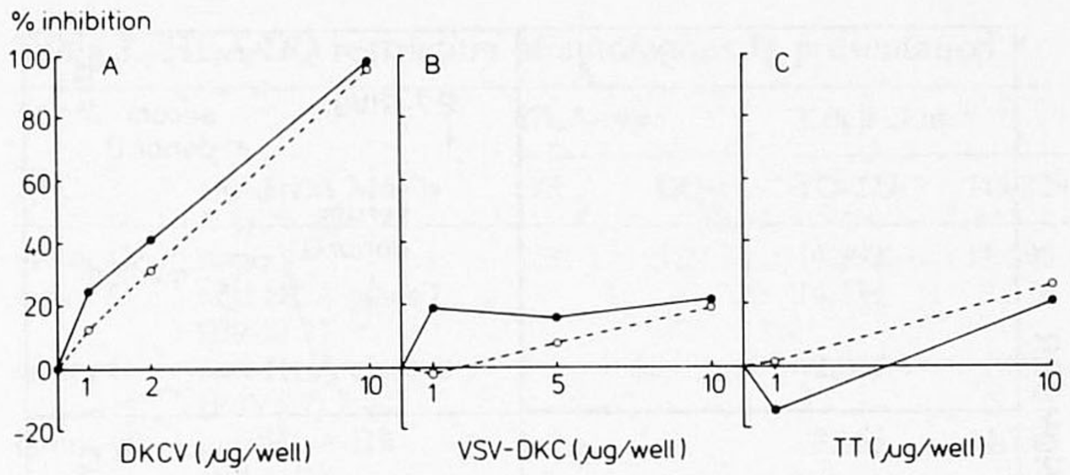

Fig. 1. Inhibition of the proliferation, induced by serum from donor O, of clone T0-227 (● - - ●) and clone T0-229 (○ - - ○) by rabies virus (Fig. 1A), vesicular stomatitis virus (Fig. 1B) and tetanus toxoid (Fig. 1C). T cell clones were stimulated with serum from donor O as described in the legend to Table 1. Rabies virus (DKCV), Vesicular stomatitis virus (VSV-DKC) or tetanus toxoid (TT) were added at concentration indicated at the beginning of the experiment. Rabies virus (strain Wistar PM/WI 38-1503-3M) and vesicular stomatitis virus (strain San Juan A) were propagated in dog kidney cells and were inactivated with β-propiolactone and concentrated as previously described [21].

clones T0-227 and T0-229 by autologous antibodies to be accompanied by modulation of the T3/Ti molecular complex, as has been described for monoclonal anticlonotypic antibodies raised against human T cell clones [36, 37]. This was investigated by incubation of clones T0-229 and TK-102 with either serum from donor O, serum from donor U or with a monoclonal antibody against T3 (E7-RIV9) for 18 hrs at 37°C in the presence of APC. Incubation of T cells with E7-RIV9 was previously shown to result in the complete modulation and internalization of the T3/Ti complex within one hour [38]. Subsequently, surface expression of T3 and T4 antigens was determined using double labelling with monoclonal anti-T3-FITC conjugated antibody (anti-Leu-4) and monoclonal anti-T4-PE conjugated antibody (anti-Leu-3a) respectively by FACS analysis. The results of this experiment are shown in Figure 2. Analysis of clone T0-229 (Fig. 2A) and of the rabies virus-specific clone TK-102 (Fig. 2C) show that surface expression of T4 antigen was not affected by incubation of the cells with either monoclonal anti-T3 antibody, serum from donor O, serum from donor U or medium alone. Analysis of the same populations for surface expression of T3 antigen, showed that the incubation with monoclonal anti-T3 antibody strongly reduced the surface expression of T3 on cells of both clones T0-229 (Fig. 2B) and TK-102 (Fig. 2D). Unlike modulation with monoclonal anti-T3 antibody, incubation with serum from donor O significantly decreased surface expression of T3 antigen on clone T0-229 only (Fig. 2B) and did not influence the surface expression of T3 antigen on clone TK-102 (Fig. 2D). Treatment of cells from either clone T0-229 or clone TK-102 with

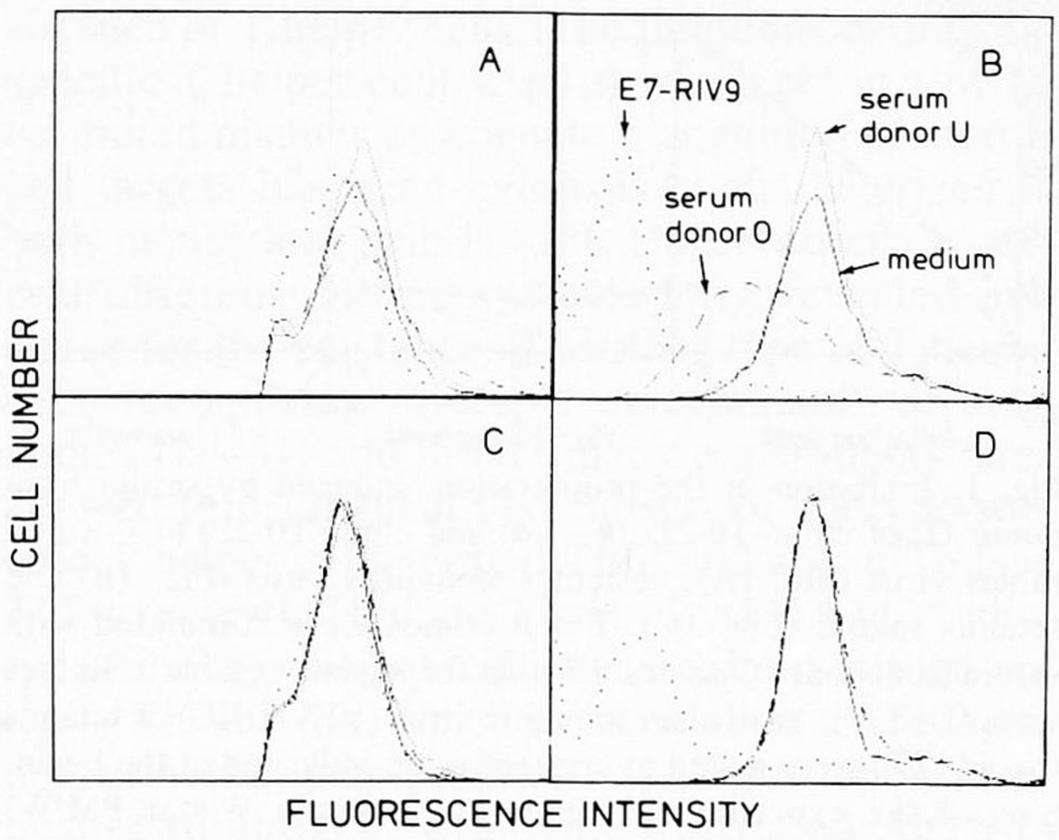

Fig. 2. FACS analysis of T4 (Fig. 2A and C) and T3 (Fig. 2B and D) surface expression of clones T0-229 (Fig. 2A and B) and clone TK-102 (Fig. 2C and D) after incubation of the cells with either medium (——), serum from donor U (" " "), serum from donor 0 (- - -) or with a monoclonal antibody against T3 (E7-RIV9) (···).

serum from donor U or medium had no effect on the surface expression of their T3 antigen. Given these findings and the functional data on proliferation listed in Table 1, the induction of proliferative response of clone T0-229 by autologous serum involves triggering through the T3/Ti complex by antibodies present in the serum from donor 0, which behave as auto-anticlonotypic antibodies. The observation that stimulation of T0-229 with serum from donor O results in a raise in cytosolic free Ca^{++} and endogeneous Il-2 production (data not shown) are in agreement with this conclusion.

Ig-induced T cell proliferation requires the presence of antigen presenting cells in culture

From a number of studies investigating the functional effect of anticlonotypic monoclonal antibodies on T cell clones, it has become clear that the induction of proliferation required the anticlonotypic antibody to be presented on a solid surface support [36, 37, 39, 40]. In our studies, the induction of proliferation in clones T0-227 and T0-229 by autologous serum Ig was found to be strictly dependent on the presence of APC (EBV transformed B cells) in culture. We investigated whether APC functioned as a solid surface support, allowing antibodies in the serum from donor O to efficient receptor triggering and cross-linking, by presenting protein A-sepharose bound serum Ig from donor O to clone T0-229. Presentation of the serum Ig of

Table 2. Presentation of autologous Ig by antigen presenting cells

manner of presentation of autologous serum Ig	T cell clones	
	TO-227	TO-229
—	1.430	960
APC donor O	14.992	11.596
prot.A-Seph beads-Ig donor O	n.d.	133
prot.A-Seph beads-Ig donor O + 10% IL-1	n.d.	644

T cells were stimulated either with autologous serum Ig (1/100 final dilution) in the presence of autologous APC or with an equivalent amount of serum Ig coupled to prot.A-sepharose by means of dimithylpilimidate-HCL for 4 hrs. IL-1 was a kind gift of Dr. L. Aarden, Central Laboratory of the bloodtransfusion service, Amsterdam. Results are expressed as mean cpm of triplicate cultures.

donor O in this manner did not initiate proliferation of clone T0-229 (Table 2). The possibility was considered that APC were required to provide a second signal necessary for proliferation [39]. However, the addition of Il-1 to cultures, which were stimulated with protein A-sepharose bound serum Ig from donor O had no effect. These results indicate that APC requirements for the serum Ig are not met by a solid surface support only. These findings are in agreement with results from experiments which failed to demonstrate a direct interaction between autologous Ig and the T cell receptor of the clones using immunofluorescence and immunoprecipitation (data not shown).

Ig-specific T cells recognize autologous Ig in association with HLA-DQ molecules

Having established that APC were necessary for the induction of clonal proliferation by autologous Ig, we investigated whether APC present serum Ig from donor O to clones T0-227 and T0-229 in association with self-MHC class I or class II molecules. The results of experiments which address this question are shown in Table 2. Inhibition of the proliferation of clones T0-227 and T0-229 induced by serum of donor O was observed in the presence of anti-class II (PdV5.2) but not anti-class I monoclonal antibody. However, a monoclonal anti-HLA-DR antibody did not block the proliferation of clones T0-229 and T0-227. These experiments provide data to support presentation of serum from donor O to the T cell clones by APC in association with either DP or DQ molecules. Therefore we analyzed the effect of monoclonal antibodies, exclusively recognizing either DP (B7.21) or DQ (SPV-L3), on the proliferative response of clones T0-227

and T0-229 to the serum of donor O. The results of these experiments show that SPV-L3 but not B7.21 completely inhibited proliferation of both clones and thus provide evidence for HLA-DQ restricted activation of clones T0-227 and T0-229 by serum Ig of donor O (Table 2). The HLA-DQ restriction was further analyzed using APC sharing different combinations of DQw1, DQw2, and DQw3 specificities. It was observed that serum Ig from donor O (DQw1, DQw2) induced a proliferative response of clones T0-227 and T0-229 only when presented by APC from donor K (DQw2) but not by APC from donor T (DQw1, DQw3). Thus induction to proliferation of T0-227 and T0-229 requires serum Ig from donor O to be presented in the context of HLA-DQw2 molecules. The use of DQ molecules as restriction elements for presentation of antigen has been reported for the *in vitro* T cell response to Candida albicans, the generation of cytotoxic T cells in mixed lymphocyte culture, the proliferative T cell response to allogeneic cells and the proliferation of T cell lines to GAT, respectively [41, 42]. However, association of DQw2 with predominant restriction determinants of proliferating and helper T cells has not been described earlier.

Table 3. HLA-DQ restriction of autologous Ig presentation

		HLA-type		T cell clones	
APC	anti-HLA MoAb	DR	DQw	TO-227	TO-229
donor O	none	2,3	1,2	14.992	11.596
donor O	anti-HLA-class I (B9-12.1)			14.439	9.354
donor O	anti-HLA-class II (PdV5.2)			2.045	1.396
donor O	anti-HLA-DR (B8.11.2)			8.653	14.730
donor O	anti-HLA-DP (B7.21)			13.060	8.208
donor O	anti-HLA-DQ (SPV-L3)			1.162	769
donor K	none	3,8	2	9.356	7.804
donor T	none	2,4	1,3	1.618	1.738

Presentation of serum Ig from donor O by APC (EBV-BC) from donor O (DR2, DR3, DQw1, DQw2), donor K (DR3, DRw8, DQw2) and donor T (DR2, DR4, DQw1, DQw3) or by protein A-sepharose-bound Ig from donor to clones TO-227 and TO-229 and inhibition of proliferation by anti-MHC antibody. The results are expressed as the mean cpm of triplicate cultures.

Interpretation

In the present study, we have shown the induction of a proliferative response of human cloned $T4^+$ cells by autologous rabies virus-specific Ig presented by APC in association with HLA-DQ molecules. We interpret these results to indicate that, in man, MHC-restricted idiotype-specific T cells exist, which may be involved in the regulation of B cell responses. A question central to this interpretation concerns the nature of the substance delivering the proliferative signal to the clones.

We have shown that IgG purified from autologous rabies virus immune serum but not autologous pre-immune serum or heterologous sera induced proliferation. This finding and the inhibition of proliferation by rabies virus antigen but not VSV antigen strongly argues against a possible role for isotypes or allotypes of Ig in the generation of proliferative responses, and rather indicate the involvement of V-regions of rabies virus-specific Ig in this process. However, we realize that isolation and a definitive characterization of the stimulatory component will greatly strengthen this conclusion and therefore we have now started the generation of autologous B cell lines producing monoclonal anti-rabies virus-specific antibody.

Numerous observations in mice have established the existence of two distinct classes of idiotype-specific Th cells [see 32 for review]. Thus in contrast to ThId, anti-IdTh are described which fail to bind idiotype, are MHC-restricted, not specific for nominal antigen, and finally do not require (re)exposure to antigen to become activated. The human T cell clones described in this study seem to meet best the criteria of anti-IdTh. Despite deliberate attempts it appeared impossible to demonstrate direct binding between the autologous Ig and the receptor of the T cell clones in immunofluorescence. Moreover, none of the experiments aimed at precipitating the surface iodinated T3/Ti molecular complex with autologous Ig, did reveal evidence for direct binding. These observations and the results of the modulation experiments [Figure 2] strongly argue against direct receptor interactions between Ig and T cells.

This conclusion is strengthened by our results showing MHC-restricted recognition of Ig from donor O by the cloned T cells (Table 3). The inhibition experiments using MoAb against HLA-classe II antigens and the experiments with HLA-DQ matched APC revealed clear evidence for Ig recognition by T cells in the context of HLA-DQ molecules. These results seem in agreement with the findings of Jorgensen and Hannestad [19], Hannestad et al. [20] and Kawahara et al. [18] showing that in mice Ig-V domain-specific Th is under Ir-gene controle supporting the idea that anti-Id Th recognize idiotype in association with MHC gene products. It was hypothesized by both groups of investigators that ligation of antigen receptors of B cells by antigen leads to activation of B cells and initiates processing of their own Ig receptors fol-

lowed by reexpression of processed Id which is then recognized by T cells in association with MHC gene products. Indeed the results of Kawahara et al. [18] strongly support this hypothesis. They showed that Ig in order to be recognized by T cells must be synthesized and presented by the H-2 compatible B cells themselves which required activation with SRBC. Although such studies have not yet been performed with our clones a similar mechanism(s) might be proposed to explain our observations.

The T cell clones were generated from PBMC of donor O primed *in vivo* and restimulated *in vitro* with rabies virus antigen. We have shown previously that in such conditions rabies virus-specific antibody is synthesized *de novo* by B cells *in vitro* [34]. Thus interaction of B cell Ig receptors with rabies virus is possible and may have resulted in processing and subsequent reexpression of processed Ig on the B cell surface leading to the activation of Ig-specific HLA-restricted T cells. As they are efficient APC [43, 44], the EBV-BC used in our *in vitro* study could have mimicked this process. The EBV-BC do not synthesize rabies virus-specific Ig themselves but they may acquire serum Ig via Fc receptors. Alternatively, serum Ig of donor O may react with the Ig receptor on EBV-BC. Although this latter possibility could more easily explain the inhibition of proliferation by rabies virus antigen it is less likely since it would imply the presence of cross-reactive anti-idiotypic receptors on the HLA-DQ matched EBV-BC used in our study which is in opposition with the finding that the T cell clones are only stimulated by autologous Ig.

Although highly attractive, the requirements for rabies virus antigen in the generation of anti-ThId seems at first slightly difficult to reconcile with the apt regulatory function of the T cell clones: the maintenance of a high level of rabies virus-specific antibody in the serum of donor O in the absence of rabies virus antigen. It should be noted that donor O had a persistent high level of neutralizing antibody against rabies virus for seven years following post exposure vaccination with rabies virus vaccine. It is quite conceivable, however, that anti-idiotypic antibodies directed against idiotypes of rabies virus-specific Ig can substitute for antigen in the activation of B cells and the subsequent triggering of anti-IdTh [45]. This idea would be consistent with the results of Eichmann et al. [17] showing that mice immunized with anti-Id (A5A) developed anti-IdTh which could assist the production of $A5A^+$ antibody in the absence of antigen. Whether our T cell clones represent functional anti-ThId for the production of rabies virus-specific antibody is currently being investigated.

Materials and Methods

Antigens

Rabies virus (strain Wistar PM/W1-38-1503-3m) and vesicular stomatitis virus (strain San Juan A) were propagated in dog kidney cells, inactivated with bèta-propiolactone and concentrated as previously described [46].

T cell cloning

Peripheral blood lymphocytes (PBL) from donor O and donor K were isolated by density gradient centrifugation on Ficoll Isopaque. Cells were cultured in flat-bottomed 24-well tissue culture plates Falcon 3074 (Becton Dickinson Oxnard, CA) at a cell density of 10^6 PBL per well in 1 ml of culture medium consisting of RPMI-1640 (Gibco, Scotland) supplemented with 10% (v/v) pooled human AB serum, 10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, penicillin (100 IU/μl) streptomycin (100 μg/ml), and 1 μg/ml rabies virus antigen (dog kidney cell vaccine, DKCV). After 7 days of culture cells were harvested and cloned by limiting dilution (0.5 cell/well) in flat-bottomed 96-well plates (Greiner no. 655180) and cultured in the presence of irradiated (3000 rds) Epstein-Barr virus transformed autologous B cells (EBV-BC) (5×10^3/well) and irradiated (3000 rds) allogeneic PBL (5×10^5/well) in culture medium supplemented with 10% conditioned medium from MLA 144 cells containing 160 U/ml IL-2 [20] and 2% (w/v) phytohaemagglutinin (PHA) (Wellcome, England). Proliferating cells were expanded in round-bottomed 96-well culture plates (5×10^3/well) in culture medium supplemented with 10% IL-2.

Epstein-Barr transformed B cell lines (EBV-BC)

EBV-BC, which were used as antigen presenting cells (APC), were established by incubation of 10^7 PBL with 1 ml supernatant fluid of marmoset cell line B95-8 for 1 hr at 37°C. Cells were washed twice and cultured in RPMI-1640, supplemented with 2% (v/v) (PHA) (Wellcome, England), 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) at a density of 5×10^6 cells/50 ml in 200 ml flasks.

HLA-typing was performed at the Department of Immunology and Bloodbank, University Hospital Leiden.

Assay for lymphocyte proliferation

Cloned T cells (10^4/well) were cultured in the presence of autologous irradiated (3000 rds) EBV-BC (2.5×10^4/well) in RPMI-1640 containing 20% (v/v) pooled human AB serum in round-bottomed 96-well plates at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were stimulated with either 1 μg of rabies virus, pre-immune serum from donor O or rabies virus immune serum from donor O, donor U, or donor B at a final dilution of 1/100. The protein A-sepharose and the anti-human IgG-Sepharose effluent and eluate fractions of the serum from donor O were added at equivalent concentrations after dialysis against RPMI-1640. After 5 days cells were pulsed for 18 hrs with 0.5 μCi (6-^{3}H) Thymidine/well (TRA.61, 1640, Amersham). Cells were harvested and incorporated ^{3}H-Thymidine was measured. Purification of serum Ig from donor by protein A-sepharose CL-4B chromatography was performed according to methods recommended by the manufacturer (Pharmacia AB, Sweden). Serum IgG was further purified from protein A-Sepharose isolated Ig by affinity chromatography using Goat anti-human IgG (Fc fragment-specific) antibodies (20832, Cappel, USA) coupled to Sepharose 4B (Pharmacia, AB, Sweden). The column was eluted with 0.5 M ammonium hydroxide containing 3 M potassium thiocyanate. The eluate containing the IgG fraction was dialysed against RPMI-1640.

Modulation of the T3/Ti complex with anti-T3 MoAb and serum of donor O

Cloned T cells (10^6) were incubated for 18 hrs at 37°C in the presence of autologous irradiated EBV-BC in RPMI-1640 containing 10% (v/v) pooled human AB serum and in the presence of either medium, serum from donor U (1/100), serum from donor O (1/100), or anti-T3 monoclonal antibody E7-RIV9 (1/100). After 18 hrs cells were harvested, washed twice in phosphate buffered saline (PBS) PH 7.2 containing 5% (v/v) Boseral (Organon Technika) and double stained with anti-Leu-3^a-PE (Becton Dickinson, 7327) and anti-Leu-4-FITC (Becton Dickinson, 92-0001) for 30 min at 4°C. Cells were analyzed on a Becton Dickinson FACS analyzer. Statistical analysis of data was performed using the Consort-30 program (Becton Dickinson).

Inhibition of proliferation with MoAb against class I and II MHC antigens

T cell clones were stimulated with serum Ig from donor O as described in legend to Table 1. Monoclonal anti-HLA-class I or II antibody was added at the beginning of the experiment at a final dilution of 1/300. The monoclonal antibodies used were: B9.12.1 monomorphic anti-class I (gift of B. Malissen), PdV5.2 monomorphic anti-class II recognizing DP and DR, and approximately half of all DQ molecules (gift of F. Koning), B8.11.2 monomorphic anti-DR recognizing exclusively all DR molecules (gift of B. Malissen), SPV-L3 recognizing exclusively all DQ molecules [47] and B7.21 with unique specificity for DP molecules (gift of F. Bach).

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Editor's Comment: The paper by UytdeHaag et al. represents a potentially novel and important finding in two areas of immunology: the specificity of idiotype-specific T cells, and their possible function in persistent antibody production. My ideas in this area have been summarized in two recent commentaries in JMCI, and will not be repeated in detail here (*JMCI, 2:*118, 1985, and *JMCI 2:*265, 1986.). In summary, two types of idiotype-specific helper T cells have been described, with different recognition characteristics and different functional effects. One type binds directly to native idiotype, and plays an accessory role in B cell activation, requiring the additional participation of an antigen-specific, MHC-restricted helper T cell for its function. The second type, normally found after immunization with idiotype in adjuvant, is a typical MHC-restricted helper T cell that has been shown by Bogen and Hannestad to recognize a fragment of the idiotypic immunoglobulin in the context of self class II MHC molecules. Based on the finding that this latter cell type can only be elicited in animals lacking expression of the idiotype in serum, and on experiments of Eichmann and co-workers showing that such cells, when mixed with idiotype-bearing B cells can directly activate such cells in the absence of antigen, we predicted that such anti-idiotypic T cells could lead to a state of persistent antibody production. Thus, the experiments of UytdeHaag et al. may represent an example of this. To prove that this mechanism is responsible for chronic production of anti-rabies antibody in this patient in the absence of antigen, it would be important to show definitively that the cloned T cells are specific for purified anti-rabies antibodies derived from this individual. Ideally, this would involve the generation of hybridomas or EBV-transformed B cell lines so that homogeneous antibodies could be used. Secondly, it should be possible to use the cloned T cell lines to stimulate the patient's B cells in vitro to secrete anti-rabies antibody in the absence of antigen. Whether antigen could stimulate this process by inducing internalization and processing of the appropriate immunoglobulin could also be tested. These issues are important in that they relate to the issue of biological importance of such idiotype-specific T cells, which could also play a role in persistent antibody production in autoimmunity. The findings in this paper are novel and intriguing, and further study to clarify these findings will be most welcome.

A Critique

The paper describes several T cell clones isolated from a healthy human donor, previously vaccinated against rabies, by stimulation with rabies virus. The clones isolated did not respond to rabies virus but they did respond to autologous serum and to material in autologous serum adherent to protein A sepharose. Responses depended on the presence of MHC class II matched presenting cells. On the basis of these data the authors claim to have described human T cell clones responding to idiotypic determinants in a HLA-restricted way.

The only evidence that the inducing material in human serum is immunoglobulin stems from protein A absorption and elution experiments. The eluted material has not been biochemically analysed. Not even the claim that the T cells respond to anti-virus antibody has been ascertained.

A Critique

This is the first example of the generation of human cloned T cell lines specific for immunoglobulin (presumably anti-rabies virus antibodies)—these cloned T cells being derived from an individual immunized with rabies virus and not immunoglobulin. While the authors haven't definitively proved that the stimulating immunoglobulin is rabies virus specific, the circumstantial evidence is strong. Most interestingly, the findings implicate idiotype specific T cells in the continued activation of virus-specific B cells, and may account in part for the high anti-viral titer seen in the T cell donor, immunized with virus 7 years previously. Such an interpretation would require a demonstration that immunoglobulin specific T cells activate B cells, in particular virus-specific B cells to secrete antibody. Effects on B cells have not been reported in this paper but presumably these studies will be pursued in future work.

This paper is focussed on the specificity and activation requirements of the cloned Ig-specific T cell. One dilemma apparent in these studies is why free virus blocks activation. The authors convincingly demonstrated that the activation of the cloned T cells was class II restricted and Ig specific, presumably requiring processing of the Ig. As this is identical to the activation requirements of most class II specific helper T cells, is difficult to understand how free virus inhibits T cell proliferation. Perhaps the binding of virus prevents APC uptake of the Ig molecule—if so it would suggest the V region is important in uptake and processing. This issue could be expanded upon in the discussion.

The Authors' Respond

The editor and the reviewers raise pertinent questions on the results reported here. One major criticism concerns the specificity of the cloned T cells. Reviewer 1 objects to our interpretation that the stimulating material isolated by chromatography on protein-A-Sepharose represents immunoglobulin. We agree with the reviewer that co-purification of "a T cell stimulating moiety" other than immunoglobulin or isolation of T cell stimulating immune complexes cannot be excluded using protein-A-Sepharose chromatography. Therefore we have performed T cell stimulation experiments with serum IgG purified by affinity chromatography on CnBr-activated Sepharose-linked goat anti-human IgG-Fc fragment specific antibody (see "Materials and Methods"). The results of these experiments are included in table 1 of the revised paper. Analysis of the purified IgG by PAGE and immunoblotting, using a rabbit anti-serum raised against the protein-A-Sepharose purified serum fraction of donor O, revealed 2 bands corresponding to H-chain and L-chain of IgG. Furthermore analysis of freshly drawn serum from donor O, which was found to contain T cell stimulating activity in C1q binding assay and indirect granulocyte phagocytosis test revealed no evidence for the presence of immune complexes.

A second criticism in the editorial and both the reviewers' comments concerns the specificity of the stimulating Ig. In the interpretation of our paper we have stated, as quoted by the editor, that it would be important to show that the cloned T cells are specific for anti-rabies virus antibodies. To that end we have started the generation of autologous EBV transformed B cell lines. Thusfar we have not obtained lines producing Ig of the relevant specificity. However, a pre-immune serum of donor O was recently traced. In contrast to all rabies virus immune sera of donor O tested thusfar its pre-immune serum failed to induce proliferation of the T cell clones. These results, which were incorporated in table 1 of the revised paper, again indicate that the cloned T cells are specific for anti-rabies virus antibody.

As his second point reviewer 2 argues that it is difficult to understand how free rabies virus can inhibit proliferation of class II restricted cloned T cells. The reviewer intuitively concluded that class II restricted activation of T cells by Ig requires processing of the Ig and that free rabies virus prevents APC uptake of the Ig molecule. We initially came to the same assumption. However, experiments focussed on the demonstration of processing of Ig by APC revealed the opposite results. Thus autolo-

gous glutaraldehyde fixed EBV transformed B cells appeared to be as efficient as unfixed EBV-BC in presenting the Ig to cloned T cells.

Furthermore EBV-BC pulsed with Ig in the presence of either leupeptin or chloroquine were as effective as EBV-BC pulsed with Ig in the absence of these drugs, in inducing T cell proliferation. Thus the activation requirements for class II restricted Ig-specific T cells in this case are not identical to those of class II restricted antigen-specific T cells, since apparently processing of Ig is not required. These results may be difficult to interpret with regard to the class II restricted activation of the cloned T cells by Ig, they are, however, in agreement with the observation that free rabies virus blocks activation.

Thus although the V region seems important for T cell activation, the exact mechanism(s) how Ig is presented by APC and is recognized by the T cells in association with class II antigens remains a matter of speculation.