

26th FORUM
IN IMMUNOLOGY**REGULATION OF ANTIBODY PRODUCTION
BY SUPPRESSOR T CELLS**Organized by **C. Leclerc** and **L. Herzenberg****INTRODUCTION****C. Leclerc** (1) and **L. Herzenberg** (2)(1) *Institut Pasteur, 75724 Paris Cedex 15 and*
(2) *Stanford University, Stanford, CA (USA)*

For many years, immunologists have been trying to understand the mechanisms responsible for immunoregulation. One of the most intriguing properties of the immune system is certainly its capacity to be specifically suppressed either "spontaneously" or experimentally. For a while, specialized regulatory T cells (*i.e.* suppressor T cells) were universally accepted as playing a key role in many of these regulatory systems. In fact, a few years ago, there was scarcely a regulatory phenomenon known that someone did not attribute to suppressor T-cell activity. However, the existence of such cells (particularly in the mouse) has been increasingly questioned recently due to difficulties in obtaining T-cell lines that specifically suppress

responses and in cloning genes for molecules that mediate specific suppression.

This Forum reopens the suppressor T-cell question from a modern perspective. As is evident from the contributions, considerable progress has been made towards understanding of the mechanisms of immunosuppression. Many points are still surrounded by controversy; however, rather than supporting the view that suppressor cells do not exist, the data discussed show how rich and promising this field of investigation has become. We thank all the participants for having discussed their most recent findings and for raising key issues that hopefully will stimulate further studies on this fascinating area of investigation.

T SUPPRESSOR LYMPHOCYTES AND ASPECTS OF IMMUNOLOGICAL TOLERANCE

E. Kölsch

*Institute for Immunology, University of Münster,
D-4400 Münster (FRG)*

The actual phenomenon of antigen-specific downregulation or suppression of immune responses by T cells is not in dispute. Overwhelming evidence supporting the concept has been accumulating within the 20 years since the discovery of the phenomenon (Gershon and Kondo, 1971). In question is the existence of T suppressor (Ts) lymphocytes as a distinct T-cell lineage. Many colleagues believe that suppression can be explained by the interaction of the already known components of the immune system. Above all, critics maintain that (1) Ts cells have not been cloned; (2) Ts cells have no conventional T-cell receptors; (3) any kind of antagonistic down-regulating interaction of lymphokines could mimic T-cell mediated suppression; and (4) many Ts-cell factors have been described but genes for such factors have not been cloned. In addition, there were misinterpretations.

1) Considerable work on suppression is connected with the I-J determinant, which was prematurely considered by critics to be an artefact because of the difficulty in mapping the gene responsible. I-J is not coded for by a gene of the MHC, despite the fact that formal genetics puts it there. As others have pointed out, this does not, however, exclude the existence of a haplotype-restricted serologically defined epitope coded for by a locus outside the MHC.

2) Preoccupation with CD4 as marker for helper, and CD8 for cytotoxic T cells does not rule out the existence of other T-cell lineages using either one of them. The combination of

both markers, or one of them in combination with yet undefined ones, might define other true lineages.

For argument's sake, in this Forum, I shall take the role of a strong proponent of the existence of special down-regulating T lymphocytes, traditionally termed Ts cells. The reason for this bias comes from three experimental systems.

Work has been carried out on mechanisms of low dose tolerance to antigens like phage fd and BSA (Kölsch *et al.*, 1975; Heuer *et al.*, 1982; Degwert *et al.*, 1987). Subimmunogenic doses of antigen induce specific Ts-cell mediated unresponsiveness, as demonstrated in adoptive transfer experiments. Ts-cell clones have shed light on the function of these cells. Their suppressive capacity can be measured *in vitro* by their influence on primed lymph node cell proliferation and anti-hapten antibody formation. *In vivo* both antigen-specific priming of T cells is inhibited, as well as the humoral anti-hapten response during the course of hapten-carrier priming. Thus, cloned Ts effector cells when injected together with antigen specifically paralyse the immune system of an animal (Degwert *et al.*, 1988). These Ts-cell clones show exquisite antigen specificity in their antigen-dependent proliferation and have functional transcripts for TCR α - and β chains which show in-frame V-J-C and V-D-J-C joining (Heuer *et al.*, 1988).

An area of longstanding interest concerns the analysis of immune reactions during tumour growth. For obvious reasons early stages of

tumorigenesis cannot be analysed, and researchers have to rely on models which mimic them. Knowing the generation time of an immunogenic transplantable tumour, it is possible to consecutively inject exponentially increasing numbers of irradiated tumour cells and measure the influence of this treatment on the anti-tumour immune status of the animal. The results are surprising: the first reaction of the immune system towards the initially low but increasing number of tumour cells is the activation of specific Ts cells. They prevent, both *in vivo* and *in vitro*, the otherwise successful induction of a cytotoxic T-cell response, apparently via inactivation of corresponding T helper (Th) cells (Haubeck and Kölsch, 1982; Haubeck *et al.*, 1988).

The third example of Ts-cell regulation comes from the response to "thymus-independent" antigens. Conventionally raised BALB/c mice respond to $\alpha(1 \rightarrow 3)$ dextran (Dex) with an IgM response expressing predominantly the public MOPC 104 idiotype. BALB/c nu/nu mice and germ-free-raised normal BALB/c, in addition, show a vigorous IgG response employing the same idiotype. Pre- or perinatal exposure of the animal to the antigen is decisive for the behaviour of the immune system in the adult (Schuler *et al.*, 1982). We have now isolated Ts-cell lines responsible for this regulatory phenomenon. They are idiotype-specific, do not require antigen and inhibit proliferation and antibody secretion of only those B-cell hybridomas expressing the MOPC 104 idiotype. *In vivo* they phase out the capacity of nu/nu mice to produce anti-Dex IgG antibodies bearing the MOPC 104 idiotype (Stäb, F. and Kölsch, E., in preparation).

The above is a short description of three seemingly disparate examples in which Ts cells are operating. Yet, all three systems are related to aspects of immunological tolerance, the first one perhaps not to self tolerance, though there are many circulating proteins in the body for which low dose BSA might be a model system. In the second case, the tumour-specific Ts cells, in contrast

to cytotoxic T cells, recognize a self determinant and not a tumour-associated antigen on the ADJ-PC5 plasmacytoma cells. This means that self-recognizing Ts cells exist in the body, in this case with adverse effects on the initiation of a cytotoxic T-cell response against tumour-associated neoantigens (Kloke *et al.*, 1986; Grooten *et al.*, 1987). Ts-cell activation as a consequence of pre- or perinatal exposure to environmental antigens, I consider a further example for their role in self tolerance. The predominant control of IgG responses might not be accidental since IgG seem to be more deleterious than IgM autoantibodies.

Though considerable selection of the repertoire takes place in the thymus, one cannot neglect data showing that specific Ts cells in the periphery prevent the appearance of autoreactivity (Miller and Calkins, 1988). It is legitimate to consider their control as part of the mechanisms for maintenance of the integrity of the immune system.

Yet, there are many unanswered questions associated with the appearance of Ts cells in the periphery. The Ts-cell repertoire is adaptive. In a yet unknown way, it evolves in ontogeny in parallel with maturation of the overall immune system, and from then on contributes to the maintenance of immunological tolerance (Miller and Calkins, 1988).

It is not so much a question of idiotype or antigen specificity of Ts cells. If they were antigen-specific, this would perhaps reduce the number of regulatory clones involved in an ongoing response. If the repertoire is greatly restricted, as in the BALB/c anti-Dex response, idiotype restriction would serve the same purpose. In addition, since Dex is a "treadmill" antigen, the idiotype determinant is the most likely structure to be processed.

Evaluation of the phenotype of Ts cells poses several difficulties. It was argued for not clonally dissected Ts-cell populations that a number of subgroups with different phenotypes interact as Ts inducers, effectors and contrasuppressors. These experiments have hardly

clarified the cellular basis of suppression. In fact, taken together in association with discrete factors of each cell type, they have contributed to the existing confusion. Ts-cell clones isolated by different groups show a heterogeneous picture. However, the three BSA-specific Ts-cell clones isolated in our laboratory are comparable in phenotype (I-A^{k+}, I-E^{k+}, CD4⁺, CD8⁺) despite differing in cross-reactivity and the use of restriction elements in antigen-specific proliferation (Heuer *et al.*, 1986). T-cell clones with such a phenotype are suspicious to those thinking of traditional T-cell line categories. It is known that *in vitro* cultivation together with differential influences of interleukins can modulate cell phenotypes, for example, induce Th1.2 expression in B lymphocytes (Snapper *et al.*, 1988). But why do all three BSA-specific Ts clones modulate their phenotype according to the same program?

It is important to learn about possible differences in the activation of Th and Ts cells of the same antigen specificity. From our data, I favour the idea that Ts cells have a lower activation threshold than Th cells. This implies that Th cells cannot be the Ts-cell inducers, though it would be economical if Th cells were involved in the activation of all effector T cells. It remains to be clarified why relatively large doses of antigen are required for *in vitro* propagation of Ts-cell clones. Furthermore, I believe that Th- and Ts-cell-inducing determinants are distinct epitopes; this has been shown for foreign protein antigens, and is of eminent importance for understanding anti-tumour responses.

Antigen recognition by the BSA-specific Ts clones described can be I-A or I-E restricted (Heuer *et al.*, 1982; Degwert *et al.*, 1987). Thus, there is no absolute requirement for the recognition of one distinct class II molecule; this is unfortunate since restriction singularity would be a good argument in favour of a separate Ts lineage.

The Ts cells studied in our laboratory have the essential features of antigen-presenting cells because they en-

dogenously express class II molecules (Degwert *et al.*, 1987; Haubeck and Kölsch, 1985). If they use such a mechanism, how can they be specific? This puzzle has been solved for BSA-specific Ts cells. Activation of their Ts function requires the presence of nominal antigen BSA, apparently for the occupation of the T-cell receptor. If this condition is fulfilled, not only BSA-specific but also Th cells with specificity for non-cross-reacting antigens can be suppressed *in vitro* (Degwert *et al.*, 1987). This is compatible with the model in which antigen presentation by Ts cells to Th cells is part of Ts function (fig. 1). The requirement of the nominal antigen is under normal *in vivo* conditions the safeguard ensuring specificity of suppression.

Analysing low-dose tolerance to BSA, we have provided the first evidence that Ts-cell and target Th-cell interaction involves a cytolytic component (Heuer *et al.*, 1982; Heuer and Kölsch, 1986). This is now widely discussed as the Ts-effector mechanism (Simpson, 1988). Since we now understand the aspect of antigen presentation in the system and some other minor differences with cytotoxic T-cell activity, we avoid the term «class-II-restricted killers». In fact, the latter cells might well use the mechanism described by us. Thus, analysis of class II antigen expression by Ts cells becomes mandatory.

As a further personal view, I would thus stress that Ts cells operate in direct cell-cell contact. Known or unknown lymphokines would be expected to play a minor role in Ts-cell mediated suppression.

Integrating our data into a general discussion, we can now deal with the objections raised in the introduction.

Argument (1) is not valid. Ts cells have been isolated and cloned, apparently most successfully under conditions where they have been proven to dominate the immune response, *e.g.* low-dose tolerance or situations which mimic Ts-cell-dominated early stages of tumorigenesis (Heuer *et al.*, 1982; Degwert *et al.*, 1987; Haubeck and Kölsch, 1985).

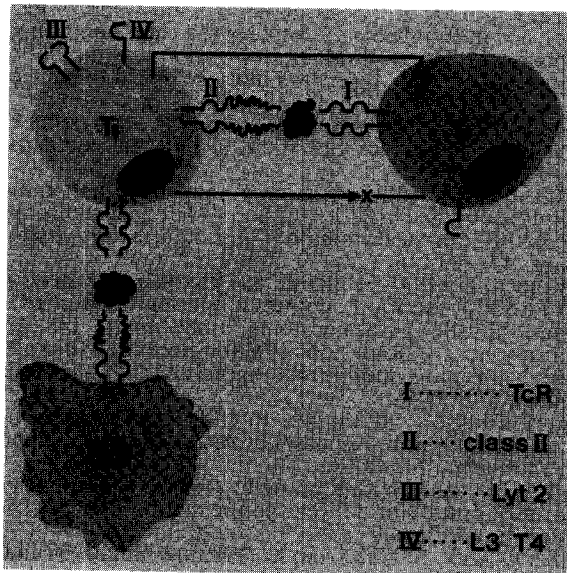


FIG. 1. — Schema to demonstrate parameters of BSA-specific Ts-cell activation and effector function.

X represents a possibly additional Ts-Th interaction molecule.

Argument (2), for unknown reasons, was predominantly based on negative data from Ts-cell hybridoma analysis (Blanckmeister *et al.*, 1985). The murine Ts-cell clones studied by us have antigen specificity and functional transcripts of TCR α - and β -genes (Heuer *et al.*, 1988). A human KLH-specific Ts-cell clone expresses determinants of the constant region of the human TCR α , β complex (Takeuchi *et al.*, 1988). Even considering the relatively small number of

known cloned Ts cells, the positive results should be appraised.

My personal feelings about (3) are ambiguous. Certainly some suppression phenomena might be caused by interleukin antagonism; but the simple theory that T-cell suppression is caused by plain IL-2 deprivation cannot be maintained (Haubeck *et al.*, 1988).

Thus, only argument (4) holds true and remains a large obstacle, though hopefully for not too long.

References.

- BLANCKMEISTER, C.A., YAMAMOTO, K., DAVIS, M.M. & HÄMMERLING, G.J. (1985), Antigen-specific, I-A-restricted suppressor hybridomas with spontaneous cytolytic activity. Functional properties and lack of rearrangement of the T-cell receptor β -chain genes. *J. exp. Med.*, **162**, 851-863.
- DEGWERT, J., BETTMANN, R., HEUER, J. & KÖLSCH, E. (1987), Isolation of a new bovine-serum-albumin-specific T suppressor cell clone and evaluation of its *in vitro* functions. *Immunology*, **60**, 345-352.

- DEGWERT, J., HEUER, J. & KÖLSCH, E. (1988), Specific *in vivo* suppression of lymph node cell proliferation and humoral immune responses by cloned antigen-specific T suppressor cells. *J. Immunol.*, **140**, 1448-1453.
- GERSHON, R.K. & KONDO, K. (1971), Infectious immunological tolerance. *Immunology*, **21**, 903-914.
- GROOTEN, J., LEROUX-ROELS, G. & FIERS, W. (1987), Specific suppression elicited by EL4 lymphoma cells in syngeneic mice. Specificity includes self-antigens on EL4. *Europ. J. Immunol.*, **17**, 605-611.
- HAUBECK, H.-D. & KÖLSCH, E. (1982), Regulation of immune responses against the syngeneic ADJ-PC-5 plasmacytoma in BALB/c mice. — III. Induction of specific T suppressor cells to the BALB/c plasmacytoma ADJ-PC-5 during early stages of tumorigenesis. *Immunology*, **47**, 503-510.
- HAUBECK, H.-D. & KÖLSCH, E. (1985), Isolation and characterization of *in vitro* and *in vivo* functions of a tumor-specific T suppressor cell clone from a BALB/c mouse bearing the syngeneic ADJ-PC-5 plasmacytoma. *J. Immunol.*, **135**, 4297-4302.
- HAUBECK, H.-D., MINKENBERG, I. & KÖLSCH, E. (1988), Selective *in vitro* activation of BALB/c T suppressor lymphocytes specific for syngeneic ADJ-PC-5 plasmacytoma cells. *Clin. exp. Immunol.*, **74**, 53-58.
- HAUBECK, H.-D., STUTENKEMPER, R. & KÖLSCH, E. (1988), Suppression of specific and non-specific cytolytic anti-tumor responses by tumor-specific T suppressor lymphocytes. *Clin. exp. Immunol.*, **74**, 47-52.
- HEUER, J., APELER, H., DEGWERT, J. & KÖLSCH, E. (1988), The primary structure of T-cell receptor α - and β -genes in an antigen-specific murine T suppressor lymphocyte clone. *Immunobiology*, **178**, 106.
- HEUER, J., BRÜNER, K., OPALKA, B. & KÖLSCH, E. (1982), A cloned T-cell line from a tolerant mouse represents a novel antigen-specific suppressor cell type. *Nature (Lond.)*, **296**, 456-459.
- HEUER, J., DEGWERT, J. & KÖLSCH, E. (1986), Expression of L3T4 molecules on Lyt-2⁺, class-II-restricted antigen-specific T suppressor lymphocytes. *Immunogenet.*, **24**, 316-323.
- HEUER, J. & KÖLSCH, E. (1986), Selective elimination through a cytolytic mechanism of bovine serum albumin specific T helper lymphocytes by T suppressor cells with the same antigen-specificity. *Europ. J. Immunol.*, **16**, 400-404.
- KLOKE, O., HAUBECK, H.-D. & KÖLSCH, E. (1986), Evidence for a T-suppressor-cell-inducing antigenic determinant shared by ADJ-PC-5 plasmacytoma and syngeneic BALB/c spleen cells. *Europ. J. Immunol.*, **16**, 659-664.
- KÖLSCH, E., STUMPF, R. & WEBER, G. (1975), Low zone tolerance and suppressor T cells. *Transplant. Rev.*, **26**, 56-86.
- MILLER, R.D. & CALKINS, C.E. (1988), Development of self-tolerance in normal mice. Appearance of suppressor cells that maintain adult self-tolerance follows the neonatal autoantibody response. *J. Immunol.*, **141**, 2206-2210.
- SCHULER, W., LEHLE, G., WEILER, E. & KÖLSCH, E. (1982), Immune response against the T-independent antigen $\alpha(1 \rightarrow 3)$ dextran. — I. Demonstration of an unexpected IgG response in athymic and germfree-raised euthymic BALB/c mice. *Europ. J. Immunol.*, **12**, 120-125.
- SIMPSON, E. (1988), Suppression of the immune response by cytotoxic T cells. *Nature (Lond.)*, **336**, 426.
- SNAPPER, C.M., HORNBECK, P.V., ATASOY, U., PEREIRA, G.M.B. & PAUL, W.E. (1988), Interleukin 4 induces membrane Thy-1 expression on normal murine B cells. *Proc. nat. Acad. Sci. (Wash.)*, **85**, 6107-6111.
- TAKEUCHI, T., SCHLOSSMANN, S.F. & MORIMOTO, C. (1988), Development of an antigen-specific CD8 suppressor effector clone in man. *J. Immunol.*, **141**, 3010-3015.

Studies by the author are supported by the Deutsche Forschungsgemeinschaft (SFB 310), the Minister für Wissenschaft und Forschung NRW IV B 5-500 002 86 and the Deutsche Krebshilfe WA/85/Köl.

PRESENT UNDERSTANDING OF SUPPRESSOR T CELLS

T. Tada, Y. Asano and K. Sano

Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo (Japan)

The availability of IL-2-dependent T-cell clones has enabled us to define CD8⁺ cytotoxic (Tc) and CD4⁺ helper (Th) T cells at the clonal level. Mouse CD4⁺ T-cell clones have been subdivided into Th₁ and Th₂ primarily by their ability to produce different lymphokines. It is generally accepted that T cells belong to either one of the these cell types.

What about the suppressor T cells (Ts)? This cell type is located in the "dead angle" of the above criteria. There are no particular CD antigens unique to Ts. Biochemical identification of lymphokines exerting a suppressor function has not been achieved. It has been extremely difficult to establish Ts clones. And, the phenomenology of Ts-mediated suppression has been complex enough to tire rationalists.

Does Ts in fact exist? This question has been asked several times, recently in an irritated and cross manner with a tone of accusing heresy. Our intention here is not to refute the skepticism about Ts, but to reappraise several features of Ts in order to encourage further investigation.

Definition of Ts.

We define Ts as *T cells specialized in inhibiting the immune response not by cytotoxicity and not by the production of known helper-type lymphokines.* There are some CD8⁺ T cells with cytotoxicity for antigen-presenting cells (APC) which recognize antigen associated with class II molecules. They may suppress the *in vitro* antibody response by eliminating APC, but they cannot be called Ts according to the above defini-

tion. Some lymphokines such as IL-4 can suppress antibody synthesis of B cells, in high doses (Asanao *et al.*, 1988), but the T cells producing IL-4 are not called Ts. Gamma interferon (γ IFN) and transforming growth factor beta (TGF β) can inhibit the proliferation of certain cells, but the T cells producing such lymphokines are not called Ts. Ts cells are at present defined as those *specialized in the role of suppressing the immune response.*

Th and Ts clones.

Are there any such examples? For the sake of simplicity, we will confine our discussion only to cloned T-cell lines acting as effector cells. All T-cell clones which we describe here are available to those who are interested in the suppressor mechanism.

In table I is a partial list of T-cell clones maintained in our laboratory. They were established from antigen-primed spleen cells by occasional stimulation with antigen and APC followed by propagation with IL-2. Th clones were selected for their helper activity for B cells in an *in vitro* secondary antibody response. They can be subdivided into Th₁ and Th₂ by their ability to produce IL-2 and γ IFN (Th₁) or IL-4 (Th₂). All the clones are L3T4⁺ and Ly-2⁻. Some L3T4⁺ T-cell clones show absolutely no helper activity for B cells. They could proliferate upon stimulation with antigen plus APC just as Th cells did. When such cells were added to the culture of primed spleen cells that were stimulated with antigen, the secondary antibody response was

TABLE I. — Properties of IL-2-dependent regulatory T-cell clones.

Code	Origin	Specificity	Type	Function	Production of:		
					IL-2	γ IFN	IL-4
2-19-2	B10	A ^b +FGG, A ^{bm12}	L3T4	Th	+	+	-
9-16	F ₁	E ^k +KLH	L3T4	Th	+	+	-
23-1-8	F ₁ → C3H	A ^k +KLH	L3T4	Th	+	+	-
28-4	F ₁	A ^k +KLH	L3T4	Th	+	+	-
28-2	F ₁	K ^k +KLH	L3T4	Th	+	nd	-
24-2	F ₁ → B6	A ^b +KLH	L3T4	Th	-	-	+
24-15-1	F ₁ → B6	A ^b	L3T4	Th	-	nd	+
8-5	F ₁	A ^b +KLH	L3T4	Th	-	-	+
MS202	C3H	A ^k	L3T4	Th	-	-	+
8-4	F ₁	A ^b +KLH	L3T4	Ts	-	+	-
9-5	F ₁	A ^k +KLH	L3T4	Ts	-	+	-
26-11-20	B6 → F ₁	A ^k +KLH	L3T4	Ts	-	+	-
25-18-5	B6 → F ₁	E ^k +KLH	L3T4	Ts	-	+	-
3D10	C3H	A ^k +KLH	Ly 2	Ts	-	+	-
13G2	B6	A ^b +casein	Ly 2	Ts	-	+	-

nd=not done.

greatly suppressed (Asano *et al.*, 1983). Such cells were defined as Ts clones.

Under no circumstances did they produce IL-2 or IL-4, but γ IFN was produced to variable degrees. However, γ IFN cannot account for the suppressor activity, as even at higher concentrations γ IFN did not suppress the response. Such Ts clones may also suppress the activity of other cloned Th cells (Asano *et al.*, 1983). Suppression by Ts is characterized by strict H-2 restriction.

We have had only two CD8⁺ Ts clones that suppress antibody responses and T-cell proliferation without detectable cytotoxic activity. Clone 3D10 constitutively produced an antigen-specific suppressor T-cell factor (TsF) that acted selectively on CD4⁺ T cells to inhibit their proliferation and helper function (Kitamura *et al.*, 1984). Unfortunately, the activity has been lost after years of cultivation. The clone produced γ IFN and IL-3 upon stimulation with antigen and APC (Koyasu *et al.*, 1985). The second CD8⁺ Ts clone is also an effector Ts. This clone

recognizes an antigenic fragment (residues 136-196) of bovine milk casein on H-2^b APC and suppresses CD4⁺ T cells and T-cell clones with H-2 restriction. At least these Ts clones of the CD4⁺ and CD8⁺ subset seem to fit to the criteria of Ts we set above.

TcR rearrangements.

All the CD4⁺ Ts clones were found to have rearrangements of TcR α - and β -chain genes and transcription of both chains. CD8⁺ Ts clones also showed successful rearrangement of TcR α - and β -genes. Clone 3D10 showed an additional γ -gene in-phase rearrangement (Heilig and Tonegawa, 1987). The other clone (13G2) expressed V _{β 8}, and anti-V _{β 8} blocked the suppressor function. These results indicate that the cloned Ts cells so far obtained express the usual TcR heterodimers for recognition of antigen. This is in contrast to reports of Ts hybridomas where a defect in TcR-gene expression has been emphasized (Hedric *et al.*, 1985).

I-J determinants.

I-J was first described as a genetic marker of Ts and TsF (Murphy *et al.*, 1976; Tada *et al.*, 1976). However, I-J was later found on other cell types including Th. Recently, I-J has been defined as an epitope which is adaptively expressed on T cells during ontogeny according to environmental H-2 (Asano *et al.*, 1987). Recent studies in our laboratory have identified I-J as a dimeric molecule of MW 84,000, composed of MW 43,000 glycopeptides. An I-J molecule with identical physicochemical properties can be precipitated from Th and Ts. I-J participation in the process of suppression has been shown by the fact that preincubation of T cells with anti-I-J reagents resulted in the inhibition of antigen-induced proliferative response and helper function of cells (Nakayama *et al.*, 1988). Successful immunoprecipitation of I-J followed by biochemical analysis will soon uncover the nature of this paradoxical molecule.

Mechanisms of suppression.

Although there exist complex regulatory processes in the induction of Ts cells, the final effector mechanism seems to be relatively simple. CD4⁺ Ts clones suppress the antibody production of B cells by inhibiting CD4⁺ Th functions. Suppression is primarily due to inhibition of early membrane signal transduction. This was studied by measuring intracellular Ca⁺⁺ in Th cells after stimulation with antigen plus APC. Fura-2-loaded Th clone was admixed with Ts cells and antigen-pulsed APC under stopped-flow conditions (Utsunomiya *et al.*, submitted). The Ca⁺⁺ influx of Th cells induced by antigenic stimulation was almost completely inhibited by the co-existence of a Ts clone having the same H-2 restriction specificity. Ts clones were able to inhibit the Ca⁺⁺ response of Th clones, but not vice versa (unidirectional). Ts clones were not able to in-

hibit the Ca⁺⁺ response of other Ts clones (target specificity).

Similar inhibition of Ca⁺⁺ response can be obtained by pretreatment of T-cell clones with anti-I-J. F(ab')₂, but not Fab of anti-I-J, induced the inhibition of the Ca⁺⁺ influx. We are currently studying how Ts or anti-I-J inhibit the Ca⁺⁺ response in target cells. Clearly, it is not by cytotoxic killing. The presence of a new type of lymphokine that acts locally on target cells in close proximity has also to be examined. Both γ IFN and TGF β were unable to inhibit the Ca⁺⁺ response.

Conclusion and future perspectives.

The above observations with IL-2-dependent T-cell clones undoubtedly indicate that T cells of both CD4⁺ and CD8⁺ subtype which specialize in suppression by non-cytotoxic mechanisms do exist. They utilize conventional TcR to recognize antigen and MHC. The relationship of TcR to previously described antigen-specific TsF is still unknown. I-J has been found on both CD4⁺ Th and Ts, and on CD8⁺ Ts, and is not a unique marker for Ts. However, I-J on the cell surface seems to play an important role in the generation of a negative signal transduction for cell activation.

I-J is a homodimeric glycoprotein of MW 86,000. Since the monomeric form of I-J has also been demonstrated, its relationship to previously described TsF should also be examined. Molecular cloning of I-J will enable the understanding of the mechanism of adaptive acquisition of I-J polymorphism on T cells and its role in the regulation of immune responses. If I-J is an anti-self receptor for suppression, it may play a role in preventing the autoreactivity of T cells positively selected to recognize self MHC. As the door to answer these questions is now being opened, can we still be so narrow-minded as to ignore the existence of Ts cells?

References.

- ASANO, Y. & HODES, R.J. (1983), *J. exp. Med.*, **158**, 1178-1190.
- ASANO, Y., NAKAYAMA, T., KUBO, M., FUJISAWA, I., KARASUYAMA, H., SINGER, A., HODES, R.J. & TADA, T. (1988), *J. Immunol.*, **143**, 419-426.
- ASANO, Y., NAKAYAMA, T., KUBO, M., YAGI, J. & TADA, T. (1987), *J. exp. Med.*, **166**, 1613-1626.
- HEDRIC, S.M., GERMAIN, R.N., BEVAN, M.J., DORF, M., ENGEL, B., FINK, P., GASCOIGN, N., HEBER-KATZ, E., KAPP, J., KAUFMANN, Y., KAYE, J., MELCHERS, F., PIERCE, C., SCHWARTZ, R.H., SORENSEN, C., TANIGUCHI, M. & DAVIS, M.M. (1985), *Proc. nat. Acad. Sci. (Wash.)*, **82**, 531-535.
- HEILIG, J.S. & TONEGAWA, S. (1987), *Proc. nat. Acad. Sci. (Wash.)*, **84**, 8070-8074.
- KITAMURA, K., NAKAUCHI, H., KOYASU, S., YAHARA, I., OKUMURA, K. & TADA, T. (1984), *J. Immunol.*, **133**, 1371-1378.
- KOYASU, S., NAKAUCHI, H., KITAMURA, K., YONEHARA, S., OKUMURA, K. & TADA, T. (1985), *J. Immunol.*, **134**, 3130-3136.
- MURPHY, D.B., HERZENBERG, L.A., OKUMURA, K., HERZENBERG, L.A. & McDEVITT, H.O. (1976), *J. exp. Med.*, **144**, 699-712.
- NAKAYAMA, T., KUBO, R.T., KUBO, M., FUJISAWA, I., KISHIMOTO, H., ASANO, Y. & TADA, T. (1988), *Europ. J. Immunol.*, **18**, 761-765.
- TADA, T., DAVID, C.S. & TANIGUCHI, M. (1976), *J. exp. Med.*, **144**, 713-725.

ANTIGEN-SPECIFIC REGULATORY T-CELL FACTORS AND THE T-CELL RECEPTOR

D.R. Green and H. Zheng

*Department of Immunology, University of Alberta, Edmonton,
Alberta T6G 2H7 (Canada)*

The five "no's" of suppression.

T suppressor (Ts) cells, herein defined as T cells which produce antigen-specific, antigen-binding, immunosuppressive factors, have fallen into disrepute despite their demonstration in a large number of systems and laboratories (reviewed in (Cone *et al.*, 1988)). The problems associated with the study of such cells and factors we have nicknamed "the five no's". Stated in more detail by others (Moller, 1988), these are: 1) no T-cell receptor (TCR) rearrangements can be seen in Ts cells; 2) no I-J gene can be found in the region of the murine MHC to which I-J maps;

3) no unique markers for Ts cells have been described; 4) no clones of Ts cells have been generated; and 5) no genes have been identified which encode antigen-specific Ts factors. While some have taken this as a strong argument against a *unique* subset of Ts cells, a great many immunologists contend that this pretty well finishes this area of study in general.

Herein, we will consider only the first "no", regarding TCR in Ts cells (argument 1), because not only has there been a sudden burst of progress in this area, but also because we find it particularly interesting. However, to state a position, we consider argument (3) ir-

relevant, argument (4) wrong, and argument (5) unjust, since if and when such genes are described, this argument will be unnecessary (argument (2) is currently being addressed by other investigators in this Forum). Either antigen-specific regulatory T-cell factors are a mass hallucination, or else they are real. If the latter is true, then perhaps new approaches can be taken to studying them. One such approach is considered herein.

Antigen-specific regulatory T-cell molecules and the TCR.

One approach to the study of antigen-specific regulatory T-cell factors has been through the production of T-cell hybridomas generated from Ts cells. Such Ts hybridomas have been made in a large number of laboratories and have been used as a source of "monoclonal" factor. However, when such hybridomas were analysed for TCR β -chain gene rearrangement, it was found that unlike helper T-cell lines and hybrids, these Ts hybrids lacked the rearranged gene (Hedrick *et al.*, 1985; Kronenberg *et al.*, 1985). In several cases, the chromosome bearing this gene was missing (Kronenberg *et al.*, 1985). These observations led to the conclusion that Ts cells do not express or use TCR.

Shortly after these observations were reported, two reports of TCR rearrangement and expression in a Ts line and a Ts hybridoma appeared (Imai *et al.*, 1986; De Santis *et al.*, 1985), but these had relatively little impact on thinking about the problem. At best, one could say that Ts cells rearrange and express conventional (α/β) TCR genes, but that this expression had little or no role in the function of the cell.

A critical difference between Ts hybridomas and other T-cell hybridomas is that the function of the former (*i.e.*, production of detectable factor) is not rapidly lost in culture. The possibility emerged, therefore, that as Ts hybridomas were maintained for extended periods in culture, fewer cells expressed TCR (through chromosome loss). It was possible that fewer cells also

sustained a capacity for production of factor.

This possibility was directly addressed in an important experiment by Weiner *et al.* (1988). They found that a cloned Ts hybridoma expressed cell surface T3 on only 1-3 % of cells growing in culture. Upon cloning these cells, they found that these T3⁺ Ts cells were the only ones capable of producing detectable factor. The T3 molecules on the surface of the cells were associated with a heterodimeric TCR. This result was confirmed for other Ts hybridomas (Kuchtoo *et al.*, 1988). Further, in this second study, the T3⁺ Ts hybrids were also found to directly bind antigen, and this binding was removed by modulating T3 from the surface. Again, a heterodimeric TCR could be precipitated from the cell surface using either anti-T3 or anti-C α antibodies.

These studies indicated that the earlier results concerning TCR in Ts hybridomas may have been confounded by a technical artifact and that TCR expression may, indeed, be important for the function of such cells. The question then arises as to whether there is any relationship between the TCR on Ts cells and the mysterious antigen-specific Ts factor.

Recent studies from Fairchild *et al.* (1988) addressed this question. They found that antigen-specific factors from a series of Ts hybridomas (or from "bulk" Ts cells) were bound by a monospecific anti-TCR antiserum. Like many antigen-specific Ts factors (Cone *et al.*, 1988) this factor was composed of two chains. One chain, capable of binding antigen, was found to react with a monoclonal anti-C α antibody, while the other non-antigen-binding chain is bound by antibodies to the β chain (Fairchild and Moorhead, personal communication). A similar observation was reported by Perrin *et al.* (1989) who found V β determinants on the non-antigen-binding chain of an antigen-specific Ts factor involved in regulation of schistosoma granuloma formation.

We have studied an antigen-specific suppressor inducer activity which is constitutively produced by a helper T-cell

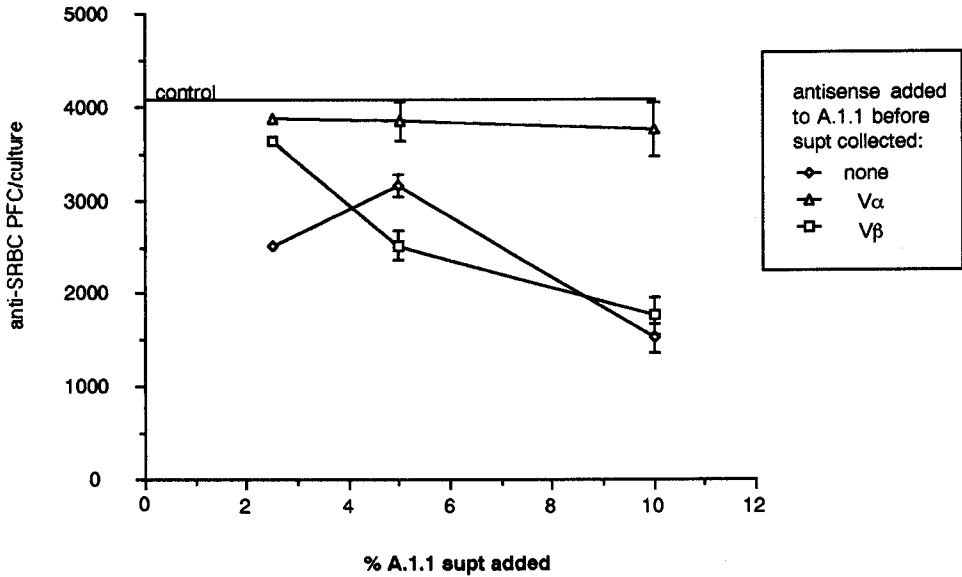


FIG. 1. — Antisense oligodeoxynucleotides to TCR-V α block production of antigen-specific suppressor inducer factor from A1.1.

A1.1 cells were cultured with 50 μ M of the indicated antisense "21mer" for 48 h and supts collected. Supernatants were tested as described previously (Zheng *et al.*, 1988). Briefly, cultures contained 10^7 C57BL/6 spleen cells, 5×10^6 sheep red blood cells (SRBC) coupled with poly18, 10 % of a supernatant from SRBC-immune T cells absorbed with SRBC (accessory supt) and the indicated amount of A1.1 supt. After 5 days, anti-SRBC PFC/culture were determined. No inhibition was observed when either A1.1 supt or accessory supernatant were added alone (not shown).

hybridoma, A1.1. These cells release lymphokines in response to antigen presenting cells (APC) plus a synthetic polypeptide antigen, poly18. The factor from these cells resembles the antigen-binding chain of a T suppressor inducer factor and can specifically recognize the poly18 antigen. The ability of helper T cells (defined by their ability to release lymphokines in response to antigen plus APC) to release such a factor had been previously observed by ourselves (Green *et al.*, 1987) and others (Koyasu *et al.*, 1985). Analysis of the antigenic fine-specificity of the cell surface TCR and the factor from A1.1 revealed a

remarkable concordance (Zheng *et al.*, 1988). Peptides capable of stimulating the T cell via the TCR were also capable of acting as targets for the soluble factor, while nonstimulatory peptides could not. This led us to postulate a relationship between the factor and the TCR of this cell. Using the monospecific anti-TCR antiserum employed by Fairchild (see above) we found that the antigen-specific factor from this cell bears TCR determinants (Zheng *et al.*, 1989). A more direct demonstration of a relationship between the factor and the TCR, however, depended on the use of a novel technique, which we consider next.

Antisense oligodeoxynucleotides to V α inhibit both cell surface TCR expression and production of antigen-specific regulatory factor in A1.1.

We have taken advantage of recent techniques employing antisense oligodeoxynucleotides to block translation of TCR chains in T cells. Such oligonucleotides have been successfully used in regulating oncogene and lymphokine gene function, as well as function of other genes (reviewed elsewhere (Zheng *et al.*, 1989)). Antisense oligodeoxynucleotides corresponding to approximately the first 7-8 codons after the translation start of TCR-V α or -V β , when added to cultures of T-cell hybridomas, are capable of blocking the re-expression of cell surface TCR following trypsin treatment. This inhibition is specific for the TCR-V regions expressed in the T-cell hybridoma. Further, the oligonucleotides do not affect expression of other, irrelevant cell surface molecules, such as class I MHC.

As discussed above, A1.1 cells constitutively produce an antigen-specific activity which appeared to be related, in some way, to the TCR. When antisense oligodeoxynucleotides corresponding to the TCR-V α or -V β of A1.1 were added to cultures of these cells, both oligonucleotides (but not control oligonucleotides) blocked the re-expression of cell surface TCR. Production of the antigen-specific factor, however, was unaffected by either control or V β antisense oligonucleotides. Notably, antisense oligonucleotides corresponding to the TCR-V α of A1.1 completely blocked production of the antigen-specific activity by the A1.1 cells (Zheng

et al., 1989). One such experiment is shown in figure 1.

Taken together with our earlier results (Zheng *et al.*, 1988; 1989) and those of Fairchild *et al.* (1988) it is now likely that TCR genes, especially α , play an important role in encoding antigen-specific regulatory molecules. Because the chemistry of such molecules is somewhat different from that of the TCR (*e.g.* 1 (Cone *et al.*, 1988)), it is likely that if TCR genes do encode any part of these factors, the products are altered at either the mRNA or protein levels. In this regard, it is interesting that alternative splicing of TCR RNA has been observed ((Behlke and Loh, 1986); A. Fotedar, personal communication), opening the possibility that such alternative splicing may play a role in this relationship.

Conclusions.

The results discussed herein strongly suggest that the presence and function of conventional TCR in Ts cells be re-examined as a possible key to the dilemma of antigen-specific regulatory T-cell molecules. Although most TCR recognize only a combination of MHC and antigenic fragments, some TCR molecules may show an affinity for antigen alone, and thereby may direct T cells to take on regulatory functions. In light of the recent results relating TCR α chains and antigen-binding, antigen-specific factors, it is interesting that for several antigens there is a strong association between V α utilization and antigen recognition. Perhaps modern immunology should take another hard look at antigen-specific regulatory T-cell factors before just saying "no".

References.

- BEHLKE, M.A. & LOH, D.Y. (1986), Alternative splicing of murine T-cell receptor β -chain transcripts. *Nature* (Lond.), **322**, 379-382.
- CONE, R.E., ZHENG, H., CHUE, B., BEAMAN, K., FERGUSON, T. & GREEN, D.R. (1988), T cell-derived antigen-binding molecules (TABM): molecular and functional properties. *Intern. Rev. Immunol.*, **3**, 205-228.

- FAIRCHILD, R.L., KUBO, R.T. & MOORHEAD, J.W. (1988), Soluble factors in tolerance and contact sensitivity to 2,4-dinitrofluorobenzene in mice. — IX. A monoclonal T-cell suppressor molecule is structurally and serologically related to the α/β T-cell receptor. *J. Immunol.*, **141**, 3342-3348.
- GREEN, D.R., CHUE, B., ZHENG, H., FERGUSON, T.A., BEAMAN, K.D. & FLOOD, P.M. (1987), A helper T-cell clone produces an antigen-specific molecule (T-ABM) which functions in the induction of suppression. *J. mol. Cell. Immunol.*, **3**, 95-108.
- HEDRICK, S.M., GERMAIN, R.N., BEVAN, M.J., DORF, M., ENGEL, I., FINK, P., GASCOIGNE, N., HEBER-KATZ, E., KAPP, J., KAUFMAN, Y., KAYE, J., MELCHERS, F., PIERCE, C., SCHWARTZ, R.H., SORENSEN, C., TANIGUCHI, M. & DAVIS, M.M. (1985), Rearrangement and transcription of a T-cell receptor β chain gene in different T cell subsets. *Proc. nat. Acad. Sci. (Wash.)*, **82**, 531-535.
- IMAI, K., DANNO, M., KIMOTO, H., SHIGEMOTO, K., YAMAMOTO, S. & TANIGUCHI, M. (1986), Sequence and expression of transcripts of the T-cell antigen receptor α chain gene in a functional, antigen-specific suppressor T-cell hybridoma. *Proc. nat. Acad. Sci. (Wash.)*, **83**, 8708-8712.
- KOYASU, S., NAKAUCHI, H., KITAMURA, K., YONEHARA, S., OKUMURA, K., TADA, T. & YAHARA, I. (1985), Production of interleukin 3 and γ -interferon by an antigen-specific mouse suppressor T-cell clone. *J. Immunol.*, **134**, 3130-3136.
- KRONENBERG, M., GOVERMAN, J., HAARS, R., MALISSEN, M., KRAIG, E., PHILLIPS, L., DELOVITCH, T., SUCIU-FOCA, N. & HOOD, L. (1985), Rearrangement and transcription of the β -chain genes of the T-cell antigen receptor in different types of murine lymphocytes. *Nature (Lond.)*, **313**, 647-653.
- KUCHROO, V.K., STEELE, J.K., BILLINGS, P.R., SELVARAJ, P. & DORF, M.E. (1988), Expression of CD3-associated antigen-binding receptors on suppressor T cells. *Proc. nat. Acad. Sci. (Wash.)*, **85**, 9209-9214.
- MOLLER, G. (1988), Do suppressor T cells exist? *Scand. J. Immunol.*, **27**, 247-250.
- PERRIN, P.J., PRYSTOWSKY, M.B. & PHILLIPS, S.M. (1989), The molecular basis of granuloma formation in schistosomiasis. — II. Analogies of T-cell-derived suppressor effector factor to the T-cell receptor. *J. Immunol.*, **142**, 985-991.
- DE SANTIS, R., GIVOL, D., HSU, P., ADORINI, L., DORIA, G. & APELLA, E. (1985), Rearrangement and expression of the α and β T-cell antigen receptor in functional murine suppressor T-cell clones. *Proc. nat. Acad. Sci. (Wash.)*, **82**, 8638-8642.
- WEINER, D.B., LIU, J., HANNA, B., BLUESTONE, J.A., COLIGAN, J.E., WILLIAMS, W.W. & GREENE, M.I. (1988), CD3-associated heterodimeric polypeptides on suppressor hybridomas define biologically active inhibitory cells. *Proc. nat. Acad. Sci. (Wash.)*, **85**, 6077-6081.
- ZHENG, H., BOYER, M., FOTEDAR, A., SINGH, B. & GREEN, D.R. (1988), An antigen-specific helper T-cell hybridoma produces an antigen-specific suppressor inducer molecule with identical antigenic fine specificity. *J. Immunol.*, **140**, 1351-1358.
- ZHENG, H., FOTEDAR, A., SINGH, B. & GREEN, D.R. (1989), An antigen-specific immunoregulatory activity from a helper T-cell hybridoma: possible relationship to the T cell receptor, in "Cellular basis of immune modulation" (Kaplan, J.G., Green, D.R. & Bleakley, R.C.) (pp. 379-382). Alan R. Liss, Inc., New York.
- ZHENG, H., SAHAI, B.M., KILGANNON, P., FOTEDAR, A. & GREEN, D.R. (1989), Specific inhibition of cell-surface T-cell receptor expression by antisense oligodeoxynucleotides and its effect on the production of an antigen-specific regulatory T-cell factor. *Proc. nat. Acad. Sci. (Wash.)* (in press).

CHARACTERISTICS OF CARRIER-SPECIFIC REGULATORY T CELLS

K. Ishizaka, M. Iwata and K. Katamura

Johns Hopkins University School of Medicine
at the Good Samaritan Hospital,
Baltimore, MD 21239 (USA)

At present, many cellular immunologists and molecular biologists are suspicious about the presence of regulatory T cells, which were believed to produce T-cell factors having affinity for nominal antigen. Our interest in regulatory T cells and T-cell factors originated from unexpected findings in the regulation of the IgE antibody response. We found that glycosylation-inhibiting factor (GIF), which is involved in the selective formation of IgE-suppressive factors, is derived from I-J⁺, Lyt2⁺ T cells; when BDF1 mice were treated with i.v. injections of ovalbumin (OVA) in order to generate "antigen-specific suppressor T (Ts) cells", the GIF from such OVA-treated mice bound to OVA-coupled Sepharose (Jardieu *et al.*, 1984).

Subsequent work indicated that OVA-binding GIF from a T-cell hybridoma suppressed *in vivo* antibody response to DNP-OVA in an antigen-(carrier)-specific manner (Jardieu *et al.*, 1987), bound to anti-I-J alloantibodies and consisted of multiple (two) polypeptide chains (Jardieu and Ishizaka, 1987); one of the polypeptide chains appeared to bind to nominal antigen, while the other peptide carried the I-J-determinant and was responsible for GIF activity.

Recent experiments suggested that the antigen-binding chain of GIF binds to monoclonal antibody 14-12 which binds antigen-specific TseF (Ferguson *et al.*, 1985). Thus, antigen-specific GIF from the hybridoma appears to represent a kind of TseF. On the other hand, glycosylation-enhancing factor (GEF), which is involved in the selective formation of IgE-potentiating factors, ap-

pears to be related to augmenting factor (TaF) described by Tokuhisa *et al.* (1978), Hiramatsu *et al.* (1981) and Miyatani *et al.* (1983). Both antigen-binding GEF and TaF augment the antibody response in a carrier-specific manner, but can not replace helper T (Th) cells (Iwata *et al.*, 1987). Both factors are composed of an antigen-binding chain and a non-specific chain, the latter of which bound to alloantibodies against Ia (Miyatani *et al.*, 1983; Iwata and Ishizaka, 1988).

The first problem to be discussed is whether the cell sources of TsF (GIF) and TaF (GEF), actually represent a distinct subset of T cells. This question was raised from the findings by Hedrick *et al.* (1985) who showed that rearrangement of the V β gene of T-cell receptor (TcR) cannot be demonstrated in many Ts-cell hybridomas studied. However, subsequent work by several investigators demonstrated rearrangement of both α - and β -chain genes in some mouse Ts-cell clones (DeSantis *et al.*, 1985) and human Ts-cell hybridomas (Modlin *et al.*, 1987). Recent publications on azobenzene-specific Ts hybridomas (Weiner *et al.*, 1988) and NP-specific Ts hybridomas (Kuchroo *et al.*, 1988) indicated that the cell source of TsF bears CD3.

Our experiments on the GIF-producing hybridoma 231F1 cells and GEF-producing hybridoma 12H5 cells are in agreement with these findings. The 12H5 cells can be stained by monoclonal anti-CD3 (11-2C) and anti-TcR β chain. After enrichment of the β -chain⁺ population in the 231F1 cells, we obtained a TcR β ⁺, CD3⁺ subclone of 231F1 cells. The 231F1 cells produ-

ced IgE-suppressive factors and OVA-binding GIF (TsF) upon incubation with OVA-pulsed syngeneic macrophages (Jardieu *et al.*, 1987), while the 12H5 cells produce IgE-potentiating factor and OVA-binding GEF (TaF) upon incubation with OVA-pulsed syngeneic macrophages (Iwata and Ishizaka, 1988).

The responses of the 231F1 cells are H-2^d restricted, while the responses of the 12H5 cells are H-2^b restricted. When these cells were incubated with OVA-pulsed macrophages of histoincompatible strains, IgE-binding factors (IgE-BF) were not detected in culture supernatants, and GIF/GEF in the supernatants failed to bind to OVA-Sepharose. It appears that the hybridomas constitutively produce non-specific GIF/GEF (GIF/GEF chain) but not an antigen-binding chain unless the cells were stimulated with OVA-pulsed antigen-presenting cells. Furthermore, treatment of the 231F1 cells with either anti-CD3 (11-2C) or anti-TcR β , supplied by Dr Ralph Kubo, followed by culture of the antibody-treated cells in protein-A-coated wells resulted in the formation of IgE-BF and OVA-binding GIF. Similar treatment of 12H5 cells resulted in the formation of IgE-BF and OVA-binding GEF. These findings suggest that both hybridomas bear TcR: $\alpha\beta$ and that stimulation of the cells through T-cell receptors resulted in the formation of antigen-binding T-cell factors. It appears that regulatory T cells may represent a subset of TcR: $\alpha\beta$ -bearing T cells.

Since the hybridomas produced neither IL-2 nor IL-4 upon antigenic stimulation, we suspect that regulatory T cells, represented by the 231F1 and 12H5 hybridomas, are distinct from Th cells. However, Green *et al.* (1987) reported that D10G4 cells, a well-established Th-cell clone, produced an antigen-binding chain of TsIF upon incubation with UV-irradiated antigen-pulsed macrophages, and Zheng *et al.* (1988) reported that a Th-cell hybridoma, A11, that responds to a synthetic polypeptide in the context of I-A^d also formed an antigen-binding chain of TsIF. It appears that the

"antigen-binding chain" could be produced by various T-cell subsets, including Th cells. It has been believed that the I-J⁺ chain in TsF and the Ia(t)⁺ chain of TaF determine MHC-restriction of the T-cell factors.

Our experiments indicate that I-J⁺ chain has GIF activity and represents a derivative of phospholipase inhibitory protein (Jardieu *et al.*, 1987). On the other hand, Ia(t)⁺ chain in TaF may be non-specific GEF, which has kallikrein-like enzymatic activity (Iwata and Ishizaka, 1988). We suspect that the biochemical functions of I-J⁺ GIF chain and Ia(t)⁺ GEF chain play an essential role in the immunoregulatory effects of TsF/TaF. If this is the case, Ts cells would represent the T-cell subset which produce both the antigen-binding chain and GIF, while Ta cells would be the T cells that produce both the antigen-binding chain and GEF. It is quite possible that the regulatory T cells represent phenotypes of T cells, rather than a distinct lineage. This concept may explain the lack of a cell surface marker which is characteristic of Ts cells.

Two major questions emerge which concern Ts cells and Ta cells. Several investigators succeeded in enriching Ts cells by adherence to antigen-coated tissue culture dishes. It is also known that antigen-specific factors derived from the cells bind to antigen-coupled Sepharose. T-cell receptors on the 231F1 cells and 12H5 cells recognize a fragment of processed antigen (OVA) in association with a product of MHC. Incubation of these hybridomas with nominal antigen or antigen-coated wells failed to induce the formation of IgE-BF.

Failure of the cells to respond to nominal antigen, however, does not exclude the existence of a possible relationship between T-cell factors and TcR. Zheng *et al.* (1989) reported that in A11 cells, all synthetic peptides capable of stimulating the cells to release lymphokine were similarly effective as targets for the A11-derived "antigen-binding factor". Fairchild *et al.* (1988) reported that their TsF bind to polyclonal anti-TcR. We have also

obtained evidence that OVA-binding GIF (TsF) from the 231F1 cells bind to both monoclonal anti-TcR α , and anti-TcR β , which were kindly supplied by Dr R. Kubo. Zheng *et al.* (1989) recently demonstrated that anti-sense oligonucleotide for TcR of the A11 cells prevent not only the expression of TcR but also the formation of antigen-binding factor by the cells. These findings collectively suggest that a structural gene encoding the TcR chains may be utilized for the synthesis of antigen-binding T-cell factors, and that the factors may share a common structure with the α -chain and/or β -chain of TcR.

Siliciano *et al.* (1986) reported that TcR: α B on human T-cell clones specific for fluorescein isocyanate can bind hapten-conjugated polyacrylamides, and suggested that hapten itself has a weak affinity for TcR. It is quite possible that TcR on NP-specific Ts and GAT-specific Ts cells may bind NP and GAT group, respectively, without the participation of MHC-products. If the same principles apply to Ts cells specific for a protein antigen, it is expected that TcR on Ts cells is specific for external structures on the native antigen molecules.

In some protein antigen systems, such as fibrinogen (Lee *et al.*, 1988) and influenza virus haemagglutinin (Mills *et al.*, 1986), processing is not required for antigen presentation. In the OVA system, most of the OVA-specific Th hybridomas from OVA-primed BALB/c mice responded to a peptide representing amino acids 323-339 in the context of I-A^d (Shimonkevitz *et al.*, 1984). The amino acid sequence of the peptide implies that the peptide is hidden in the molecules. If our hypothesis is correct, one may expect that Ts cells may recognize external structures in OVA molecules in association with a MHC product. Recent experiments in

collaboration with Dr H. Grey have shown that both the 231F1 cells and 12H5 cells formed IgE-BF when they were incubated with syngeneic macrophages together with a synthetic peptide representing amino acids 307-317 in the OVA molecule, but not with the peptide 323-339.

Furthermore, our preliminary experiments showed that the interaction between the OVA-binding factors and OVA-Sepharose was inhibited by P307-317 but not by P323-339, suggesting that OVA-binding molecules share common specificity with TcR on the cell source. It was noted that 8 out of 11 amino acids in the peptide are hydrophilic, suggesting that the peptide may represent an external structure in the native OVA molecules.

A fundamental question is whether the specificity of TcR on Ts cells is always distinct from those on Th cells. We found that some of the GEF/GIF-producing T-cell hybridomas responded to P323-339 in the context of a MHC product for the formation of IgE-BF. As expected from the hypothesis described above, GEF/GIF produced by the cells upon antigenic stimulation, failed to bind to the native OVA. We suspect that T-cell factors might recognize the epitope represented by P323-339. An important question yet to be answered is whether these cells and/or GIF from such cells can regulate the immune response to native OVA.

Speculation about the mechanisms of immunosuppression by Ts cells and TsF depends highly on the outcome of future experiments where we would like to determine whether some "antigen-specific GIF" may bind to the epitope identical to that recognized by TcR on Th cells, and if so, whether such GIF may or may not suppress the antibody response.

References.

- DESANTIS, R., GIVOL, D., HSU, P., ADORINI, L., DORIA, G. & APPELLA, E. (1985), *Proc. nat. Acad. Sci. (Wash.)*, **82**, 8638.
FAIRCHILD, R.T., KUBO, R.T. & MOORHEAD, J.W. (1988), *J. Immunol.*, **141**, 3342.

- FERGUSON, T.A., BERMAN, K.D. & IVERSON, M. (1985), *J. Immunol.*, **134**, 3163.
- GREEN, D.R., CHUE, B., FERGUSON, T.A., BEAMAN, K.D. & FLOOD, P.M. (1987), *J. mol. Cell. Immunol.*, **3**, 95.
- HEDRICK, S.M., GERMAIN, R.N., VEVAN, M., DORF, M., ENGEL, I., FINK, P., GASCOIGNE, N., HOBER-KATZ, E., KEPP, J., KAUFMAN, Y., KAYE, J., MELCHER, F., PIERCE, D., SCHEARTZ, R.H., SORENSEN, C., TANIGUCHI, M. & DAVIS, M.M. (1985), *Proc. nat. Acad. Sci. (Wash.)*, **82**, 531.
- HIRAMATSU, K., MIYATANI, S., KIM, M., YAMEDA, S., OKUMURA, R. & TADA, T. (1981), *J. Immunol.*, **127**, 1118.
- IWATA, M., FUKUTONI, Y., HASHIMOTO, T., SATO, Y., SATO, H. & ISHIZAKA, K. (1987), *J. Immunol.*, **133**, 2561.
- IWATA, M. & ISHIZAKA, K. (1988), *J. Immunol.*, **141**, 3270.
- JARDIEU, P., AKASAKI, M. & ISHIZAKA, K. (1987), *J. Immunol.*, **138**, 1493.
- JARDIEU, P. & ISHIZAKA, K. (1987), *UCLA Symp. Mol. Cell. Biol.*, **41**, 595.
- JARDIEU, P., UEDE, T. & ISHIZAKA, K. (1984), *J. Immunol.*, **133**, 3266.
- KUCHROO, V.K., STEELE, J.K., BILLINGS, P.R., SELVARAJ, P. & DORF, M.E. (1988), *Proc. nat. Acad. Sci. (Wash.)*, **84**, 9209.
- LEE, P., MATSUEDA, G. & ALLEN, P. (1988), *J. Immunol.*, **140**, 1063.
- MILLS, H.G., SKEBEL, J.J. & THOMAS, D.B. (1986), *Europ. J. Immunol.*, **16**, 276.
- MIYATANI, S., HIRAMATSU, K., NAKAJIMA, P.B., OWEN, F.L. & TADA, T. (1983), *Proc. nat. Acad. Sci. (Wash.)*, **80**, 6336.
- MODLIN, R.L., BRENNER, M., KRANGE, M.S., DUBY, A.D. & BLOOM, B.R. (1987), *Nature (Lond.)*, **329**, 541.
- SHIMONKEVITZ, R., COLON, S., KAPPLER, J.W., MARRACK, P. & GREY, H.M. (1984), *J. Immunol.*, **133**, 2067.
- SILLICIANO, R.F., HEMSATH, J., PRATT, J.C., DINTZIS, R.Z., ACUTO, O., SHIN, H.S. & REINHERZ, E.L. (1986), *Cell*, **47**, 161.
- TOKUHISA, T., TANIGUCHI, M., OKUMURA, K. & TADA, T. (1978), *J. Immunol.*, **120**, 414.
- WEINER, D.B., LIU, J., HANNA, N., BLUESTONE, J.A., COLIGAN, J.E., WILLIAMS, W.V. & GREENE, M.I. (1988), *Proc. nat. Acad. Sci. (Wash.)*, **85**, 6077.
- ZHENG, H., BOYER, M., FOTEDAR, A., SINGH, B. & GREEN, D.R. (1988), *J. Immunol.*, **140**, 1351.
- ZHENG, H., SAHAI, B., KILGANNON, P., FOTEDAR, A. & GREEN, D.R. (1989), *Proc. nat. Acad. Sci. (Wash.)* (in press).

SPECIFICITY AND INTERACTIONS OF CD8⁺ T SUPPRESSOR CELLS

U. Krzych, N. Nanda and E. Sercarz

*Department of Microbiology,
University of California,
Los Angeles, CA (USA)*

One of the fundamental issues in immunology is the extent and diversity of specificity repertoires and their comparison among different lymphoid subpopulations. The repertoires of T proliferative cells and B cells have been well studied, while that of CD8⁺ T suppressor (Ts) cells has lagged

behind. Actually, the stable specificity of the CD8⁺ Ts cell instills some confidence that there really are cell types with distinctive expressed repertoires that engage in suppressive activity (it should be remembered to use the plural when considering Ts cells and to clearly distinguish between the CD4⁺ in-

ducer (Tsi) and the CD8⁺ precursor/effector cell (Tse)). In our experience with two quite different protein antigens, *E. coli* β -galactosidase (GZ) and hen eggwhite lysozyme (HEL), particular determinants which activated CD8⁺ Ts did so reproducibly, and these determinants were always different from those that induced CD4⁺ T-cell proliferation and help. In this short overview, we would like to consider the specificity of CD8⁺ Tse and their relationship to CD4⁺ T helper (Th) cell populations.

I. — T suppressor effectors (Tse) and T suppressor inducers (Tsi).

a) *Early partition of Tse and Tsi.* — One of the mysteries in our study of Ir-gene control of the anti-HEL response was the existence of reactivity in putative non-responder H-2^b mice in the popliteal lymph nodes (PLN) after footpad priming (Araneo *et al.*, 1979); intraperitoneal (i.p.) or intravenous (i.v.) priming led to no response. It was of great importance for simplifying the study of CD8⁺ Tse that in fact, Tse were not detectable in the draining PLN soon after immunization although they could be readily demonstrated later as well as among splenic CD8⁺ T cells after i.p. or i.v. injection. In the HEL system, the splenic Tse required the presence of fresh PLN Tsi in order to suppress Th. Presumably, one of the first activities of Tse in the spleen is the down-regulation of Tsi (Green *et al.*, 1981). This anatomical separation of the 2 cell populations has permitted a simplified analysis of cell mixture experiments and was used in most of the experiments to be mentioned.

b) *The restriction specificity of CD8⁺ Tse.* — Although suppressor inducers and effectors are usually phenotyped as CD4⁺ and CD8⁺ respectively, there is no clear picture of the restriction molecules being used by these cells. The unselected suppressor populations (inducers and effectors) have been shown to be restricted to MHC class II molecules, though only in a few systems (Baxenavis *et al.*, 1984;

Nanda, 1989). It is likely that the CD4⁺ inducers in these systems provide the class-II restrictions. Why are the effectors CD8⁺, when there are few known examples of suppression being restricted to class I molecules? The CD8 molecules are clearly tightly linked to the phenomenon of "down-regulation" as even the cloned Ts cells in different systems are either CD8⁺, or CD4⁺CD8⁺, in spite of being restricted to the class II molecules (Modlin *et al.*, 1986; Ottenhoff *et al.*, 1986; Nanda *et al.*, 1989). If the effector function of Tse is mediated by their direct interaction with inducers (Tsi) and cellular targets (Th or CTL) of suppression, the CD8 molecules may indeed be important. It has recently been shown that CD8 molecules are able to directly bind class I molecules (Rosenstein *et al.*, 1989) and as the latter are expressed on the surface of all cells of the immune system, the CD8 molecules may play a role in providing the extra "adhesiveness" to facilitate the multicellular interaction required for the function of Ts cells; this would amount to "pseudo-restriction" to class I molecules, as no class I antigen complex is formed.

II. — Amputation of suppressor T-cell-inducing determinants (SD).

a) *Immunogenicity revealed by amputation.* — Induction of Tse activity with fragments and peptides of HEL or GZ had shown that in every case, the SD was different from Th-cell-inducing determinants (HD), and non-overlapping with them (Adorini *et al.*, 1979; Krzich *et al.*, 1988). Thus, in explaining non-responsiveness to the whole molecule, it was assumed that the CD8⁺ Tse induced by SD were able to nullify Th induced by HD. Specificity controls indicated that the SD and HD had to be on the same molecule for suppression to take place. It followed that if the SD could be removed or altered, this might be a mechanism to reveal HD on apparently non-immunogenic molecules. This approach was attempted with HEL (a.a.1-129) in two stages.

In a first set of experiments, the "amputation" was major, as removal of 1-12 and 106-129 uncovered T-cell inducing activity in the residual 13-105 fragment (Yowell *et al.*, 1979). From other evidence, it was already expected that SD included residue 3, so a less ambitious "circumcision" was attempted to just remove the amino terminal lysal-phe from HEL (Wicker, Katz *et al.*, 1984; Wicker, Benjamin *et al.*, 1984). Again, the residual molecule (a.a.4-129) could induce both proliferation and help, indicating that the SD was localized on HEL at the amino terminus, and that if other SD existed, they were not active in preventing responsiveness of the remainder of the molecule. Apparently, a single SD could prevent responsiveness to the molecule as a whole.

b) *Complexities in the interpretation of amputation experiments.* — An amputation experiment apparently can reveal underlying responsiveness to some portions of a molecule, and it can also reveal the location of a suppressor T-cell-inducing determinant. However, without substantiating evidence from peptide induction of Ts followed by cell-mixing experiments (Adorini *et al.*, 1979), it may be hazardous to use amputation as direct evidence for Ts. We have reviewed a variety of studies that lead to an alternative type of explanation for changes in T-cell response pattern with change in the structure of the immunogen (Sercarz, Wilbur, Gammon *et al.*, 1986; Sercarz, Wilbur, Sadegh-Nasseri *et al.*, 1986). These studies with lysozyme (Shastri, Miller *et al.*, 1986; Shastri, Gammon *et al.*, 1986) and with other proteins (Kovac and Schwartz, 1985; Shivakumar *et al.*, 1989) show that changes in the molecular context of a determinant, even in residues quite distant from the site of T-cell recognition, will strongly influence whether T cells will be activated or not. Therefore, in amputation studies, it is possible that the derivatized molecule might induce a far different repertoire of T cells, directed against different determinants on the molecule, a repertoire which existed unsuppressed in any case.

c) *Amputation and immune tolerance.* — The lack of overlap between HD and SD in our systems permitted us to address the puzzling question of whether tolerance can be induced by clonal inactivation or by suppression. The crux of the approach is that a minimal HD should not be able to induce suppression. Therefore in an animal tolerant to a multideterminant molecule such as native HEL, we could ask whether a minimal HD from the molecule removed from suppressive influence could induce a T-cell proliferative response. The result is a complex one: first, latent reactivity to a large fragment of the molecule, 13-105, was found upon immunization, indicating that Ts cells had probably been active in regulating the response to the whole molecule (Oki and Sercarz, 1985) but could no longer be implicated in regulation of the response to the amputated fragment. Second, upon challenge of the HEL tolerant animal with certain minimal HD, no T-cell proliferative response could be detected: since in this case, Ts could not be involved, the evidence pointed to direct clonal inactivation. In fact, recent work indicates that under such circumstances, only T cells directed against the dominant determinants are tolerized (Gammon and Sercarz, unpublished), while T cells directed against subdominant determinants are spared.

III. — Tse interaction with Th in the β -galactosidase (GZ) system.

The GZ system provides a possibility of careful dissection of specificities underlying target-effector partnerships: its amino acid sequence is known (Fowler and Zabin, 1978), a repository of small subunit peptides is available for priming and analysis of interacting T-cell populations, and several HD and SD have been enumerated. During the past decade we have been investigating the fine specificity of the T-cell repertoire directed against HD and SD on this large tetrameric protein (monomer molecular weight = 116 kDa). Several lessons were learned from these experiments that are summarized below.

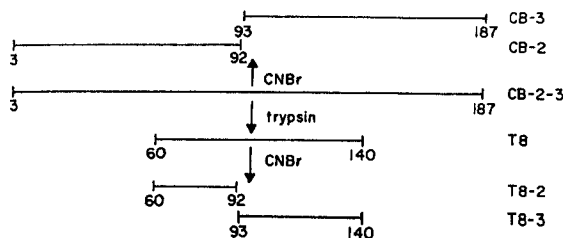
a) *Not all T-proliferation-inducing determinants have regulatory implications.* — A majority of cyanogen bromide (CB) cleavage peptides representing approximately 70 % of the molecule, prime for T-cell responses recalled by GZ in culture. However, not all of these T cells are used for functions such as help and suppression, at least in our assay system, in which the readout was anti-fluorescein-isothiocyanate (FITC) plaque-forming cells to GZ-FITC. Only native GZ and two cyanogen bromide peptides, CB-2 (a.a.3-92) and CB-10 (a.a.387-418), primed for Th cells while only CB-2 and CB-3 (a.a.93-187) primed for Ts cells. The restricted Th expression probably reflects constraints imposed by the specificity of anti-FITC B cells which are the recipients of helper activity. Our evidence (Krzych *et al.*, 1982 and 1985) supports the idea that HD and B-cell determinants (BD) must be in close proximity, so that a single processed antigenic fragment could contain *both* determinants for a successful collaboration. A corollary of this dictum is that in order for suppression to become visible, an HD-BD pair must be close at hand.

b) *Th and Ts cells have separate, non-overlapping specificities.* — Immunization with CB-2 results in activation of both Th and Ts. Probing for fine T-cell specificities with smaller trypsin-cleaved peptides revealed that T6 (a.a.44-52) contains Th-inducing activity, whereas T4-induced (a.a.27-37) Ts cells capable of suppressing the T6-Th. The distinctness of the HD and SD suggests that the specificity repertoires of Th and Ts subpopulations are quite dif-

ferent owing to the selection of different TcR-V gene families, or to the alternative view that the MHC plays a selective role.

c) *Not all inducible Th cells serve as targets for every Ts cell.* — Only certain SD-HD pairs enlist T cells which can enter into a helper-suppressor relationship. For example, GZ-induced Th are suppressible by all Ts cells, whereas CB-2-Th are affected by CB-2-Ts, but not at all by CB-3-Ts. Meanwhile, CB-10-Th are completely insensitive to any of these Ts cells (Krzych *et al.*, 1985). The lack of sensitivity of CB-10-induced Th cells to suppression strongly implies a regional effect in the interaction between Ts and Th. It is compelling to postulate that fragmentation of the large GZ molecule into smaller overlapping fragments occurs and that only when these fragments contain the *combination* of epitopes for recognition by Th, Ts and B cells will the regulatory effect (suppression) become evident.

Availability of the large CB-2-3 (a.a.3-187) peptide fragment, from incomplete CnBr cleavage of GZ, provided yet another source of Th-inducing determinants. Interestingly, the sensitivity of CB-2-3-Th to Ts cells parallels that of GZ-induced Th. Do these results imply that CB-2-3-Th cells arise as a dominant Th population after GZ priming? We have explored this question using smaller, trypsin-cleaved peptides from the region of CB-2-3. Specifically, peptide T8 (a.a.60-140), which overlaps CB-2 and CB-3, and its component peptides, T8-2 (a.a.60-92) and T8-3 (a.a.93-140) were used for Th and



Ts induction to establish possible hierarchies of Th and Ts interactions. T8 proved to be unable to induce Th cells: this was most surprising since 60-140 contains the intact critical central region, including the bond between residues 92 and 93 intact, unlike CB-2 and CB-3 which are split at methionine 92 (CB-2 and CB-3 no longer induce the GZ-specific immunodominant Th, as expected). Further, as mentioned earlier (section IIIb), tryptic peptide priming induces Th of a different specificity, which are not seen after priming with native GZ. Presumably, a hierarchy of Th engagement occurs depending on the structure of the priming antigen. While the exact mechanism of this processing-dependent dominance hierarchy is still unknown, it requires the availability of determinants for MHC interaction, as well as the existence of a T-cell repertoire of the appropriate specificity.

d) *Expression of Ts and Th dominance and antigen processing.* — By testing the susceptibility of the group of induced Th to the available Ts, it became apparent that peptide-induced Th were not of the same specificity as Th induced by GZ or CB-2-3. Similarly, the pattern of induction of Ts by different fragments within the CB-2-3 region, could be used as evidence for parallel hierarchies of Ts dominance (Shivakumar and Sercarz, 1989). For example, despite the fact that peptide T8-3, like T8, contains an SD, the Th target specificity of T8-3-Ts is different from that of T8-Ts: while maintaining effective suppression of GZ-Th targets, T8-3-Ts no longer suppressed CB-2- or T8-2-induced Th.

This shift in Ts repertoire by different inducing molecules suggests a hierarchy of Ts usage, which must be examined further. We think that it relates to antigen processing: in the processing of structurally complex antigens, buried determinants in the native antigen will be surrounded by unique sets of residues, perhaps from distant regions of the primary sequence. The pattern of display of potential determinants for activation of different T-cell subpopulations may very well be directly dependent on their different submolecular contexts, as mentioned above in the lysozyme system (section IIb). It has been postulated that binding of MHC to the unfolding antigen molecule in the endosome could also alter the subsequent route of processing (Sercarz, Wilbur, Sadegh-Nasseri *et al.*, 1986): while certain sites may never get exposed, other parts of the molecule may indeed become more accessible.

It may be very difficult to predict nearest neighbour relationships or the pathways of fragmentation of a molecule. As an example, a head of garlic naturally fragments into cloves, whereas an onion can be carefully peeled into concentric dome-shaped layers. We believe that the dominant "preferential partnerships" of certain Ts and Th arise from the topology of SD and HD established by the varied fragmentation patterns that result from different forms of an immunogen. Although the statement "once a suppressor determinant, always a suppressor determinant" holds in the abstract, for a particular MHC haplotype, the SD may never have a chance to confront a Tse to initiate an induction event.

References.

- ARANELO, B.A., YOWELL, R.L. & SERCARZ, E.E. (1979), *J. Immunol.*, **123**, 961-967.
 ADORINI, L., HARVEY, M.A., MILLER, A. & SERCARZ, E.E. (1979), *J. exp. Med.*, **150**, 293-306.
 BAXENAVIS, C., ISHII, N., NAGY, Z. & KLEIN, J. (1984), *Scand. J. Immunol.*, **16**, 25.
 FOWLER, A.V. & ZABIN, I. (1978), *Proc. nat. Acad. Sci. (Wash.)*, **253**, 588.
 GREEN, D.R., GERSHON, R.K. & EARDLEY, D.D. (1981), *Proc. nat. Acad. Sci. (Wash.)*, **78**, 3819.
 KOVAC, Z. & SCHWARTZ, R.H. (1985), *J. Immunol.*, **134**, 3233.
 KRZYCH, U., FOWLER, A.V., MILLER, A. & SERCARZ, E.E. (1982), *J. Immunol.*, **128**, 1529-1534.

- KRZYCH, U., FOWLER, A., MILLER, A. & SERCARZ, E.E. (1988), *FASEB J.*, **2**, 141-144.
- KRZYCH, U., FOWLER, A.V. & SERCARZ, E.E. (1985), *J. exp. Med.*, **162**, 311-323.
- MODLIN, R.L. *et al.* (1986), *Nature* (Lond.), **322**, 459-461.
- NANDA, N.K. (1989), *Immunology* (in press).
- NANDA, N.K., THOMSON, E., BLUESTONE, J.A. & MASON, I. (1989), Manuscript in preparation.
- OKI, A. & SERCARZ, E.E. (1985), *J. exp. Med.*, **161**, 897-911.
- OTTENHOFF, T.H.M., ELFERINK, D.G., KLATSER, P.R. & DE VRIES, R.R.P. (1986), *Nature* (Lond.), **322**, 462-464.
- ROSENSTEIN, Y., RATNOFSKY, S., BURAKOFF, S.J. & HERRMANN, S.H. (1989), *J. exp. Med.*, **169**, 149-160.
- SERCARZ, E.E., WILBUR, S., GAMMON, G., SADEGH-NASSERI, S., SHASTRI, N. & MILLER, A. (1986), *Ann. Inst. Pasteur/Immunol.*, **137 D**, 329-333.
- SERCARZ, E., WILBUR, S., SADEGH-NASSERI, S., MILLER, A., MANCA, F., GAMMON, G. & SHASTRI, N. (1986), *In Progress in Immunology VI. Sixth International Congress of Immunology*, **6**, 227-237.
- SHASTRI, N., GAMMON, G., HORVATH, S., MILLER, A. & SERCARZ, E.E. (1986), *J. Immunol.*, **137**, 911-915.
- SHASTRI, N., MILLER, A. & SERCARZ, E.E. (1986), *J. Immunol.*, **136**, 371-376.
- SHIVAKUMAR, S., SERCARZ, E.E. & KRZYCH, U. (1989), *Europ. J. Immunol.* (in press).
- WICKER, L.S., BENJAMIN, C.D., MILLER, A. & SERCARZ, E.E. (1984), *Europ. J. Immunol.*, **14**, 447-453.
- WICKER, L.S., KATZ, M., SERCARZ, E.E. & MILLER, A. (1984), *Europ. J. Immunol.*, **14**, 442-447.
- YOWELL, R.L., ARÁNEO, B.A., MILLER, A. & SERCARZ, E.E. (1979), *Nature* (Lond.), **279**, 70-71.

Supported by NIH-AI-11183.

T-CELL-INDUCED CHRONIC IMMUNOGLOBULIN ALLOTYPIC SUPPRESSION IN MOUSE

P. Benaroch (*) and G. Bordenave

*Unité d'Immunophysiologie Moléculaire, Institut Pasteur,
25, rue du Dr Roux, 75724 Paris Cedex 15*

Introduction.

The dispute between cytotoxic T cell versus suppressive T (Ts) cell proponents has been fully and even passionately substantiated in recent issues of the *Scandinavian Journal of Immunology* (Eichmann, 1988; Janeway, 1988; Mitchinson, 1988; Möller, 1988;

Pereira *et al.*, 1988; Tada, 1988). We believe that the key to this problem resides in a convincing *in vivo* demonstration, since, in this field, each *in vitro* observation necessitates a strict correlation at the *in vivo* level. Obviously, this requirement is one of the major difficulties to overcome.

(*) Philippe Benaroch is a recipient of a fellowship from the Ligue Nationale Française Contre le Cancer.

The concept of T_s cells cannot be discarded without strong evidence as we feel that this kind of activity is likely to explain at least a part of the down-regulation of immunoglobulin (Ig) production. Cytotoxic T cells are clearly required in dramatic situations such as those involving myelomas and virus-infected cells. However, cell lysis is the only mechanism of T-cell-mediated negative regulation of Ig production seems to be a concept that is difficult to conceive.

One of the advantages of our experimental model is that it deals with an unambiguous case of T-cell-mediated downregulation of Ig production. It enables *in vivo* induction, in 100 % of treated animals, of complete and chronic inhibition of expression of a whole, large set of Ig: an allotype, when heterozygous mice are concerned; and an isotype, when homozygous mice are studied, regardless of antibody function.

Dray's pioneering work.

Before briefly reviewing some of our data, it is necessary to define the terminology we use.

A few years after the discovery by J. Oudin (1956a; 1976b; 1960a; 1960b), using rabbit Ig, of what he termed allotypy of proteins, S. Dray, again using the rabbit, made the first observation of allotypic suppression (Dray, 1962), namely that perinatal exposure of heterozygous rabbits to antibodies directed against paternally inherited Ig allotypes could lead to chronic abrogation of their expression. These early experiments opened the door to manipulation of the immune system by means of antibodies.

The term "Ig allotypic suppression" was thus coined at an epoch where T cell/B cell dichotomy was not yet suspected. Therefore, the use of the word "suppression" was devoid of any meaning concerning the mechanism ensuring the "chronicity" of the non-expression of the paternally inherited allotypes. It should be noted that this

question was already raised at this time, as the suppression lasted a long time after clearance of the anti-allotypic antibodies which had induced it. This expression was preserved when the L. and L. Herzenberg group successfully transposed S. Dray's protocol in the mouse (Jacobson and Herzenberg, 1972; Jacobson *et al.*, 1972), and showed that the chronicity of this antibody-induced allotypic suppression was ensured by T cells (Herzenberg *et al.*, 1973). Naturally, we continue to use this terminology in honour of S. Dray's precedence. However, this does not mean, for the moment, that we are dealing with T_s-cells rather than cytotoxic T cells.

The cellularly induced Ig allotypic suppression.

Starting from the work of A. Nisonoff's school (Dohi and Nisonoff, 1979; Sy *et al.*, 1979) who succeeded in inducing idiotypic suppression in mouse by using T cells educated against a given idio type, we tried to establish an induction system of allotypic suppression by means of T cells with deliberately induced anti-allotypic activity. This was successfully achieved first in the rabbit (Benaroch and Bordenave, 1984) then in the mouse (see below). The T-cell education was realized by *i.v.* injection of foreign allotype-coated autologous lymphocytes into the T-cell donor.

With regard to Herzenberg's antibody-induced allotypic suppression in mouse which was shown to have its chronicity mediated by autoimmune T cells, we tried to induce allotypic suppression directly by means of normal T cells. We wondered whether using hybrid mice obtained by mating Igh congenic partners, the injection at birth of T splenocytes from one parental strain would induce an allotypic suppression against Igh allotypes inherited from the other parental strain and vice versa.

Indeed, when injected into appropriate Igh^{a/b} newborn mice, normal T splenocytes from BALB/c and BC8

mice (strains of the Igh^a haplotype) were able to induce a suppression of the expression of IgG2a of the Igh^b haplotype (IgG2a^b) in a certain proportion of them (Benaroch and Bordenave, 1987). The suppression was complete, chronic and its spontaneous release was not observed. This activity of T cells from normal Igh^a mice was greatly enhanced when the mice were sensitized either with IgG2a^b-coated autologous splenocytes or with B splenocytes from the Igh^b congenic strain. T splenocytes from such sensitized mice (Tsens) enabled the induction of a chronic IgG2a^b suppression, not only in 100 % of treated Igh^{a/b} mice (Benaroch and Bordenave, 1988) but also in 100 % of treated Igh^{b/b} mice (Benaroch *et al.*, 1988). It has to be stressed that anti-allotypic antibodies were not detectable in sensitized mice or in Tsens recipients.

In B cells devoted to the expression of IgG2a^b, this suppression operates downstream of allelic exclusion; as in Igh^{a/b} heterozygotes, the expression of the IgG2a^a allelic gene is not attained, and also downstream of the isotypic switch, as expression of IgM^b, IgD^b, IgG2b^b and IgA^b is untouched.

We established that two Tsens, CD4 + CD8⁻ Tsens and CD4⁻ CD8⁺ Tsens, were necessary for the induction of suppression (Benaroch and Bordenave, 1989). We succeeded in attempting to abrogate this suppression *in situ* in Igh^{b/b} homozygotes. Indeed, injections of cytotoxic rat anti-mouse CD8 monoclonal antibody (mAb) into IgG2a^b-suppressed Igh^{b/b} homozygotes, quickly reversed the suppression (the day following the last of 7 daily injections we found up to 0.5 mg of IgG2a^b per ml of serum of treated animals) while injections of equivalent amounts of cytotoxic rat anti-mouse CD4 mAb remained without effect. The corollaries of this observation are (1) that CD4⁻ CD8⁺ lymphocytes are essential for the maintenance of the suppression (Benaroch *et al.*, 1988), and (2) that this suppression does not result from a clonal deletion of the B cells capable of producing IgG2a^b. As in other systems (Dorf and Benacerraf, 1984; Tada, 1984), it is possible to imagine that

CD4⁺ CD8⁻ Tsens provide specific signals to effector CD4⁻ CD8⁺ Tsens which induce the inhibition of IgG2a^b expression. For the CD4⁻ CD8⁺ cells maintaining the suppression (whatever their origin), the permanent triggering given by the tendency of their target to reappear would replace the initial signal provided by CD4⁺ CD8⁻ cells. Besides, this permanent triggering is also suggested by our ease (as with Tsens) in inducing this suppression in naive syngeneic recipients by transfer of T splenocytes from IgG2a^b-suppressed adults.

It is noteworthy that this suppression is rather isotypic than allotypic in Igh^{b/b} homozygotes. In addition, for maintenance, this isotypic suppression does not require continual injection of these antibodies, as is usual when induced with antiisotypic antibodies.

The problem of IgG2a^b recognition by T cells.

In the course of the sensitization of BALB/c T-cell donors by B cells from BALB/c Igh^b congenic mice, we showed that among these B cells those responsible for sensitization against IgG2a^b were probably either the minor IgG2a^b-bearing cell population or other cells having, passively or not, adsorbed this allotype, or both. In addition, the success obtained in sensitizing BALB/c mice by means of IgG2a^b-coated autologous splenocytes could also be interpreted as direct T cell recognition of IgG2a^b-bearing cells. Nevertheless, considering the suppression induction, the presence of soluble IgG2a^b (transmitted from mother to foetus through the placenta) in the blood of newborn (Igh^{b/a} heterozygotes or Igh^{b/b} homozygotes) recipients of Tsens, during the suppression induction phase, did not preclude this induction but significantly delayed it (around 19 weeks of age versus 6 weeks of age — the time of the first bleeding — with Igh^{a/b} hybrids). We also observed that it was difficult, but in a few cases not impossible, to abrogate this suppression by massive administration of soluble

IgG2a^b in IgG2a^b-suppressed Igh^{a/b} adults (Benaroch and Bordenave, 1989). Consequently, if Tsens and CD4⁻CD8⁺ cells maintaining the suppression were directed against IgG2a^b-bearing cells, these results could indicate that they would recognize the allotype (or peptides derived from it) in a particular configuration, perhaps in association with other cell membrane structures like the MHC products. Cell candidates for processing and presenting antigens, and even endogenous protein molecules, have greatly increased in the past few years (Bikoff and Birshtein, 1986; Bikoff *et al.*, 1988; Lanzavecchia, 1985; Lanzavecchia *et al.*, 1988). The delay observed in inducing this allotypic suppression in Tsens recipients carrying soluble IgG2a^b in their blood could be ascribed to a kind of Tsens dilution by Tsens action on diverse targets, such as any other cells — and Tsens themselves — having processed and correctly presented IgG2a^b. Such a situation was reported earlier by M. Bosma's team (Snodgrass *et al.*, 1981) who prepared T cells cytolytic for IgG2a^b-bearing myeloma cells and who demonstrated the autolytic capacity of these cells when appropriately incubated with soluble IgG2a^b.

We do not yet have an argument for a given MHC restriction of the action of Tsens, or of T cells maintaining the suppression. Nevertheless, as we obtained the same kind of results with two different couples of Igh congenic mouse strains, one being H₂^d (BALB/c-CB20) and the other H₂^b (C57BL/6-BC8), we can say that if there is an IgG2a^b (or derived peptide) presentation by cells in association with H₂ products, this presentation can be efficiently achieved (inside Igh congenic couples) with at least two different H₂ haplotypes.

Some remarks about the allotypic suppression abrogation.

The facility to abrogate suppression in Igh^{b/b} homozygotes and the subsequent high level of the observed IgG2a^b production (around 0.5 mg/ml

of serum, 8 days after the beginning of the anti-CD8 mAb treatment) surprised us. This may have resulted either from a release of the suppressed allotype by accumulated B cells blocked in their secretion, or from allotype production by B cells quickly descending from precursors.

Our general view of suppressive T-cell action involves accumulation of their target, as opposed to the effect of cytotoxic T cells which immediately kill targets. As a consequence, in our experimental model, we can expect to observe a certain amount of B cells blocked in IgG2a^b production or secretion if T cells maintaining the suppression directly recognize IgG2a^{b+} B cells. Awaiting a more specific criterion, this perhaps represents our first attempt at distinguishing between suppressive or cytotoxic activity.

In a system like ours, there is permanent B-cell renewal from precursors sheltered from suppression (as suppression is exerted downstream of allelic exclusion, this renewal is attested, in IgG2a^b-suppressed Igh^{a/b} hybrids, by the constant production of the IgG2a^a allelic allotype). Therefore, escaping from suppression does not furnish an argument for suppressive versus cytotoxic activity. Obviously, removing the blocking agent, be it suppressive or cytotoxic, will lead to the same result: the resurgence of the function, here IgG2a^b production.

The *in vivo* distinction of regulation mediated by cytotoxic T cells rather than suppressive T cells is hard to establish. For example, what can T cells which totally block the capacity of B cells to secrete an Ig and cause, without direct lysis, slowly but surely, the death of these B cells before they have expressed their function be called? As cell death can be considered a kind of suppression, cytotoxic T cells could be considered members of a large suppressive T-cell family. In opposition to cytotoxic T cells, a minimal definition of suppressive T cells could be the following: T cells capable of down regulating the expression of a given Ig-producing B cell without quickly killing

it. The adverb "quickly" is rather subjective here as it is implicitly related to the time required in the usual *in vitro* chrome-release assay to obtain lysis, and to our lack of knowledge about *in vivo* cell death and *in vivo* lytic mechanisms. Again, perhaps we can hope to distinguish between suppressive T cell and cytotoxic T cell *in vivo* action, by an accumulation of target B cells.

Some considerations about the restriction of this suppression to the IgG2a^b allotype.

Considering, as we have done, only complete inhibition of Ig production and not quantitative variations in the production, it appears that this allotypic suppression is restricted to IgG2a^b. Using the technique of sensitization by means of allotype-coated autologous splenocytes, we were unable to detect, in Igh^a BALB/c mice, any T-cell activity against IgM^b, IgG2b^b or IgA^b (unpublished results). The same holds true for T-cell activity against IgG2a^a in Igh^b BALB/c congenic mice. Contrary to what we thought we observed at the beginning, IgG2a^b suppression has no repercussion on IgG2b^b expression. Moreover, sensitization of Igh^a BALB/c mice with IgG2b^b-coated autologous splenocytes did not enhance their T-cell activity against IgG2a^b (unpublished results). Despite high nucleotide sequence homology between $\gamma 2a^b$ and $\gamma 2b^b$ genes, up to 94 % according to the CH domains (Ollo and Rougeon, 1983), antiallotypic antibodies prepared in Igh^a BALB/c mice against IgG2a^b or IgG2b^b (antiallotypic antibodies which reflect the corresponding B-cell repertoire of these mice) mostly recognize differences between the two allotypes (Lieberman, 1978). Still concerning the assumption of direct recognition of IgG2a^b-bearing B cells by T_sens (IgG2a^b being the entire membrane Ig or pieces of the endogenous processed and presented allotype), we can for the moment conclude that the T-cell repertoire of Igh^a BALB/c mice against IgG2a^b also only recognizes differences between IgG2a^b and IgG2b^b.

Contrary to what happens in mouse antibody-induced allotypic suppression where the cellular mechanism ensuring the chronicity of the suppression is obviously an auto-immune process (Herzenberg *et al.*, 1973), in our system we still have to demonstrate — and work is in progress — the origin (donor or recipient) of the T cells maintaining the suppression. Nevertheless, even in the case of donor origin, it is noticed using the C57BL/6-BC8 Igh congenic couple, we are already very close to an autoimmune situation. Indeed, T cells from BC8 mice, theoretically having the same potential T-cell repertoire as C57BL/6 mice, are able to completely block the expression of a C57BL/6 component, IgG2a^b.

It is of interest to look at the reasons behind this ability to induce suppression restricted to the IgG2a^b allotype. A possible explanation could be a regulation sequelae existing in Igh^a BALB/c mice from a period of time where the two genes, $\gamma 2a^a$ and $\gamma 2a^b$, were tandemly organized, a situation which is still observed in foetal mice (Jouvin-Marche *et al.*, 1989; Lieberman and Potter, 1969; Shimizu, 1982). This means that if the products of these two genes have a "present" of allotypes, they have a "past" of isotypes. Another possibility could be that this T-cell reactivity against IgG2a^b was fortuitous, and was the result of cross-reactivity with a T-cell function which is at present unknown.

Conclusion.

Even if restricted to IgG2a^b expression, this experimental model offers two remarkable *in vivo* possibilities. First, it enables the induction, in 100 % of the treated animals, of the total disappearance of IgG2a^b production. Second, this IgG2a^b production can subsequently be induced to reappear at levels as high as 0.5 mg/ml of serum (in Igh^{b/b} homozygotes). Thus, it provides us with a clear and powerful tool for studying what could be an example of down-regulation mediated by non-cytolytic T cells.

References.

- BENAROCHE, P. & BORDENAVE, G. (1984), Induction of immunoglobulin allotypic suppression in the rabbit: a cellular approach. *Proc. nat. Acad. Sci. (Wash.)*, **81**, 2859-2863.
- BENAROCHE, P. & BORDENAVE, G. (1987), Normal T splenocytes are able to induce immunoglobulin allotypic suppression in F1 hybrid mice. *Europ. J. Immunol.*, **17**, 167-171.
- BENAROCHE, P. & BORDENAVE, G. (1988), Enhancement in Igh^a mouse strains of the "natural" suppressive activity of normal T splenocytes against the expression of Igh-1b allotype. — I. Molecular aspects of the chronic suppression obtained. *Europ. J. Immunol.*, **18**, 51-58.
- BENAROCHE, P., GEORGATSOU, E. & BORDENAVE, G. (1988), Cellular induction of chronic allotype suppression of IgG2a in Igh^{b/b} homozygous mice and its abrogation by *in vivo* treatment with anti-CD8 monoclonal antibody. *J. exp. Med.*, **168**, 891-904.
- BENAROCHE, P. & BORDENAVE, G. (1989), T-cell-induced Ig allotypic suppression in mice. — II. Both CD4⁺CD8⁻ and CD4⁻CD8⁺ T-cell subsets from sensitized Igh^a mice are required to induce suppression of Igh-1b-allotype expression. *J. Immunol.*, **142**, 1-7.
- BIKOFF, E. & BIRSSTEIN, B.K. (1986), T-cell clones specific for IgG2a of the a allotype: direct evidence for presentation of endogenous antigen. *J. Immunol.*, **137**, 28-34.
- BIKOFF, E., YU, H. & ECKHARDT, L.A. (1988), T-cell recognition of endogenous IgG2a expressed in B lymphoma cells. *Europ. J. Immunol.*, **18**, 341-348.
- DOHI, Y. & NISONOFF, A. (1979), Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. *J. exp. Med.*, **150**, 909-918.
- DORF, M.E. & BENACERRAF, B. (1984), Suppressor cells and immunoregulation. *Ann. Rev. Immunol.*, **2**, 127-157.
- DRAY, S. (1962), Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling γ -globulin allotypic specificities. *Nature (Lond.)*, **195**, 677-680.
- EICHMANN, K. (1988), Suppression needs a new hypothesis. An answer to Göran Möller. *Scand. J. Immunol.*, **28**, 273-276.
- HERZENBERG, L.A., CHAN, E.L., RAVITCH, M.M., RIBLET, R.J. & HERZENBERG, L.A. (1973), Active suppression of immunoglobulin allotype synthesis. — III. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node, and bone marrow. *J. exp. Med.*, **137**, 1311-1324.
- JACOBSON, E.B. & HERZENBERG, L.A. (1972), Active suppression of immunoglobulin allotype synthesis. — I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL \times BALB/c)F1 mice. *J. exp. Med.*, **135**, 1151-1162.
- JACOBSON, E.B., HERZENBERG, L.A., RIBLET, R. & HERZENBERG, L.A. (1972), Active suppression of immunoglobulin allotype synthesis. — II. Transfer of suppressing factor with spleen cells. *J. exp. Med.*, **135**, 1163-1176.
- JANEWAY, C.A. Jr (1988), Do suppressor T cells exist? A reply. *Scand. J. Immunol.*, **27**, 621-623.
- JOUVIN-MARCHE, E., GONCALVES MORGADO, M., LEGUERN, C., VOEGTLE, D., BONHOMME, F. & CAZENAVE, P.A. (1989), The mouse Igh-1^a and Igh-1^b H chain constant regions are derived from two distinct isotopic genes. *Immunogenet.*, **29**, 92-97.
- LANZAVECCHIA, A. (1985), Antigen-specific interaction between T and B cells. *Nature (Lond.)*, **314**, 537-539.
- LANZAVECCHIA, A., ROOSNEK, E., GREGORY, T., BERMAN, P. & ABRIGNANI, S. (1988), T cells can present antigens such as HIV gp120 targeted to their own surface molecules. *Nature (Lond.)*, **334**, 530-532.
- LIEBERMAN, R. & POTTER, M. (1969), Crossing over between genes in the immunoglobulin heavy chain linkage group of the mouse. *J. exp. Med.*, **130**, 519-541.
- LIEBERMAN, R. (1978), Genetics of IgCH (allotype) locus in the mouse. *Springer Seminarian Immunopath.*, **1**, 7-30.
- MITCHISON, N.A. (1988), Suppressor activity as a composite property. *Scand. J. Immunol.*, **28**, 271-273.
- MÖLLER, G. (1988), Do suppressor T cells exist? *Scand. J. Immunol.*, **27**, 247-250.
- OLLO, R. & ROUGEON, F. (1983), Gene conversion and polymorphism: generation of mouse immunoglobulin γ 2a-chain alleles by differential gene conversion by γ 2b-chain gene. *Cell*, **32**, 515-523.
- OUDIN, J. (1956a), Réaction de précipitation entre des sérums d'animaux de même espèce. *C.R. Acad. Sci. (Paris) (Sér. D)*, **242**, 2489-2490.
- OUDIN, J. (1956b), L'allotypie de certains antigènes protéidiques du sérum. *C.R. Acad. Sci. (Paris) (Sér. D)*, **242**, 2606-2608.

- UDIN, J. (1960a), Allotypy of rabbit serum proteins. — I. Immunochemical analysis leading to the individualization of seven main allotypes. *J. exp. Med.*, **112**, 107-124.
- UDIN, J. (1960b), Allotypy of rabbit serum proteins. — II. Relationships between various allotypes: their common antigenic specificity, their distribution in a sample population. Genetic implication. *J. exp. Med.*, **112**, 125-142.
- PEREIRA, P., LARSSON-SCIARD, E.L., COUTINHO, A. & BANDEIRA, A. (1988), Suppressor versus cytolytic CD8⁺ T lymphocytes: where are the artefacts? *Scand. J. Immunol.*, **27**, 625-627.
- SHIMIZU, A., HAMAGUCHI, Y., YAOITA, Y., MORIWAKI, K., KONDO, K. & HONJO, T. (1982), Japanese wild mouse, *Mus musculus molossinus*, has duplicated immunoglobulin γ 2a genes. *Nature (Lond.)*, **298**, 82-84.
- SNODGRASS, H.R., BOSMA, M.J. & WILSON, D.B. (1981), T lymphocytes specific for immunoglobulin allotype. — II. Cloned Igh-1b-specific cytolytic T cells. *J. exp. Med.*, **154**, 491-500.
- SY, M.S., BACH, B.L., BROWN, A., NISONOFF, A., BENACERRAF, B. & GREENE, M.I. (1979), Antigen and receptor-driven regulatory mechanisms. — II. Induction of suppressor T cells with idiotype-coupled syngeneic spleen cells. *J. exp. Med.*, **150**, 1229-1240.
- TADA, T. (1984), Help, suppression and specific factors, in "Fundamental Immunology" chapter 18 (W.E. Paul) (pp. 481-517). Raven Press, New York.
- TADA, T. (1988), But still it moves! An answer to Professor Göran Möller. *Scand. J. Immunol.*, **27**, 623-624.

This work was supported by grants 13540 from the Institut Pasteur, U.A. 040.359 from the Centre National de la Recherche Scientifique, and from the Fondation pour la Recherche Médicale.

SUPPRESSION IN AN ADOPTIVE HAPTEN-CARRIER SYSTEM

H. Pritchard-Briscoe and R.H. Loblay

*Clinical Immunology Research Centre, University of Sydney,
NSW 2006 (Australia)*

Möller has recently articulated (1988) the growing disquiet of many immunologists concerning the existence and nature of suppressor T (Ts) cells. We shall focus our discussion on the questions raised by him, indicating those areas where we feel our own data can provide answers based on the observation of robust biological phenomena, and other areas where the available data are "soft", or do not allow firm conclusions to be drawn.

Suppression as an *in vivo* biological phenomenon.

There is general agreement that under appropriate conditions T cells can be shown to inhibit immune responses. Our own studies, spanning more than a decade, have used human gammaglobulin (HGG) to induce suppressor cells, the effects of which are assayed in an adoptive hapten-carrier system (Basten *et al.*, 1975). Thus, spleen cells

transferred from mice recently immunized or tolerized to HGG can suppress the secondary anti-hapten antibody response of cells transferred from hapten- and carrier-primed donor animals. This effect is mediated by purified CD8⁺ T cells, but not other cell types, and can be abrogated by pretreatment of the cells or donor animals with anti-thy-1, anti-Ly-2, and an antiserum raised by cross-immunizing B10A.3R and B10A.5R mice (anti-“I-J”). Normal mouse serum and anti-L3T4 have no effect. Suppression in this system is highly specific for the inducing carrier (HGG), and is capable of inhibiting the response of B cells primed to any hapten in the presence of the appropriate hapten-carrier conjugate. The antigen specificity of suppression is thus exactly analogous to that of helper T (Th) cells.

In addition, suppression, like all other recognized T-cell functions, exhibits memory for the inducing antigen. Thus, when HGG primed or tolerant mice are boosted with deaggregated antigen the subsequent suppression observed in adoptive transfer is accelerated in onset, more profound and more prolonged compared with the primary effect in animals never previously exposed to HGG (Loblay *et al.*, 1984; 1983). This “secondary” suppressive effect is also antigen-specific, can be mediated by 5- to 10-fold fewer T cells, and has proved to be a very potent, long-lived and robust phenomenon in our hands. The clear implication of these findings is that suppression must be mediated by cells with clonally distributed receptors for antigen, notwithstanding observations of a lack of productive rearrangement of T-cell receptor genes in “suppressor” clones, hybridomas and lymphomas (Moller, 1988).

What do we know about how suppression works?

In our system it is clear that suppression acts directly at the B-cell level. Hapten-primed B cells were mixed with helper cells specific for two different

carriers, HGG and KLH, together with either normal cells or suppressor cells specific for HGG. The recipients were challenged with KLH as well as HGG, the hapten being coupled to one or other, or both. Animals challenged with uncoupled HGG and hapten-coupled KLH responded normally, whereas the response of those challenged with hapten coupled to both carriers was suppressed. Thus, the necessary and sufficient condition for suppression was that the hapten be coupled to the inducing carrier, HGG (Basten *et al.*, 1975). This observation is not compatible with suppression acting at the helper cell level.

The nature of the suppressive effect on B cells was investigated in a number of ways. Firstly, delaying the injection of suppressor cells by varying times after co-transfer of helper and B cells, with and without additional boosts of antigen, showed that suppression can only operate during the first 24 h after antigenic stimulation. Thereafter, there was little or no effect on the magnitude of the subsequent plaque-forming cell (PFC) response, suggesting that once activated, B cells are no longer susceptible to suppression (Loblay, unpublished).

To determine whether suppression could be saturated by excess helper cells or B cells, a series of cell titration experiments was performed. Selectively increasing the number of B cells resulted in a linear increase in the PFC response in both suppressed and control groups, but the percent suppression remained constant. Titration of increasing numbers of helper cells into the system showed that it was not possible to compete out the suppressive effect. Furthermore, when the number of helper and suppressor cells were increased in parallel suppression invariably supervened beyond a certain cell number, an effect which was shown to be antigen-specific and which could be abrogated by treatment of the cells with anti-“I-J” before transfer (Loblay and Basten, 1986).

Taken together, the findings suggest that help and suppression act in a mutually exclusive, non-competitive

manner to regulate B-cell activation in the effector phase of the antibody response. Thus, once triggered B cells become refractory to suppression, whereas suppressed B cells are refractory to helper signals even when the latter are present in excess. This is in contrast to the findings of Lynch (1987) who have observed suppression of immunoglobulin synthesis, but not cell growth, using a hybridoma line as the target. The apparent action of suppressor cells on helper cell targets in other models may be due to the need for a regulated T-cell induction step *in vitro* (Feldmann *et al.*, 1977).

Is suppression MHC-restricted?

We have examined the question of whether suppression is MHC-restricted in a long series of experiments with radiation chimeras (Pritchard-Briscoe *et al.*, in preparation). By appropriately varying the genotype and MHC environment of the hapten- and carrier-primed cells in different donor animals, and transferring them to suitable adoptive recipients together with similarly manipulated cells from suppressor donors, it was possible to place a putative MHC-recognition barrier between any desired combination of these interacting cells. As expected, interactions between Th cells and B cells were clearly MHC-restricted. However, suppression was not, regardless of whether the recognition barrier was placed between suppressor cells and Th cells or between suppressor cells and B cells.

In this context it is interesting to note that the antigen-specific suppressor memory cells described above are CD4⁻8⁻ (Andelstein *et al.*, 1989). This suggests that they may not require accessory recognition of MHC for activation, a finding which fits well with their apparent lack of MHC restriction in our system. Furthermore, as we have speculated elsewhere (Andelstein *et al.*, 1989) it raises the question of whether, unlike other T cells, suppressor cells may use $\gamma\delta$ receptors.

Finally, by mixing-and-matching hapten-primed cells, carrier-primed cells

and suppressor cells from either B10A.3R or B10A.5R donors, we were able to show that suppression operates in an unrestricted fashion across an "I-J" barrier (Loblay, unpublished). This is despite the fact that in appropriate strains suppression can be completely abrogated by pretreatment of the transferred cells with antisera raised between B10A.3R and B10A.5R mice. It follows that, whatever "I-J" may be, it does not act as a restriction element.

Is suppression really a manifestation of cytotoxic T-cell activity?

The fate of "suppressed" B cells was studied by removing them from the suppressive environment of their adoptive hosts and restimulating them with an alternative source of help (Loblay, unpublished). Hapten- and carrier-primed cells were transferred in the usual way, together with either HGG-specific suppressor cells or virgin spleen cells, and were stimulated with hapten coupled to HGG. A week later the recipients were sacrificed for PFC assay, and the remainder of their spleen cells were transferred into freshly irradiated animals previously primed to a non-cross-reacting carrier, but never before exposed to HGG or to the hapten. The intention was to provide a source of irrelevant radioresistant help to rescue any surviving "suppressed" B cells, whilst preventing them from having any further interaction with HGG-specific suppressor cells by depriving them of hapten coupled to the relevant carrier, HGG. Not only were the previously suppressed spleen cells able to respond under these circumstances, but the magnitude of the anti-hapten response was doubled by comparison with that of the previously unsuppressed control cells.

From these results it appears that help produces a temporary depletion of the memory B-cell pool, probably by driving cells to divide and differentiate, whereas suppression inhibits the activation of memory B cells, presumably leaving them in a dormant state from

which they can later be aroused by an appropriate antigenic stimulus. Thus, suppression is a reversible phenomenon, at least when B cells are the target of action. This, together with the lack of MHC restriction of suppression, argues strongly against cytotoxicity as its principal mechanism.

Why has it been so difficult to clone suppressor cells?

Despite much effort, and some claims to the contrary, it has not yet been possible to generate a stable T-cell clone with all the attributes of suppressor cells as described here. We have spent three fruitless years attempting to clone antigen-specific Ts cells and the closest we have come is isolation of an HGG-specific, IL-2-dependent CD4+ clone which can act as a helper cell when transferred in low cell numbers, but which appears to act as an *in vivo* inducer of suppression at higher numbers (Crosbie and Loblay, in preparation).

We have come to believe that suppressor cells, unlike other T cells, may be inherently impossible to clone. Assuming suppressor cells act by releasing one or more lymphokines capable of inhibiting the activation and proliferation of any nearby target cell, it stands to reason that, when cultivated *in vitro*, suppressor cells will begin to inhibit one another once they reach a critical cell density. After all, there is no reason why suppressor cells should be exempt from regulatory restraint. It seems likely, therefore, that because of their physiological properties suppressor cells can only be grown *in vitro* if they are grossly abnormal. We therefore disagree with Moller's view (Moller, 1988) that abnormal T cell receptor gene expression in "suppressor" hybridomas and lymphomas argues against the existence of Ts cells *in vivo*.

In vivo veritas.

Moller has also drawn attention to the lack of any consensus about the nature of suppressor "factors". In the absence of an ability to isolate suffi-

ciently large numbers of uncontaminated suppressor cells *in vitro*, this is hardly surprising. Almost all such factors have been described using *in vitro* experimental models where inhibition can occur for a wide variety of reasons, only some of which are of biological significance. Our own attempts to transfer suppression *in vivo* with both serum and supernatants have been unsuccessful, leading us to conclude that if suppression is mediated by soluble factors they must act at very short range in the tissue microenvironment and/or be very unstable.

On the other hand, our results in studying the *in vivo* requirements for induction of suppression are more consistent with the *in vitro* data from other laboratories (Tada, 1984). Injection of antibodies to various T-cell markers showed that *in vivo* induction of primary suppression can be abrogated by anti-Ly-1, anti-Ly-2 and anti-L3T4, but not anti-"I-J", and that suppressor memory induction can be abrogated by anti-Ly-1, anti-Ly-2 and anti-"I-J" (Pritchard-Briscoe *et al.*, in preparation). Adoptive mixing experiments with depleted cell populations suggests that in both cases at least two cell types are involved, but the data do not allow us to determine whether a "feedback circuit" is involved.

Complexity *per se* is not an argument against the existence of suppressor cells, but there has clearly been a tendency for regulatory theories to become unnecessarily elaborate as a result of manipulation of responses *in vitro*. As outlined above, help and suppression are sensitive to the cell numbers as well as ratios used in mixing experiments, and under some circumstances mixed cell populations can behave in an apparently paradoxical way (Loblay and Basten, 1986). No doubt, observations such as this originally led Gershon to postulate the existence of "contrasuppressor" cells (Gershon, 1974). A more parsimonious explanation can be put forward to account for such phenomena by assuming that (1) there are only two classes of regulatory signals, stimulatory and inhibitory, acting on target cells in a non-

competitive way, as outlined above, (2) target cells can be "switched off" more rapidly than "on", and (3) interactions between different cell types are determined stochastically.

Do Ts cells exist as a specialized subset?

The question of whether some CD8⁺ T cells are uniquely and irreversibly committed to act as suppressor cells is not possible to answer at present. Clearly, suppression can be distinguished from cytotoxicity by functional criteria (including sensitivity to anti-"I-J", whatever this may eventually prove to be). If cytotoxic and suppressor cells are separate lineages it is not yet clear whether they are functionally committed before leaving the thymus, or whether commitment takes place after encounter with antigen, under the influence of certain types of antigen-presenting cells, for example.

On the other hand, although it is very unlikely that T cells can perform suppression and cytotoxicity at the same time, the possibility remains that an individual cell (or its progeny) might switch back and forth between these two functions, depending on what "inducer" or other signals are encountered in a given microenvironment.

Similar uncertainties apply to the question of whether there is a specialized subset of DTH cells which can be distinguished from CD4⁺ helper cells. Help and DTH are certainly distinguishable functionally, being regulated in a reciprocal manner by suppressor cells (Nash *et al.*, 1981). However, like CD8⁺ "suppressor/cytotoxic" cells, the real differences may lie at the level of cytokine gene regulation, cellular function being determined principally by the array of lymphokines secreted in response to different stimuli in a particular microenvironment.

References.

- ADELSTEIN, S., PRITCHARD-BRISCOE, H., LOBLAY, R.H. & BASTEN, A. (1989), Suppressor T-cell memory. *Curr. Top. Microbiol. Immunol.* (in press).
- BASTEN, A., MILLER, J.F.A.P. & JOHNSON, P. (1975), T-cell-dependent suppression of an anti-hapten antibody response. *Transplant. Rev.*, **26**, 130-169.
- FELDMANN, M., BEVERLEY, P.C.L., WOODY, J. & MCKENZIE, I.F.C. (1977), T-T interaction in the induction of suppressor and helper T cells: analysis of membrane phenotype of precursor and amplifier cells. *J. exp. Med.*, **145**, 793-801.
- GERSHON, R.K. (1974), Lack of activity of contra-suppressor T cells as a mechanism of tolerance, in "Immunological tolerance. Mechanisms and potential therapeutic applications" (D.H. Katz & B. Benacerraf) (pp. 441-465). Academic Press, New York, London.
- LOBLAY, R.H., PRITCHARD-BRISCOE, H. & BASTEN, A. (1984), Suppressor T-cell memory. Induction and recall of HGG-specific memory suppressor T cells and their role in regulation of antibody production. *Aust. J. exp. Biol. med. Sci.*, **62**, 11-25.
- LOBLAY, R.H., FAZEKAS DE ST. GROTH, B., PRITCHARD-BRISCOE, H. & BASTEN, A. (1983), Suppressor T-cell memory. — II. The role of memory suppressor T cells in tolerance to human gammaglobulin. *J. exp. Med.*, **157**, 957-973.
- LOBLAY, R.H. & BASTEN, A. (1986), Why is the immune system not overwhelmed by suppression? A reductionist paradox, in "Paradoxes in Immunology" (G.W. Hoffmann, J.G. Levy & G.T. Nepom) (pp. 115-122). CRC Press Inc., Boca Raton, Florida.
- LYNCH, R.G. (1987), Immunoglobulin-specific suppressor T cells. *Advanc. Immunol.*, **40**, 135-151.
- MOLLER, G. (1988), Do suppressor T cells exist? *Scand. J. Immunol.*, **27**, 247-250.
- NASH, A.A. & GELL, P.G.H. (1981), The delayed hypersensitivity T cell and its interaction with other T cells. *Immunol. Today*, **2**, 162-165.
- TADA, T. (1984), Help, suppression, and specific factors, in "Fundamental immunology" (W.E. Paul) (506-509). Raven Press, New York.

SUPPRESSOR T CELLS: AN APPRAISAL OF THEIR ROLE BY *IN VIVO* ELIMINATION

C. Leclerc

*Laboratoire de Biologie des Régulations Immunitaires,
Institut Pasteur, 75724 Paris Cedex 15*

Rather than debate the existence of T suppressor cells which, like the Loch Ness Monster, receive periodic and passionate attention, I have preferred to discuss their *in vivo* activity. It might appear strange to discuss the role of a cell population without being sure of its existence. However, I believe that it is currently the only way to proceed any further. Indeed, one can assume that only some of the many heterogeneous putative suppressor T cells described so far are responsible for *in vivo* suppression. Many T cells have properties which, under appropriate conditions, make them suppressive *in vitro*. It is, however, difficult to establish whether these activities play a role *in vivo*. Therefore, experimental systems are needed in which suppressor T cells could be identified unequivocally and demonstrated *in situ* to be the effector cells responsible for the suppression.

Assessment of the suppressive role of CD8⁺ T cells by *in vivo* elimination.

Interestingly, although numerous studies have demonstrated the suppressive activity of CD8⁺ T cells in suppression transfer experiments, only a few reports have clearly demonstrated their role *in situ*.

A suppressive role of CD8⁺ T cells has recently been demonstrated in virus-triggered immune suppression (Leist *et al.*, 1988). Infection of mice with lymphocytic choriomeningitis virus (LCMV) strongly suppresses antibody responses against a second infectious

agent. However, this suppression was not observed in mice infected with LCMV and treated with a monoclonal antibody (mAb) to eliminate cytotoxic T CD8⁺ cells. In this model and presumably in other viral infections, the suppression could therefore be due to killing by virus-specific cytotoxic T cells of virus-infected cells essentially involved in the immune responses such as helper T cells or antigen-presenting cells (APC).

Most interestingly, Benaroch *et al.* (1988; Benaroch and Bordenave, 1989 and this Forum) have recently succeeded in breaking mouse chronic allotypic suppression by anti-CD8⁺ *in vivo* treatment. *In vivo* treatment with cytotoxic anti-CD4 or anti-CD8 mAb of mice subjected to chronic Igh-1b suppression clearly showed that CD8⁺ lymphocytes were essential for the maintenance of the suppression. Whether the effector CD8⁺ T cells are classical cytotoxic T cells or belong to a separate T-cell subset remained to be determined in this model. However, the involvement of CTL in allotype suppression has been previously suggested by Snodgrass *et al.* (1981) who proposed that suppressor T cells may act directly upon allotype-bearing B cells. Moreover, they were able to demonstrate allotypic-specific cytotoxic T cells which were lytic for target myeloma cells expressing the suppressed allotypes, but were ineffective against target cell lines expressing different allotypes or isotypes (Snodgrass *et al.*, 1981).

It is therefore interesting to note that in these models, where suppression

could be abrogated by *in vivo* elimination of CD8⁺ T cells, the involvement of CTL was previously suggested or even demonstrated. That could indicate that, *in some models*, classical CTL could indeed be final effectors of the suppression. The recent demonstration by Shinohara *et al.* (1988) of class-II-restricted CD8⁺ cytolytic T lymphocytes specific for soluble antigens, is interesting in this respect. These CTL have been shown to be involved in specific lysis of antigen-binding B cells in the presence of physiological concentrations of native antigen and thus could play an important regulatory role. This could indicate that their exist separate CD8⁺ CTL subsets which could be identified by functional parameters (*i.e.* lymphokine secretion or restriction elements), as was recently done for T helper CD4⁺ T cells. Therefore, establishment of suppressor T-cell lines in models where CD8⁺ T cells are clearly suppressive effectors will certainly provide important information on the mechanisms by which T cells suppress the immune response.

In both of these studies, IgG2b cytotoxic rat mAb were used. However, in an earlier study, MacDonald *et al.* (1986) reported reversal of ConA-induced immunosuppression of the IgG-anti sheep red blood cell response following *in vivo* treatment with a non-cytotoxic rat IgG2a mAb directed against Lyt-2. Interestingly, this treatment did not eliminate Lyt-2⁺ cells since, after overnight culture *in vitro*, T cells from anti-Lyt-2-treated mice reexpressed surface Lyt-2 at normal level. Therefore, the modulation of Lyt-2 at the surface of suppressor T cells appears to be sufficient to downregulate the function of these cells.

CD4⁺ suppressor T cells in autoimmune diseases and parasitic infection.

The same strategy of *in vivo* depletion of CD8⁺ T cells had been used in two experimental autoimmune diseases. A number of studies had suggested that suppressor cells and, in particular, CD8⁺ suppressor cells, may be impor-

tant in the recovery phases in experimental allergic encephalomyelitis. However, Sedgwick (1988) have recently demonstrated that Lewis rats depleted of CD8⁺ T cells acquire and subsequently recover from passively and actively induced EAE in a comparable fashion to normal animals.

Similar conclusions were recently published for experimental autoimmune thyroiditis (EAT). EAT can be induced in susceptible mice by injection of murine thyroglobulin (MTg) with adjuvant. The development of EAT could be suppressed if animals were first injected with soluble MTg (Kong *et al.*, 1982). These authors also showed that the induced suppression is transferable with T cells. More recently, Parish *et al.* (1988) have demonstrated that pretreatment of animals with anti-L3T4 or anti-Ly-1 prior to tolerization prevent induction of *in situ* tolerance, whereas pretreatment with anti-Lyt-2 antibody has no effect. Treatment of animals with anti-L3T4 following tolerization also prevented demonstrable tolerance (Parish *et al.*, 1988). When donor mice were tolerized and were then treated with different antibodies prior to transfer, it was again the anti-L3T4 antibody which prevented the successful transfer of suppression, anti-Lyt-2 antibody having no effect. Thus, both *in situ* and in transferable tolerance, the cells mediating the effect appear to be L3T4⁺, Lyt-2⁻ T cells. Recently, a suppressor T-lymphocyte CD4⁺ cell line for autoimmune encephalomyelitis had been established by Ellerman *et al.* ((1988) and this Forum). These T cells, when admixed with guinea-pig myelin basic-protein-specific T helper cells, prevented the adoptive transfer of EAE.

CD4⁺ suppressor T cells have also been clearly demonstrated in leishmanial infection. Early studies by Liew and co-workers have clearly demonstrated that the genetic susceptibility of BALB/c mice to *Leishmania tropica* infection involves suppression of cell-mediated immune response by antigen-specific suppressor T-cell generation ((Howard *et al.*, 1980) and this Forum). These Ts cells have been described as of the Thy-1⁺, Lyt-1+2-

and I-J⁻ phenotype by *in vivo* transfer experiments (Liew *et al.*, 1982). Ts from BALB/c mice with progressive infection were cloned and were shown to specifically suppress *in vivo* induction of delayed-type hypersensitivity to *L. tropica* antigens and to exacerbate disease development upon adoptive transfer. These cells were Lyt-1⁺ I-J⁻ (Liew, 1983). Moreover, depletion of CD4⁺ T cells by *in vivo* treatment with anti-L3T4 antibody enables BALB/c mice to heal subsequent *Leishmania* major infection (Titus *et al.*, 1985). Recently, it was shown that this treatment was associated with the disappearance of mRNA for IL-4 and the appearance of mRNA for IFN- γ (Heinzel *et al.*, 1989). Therefore, the suppressive activity previously demonstrated by transfer experiment could be attributed to Th2 cells, whereas Th1 cells would have protective activity (Scott *et al.*, 1988).

It is interesting to note that the demonstration of the *in vivo* suppressive activity of CD4⁺ T cells had been done for experimental systems in which the outcome of infection or the induction of an autoimmune disease were scored. This could indicate that, in both cases, the final response depends upon the balance between Th subsets induced. Therefore, the so-called suppressive activity of CD4⁺ T cells could be related to their functional diversity. Depending upon the Th1 or Th2 subset which will be activated, cellular or humoral immunity could either be stimulated or suppressed. Therefore, operationally, Th2 cells could be suppressive for cell-mediated immunity, whereas Th1 cells could be suppressive for antibody responses. Therefore, at least in some experimental systems, classical T helper cells could be final effectors of the suppression.

***In vivo* elimination of CD8⁺ T cells does not abrogate epitopic suppression.**

That CD8⁺ CTL cells or CD4⁺ T helper cells could be final effector cells in some experimental systems (mostly infectious or autoimmune

disease) does not imply that suppressor T cells do not exist as a separate cell population. Moreover, the previously described experimental systems reflect more the balance between the induction of several aspects of immunity rather than the regulation of a particular immune response. In contrast, epitope-specific regulation (epitopic suppression) represents an adequate model for studying fine regulation of immune responses.

Epitopic suppression occurs when a host is immunized with a hapten conjugated to an immunogenic carrier to which the animal has been previously immunized. As demonstrated by L. Herzenberg and Tokuhisa (1982), a strong secondary antibody response to the carrier is produced but the host resists IgG production to the linked haptenic epitope. Initial studies of epitopic suppression were conducted employing DNP-KLH (dinitrophenol keyhole limpet haemocyanin). Priming animals with KLH before their immunization with DNP-KLH induces a persistent epitope-specific suppression. This suppression occurs for doses of KLH which are optimally immunogenic. Following these studies, we have demonstrated that epitopic suppression could apply to other models such as synthetic vaccines built by linkage of synthetic peptides to natural immunogenic carriers such as tetanus toxoid (Schutze *et al.*, 1985). Moreover, Sun and Waltenbaugh (1986) have demonstrated that this regulatory system not only suppresses antibody responses against low molecular weight molecules, but also suppresses responses against macromolecular immunogens. These studies concerned the antibody response induced by foreign antigens, but it has been demonstrated that such a regulatory system could influence antibody response against self-antigens (Rauch *et al.*, 1981). Most studies on epitope-specific suppression have concerned humoral response; however, very recently, we have demonstrated that a similar regulatory system influences the *in vivo* development of cytotoxic T lymphocytes directed against allogeneic cells (Schutze *et al.*, submitted for publication).

Herzenberg *et al.* have reported that carrier-specific Ts cells generate suppression for IgG antibody production to epitopes such as DNP presented on carrier molecules in carrier/hapten-carrier immunized animals. This has been demonstrated by injection of spleen cells or carrier-primed mice to naive recipient (Herzenberg and Yokuhisa, 1982). These carrier-specific Ts were not fully characterized in the Herzenberg report, but in studies which followed, Sun and Waltenbaugh studied the surface phenotype of *in vivo*-derived specific Ts. Transfer studies have indicated that the effector cells of epitope-specific suppression are Lyt-2⁺ T cells (Sun and Waltenbaugh, 1986). Moreover, following carrier/hapten-carrier immunization, subsequent anti-hapten responses remain suppressed even upon immunization with the hapten linked to an unrelated carrier, which suggests the presence of an epitope-specific suppressor population. Indeed, *in vitro* studies have indicated that the effector cells responsible for suppression are suppressor T cells some (Tagawa *et al.*, 1984) or most of them (Schutze *et al.*, 1987) bearing surface Lyt-2.

We have recently studied the role of Lyt-2⁺ T cells in epitopic suppression in BALB/c mice depleted of the Lyt-2⁺ T-cell population with a cytotoxic rat anti-CD8 mAb. The effect of this treatment was followed both by cytofluorographic analysis and by functional studies. The injection of the anti-CD8 mAb have induced a severe long-lasting depletion of splenic CD8⁺ cells as well as an almost total inhibition of CTL responses against alloantigens.

BALB/c mice were treated with the anti-Lyt-2 mAb either at the time of KLH priming (induction of suppression) or of TNP-KLH immunization (expression of suppression). In both instances, the elimination of Lyt-2⁺ T cells did not affect the suppression of the anti-TNP responses (Leclerc *et al.*, in preparation). We have performed the same experiment in a second experimental model where the suppression was induced by high dosage of KLH. Huchet (1986) have previously demonstrated that *in vivo* administration of high doses

(4 mg) of KLH before TNP-KLH immunization nearly abolished the IgG anti-TNP responses. In contrast to the Herzenberg protocol in which the suppression affects mostly IgG2a anti-TNP responses, this protocol suppresses both IgG1 and IgG2a responses (Leclerc *et al.*, in preparation). The involvement of suppressor T cells in this model has been demonstrated. Once again, *in vivo* depletion of CD8⁺ T cells at the time of KLH administration or of TNP-KLH immunization did not affect the suppression of the TNP responses. It is interesting to note that, in an attempt to identify the carrier-induced Ts, Sun and Waltenbaugh (1986) have established carrier-specific T-cell lines which may behave as epitope-specific suppression inducers. However, the analysis of these lines indicate that they are Thy-1⁺, L3T4⁺, Lyt-2⁻. Interestingly, our preliminary experiments indicate that CD4⁺ depletion at the time of carrier priming could abrogate the epitopic suppression.

It is clearly too early to explain these discrepancies between *in vivo* and *in vitro* studies. However, these data could indicate that these CD8⁺ suppressor T cells, in contrast to CD8⁺ CTL, are not sensitive to *in vivo* depletion by this rat mAb. Conversely, these results could indicate that suppressor T cells are, in this model, associated with the unresponsiveness state but are not responsible for the suppression.

Conclusions.

Suppressor T cells have been demonstrated in many experimental systems using either *in vitro* cell co-culture experiments or *in vivo* cell transfers. Clearly, these experimental systems are not discriminatory enough. Indeed, in most cases, the suppressor/effector cell ratios used or the number of injected suppressed cells are very high. This could favour the description of minor-associated phenomena, arising with the unresponsiveness state but not responsible for this suppression. For instance, cytotoxic T cells specific for soluble antigens

could be induced following administration of tolerogenic doses of this antigen without being responsible for the tolerance state. This type of correlative studies has favoured the description, in the past, of a large variety of putative suppressor T cells. This overestimation of these epiphenomena could explain the great heterogeneity of suppressor T cells described in the literature. Therefore, I strongly believe it is im-

perative to firmly establish, in *each experimental system*, the activity of these Ts cells by the *in situ elimination approach*. Clearly, there exists several immunoregulatory pathways. One important test will be to identify and classify these various pathways. The *in vivo* approach will at least permit identification of the situations in which CD4⁺ or CD8⁺ T cells are the true final effector suppressive cells.

References.

- BENAROCH, P. & BORDENAVE, G. (1989), T-cell-induced Ig allotypic suppression in mice. — II. Both CD4⁺ CD8⁻ and CD4⁻ CD8⁺ T-cell subsets from sensitized Igh^a mice are required to induce suppression of Igh-1b allotype expression. *J. Immunol.*, **142**, 1-7.
- BENAROCH, P., GEORGATSOU, E. & BORDENAVE, G. (1988), Cellular induction of chronic allotype suppression of Ig2a in Igh^{b/b} homozygous mice and its abrogation by *in vivo* treatment with anti-CD8 monoclonal antibody. *J. exp. Med.*, **168**, 891-904.
- ELLERMAN, K.E., POWERS, J.M. & BROSTOFF, S.W. (1988), A suppressor T-lymphocyte cell line for autoimmune encephalomyelitis. *Nature (Lond.)*, **331**, 265-267.
- HEINZEL, F.P., SADISK, M.D., HOLADAY, B.J., COFFMAN, R.L. & LOCKSLEY, R.M. (1989), Reciprocal expression of interferon- γ or interleukin-4 during the resolution of progression of murine leishmaniasis. Evidence for expansion of distinct helper T-cell subsets. *J. exp. Med.*, **169**, 59-72.
- HERZENBERG, L.A. & TOKUHISA, T. (1982), Epitope-specific regulation. — I. Carrier-specific induction of suppression for IgG anti-hapten antibody responses. *J. exp. Med.*, **155**, 1730-1740.
- HOWARD, J.G., HALE, C. & LIEW, F.Y. (1980), Immunological regulation of experimental cutaneous *Leishmaniasis*. — III. Nature and significance of specific suppression of cell-mediated immunity in mice highly susceptible to *Leishmania tropica*. *J. exp. Med.*, **152**, 594-607.
- HUCHET, R. (1986), Features of KLH-induced suppression *in vivo*: characterization of two pathways of suppression. *Cell. Immunol.*, **98**, 188-199.
- KONG, Y.M., OKAYASU, I., GIRALDO, A.A., BEISEL, K.W., SUNDICK, R.S. & ROSE, N.R. (1982), Tolerance to thyroglobulin by activating suppressor mechanisms. *Ann. N.Y. Acad. Sci.*, **392**, 191-209.
- LEIST, T.P., RUEDI, E. & ZINKERNAGEL, R.M. (1988), Virus triggered immune suppression in mice caused by virus-specific cytotoxic T cells. *J. exp. Med.*, **167**, 1749-1754.
- LIEW, F.Y. (1983), Specific suppression of responses to *Leishmania tropica* by a cloned T-cell line. *Nature (Lond.)*, **305**, 630-632.
- LIEW, F.Y., HALE, C. & HOWARD, J.G. (1982), Immunologic regulation of experimental cutaneous leishmaniasis. — V. Characterization of effector and specific suppressor T cells. *J. Immunol.*, **128**, 1917-1922.
- MACDONALD, T.T., CALKINS, C.E. & ROTHERMEL, A.L. (1986), Modulation *in vivo* of Lyt-2 antigen expression on T cells by anti-Lyt-2 antibody: effects on ConA-induced unresponsiveness. *Immunology*, **59**, 181-186.
- PARISH, N.M., ROITT, I.M. & COOKE, A. (1988), Phenotypic characteristics of cells involved in induced suppression to murine experimental autoimmune thyroiditis. *Europ. J. Immunol.*, **18**, 1463-1467.
- RAUCH, H.C., MOWICKI-MONTGOMERY, I. & SWENBORG, R.H. (1981), Inhibition of experimental allergic encephalomyelitis by carrier administered prior to challenge with encephalitogenic peptide-carrier conjugate. *Europ. J. Immunol.*, **11**, 335-338.
- SCHUTZE, M.P., LECLERC, C., JOLIVET, M., AUDIBERT, D. & CHEDID, L. (1985), Carrier-induced epitopic suppression, a major issue for future synthetic vaccine. *J. Immunol.*, **135**, 2319-2322.
- SCHUTZE, M.P., LECLERC, C., VOGEL, F.R. & CHEDID, L. (1987), Epitopic suppression in synthetic vaccine models: analysis of the effector mechanisms. *Cell. Immunol.*, **104**, 79-90.

- SCOTT, P., NATOVITZ, P., COFFMANN, R.L., PEARCE, E. & SHER, A. (1988), Immunoregulation of cutaneous leishmaniasis T-cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to different parasite antigens. *J. exp. Med.*, **168**, 1675-1684.
- SEDGWICK, J.D. (1988), Long-term depletion of CD8⁺ T cells *in vivo* in the rat: no observed role for CD8⁺ (cytotoxic/suppressor) cells in the immunoregulation of experimental allergic encephalomyelitis. *Europ. J. Immunol.*, **18**, 495-502.
- SHINOHARA, N., WATANABE, M., SACHS, D.H. & HOZUNI, N. (1988), Killing of antigen-reactive B cells by class-II-restricted soluble antigen-specific CD8⁺ cytolytic T lymphocytes. *Nature (Lond.)*, **336**, 481-484.
- SNODGRASS, H.R., BOSMA, M.J. & WILSON, D.B. (1981), T lymphocytes specific for immunoglobulin allotype. — II. Cloned IgH-1^b specific cytotoxic T cells. *J. exp. Med.*, **154**, 491-500.
- SNODGRASS, H.R., WILSON, D.B. & BOSMA, M.J. (1981), T lymphocytes specific for immunoglobulin allotype. — I. IgH-1^b-specific T cells demonstrated by suppression *in vivo* and cytotoxicity *in vitro*. *J. exp. Med.*, **154**, 480-490.
- SUN, L. & WALLENBAUGH, C. (1986), Epitope-specific regulation in Ir gene systems. — I. Conditions for the induction of epitope-specific suppressors to poly(Glu⁶⁰Ala³⁰Tyr¹⁰). *Cell. Immunol.*, **98**, 375-385.
- TAGAWA, M., TAKUHISA, T., ONO, K., TANIGUCHI, M., HERZENBERG, L.A. & HERZENBERG, L.A. (1984), Epitope-specific regulation. — IV. *In vitro* studies with suppressor T cells induced by carrier/hapten-carrier immunization. *Cell. Immunol.*, **86**, 327-336.
- TITUS, R.G., CEREDIG, R., CEROTTINI, J.C. & LOUIS, J.A. (1985), Therapeutic effect of anti-L3T4 monoclonal antibody GK1-5 on cutaneous leishmaniasis in genetically susceptible BALB/c mice. *J. Immunol.*, **135**, 2108-2114.

This work was supported by grants from the Institut Pasteur and from the Association pour le Développement de la Recherche sur le Cancer.

I would like to thank Drs. A. Galelli and M.P. Schutze for critical reading of the manuscript and F. Georges for preparation of the manuscript.

ARE T_s CELLS INVOLVED IN ISOTYPE-SPECIFIC REGULATION?

J.L. Teillaud and W.H. Fridman

Unité INSERM 255, Institut Curie, 26, rue d'Ulm, 75231 Paris Cedex 05

Introduction.

The serum level of the different isotypes of immunoglobulins in naive animals and the isotypic profile of antibody responses are strictly controlled (Van Loghem, 1978). Isotypic dysregulation can lead to potentially harmful diseases such as hyper-IgE syndrome or

IgA nephropathy (Geha *et al.*, 1981; Lopez-Trascasa *et al.*, 1980), indicating that isotype control is of major importance. Although the existence of isotypic regulation is fully recognized by immunologists, the precise molecular and cellular mechanisms involved in this phenomenon are still obscure and controversial. On the one hand, isotype

control can affect B cells to trigger switch events leading to the production of a given Ig class or subclass. On the other hand, isotype regulation can affect B cells already committed to the production of a particular isotype, modifying the amounts of Ig produced and possibly the whole isotypic profile of a particular antibody response. In both cases, soluble mediators produced by T-cell subpopulations have been implicated.

Helper T (Th) cells that are suppressor T (Ts) cells and vice versa.

Recent data support the notion that well-characterized cytokines such as IL4, IL5, and IFN- γ regulate the isotypic profile of antibodies produced upon stimulation of B cells by LPS (Paul and Ohara, 1987; Harriman *et al.*, 1988; Snapper and Paul, 1987). IL5 may enhance the production of IgA; IL4 leads to the production of IgG1 and IgE, depending on the dose; while IFN- γ induces the secretion of IgG2a. Thus, these lymphokines act as molecules that promote the production of particular isotypes. However, close examination of the effects of IL4 and IFN- γ on the secretion of other isotypes shows that they also act as potent isotype inhibitors. IFN- γ blocks IgG1, IgG3, IgG2b and IgE, while IL4 inhibits IgG3, IgG2a, IgG2b and IgM. IFN- γ totally abrogates the IgG1 induction by IL4, while IL4 does not affect IgG2a induction by IFN- γ . T cells producing IFN- γ are therefore Th cells (referred to as Th1 (Mosmann and Coffman, 1987) for IgG2a production but act as (Ts) cells for the secretion of other Ig isotypes.

The claim that another subset of T cells (Th2) produce (Mosmann and Coffman, 1987) implies that Th1 acts directly, or via a cascade of cellular and molecular events, on Th2 cells as a Ts cell. Th2 cells that produce IL4 are themselves Ts cells for the secretion of different isotypes. The T-cell isotype regulatory functions can therefore only be defined by the Ig end-product that is examined. At this point, it should be

noted that isotype regulation is one of the essential components of the immune system, since the production of different isotypes allows different effector functions to take place. For instance, the protective effects of antibodies in viral infections and against tumour cells depend strongly on the isotypes produced (allowing or not effective cell antibody-dependent cellular cytotoxicity, ADCC, or complement fixation (Johnson *et al.*, 1985; Nussenzweig *et al.*, 1964). Thus, a dichotomic definition of T cells that strictly distinguishes between Th and Ts does not reflect complex T-cell isotype regulatory pathways.

When examining the isotype regulation exerted by cytokines such as IFN- γ and IL4, it is important to understand how the isotype specificity is acquired. These cytokines do not interact directly with Ig classes or subclasses. For example, surface IgG are not required for the IgG1-inducing effects of IL4, as the depletion of surface IgG-positive B cells does not prevent this induction (Snapper and Paul, 1987). Thus, IL4 and IFN- γ could act by controlling the switch recombination events (for instance, by inducing specific recombinases or chromosomal accessibility to recombinases at specific sites). Alternatively, they could either induce the production of isotype regulatory factors or recruit cells producing such factors. These factors must in turn fulfill at least two functions: first, they should interact with Ig in an isotype-specific manner; second, their expression and production should be modulated, directly or indirectly, by IL4 and IFN- γ . Does any experimental data favour the existence of such T cells and T-cell-derived products?

Isotype regulation by FcR⁺ T cells and immunoglobulin-binding factors (IBF).

Obvious candidates for the isotype-specific effector molecules are the molecules that bind to the Fc region of Ig, either as membrane receptors (FcR) on a variety of cell types including a T-cell subpopulation, or as liquid-phase molecules which have been termed

immunoglobulin-binding factors (IBF). All of these molecules bind Ig in an isotype-restricted manner and their expression is modified upon interaction with cytokines IL4 and IFN- γ . For instance, the expression of Fc ϵ RII and the production of IgE-BF are increased upon incubation with IL4, which led to the hypothesis of an IgE-BF-mediated IL4 effect on IgE production (Bonnefoy *et al.*, 1988). Early experiments showed that allo-activated T cells express Tc γ R and secrete molecules in culture supernatants which, like surface Fc γ R, bind IgG in an isotype-specific manner (Gisler and Fridman, 1975; Néauport-Sautès *et al.*, 1975). The secreted molecules have therefore been termed IgG-BF.

Although highly debated for several years, these two observations, which have been extended to the whole family of FcR, have stood up to the controversy. The first cloning of an Fc γ R gene was performed using cells from a mouse T-cell line (Hibbs *et al.*, 1986), and the presence of liquid-phase IgE-BF and IgG-BF, derived from Fc ϵ R and Fc γ R respectively, has been demonstrated with the use of monoclonal antibodies (Huff *et al.*, 1984; Sarfati *et al.*, 1986; Daéron *et al.*, 1986). The functional properties of IBF and of FcR⁺ T cells have been studied extensively. FcR⁺ T cells are directly associated with isotype-regulatory properties. A large set of data implies that T α , T ϵ , and T γ cells are directly responsible for the down- and/or up-regulation of IgA, IgE, and IgG production, respectively (Yodoi *et al.*, 1983; Endoh *et al.*, 1981; Ishizaka, 1984; Young *et al.*, 1986; Fridman *et al.*, 1981). However, studies on IBF have been hampered by the fact that only partial purification of these molecules, mainly based on their Ig-binding ability, have been achieved so far. A general consensus has nevertheless emerged that these cells and these molecules, whatever their exact molecular forms, can act as potentiating or inhibitory triggers of Ig production. IgG-BF suppress the secondary *in vitro* antibody responses to sheep red blood cells (Fridman *et al.*, 1981), while IgE-BF appear to inhibit or potentiate IgE production,

depending on its glycosylation (Ishizaka, 1984). IgA-BF seem to enhance or decrease IgA production in various experimental systems (Yodoi *et al.*, 1983; Endoh *et al.*, 1981; Kiyono *et al.*, 1985; Millet *et al.*, 1988).

Again, one cannot describe a strict dichotomy between isotype-regulatory Th and Ts cells since a given T-cell clone can express and release different FcR and IBF, as it has been shown with T-cell hybridomas (Yodoi *et al.*, 1983; Daéron *et al.*, 1985). Such IBF may have different glycosylation patterns and/or may correspond to different peptides derived from a single or several types of surface FcR, acting either positively or negatively on isotype production. In the following section, we will only discuss data concerning the inhibitory activity of IBF and FcR T cells.

Isotype suppression is a non-cognate process.

Although the first evidence of isotype suppression came from *in vitro* and *in vivo* manipulations of normal B cells, investigators have focused their efforts on the establishment of experimental models using tumour hybridoma and myeloma cells to study the effects of IBF. Such systems allow the study of well-characterized, cloned cell lines and possibly somatic variants derived from these cell lines with useful phenotypic and genotypic characteristics. From various reports published so far (Uede *et al.*, 1984; Suemura *et al.*, 1983; Roman *et al.*, 1988; Mathur *et al.*, 1987; Teillaud *et al.*, 1987), it appears that: 1) the suppressive effect triggered by FcR⁺ T cells is a non-cognate process; 2) tumour B cells are susceptible to IBF and FcR⁺ T cells, with no requirement for third party cells; 3) at least one of the targeted molecules on B-cell membrane is surface Ig; and 4) the decrease in Ig production observed by PFC assays, immunofluorescence or ELISA is due to a decrease in mRNA, possibly through a blocking of the transcription itself.

Many points, however, are still unclear or unresolved. Some researchers

have observed that only the mRNA encoding heavy chains of Ig are inhibited, while others have reported a decrease in mRNA encoding both heavy and light chains. If interactions between IBF and surface Ig are a prerequisite for triggering an inhibitory signal into B cells, it is important, also to understand the difference between this interaction and that of anti-Ig antibodies that do not promote Ig suppression. We have recently proposed that this may occur by IBF-mediated cross-linking of surface Ig and FcR present on B cells through a homophilic interaction between FcR and IBF (Teillaud *et al.*, 1989).

Moreover, nothing is known about the subsequent cytoplasmic (such as internalization of IBF) or nuclear events that lead to Ig inhibition. For instance, the induction or repression of DNA-binding proteins acting on promoter and/or enhancer regions of both heavy and light Ig genes could be triggered by IBF. The view that IBF affects only the quantitative production of Ig by terminally-differentiated B cells has also been challenged, as T α cells have been shown to induce the switch from cells with surface IgM to IgA-producing cells (Kawanishi *et al.*, 1983).

Are IBF also growth-regulatory molecules?

Recently, it has been suggested that IBF may interfere with proliferation. It

has been reported that Fc ϵ RII is related to the receptor for the low molecular weight B-cell growth factor (BCGF) (Gordon *et al.*, 1986). IgE-BF, derived from Fc ϵ RII may itself represents an autocrine growth factor with BCGF and IL1 activities (Swendeman and Thoeley-Lawson, 1987). IgA-BF, IgG-BF and T γ cells also inhibit tumour B-cell proliferation *in vitro* (Muller and Hoover, 1985; Teillaud *et al.*, 1987; Teillaud *et al.*, 1989). It has further been proposed that the cytostatic effect of IgA-BF on IgA-secreting myeloma cells is related to a coordinate suppression of α -heavy chain and *c-myc* gene expression (Roman *et al.*, 1988).

Although the growth-regulatory properties of IBF have been poorly documented so far, they could be of invaluable interest. First, it may be that IBF represent anti-growth factors, opposing the effects of B-cell growth factors such as IL6. Second, these molecules *in vivo* may be involved in the control of the proliferation of and the Ig secretion by tumour B cells. For instance, patients with multiple myeloma develop a large number of FcR⁺ peripheral T cells, which may in fact produce IBF targeted to block the growth of and the Ig secretion by myeloma cells. If proven true, this possibility could lead to successful clinical trials with IBF, which would be the best argument for the existence of T-cell produced suppressor molecules.

References.

- BONNEFOY, J.Y., DEFRANCE, T., PERONNE, C., MENETRIER, C., ROUSSET, F., PENE, J., DE VRIES, J.E. & BANCHEREAU, J. (1988), Human recombinant interleukin 4 induces normal B cells to produce soluble CD23/IgE-Binding factor analogous to that spontaneously released by lymphoblastoid B-cell lines. *Europ. J. Immunol.*, **18**, 117.
- DAÉRON, M., NÉAUPORT-SAUTÈS, C., BLANK, U. & FRIDMAN, W.H. (1986), 2.4G2, a monoclonal antibody to macrophage Fc γ receptors and IgG-binding factors. *Europ. J. Immunol.*, **16**, 1545.
- DAÉRON, M., YODOI, J., NÉAUPORT-SAUTÈS, C., MONCUIT, J. & FRIDMAN, W.H. (1985), Receptors for immunoglobulin isotypes (FcR) on murine T cells. — I. Multiple FcR expression on T lymphocytes and hybridoma T-cell clones. *Europ. J. Immunol.*, **15**, 662.
- ENDO, M., SAKAI, H., NAMOTO, Y., TOMINO, Y. & KANESHIGE, H. (1981), IgA-specific helper activity of T α cells in human peripheral blood. *J. Immunol.*, **127**, 2612.

- FRIDMAN, W.H., RABOURDIN-COMBE, C., NÉAUPORT-SAUTÈS, C. & GISLER, R.H. (1981), Characterization and function of T cell Fc γ receptor. *Immunol. Rev.*, **56**, 51.
- GEHA, R.S., REINHERZ, E.L., LEUNG, D., MCKEE, K.T., SCHLOSSMAN, S.F. & ROSEN, F.S. (1981), Deficiency of suppressor T cells in the hyper-immunoglobulin E syndrome. *J. clin. Invest.*, **68**, 783.
- GISLER, R.H. & FRIDMAN, W.H. (1975), Suppression of *in vitro* antibody synthesis by immunoglobulin-binding factor. *J. exp. Med.*, **142**, 507.
- GORDON, J., WEBB, A.J., WALKER, L., GUY, G.R. & ROWE, M. (1986), Evidence for an association between CD23 and the receptor for a low molecular weight B-cell growth factor. *Europ. J. Immunol.*, **16**, 1627.
- HARRIMAN, G.R., KUNIMOTO, D.Y., ELLIOTT, J.F., PAETKAV, V. & STROBER, W. (1988), The role of IL-5 in IgA B-cell differentiation. *J. Immunol.*, **140**, 3033.
- HIBBS, M.L., WALKER, I.D., KIRSZBAUM, L., PIETERSZ, G.A., DEACON, N.J., CHAMBERS, G.W., MCKENZIE, I.F.C. & HOGARTH, P.M. (1986), The murine Fc receptors for immunoglobulin: purification, partial amino acid sequence and isolation of cDNA clones. *Proc. nat. Acad. Sci. (Wash.)*, **83**, 6980.
- HUFF, T.F., YODOI, J., UEDE, T. & ISHIZAKA, K. (1984), Presence of an antigenic determinant common to rat IgE-potentiating factor, IgE-suppressive factor, and Fc γ receptors T and B lymphocytes. *J. Immunol.*, **132**, 406.
- ISHIZAKA, K. (1984), Regulation of IgE synthesis. *Ann. Rev. Immunol.*, **2**, 159.
- JOHNSON, W.J., STEPLEWSKI, Z., KOPROWSKI, H. & ADAMS, D.O. (1985), in "Mechanism of cell-mediated cytotoxicity II" (P. Henkart & E. Mertz) (p. 75). Plenum Press, New York.
- KAWANISHI, H., SALTZMAN, L.E. & STROBER, W. (1983), Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. — I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells *in vitro*. *J. exp. Med.*, **157**, 433.
- KIYONO, H., MOSTELLER-BARNUM, L.M., PITTS, A.M., WILLIAMSON, S.I., MICHALEK, S.M. & MCGHEE, J.R. (1985), Isotype-specific immunoregulation. IgA-binding factors produced by Fc α -receptor-positive T-cell hybridomas regulate IgA responses. *J. exp. Med.*, **161**, 731.
- LOPEZ-TRASCASA, M., EGIDO, J., SANCHO, J. & HERNANDO, L. (1980), IgA glomerulonephritis (Berger's disease): evidence of high serum levels of polymeric IgA. *Clin. exp. Immunol.*, **42**, 247.
- MATHUR, A., KAMAT, D.M., VAN NESS, B.G. & LYNCH, R.G. (1987), Thymus-dependent *in vivo* suppression of IgE synthesis in a murine IgE-secreting hybridoma. *J. Immunol.*, **139**, 2865.
- MILLET, I., SAMARUT, C. & REVILLARD, J.P. (1988), Class-specific suppression of human B-cell maturation by IgA-binding factors. *Europ. J. Immunol.*, **18**, 545.
- MOSMANN, T.R. & COFFMAN, R.L. (1987), Two types of mouse helper T cell clone. *Immunol. Today*, **8**, 223.
- MULLER, S. & HOOVER, R.G. (1985), T cells with Fc receptors in myeloma: suppression of growth and secretion of MOPC-315 by T α cells. *J. Immunol.*, **134**, 644.
- NÉAUPORT-SAUTÈS, C., DUPUIS, D. & FRIDMAN, W.H. (1975), Specificity of Fc receptors of activated T cells. Relation with released immunoglobulin-binding factor. *Europ. J. Immunol.*, **5**, 849.
- NUSSENZWEIG, R.S., MERRYMAN, C. & BENACERRAF, B. (1964), Electrophoretic separation and properties of mouse anti-hapten antibodies involved in passive cutaneous anaphylaxis and passive hemolysis. *J. exp. Med.*, **120**, 315.
- PAUL, W.E. & OHARA, J. (1987), B-cell stimulatory factor-1/interleukin 4. *Ann. Rev. Immunol.*, **5**, 429.
- ROMAN, S., MOORE, J.S., DARBY, C., MULLER, S. & HOOVER, R.G. (1988), Modulation of Ig gene expression by Ig-binding factors. Suppression of α -H chain and λ -2-L chain mRNA accumulation in MOPC 315 by IgA-binding factor. *J. Immunol.*, **140**, 3622.
- SARFATI, M., NUTMAN, T., FONTEYN, C. & DELESPESE, G. (1986), Presence of antigenic determinants common to Fc IgE receptors on human macrophages, T and B lymphocytes and IgE-binding factors. *Immunology*, **59**, 569.
- SNAPPER, C.M. & PAUL, W.E. (1987), Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science*, **236**, 944.
- SUEMURA, M., ISHIZAKA, A., KOTABAKE, S., SUGIMURA, K., MAEDA, K., NAKANISHI, K., KISHIMOTO, S., YAMAMURA, Y. & KISHIMOTO, T. (1983), Inhibition of IgE production in B hybridomas by IgE class-specific suppressor from T hybridomas. *J. Immunol.*, **130**, 1056.
- SWENDEMAN, S. & THORLEY-LAWSON, D.A. (1987), The activation antigen BLAST-2, when shed, is an autocrine BCGF for normal and transformed B cells. *EMBO J.*, **6**, 1637.

- TEILLAUD, J.L., AMIGORENA, S., MONCUIT, J., SAUTES, C. & FRIDMAN, W.H. (1987), Immunoglobulin-G-binding factors (IgG-BF) inhibit IgG secretion by, as well as proliferation of, hybridoma B cells. *Immunol. Letters*, **16**, 139.
- TEILLAUD, J.L., BRUNATI, S., AMIGORENA, S., MATHIOT, C., SAUTES, C. & FRIDMAN, W.H. (1989), Control of B-cell function by Fc γ -receptor-positive T cells and immunoglobulin-binding factors, in "Contr. Microbiol. Immunol." (P. Del Guercio). Karger AG Publ., Basel (in press).
- UEDE, T., HUFF, T.F. & ISHIZAKA, K. (1984), Suppression of IgE synthesis in mouse plasma cells and B cells by rat IgE-suppressive factor. *J. Immunol.*, **133**, 803.
- VAN LOGHEM, E. (1978), Genetics of human immunoglobulins: classification, distribution and association with disease, in "CRC handbook series in clinical laboratory science" (D. Seligson) (p. 259). CRC Press, Cleveland, OH.
- YODOI, J., ADACHI, M., TESHIGAWARA, K., MIYAMI-INABA, M., MASUDA, M. & FRIDMAN, W.H. (1983), T cell hybridomas co-expressing Fc receptors (FcR) for different isotypes. — II. IgA-induced formation of suppressive IgA-binding factor(s) by a murine T hybridoma bearing Fc γ R and Fc α R. *J. Immunol.*, **131**, 303.
- YOUNG, M., GEHA, R.S., MAKSAD, K.N. & LEUNG, D.Y.M. (1986), Characterization of human T-cell-derived IgE-potentiating factor. *Europ. J. Immunol.*, **16**, 985.

SUPPRESSOR CELLS FOR CELL-MEDIATED IMMUNITY IN INFECTIOUS DISEASES

F.Y. Liew

*Department of Experimental Immunobiology,
Wellcome Research Laboratories, Beckenham, Kent BR3 3BS (GB)*

The need to control infectious diseases has been a major impetus in the development of immunology. However, the host immune response to infection is extremely complex and often consists of a self-destructive element of autoimmunity. The classic examples are Chagas' disease and leprosy. Hence, specific and generalized immune suppression has been reported in several acute and chronic infections. The suppressor mechanism makes good biological sense in achieving a fine balance between host-protective immunity and the possible risk of autoimmune disease.

The suppressor element has been variously attributed to suppressor T (Ts) cells, suppressive macrophages, deficient in IL-2 production or IL-2 receptor, or soluble suppressor substances. In

this brief discussion, I shall concentrate on the suppression of cell-mediated immunity and to illustrate it with a convenient infectious disease model, the murine experimental cutaneous leishmaniasis.

Leishmaniasis.

Leishmaniasis is caused by species of the intracellular protozoan parasite belonging to the genus *Leishmania*. There are three main categories of leishmaniasis: cutaneous leishmaniasis (Oriental Sore), mucocutaneous leishmaniasis (Espundia) and visceral leishmaniasis (Kala azar). The parasites are transmitted by female sandflies and the flagellated promastigotes develop in

the gut of the sandfly and in cell-free cultures. Transformation into the amastigote stage occurs within the mammalian macrophage.

The use of inbred mouse strains has greatly advanced our understanding of the immunological control of leishmaniasis. Mice are susceptible to most species of leishmania that are pathogenic to man and a spectrum of disease pattern can be obtained according to the genetic background of the host.

Cell-mediated immunity in leishmaniasis.

Cell-mediated immunity (CMI) rather than humoral antibody plays a causal role in the acquired immunity to leishmaniasis (reviewed in Howard, 1985). Anti-leishmanial antibodies have been shown *in vitro* to lyse promastigotes in the presence of complement and to promote phagocytosis. However, there is little evidence for a corresponding *in vivo* role for antibody in determining the outcome of leishmanial infection. Treatment of mice from birth with anti-IgM antibody can profoundly affect the outcome of *L. major* infections. This effect is likely to be due to the depletion of the antigen-presenting function of B cells rather than the abrogation of antibody production, since reconstitution with specific antibody could not restore the effects of the anti-IgM treatment (Liew *et al.*, unpublished).

In contrast to humoral immunity, the case for a causal role of CMI in acquired resistance to leishmaniasis is based on a range of impressive clinical and experimental evidence. Resistant CBA mice rendered relatively T-cell deficient by thymectomy followed by irradiation and reconstitution with syngeneic bone marrow cells are less able to control *L. major* infection. Athymic mutants of the highly resistant CBA and C57BL mice are totally unable to control *L. major* infection which progresses and visceralizes. Normal resistance, however, can be fully restored by reconstitution with normal syngeneic

T cells. Acquired immunity against *L. major* as a result of recovery from infection or prophylactic immunization can also be transferred by T cells and not B cells. Treatment of resistant C3H mice from birth with anti-IgM antibody rendered them defective in antibody response and also susceptible to *L. major* infection. However, lesion progression in these treated mice can be arrested and the disease outcome reversed by adoptive transfer of T cells alone from normal C3H donors without any restoration of humoral antibody formation. These results, therefore, provide a forceful argument for a pivotal role of CMI in acquired resistance to leishmaniasis.

There now appears to be a broad consensus that T cells conferring protective immunity primarily belong to the CD4⁺ subset. This is supported by experimental evidence from several laboratories using adoptive transfer and replacement studies with the murine *L. major* models (reviewed in Liew, 1989). Lymphokines such as macrophage IFN- γ produced by specifically sensitized T cells are deemed to be essential for the activation of infected macrophages to eliminate intracellular amastigotes.

Recent evidence suggests that CD8⁺ T cells may also be protective against *L. major* infection (Titus *et al.*, 1987). Resistant CBA mice became less able to heal from *L. major* infection after repeated CD8 monoclonal antibody (mAb) treatment *in vivo*. However, the effect of CD8 mAb was far less impressive than that of CD4 mAb treatment. The extreme susceptibility of athymic nude BALB/c mice to *L. major* infection could only be reversed by CD4⁺ and not by CD8⁺ T cells (Moll *et al.*, 1988). Thus far, CD8⁺-specific cytotoxic T cells have not been convincingly demonstrated in leishmaniasis.

Suppression of protective immunity.

Clinically, patients with visceral leishmaniasis do not develop leishmanial-specific skin reaction or proliferative T-cell response. These

reactions are restored following successful chemotherapy (Carvalho *et al.*, 1981). In experimental models, the reduced spleen cell activation by phytohaemagglutinin during *L. donovani* and *L. major* infections in BALB/c mice compared with that of normal controls is associated with impaired IL-2 production attributable to the presence of a population of macrophage-like adherent suppressor cells (Reiner and Finke, 1983). A similar non-specific, macrophage-mediated suppression of IL-2 production in clinical visceral leishmaniasis has also been demonstrated (Cillari *et al.*, 1988). In contrast, the antigen-specific suppression of delayed-type hypersensitivity (DTH) occurs earlier on and is mediated by CD4⁺ T cells. There has been much controversy over the identity of these CD4⁺ suppressor cells because they do not have the conventional CD8⁺ suppressor T-cell phenotype and their suppressive activity is defined mainly in operational terms. Recent studies clearly demonstrate that the Ts cells described earlier are not the conventional Ts cells for antibody response, although operationally they interfere with the host protective immunity. Studies leading to this conclusion are described below (reviewed in Liew, 1989).

The majority of inbred mouse strains are resistant to *L. major* infections. BALB/c mice, however, are exceptionally susceptible to this infection in that they develop uniformly fatal disseminating disease even with a minimal infecting dose. The failure of BALB/c mice to contain *L. major* infection is not due to any intrinsic inability of these mice to develop effective CD4⁺ T cells against the parasites. In fact, BALB/c mice can be rendered resistant to *L. major* infection by prior sublethal whole body γ -irradiation, treatment from birth with anti- μ antibody, injection with CD4 mAb or cyclosporin A. The recovered mice develop a classical tuberculin-type of DTH and their splenic and lymph node T cells can adoptively transfer resistance in otherwise highly susceptible BALB/c recipients. The protective T cells, like those found in the recovered resistant mice, are CD4⁺ and produce IFN- γ

when cultured with leishmanial antigens *in vitro*.

The prophylactic effect of sublethal irradiation or anti- μ treatment can be reversed by the injection, into these treated BALB/c mice, with T cells from normal syngeneic or, even more readily, T cells from mice with progressive *L. major* infection. At a population level, the disease-promoting T cells (operationally called Ts cells) from mice with progressive disease are functionally opposite to the protective T cells obtained from recovered mice (here referred to as Tr cells) (table I). These Ts cells also express the CD4⁺ phenotype. They are extremely potent, as few as 10⁶ cells can reverse the disease-resisting capability of γ -irradiated BALB/c mice. Ts cells do not mediate the classical tuberculin DTH. Instead, they can suppress the expression of DTH of Tr cells. What then are the characteristics and mechanism of interaction between Tr cells and Ts cells, both of which are CD4⁺?

Heterogeneity of CD4⁺ T cells.

Since both host-protection and disease-promotion are mediated by CD4⁺ T cells, it has been argued that the two phenomena are the function of the same population of T cells. The difference merely reflects a differential quantitative requirement of the helper T (Th) cells (Louis *et al.*, 1986). In other words, too many protective T cells are detrimental, a suggestion reminiscent of the "too much help leads to suppression" concept. However, recent reports provided compelling evidence that the disease-promoting CD4⁺ T cells are functionally distinct from the host-protective CD4⁺ T cells. T cells derived from resistant strains of mice or protected BALB/c mice secrete IFN- γ in response to leishmanial antigens, whereas T cells that promote disease are devoid of such activity. The disease-promoting T cells produce IL-3, IL-4 and IL-5. Finally, the most direct evidence was provided by T-cell clones. A CD4⁺ T-cell clone was derived from BALB/c mice with the potential to

TABLE I. — Comparison of the protective and counter-protective CD4⁺ T cells in leishmaniasis with Th₁ and Th₂ cells.

	Th ₁ /inflammatory (a)	Protective (b)	Th ₂ /helper	Counter protective
<i>Function measured</i>				
Help for specific antibody	—	+	+	+
Cytotoxicity	+	—	—	—
DTH	+	+	—	—
<i>Cytokine released</i>				
IL-2	+	+	—	±
IL-3	+	—	+	+
IL-4	—	—	+	+
IFN- γ	+	+	—	—

(a) From Bottomly (1988).

(b) From Liew (1989).

develop exacerbated *L. major* infection. The cell line significantly enhanced disease development in recipient mice infected with *L. major*. Recently, T cells from BALB/c mice protectively immunized with chemically mutagenized avirulent clones of *L. major* or purified *L. major* antigen were cloned. These CD4⁺ T-cell clones can adoptively transfer protection.

The heterogeneity of CD4⁺ T cells in humans, rats and mice has been recently reviewed (Mosmann and Coffman, 1987; Bottomly, 1988; Powrie and Mason, 1988). The comparison between the leishmanial and the other murine systems in terms of information available for leishmaniasis is summarized in table I. Based on lymphokine secretion, the protective T cells appear to fit into the Th₁/inflammatory cell category, whilst the counter-protective cells seem to be equivalent to the Th₂/helper group. The exception is IL-3, which is secreted by both Th₁ and Th₂ cells and is only produced by the counter-protective T cell. Functionally, however, there are several major discrepancies, including help for specific antibody production, cytotoxicity and DTH reactivity. These differences suggest that the heterogeneity of CD4⁺ T cells may extend beyond the Th₁ and Th₂ classification.

Relationship between protective and disease-promoting T cells.

The next important question is how do the different subsets of T cells in leishmaniasis influence each other in such a way that their balance determines the outcome of the disease. In a recent series of experiments (F.Y. Liew, S. Millott and H. Ziltener, submitted for publication), it was demonstrated that the antigen-specific culture supernatant of lymphoid cells from BALB/c mice with progressive disease can inhibit the MAF (macrophage-activating factor) activity of the culture supernatant of lymphoid cells from mice recovered from *L. major* infection. Thus, the ability of macrophages to kill a tumour cell line or intracellular leishmania parasites after culturing with the supernatant of Tr/Th₁ cells was completely abolished if the culture also contained supernatant of Ts/Th₂ cells. Furthermore, the active ingredient of MAF appears to be IFN- γ , whereas the MAF-inhibiting factors are IL-3 and IL-4. The whole system can be reproduced with recombinant IFN- γ , IL-3 and IL-4, and the MAF-inhibiting activity of the suppressive supernatant can be reversed by specific anti-IL-3 and anti-IL-4 antibodies. It thus appears that the two subsets of CD4⁺ T cells

modulate the outcome of the disease by influencing the ability of macrophage to kill the intracellular parasite. This may be a means by which the different CD4⁺ T cells regulate the immune response in general, by influencing the antigen-processing of antigen-presenting cells.

Conclusion and discussion.

That humoral response can be helped by CD4⁺ T cells seems well established. The fact that an immune response can also be down regulated is not much in dispute. What is unclear is the mechanism of such down regulation. Recent findings in the leishmanial system discussed above strongly suggest the following: 1) the IL-2 production by mitogen- or antigen-induced CD4⁺ T cells can be non-specifically inhibited by macrophages; 2) the suppressive macrophages probably mediate the inhibition via PGE₂; 3) the host-protective immunity mediated by IFN- γ -secreting, antigen-specific, CD4⁺ (Th₁/inflammatory) T cells can also be inhibited; 4) the inhibition here is mediated by antigen-specific CD4⁺ T (Th₂/helper) cells; 5) the Th₂ cells are therefore operationally Ts cells for CMI; and 6) the suppression is manifested in the arena of macrophages via the lymphokines (IL-3 and IL-4) secreted by the different subsets of CD4⁺ T cells.

These observations raise several important questions.

1) How do the IL-3 and IL-4 oppose the macrophage-activating mechanism of IFN- γ ? There is at present no answer. However, it does seem unlikely that IL-3/IL-4 down regulate or compete with IFN- γ receptor on macrophages.

2) Is this a general phenomenon? It may be a general mechanism for the suppression of CMI but is unlikely to be applicable to the suppression of humoral response, since Th₂ itself mediates help for antibody synthesis. Ts cells for DTH, on the other hand, have predominantly been reported to be CD4⁺ T cells. IL-3 has been shown to

oppose the action of IFN- γ in inducing Ia antigen expression on T-dependent mast cells. It is conceivable that IL-3/IL-4 could down regulate class-II MHC expression on macrophages activated by IFN- γ . Hence, the suppression may be due mainly to deprivation of effective antigen presentation in the expression of CMI such as DTH.

3) Where do the suppressor factors fit into the scheme? Of all the confusion in the suppressor system, antigen-specific suppressor factors (TsF) are perhaps the weakest links. It may well be that like the helper factors, they are in fact the various lymphokines (known or yet to be discovered) secreted by different subsets of T cells following specific or mitogenic stimulation.

4) Are Ts cells (Th₂) and effector cells (Th₁) of different lineages? CD4 T cells can be divided into two categories according to the expression of CD45 antigens. All CD45-related cDNA clones isolated from human, rat and mouse cells are derived from a single gene, suggesting alternative mRNA processing as a mechanism for the regulation of post-thymic T-cell maturation. In humans, it is thought that the two populations represent transitional stages of the same lineage of cells: following activation *in vitro* the majority of CD45⁺ (UCHL-1⁻) T cells gradually lose their CD45 determinant (Merkenschlager *et al.*, 1988). Only the CD45⁻ population provides help for B cells in the PWM-driven systems. However, suppressor inducer and effector functions have been associated with the CD45⁺ T cells (Morimoto *et al.*, 1985). In the mouse, both Th₁ and Th₂ clones can be maintained with continuous antigen and IL-2 stimulation. The relationship between Th₁/Th₂ and CD45 determinants in the murine system awaits further clarification. My personal feeling is that both Th₁ and Th₂ can exist as independent mature populations with distinct functions. An alternative view would be that Th₁ and Th₂ have different antigen recognition repertoires. In leishmaniasis, the host-protective (Th₁ equivalent) and disease-promoting (Th₂ equivalent) T-cell lines have been established with non-cross-

reactive antigens (Scott *et al.*, 1988). Furthermore, the two populations of T cells can be differentially induced by non-cross-reacting peptides corresponding to various glycoproteins of the

parasite (Liew *et al.*, in preparation). If this is found to be generally applicable, the selective induction of different subsets of T cells would have important implications.

References.

- BOTTOMLY, K. (1988), A functional dichotomy in CD4⁺ T lymphocytes. *Immunol. Today*, **9**, 268-274.
- CARVALHO, E.M., TEIXEIRA, R. & JOHNSON, W.D. (1981), Cell-mediated immunity in American visceral leishmaniasis: reversible immunosuppression during acute infection. *Infect. Immun.*, **33**, 498-503.
- CILLARI, E., LIEW, F.Y., LO CAMPO, P., MILANO, S., MANSUETO, S. & SALERNO, A. (1988), Suppression of IL-2 production by cryopreserved peripheral blood mononuclear cells from patients with active visceral leishmaniasis in Sicily. *J. Immunol.*, **140**, 2721-2726.
- HOWARD, J.G. (1985), Host immunity to leishmaniasis, in "Leishmaniasis" (pp. 140-162). Elsevier Science Publishers B.V., Amsterdam.
- LIEW, F.Y. (1982), Regulation of delayed-type hypersensitivity to pathogens and alloantigens. *Immunol. Today*, **3**, 18-23.
- LIEW, F.Y. (1989), Functional heterogeneity of CD4⁺ T cells in leishmaniasis. *Immunol. Today*, **10**, 40-45.
- LOUIS, J.A., MENDONCA, S., TITUS, R.G., CEROTTINI, J.C., CERNY, A., ZINKERNAGEL, R., MILON, G. & MARCHAL, G. (1986), The role of specific T-cell subpopulations in murine cutaneous leishmaniasis, in "Progress in immunology" (pp. 762-769). Academic Press, New York, London.
- MERKENSCHLAGER, M., TERRY, L., EDWARDS, R. & BEVERLEY, P.C.L. (1988), Limiting dilution analysis of proliferative responses in human lymphocyte populations defined by the monoclonal antibody UCHL1: implications for differential CD45 expression in T-cell memory formation. *Europ. J. Immunol.*, **18**, 1653-1661.
- MOLL, H., SCOLLAY, R. & MITCHELL, G.F. (1988), Resistance to cutaneous leishmaniasis in nude mice injected with L3T4⁺ T cells but not with Ly-2⁺ T cells. *Immunol. Cell Biol.*, **66**, 57-63.
- MORIMOTO, C., LETVIN, N.L., DISTASO, J.A., ALDRICH, W.R. & SCHLOSSMAN, S.F. (1985), The isolation and characterization of the human suppressor inducer T-cell subset. *J. Immunol.*, **134**, 1508-1515.
- MOSMANN, T.R. & COFFMAN, R.L. (1987), Two types of mouse helper T-cell clone — implications for immune regulation. *Immunol. Today*, **8**, 223-227.
- POWRIE, F. & MASON, D. (1988), Phenotypic and functional heterogeneity of CD4⁺ T cells. *Immunol. Today*, **9**, 274-277.
- REINER, N.E. & FINKE, J.H. (1983), Interleukin 2 deficiency in murine *Leishmania donovani* and its relationship to depressed spleen cell responses to phytohemagglutinin. *J. Immunol.*, **131**, 1487-1491.
- SCOTT, P., NATOVITZ, P., COFFMAN, R.L., PEARCE, E. & SHER, A. (1988), Immunoregulation of cutaneous leishmaniasis. T-cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. *J. exp. Med.*, **168**, 1675-1684.
- TITUS, R.G., MILON, G., MARCHAL, G., VARSALLI, P., CEROTTINI, J.-C. & LOUIS, J. (1987), Involvement of specific Lyt-2⁺ T cells in the immunological control of experimentally induced murine cutaneous leishmaniasis. *Europ. J. Immunol.*, **17**, 1429-1433.
- WONG, G.H.W., CLARK-LEWIS, I., HAMILTON, J.A. & SCHRADER, J.W. (1984), P-cell stimulating factor and glucocorticoids oppose the action of interferon- γ in inducing Ia antigens on T-dependent mast cells (P cells). *J. Immunol.*, **133**, 2043-2050.

SUPPRESSOR CELLS IN RATS THAT HAVE RECOVERED FROM AUTOIMMUNE DISEASE

S.W. Brostoff

*The Immune Response Corporation
6455 Nancy Ridge Drive
San Diego, CA 92121
(USA)*

In addressing the subject of this Forum, I would like to diverge slightly from the main theme to discuss observations (and their implications) relating to the recovery and subsequent resistance of Lewis rats to experimental autoimmune encephalomyelitis (EAE). Although this animal disease model is considered to be T-cell-mediated, the control of T-cell help for antibody responses may involve similar mechanisms.

Rats immunized with myelin basic protein (MBP) and complete Freund's adjuvant (CFA) develop clinical signs of EAE 10 or 11 days post-injection. Following a clinical course of disease lasting 4-6 days, the animals recover and, starting 28 days post-injection, are refractory to subsequent immunization with MBP in CFA. There appear to be different mechanisms involved in the short-term recovery and long-term resistance of these animals. A variety of studies have provided support for both CD8⁺ cytotoxic T lymphocytes (CTL) and an immunoregulatory CD4⁺ cell population being involved in the long-term resistance, whereas endogenous corticosteroid appears to play an essential role in the short-term recovery.

The role of CD8⁺ CTL in the resistance of recovered rat to EAE is controversial. Wekerle and coworkers (Sun *et al.*, 1988) have recently isolated a line of CD8⁺ CTL from the spleens of rats recovered from EAE that was induced by adoptive transfer of an MBP-specific CD4⁺ T-cell line. Subsequently, the T-cell line was used in attenuated

form to select suppressor CD8⁺ CTL from recovered animals. The isolated CD8⁺ line could then be used to suppress EAE *in vivo*. Cohen and coworkers (Lider *et al.*, 1988) have vaccinated with effector T-cell clones to induce resistance to EAE and then demonstrated that CD8⁺ cells from these animals could suppress the response of the immunizing effector clone to its antigen. There is no doubt that in these studies the CD8⁺ CTL could downregulate EAE, but in both cases the CTL population was selected using attenuated effector cells. It is hard to reconcile a major role for these selected CD8⁺ CTL in the long-term resistance of the recovered rats, since Sedgwick (1988) has shown clearly that depletion of CD8⁺ cells with monoclonal antibodies (mAb) does not affect the disease process or recovery. An endocrinological mechanism, which depends on the level of endogenous adrenal corticosteroids, rather than an immunological mechanism, seems to be responsible for the short-term recovery, since adjustments of corticosteroid levels in adrenalectomized rats can dramatically alter the disease duration and severity (McPhee *et al.*, 1989).

A cell population likely to be of more relevance to the normal recovery process is one which our laboratory has recently reported to be present in Lewis rats recovered from EAE (Elerman *et al.*, 1989). Using cyclosporin, we have been able to isolate a CD4⁺-cell line (CsLn-1) with the ability to downregulate EAE effector cells. This population

of cells seems to mediate its activity, at least in part, by the release of a soluble factor (Brostoff *et al.*, 1989). This soluble suppressor activity does not seem to be antigen-specific, since supernatants of this cell line can down-regulate cell lines responding not only to MBP, but also to P₂ protein (which has no sequence homology to MBP). We have also noted that EAE effector cell lines that have lost their ability to transfer EAE show quite similar characteristics and appear to release the same soluble suppressor activity (Ellerman & Brostoff, unpublished). Fritz and coworkers (Barzaga-Gilbert *et al.*, 1989) have observed a similar phenomenon with non-encephalitogenic MBP-specific cell lines. Supernatants of such cell lines down-regulate MBP-specific T-cell lines as well as ovalbumin-specific cell lines. Reports by Swanborg and coworkers (McDonald and Swanborg, 1988; Karpus and Swanborg, 1989) have described a population of nylon-adherent CD4⁺ post-recovery suppressor cells which appears to mediate its activity through a soluble factor which inhibits gamma interferon release by the effector cells.

Although MBP is used to stimulate CsLN-1, we have preliminary results indicating that CsLN-1 can also be induced to proliferate and to produce its soluble activity using MBP-specific lymphoblasts isolated from MBP-immunized rats in place of antigen plus antigen-presenting cell (Ellerman and Brostoff, unpublished). The immunoregulation afforded by these cells may be an anti-idiotypic (or anti-clonotypic) response to the effector cell population. Based upon the above observations, CsLN-1 appears to be a mixture of effector and suppressor cells. The appearance of suppressor cells in effector cell lines is likely due to the fact that stimulation with antigen of the effector cells also drives the immunoregulatory cell to propagate in response to the effector cell idiotype. Curiously, both suppressor and effector cells are CD4⁺ (although 20-30% become weakly CD8⁺, *i.e.* double-positive, upon activation). The general difficulty in isolating suppressor populations in other systems may be due

to the absence of the effector cells to which they are directed.

In discussing the immunoregulation provided by the CD4⁺-cell population, several recent observations should be considered. It is clear that the CD4⁺ population can be divided into subsets in rats (Powrie and Mason, 1988) as well as in humans (Morimoto *et al.*, 1985) and in mice (Mosmann *et al.*, 1986). These subsets display differences in function, lymphokine release and susceptibility, and may well represent different stages of maturation (Sanders *et al.*, 1988). What also seems clear is that in cultured cells the frequency of encounter with antigen and time between encounters (*i.e.*, time left in a resting state) affect the ability of the cultured cells to be stimulated, as well as their response to and release of interleukins (Huhn *et al.*, 1988). We have noted that suppressor activity of CsLN-1 is greatly affected by the length of time the cells are allowed to rest. Different cycling times lead preferentially to a release of either soluble suppressive activity or stimulatory activity (Ellerman & Brostoff, unpublished). Thus, the same cell line can be both suppressive and stimulatory depending on its previous history. Similarly, cell surface molecules known to participate in cell activation can also mediate suppression of the same cell. The use of a mAb against CD4 to suppress an *in vivo* immune response was first shown in the rat model of EAE (Brostoff and Mason, 1984). The suppression was shown clearly to be mediated by the CD4 molecule, since the CD4⁺ population was not depleted by the treatment. Others have shown that mAb directed at CD3 can either inhibit or stimulate depending upon whether the antibody is soluble or aggregated. Thus, molecules involved in cell-cell contact may be able to up-regulate or down-regulate depending on the circumstances.

It is apparent that cells and factors exist that both activate and suppress the immune system. This clearly implicates cell surface molecules in the regulatory process either as receptors for the soluble factors or as mediators of direct cell-

cell contact. A particular cell surface molecule, depending on its interaction, may either up-regulate or down-regulate an individual cell. Consequently, the individual cells themselves may serve as either effector or suppressor cells depending upon such factors as their stage of maturation (naive, memory, suppressor-inducer?), state of activation, prior history of exposure to antigen, level of expression of cell surface molecules (receptors, ligands), autocrine and paracrine production (both stimulatory and suppressive), etc. One might also speculate that some soluble factors or

cells may prove to have both up- and down-regulatory ability depending upon the receptor (or cell) with which they interact. The isolation of clones of cells which exhibit specific functions *in vitro* and can have measurable effects when introduced into test animals *in vivo* has been documented in many instances. More experiments are needed in which related clones of cells are combined in a controlled manner in order to obtain a better understanding of the parameters that are involved in immunoregulation and especially in suppression.

References.

- BARZAGA-GILBERT, E., SKEEN, M.J. & FRITZ, R.B. (1989), Suppression of passive experimental allergic encephalomyelitis (EAE) by non-encephalitogenic myelin basic protein (MBP)-specific T-cell line. *FASEB J.*, **3**, A1361.
- BROSTOFF, S., ELLERMAN, K., OLEE, T. & POWERS, J. (1989), Supernatants from suppressor cell line CsLN-1 inhibit the transfer of experimental allergic encephalomyelitis (EAE), in "Progress in Leukocyte Biology 9: Cellular Basis of Immune Modulation" (J.G. Kaplan, D.R. Green & R.C. Bleackly) (pp. 395-398). Alan R. Liss, New York.
- BROSTOFF, S.W. & MASON, D.W. (1984), Experimental allergic encephalomyelitis: successful treatment *in vivo* with a monoclonal antibody that recognizes T helper cells. *J. Immunol.*, **133**, 1938-1942.
- ELLERMAN, K.E., POWERS, J.M. & BROSTOFF, S.W. (1988), A suppressor T-lymphocyte cell line for autoimmune encephalomyelitis. *Nature (Lond.)*, **131**, 265-267.
- HUHN, H., RESKE-KUNZ, A.B. & RUDE, E. (1988), Cloned T helper cells reverting to a resting state develop increasing sensitivity in their antigen-mediated interaction with accessory cells. *Europ. J. Immunol.*, **18**, 1745-1752.
- KARPUS, W.J. & SWANBORG, R.H. (1989), Selective inhibition of the production of gamma interferon by CD4⁺ suppressor T cells. *FASEB J.*, **3**, A1084.
- LIDER, O., RESHEF, T., BERAUD, E., BEN-NUN, A. & COHEN, I.R. (1988), Antidiotypic network induced by T-cell vaccination against experimental autoimmune encephalomyelitis. *Science*, **239**, 181-183.
- MCDONALD, A.H. & SWANBORG, R.H. (1988), Antigen-specific inhibition of immune interferon production by suppressor cells of autoimmune encephalomyelitis. *J. Immunol.*, **140**, 1132-1138.
- MACPHEE, I.A., ANTONI, F.E. & MASON, D. (1989), Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J. exp. Med.*, **169**, 431-445.
- MORIMOTO, C., LETVIN, N.L., DISTASO, J.A., ALDRICH, W.R. & SCHLOSSMAN, S.F. (1985), The isolation and characterization of the human suppressor inducer T-cell subset. *J. Immunol.*, **134**, 1508-1515.
- MOSMANN, T.R., CHERWINSKI, H., BOND, M.W., GIEDIN, M.A. & COFFMAN, R.L. (1986), Two types of murine helper T-cell clones. — I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.*, **136**, 2348-2357.
- POWRIE, F. & MASON, D. (1988), Phenotypic and functional heterogeneity of CD4⁺ T cells. *Immunol. Today*, **9**, 274-277.
- SANDERS, M.E., MAKGOBA, M.W. & SHAW, S. (1988), Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol. Today*, **9**, 195-199.

- SEDGWICK, J.D. (1988), Long-term depletion of CD8⁺ T cells *in vivo* in the rat: no observed role for CD8⁺ (cytotoxic/suppressor) cells in the immunoregulation of experimental allergic encephalomyelitis. *Europ. J. Immunol.*, **18**, 495-502.
- SUN, D., QIN, Y., CHLUBA, J., EPPLIN, J.T. & WEKERLE, H. (1988), Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. *Nature* (Lond.), **332**, 843-845.

MURINE SUPPRESSOR T CELLS: MIRAGE OR CLOUDY REALITY?

Leonore A. Herzenberg

Department of Genetics, Stanford University, Stanford CA 94305

Recently, one of our students asked me whether I still "believe in" suppressor T cells. My first impulse was to respond with a statement to the effect that belief is a religious rather than a scientific issue; however, I realized that this rather fashionable question was meant to be taken seriously and reflects a potentially valid concern that conclusions from earlier studies (in the "pre-molecular era") are significantly flawed. In essence, during the last few years, genes for many of the molecules that were known to function in the immune system have been cloned and T-cell clones capable of many of the functions known for the immune system have been isolated. However, molecules mediating suppression have been evasive and cell lines capable of suppressing responses have been few and far between. Therefore, many investigators now wonder whether these cells and molecules actually exist.

The difficulties in evaluating the validity of the earlier studies are compounded by the wide variety of suppressor systems that were examined and the sometimes meager evidence advanced to demonstrate that responses were actually being suppressed. Nevertheless, there are several clear-cut systems in which suppression has been demonstrated. I am most familiar with two of

these systems: the allotype suppression system studied in our laboratory and the carrier-specific suppression system studied initially by the Tada group in Japan and revived in our laboratory in the context of the epitope-specific regulation of antibody responses.

In the allotype-suppression system (Herzenberg and Herzenberg, 1974; Herzenberg, 1983; Herzenberg *et al.*, 1983), (BALB/c × SJL)F₁ hybrids treated with antibodies to the paternal IgG2a allotype (Igh1b) develop a specific, chronic suppression of Igh1b allotype production. This suppression can be transferred with FACS-sorted T cells and reproducibly results in a 10- to 50-fold suppression of Igh1b production by co-transferred syngeneic spleen cells. In the carrier-specific suppression system (Tada *et al.*, 1972; Tada and Okumura, 1979; Tada and Hayakawa, 1980), mice immunized with typical protein immunogens such as KLH and CGG develop suppressor T cells that suppress *in vitro* antibody production to haptens on the protein (carrier) molecule. These suppressor cells have been shown to specifically suppress antibody production *in vitro* and to transfer suppression regularly, albeit not always, to non-irradiated hosts.

The carrier-specific suppressor cells were (are) commonly believed to sup-

press antibody production by interfering with carrier-specific help. However, our studies show that these suppressor cells and soluble factors that they produce actually act to induce an epitope-specific suppressor effector mechanism that specifically blocks antibody responses to new epitopes presented on the carrier protein (Herzenberg, 1983; Herzenberg *et al.*, 1983; 1982; Herzenberg, 1986). These findings, which showed that immunizing KLH primed animals with DNP-KLH specifically suppressed anti-DNP responses, ran contrary to the widely held notion that carrier priming augments subsequent antibody responses to haptens presented on the priming carrier. Thus, they contradicted much of the dogma of the era in which they were produced.

Since that time, a number of studies have been completed confirming and extending these findings and demonstrating their importance in practical immunology (*e.g.*, Schutze *et al.*, 1987; 1985). Paradoxically, however, this appears to have served to further erode confidence in the general concepts

of suppression and regulatory T cells. Perhaps this is because we have now begun to recognize the complexity of the mechanisms that control the immune system and how difficult it is to study those mechanisms *in vitro*.

I have little doubt that regulatory T cells capable of up-regulating or down-regulating immune responses exist. The evidence on which I base this conclusion is referred to above and is summarized in several reviews that we wrote over the last few years (Herzenberg and Herzenberg, 1974; Herzenberg *et al.*, 1983; 1982; Herzenberg, 1986). It does not bear repetition or further summary in the limited space available here. Rather, I would suggest that those interested in determining whether suppressor T cells exist give serious consideration to the detailed data summarized in these articles and presented in the papers to which they refer. In essence, I believe at least these data must be explained before critics who decry the existence of suppressor T cells can be seriously credited.

References.

- HERZENBERG, L.A. (1983), Allotype suppression and epitope-specific regulation. *Immunol. Today*, **4**, 113-117.
- HERZENBERG, L.A. (1986), Overview: Epitope-specific regulation, in "The handbook of experimental immunology", 4th Edition (D.M. Weir, L.A. Herzenberg, C.C. Blackwell & L.A. Herzenberg) Chapter 74. Blackwell Scientific Pub., Ltd., Edinburgh.
- HERZENBERG, L.A., HAYAKAWA, K., HARDY, R.R., TOKUHISA, T., OI, V.T. & HERZENBERG, L.A. (1982), Molecular, cellular and systemic mechanisms for regulating IgCH expression. *Immunol. Rev.*, **67**, 5-31.
- HERZENBERG, L. & HERZENBERG, L.A. (1974), Short-term and chronic allotype suppression in mice, in "Contemp. Topics in Immunobiol" (Cooper, M.D. & Warner, N.L.) Vol. 3 (pp. 41-75). Plenum Press, New York.
- HERZENBERG, L.A., TOKUHISA, T. & HAYAKAWA, K. (1983), Epitope-specific regulation. *Ann. Rev. Immunol.*, **1**, 609-632.
- SCHUTZE, M.P., LECLERC, C., JOLIVET, M., AUDIBERT, D. & CHEDID, L. (1985), Carrier-induced epitopic suppression, a major issue for future synthetic vaccine. *J. Immunol.*, **135**, 2319-2322.
- SCHUTZE, M.P., LECLERC, C., VOGEL, F.R. & CHEDID, L. (1987), Epitopic suppression in synthetic vaccine models: analysis of the effector mechanisms. *Cell. Immunol.*, **104**, 79-90.
- TADA, T. & HAYAKAWA, K. (1980), Antigen-specific helper and suppressor factors, in "Immunology 80: Progress in Immunology IV" (M. Fougereau & J. Dausset) (p. 389). Academic Press, New York.
- TADA, T. & OKUMURA, K. (1979), The role of antigen-specific T-cell factors in the immune response. *Advanc. Immunol.*, **28**, 1-87.
- TADA, T., OKUMURA, K. & TANIGUCHI, M. (1972), Regulation of homocytotropic antibody formation in the rat. — VII. Carrier functions in the anti-hapten homocytotropic antibody response. *J. Immunol.*, **108**, 1534-1541.

DISCUSSION.

G. Bordenave:

The results and opinions exposed in this Forum instill confidence in us about the solution to the dispute "cytotoxic T cells versus suppressor T cells". Perhaps we might regret that proponents of suppressor T cells in the regulation of idiotype expression were very few to participate in light of their contribution to this field.

We would like to add some information to the comments of Claude Leclerc in her overview paper, concerning mouse immunoglobulin allotypic suppression. M. Bosma's team obtained CTL clones from BALB/c mice immunized against IgG2a^b. These clones, endowed with cytotoxic activity demonstrable *in vitro* towards IgG2a^b-bearing myeloma cells, were derived from splenocytes triggered *in vitro* with the antigen: the myeloma cells. The injection of such CTL into irradiated BALB/c Igh^a mice reconstituted with splenocytes from CB17 mice (the Igh^b congenic strain) led to suppression (studied over a 3-4 week period) of IgG2a^b expression by these splenocytes. In contrast, L. and L. Herzenberg (*J. exp. Med.*, 1976, 144, 330-344), in their system of antibody-induced allotypic suppression making use of SJL-related hybrids, demonstrated that the chronicity of the suppression was ensured by suppressor T cells acting on T cells which help B cells to produce IgG2a^b. Is our model of cellular-induced allotypic suppression closer to M. Bosma's scheme than to that of L. and L. Herzenberg? We do not know for the moment, but soon hope to be able to choose between a suppressor and a cytotoxic *in vivo* activity.

S.W. Brostoff:

In reading over the contributions to this Forum, it strikes me that there is a great diversity of systems in which suppressive effects can be measured, which

makes it difficult to decide which effects are epiphenomena and which are central to immunoregulation. Certainly one can learn a great deal about the individual reactions of lymphocyte subsets by studying them carefully *in vitro*. However, I must agree with Claude Leclerc that further study of the *in vivo* effects are needed to help us determine the relationship of the various *in vitro* activities. We are still at the stage where we are having trouble distinguishing the "noise" from the "signal" in immunoregulation. There is, by necessity, a certain threshold of activity that must be reached before a given response results in either activation or suppression. Suppression cannot have too low a threshold, since overactive suppression can shut down the immune response, leaving the host at risk. Thus suppression may involve a series of events acting in concert to keep the immune system under control without shutting it down completely. The suppression phenomenon may be one in which the whole of immunoregulation may be quite different from the sum of its individual parts.

D.R. Green and H. Zheng:

Controversy is a necessary aspect of science, often forcing us to reevaluate experimental results and redefine our interpretations of these results. Perhaps more importantly, controversy can drive the development of new systems for analysis. Regardless of how things turn out in the end, we learn something.

The controversy surrounding suppressor T cells comes, we think, from three sources. One, as pointed out by Lee Herzenberg, is that the application of molecular biological analysis to suppressor T cells has yielded conflicting or negative results, and therefore, our understanding of suppression has not progressed at this level. A second source is found in our current understanding of T-cell function, in which old ideas of a T-cell or T-cell factor which directly

recognizes antigen simply do not neatly fit. The third source is the general disagreement about just what a suppressor cell is, a disagreement which can be seen in this Forum.

The general question, "Do suppressor T cells exist?" may be too vague to be usefully answered. In considering the papers in this Forum, this question seems to have been interpreted in at least three ways, each of them yielding what may be a more useful question: 1. Do T cells play an active role in suppressing immune response? 2. Do T cells suppress immune responses by means other than those employing defined lymphokines or cytotoxic mechanisms? 3. Is there a unique population of T cells whose function it is to suppress immune responses?

The first question is the easiest. As pointed out by F.Y. Liew, the regulatory interactions among subpopulations of CD4⁺ helper T cells makes it clear that T cells can, indeed, suppress immune responses. It has now been clearly shown that γ -interferon inhibits the function of Th2 cells, and it is very likely that a product of Th2 cells can, in turn, inhibit Th1 cells. Further, it is also clear from a number of studies over the past decade that CD8⁺ T cells can regulate immune responses via cytotoxic mechanisms. Thus, while it is probably not disputed that T cells can suppress immune responses, it may not be necessary (based on these types of findings) to look for novel mechanisms of suppression. One gets the feeling, though, that those on either side of the controversy would rather not assert that these sorts of effects are indicative of "suppressor T cells".

This brings us to our second question. Can T cells suppress immune responses via other, perhaps poorly understood, mechanisms? Studies, discussed in this Forum on allotype suppression, epitope-specific suppression and suppression in adoptive hapten-carrier systems all indicate that the answer to this question is probably "yes". What these mechanisms may be are less well understood. One possible mechanism invokes the specter of antigen-specific T suppressor factors,

considered in the discussions by Kimi Ishizaka and colleagues and by ourselves.

Both of our groups have reached a conclusion which conflicts with earlier findings on the role of the T-cell receptor (TCR) in suppressor T-cell hybridomas which make such antigen-specific factors. Recent findings by a number of groups (many not included in this Forum) show that Ts hybrids utilise conventional TCR. Failure to detect TCR in Ts hybrids was seen in earlier studies and was likely due to a technical artifact. We have raised the possibility that the TCR may be intimately related to the antigen-specific factor. It is very likely that, with the demonstration of TCR in factor-producing Ts cells, we will see some new and interesting progress in this area.

Clearly, however, a description of an antigen-specific factor (even if we "believe" in it) does not imply that it is an important mechanism of regulation. However, such factors have been demonstrated to function very efficiently *in vivo* (e.g., Steele *et al.*, 1987) while antibodies to such factors have been shown to block several *in vivo* phenomena, such as tolerance induction in adults (Ferguson *et al.*, 1988a; Horvat *et al.*, 1989; Ferguson *et al.*, 1988b), some Ir gene effects (Ferguson *et al.*, 1988b), and suppression of effective anti-tumour immunity (Kim *et al.*, 1987). Such studies are in the spirit of the discussion by Claude Leclerc, who urges investigations into the roles of T-cell subpopulations in *in vivo* regulation through similar antibody experiments.

The third question is perhaps the most difficult: do suppressor cells represent a unique T-cell subpopulation? We cannot comment on this from our own work, since the cells we study as a source of antigen-specific regulatory factors often have other apparent functions. Clearly, however, there are regulatory T-cell lines such as those described by E. Kolsch and by Tomio Tada, *et al.*, which appear to represent novel T-cell subpopulations. In one case, the mechanism of suppression is a cytotoxic one, in the other the

mechanism is less clear. Maybe this question should be asked another way: are there unique T-cell types which have suppressive function? When asked this way, the answer may well be "yes".

One message that comes through the papers in this Forum is that there are immunologic phenomena which are not readily explained by conventional wisdom. Our job as scientists is to try to explain them. For this purpose, the concept of a suppressor T cell has often been useful. We run into problems,

however, when we assume that an explanation of one phenomenon of suppression explains them all. The controversy surrounding suppressor T cells has served its function, forcing us to regroup and re-evaluate our systems and definitions. In many immunologist's minds, unfortunately, a discussion of the study of suppressor T cells appears to be a dead issue. But, as Tomio Tada has pointed out, "still, it moves!".

So, lets keep moving.

References.

- FERGUSON, T.A., IVERSON, G.M. & FLOOD, P.M. (1988a), Infectious and noninfectious tolerance are blocked by a monoclonal antibody to T suppressor factor. *Cell. Immunol.*, **115**, 403-412.
- FERGUSON, T.A., PTAK, W., IVERSON, G.M. & FLOOD, P. (1988b), The role of suppression in immunoregulation: *in vivo* analysis using a monoclonal antibody to T suppressor factors. *Europ. J. Immunol.*, **18**, 1179-1186.
- HORVAT, B., FERGUSON, T.A., RODRIGUEZ, A., REUTER, P., FRIEDMAN, A. & FLOOD, P.M. (1989), The role of suppressor T cells in immunologic responses analyzed with monoclonal anti-T suppressor cell antibodies, in "Cellular basis of immune modulation" (J.G. Kaplan, D.R. Green, R.C. Bleakley) (pp. 353-366). Alan R. Liss, New York.
- KIM, B., WAMAKA, P., IVERSON, G.M. & IMBEMBO, A.L. (1987), Immunotherapy of pulmonary metastases using monoclonal antibody to T-cell suppressor factor and interleukin 2. *Arch. Surg.*, **122**, 1455-1459.
- STEELE, C.K., CHU, N.P., CHAN, A., NORTH, J. & LEVY, J.G. (1987), Isolation of an antigen-specific T suppressor factor that suppresses the *in vivo* response of DBA/2 mice to ferredoxin. *J. Immunol.*, **139**, 469-475.

E. Kölsch :

Among the data discussed in this Forum, the experiments on the activity of carrier-specific Ts cells by Drs H. Pritchard-Briscoe and R.H. Loblay are closest and most comparable to our work on BSA-specific Ts cells. Yet the conclusions drawn are completely different. On the basis of an "antigenic competition" type of experiment P.-B. and L. come to the conclusion that B lymphocytes are the direct target of suppression. However, to my mind, the data do not exclude an intermediate cell, e.g. T helper cell, as the primary

target, and I could incorporate their findings into the model proposed in my contribution. Being as it is, from our experiments on the action of BSA-specific Ts cells we conclude for another classical carrier-hapten system that the T helper cells are the primary target of Ts cell action. If data from two, not too unrelated systems are interpreted, how can one expect a broad consensus on Ts cells.

This statement is true for other aspects.

1) Reading the contributions to this Forum, Ts cells can apparently have any

phenotype. Different reasons could be responsible for that. First, the differences might be real, and what we call Ts cells, in the sense T. Tada and co-authors have defined them, might have different ontogenic sources. Secondly, we might often deal with aberrant phenotypes of cloned T cells. The third possibility is that one has looked at a momentary picture of Ts cells which, in our experience, can modulate surface markers like CD4 and CD8. Thus, one can get an incomplete picture if the phenotype is not followed up over one to two weeks after antigenic restimulations.

2) The majority of people working on Ts cells apparently agree that the function of Ts cells is not comparable to that of cytotoxic T cells, if measured in a standard ⁵¹Cr-release assay. Yet this does not mean that the suppressive mechanism which arrests the target cell is without cytolytic consequence. It could lead to a slow disintegration of the target both *in vivo* and *in vitro*. Some data speak for this possibility.

The way Ts cells recognize antigens no longer seems to be a mystery. Work in progress in several laboratories suggests that the fine specificity of antigen recognition has its molecular basis in a "conventional" TCR.

What is the personal conclusion drawn from the discussions in this Forum? The term T suppressor cells apparently comprises cells responsible for two distinct phenomena. The first I would call safeguard in the periphery and relate it to immunological tolerance. To this category belong carrier-, epitope- and perhaps allotype-specific Ts cells, as well as those appearing after recovery from autoimmunity. The other set of Ts cells is responsible for the fine tuning of the immune response with either the marvellous potential to modulate DTH versus humoral responses or the capacity of selectively up- and downregulating production of antibodies of given immunoglobulin classes. "It looks as if the two Ts cell types use quite different effector mechanisms."

H. Pritchard-Briscoe and R.H. Loblay:

In reviewing the contributions of other authors, two issues came to our attention for comment:

Suppressor clones.

In light of our own difficulties in generating suppressor clones, we were surprised by the fact that several authors described such clones, and we wonder about their relationship to the suppressor cells we and many others have studied *in vivo*. Thus far, there has been fairly general agreement that in antibody responses the cells mediating suppression are CD8⁺ and that CD4⁺ cells act as helper cells, yet most of the clones described here are CD4⁺ (Tada, Liew, Brostoff) or CD4⁺8⁺ (Kolsch). Two possible explanations come readily to mind: 1) in models such as EAE (Brostoff) and infection (Liew), the effector cells mediating immunopathology may be CD4⁺ (DTH) cells, and it has been known for many years that suppression of DTH is mediated by CD4⁺ cells; 2) the CD4⁺ clones isolated by some authors might be "suppressor-inducers" rather than effectors of suppression (Sun and Waltenbaugh, cited by Leclerc).

On the other hand, as pointed out by Kolsch, T-cell clones isolated by different groups are heterogeneous and *in vitro* cultivation itself can result in modulation of the cell surface phenotype. He seems reassured by the fact that all three of his BSA-specific clones exhibit the same phenotype, but if there is a major selection bias inherent in growing suppressor cells *in vitro*, as discussed in our paper, it is possible that one would find systematic changes in the function and/or phenotype of clones isolated in this way.

We were also puzzled by the description of CD4⁺ clones which were "I-J⁺" and whose function was inhibited *in vitro* by monoclonal anti-"I-J" (Tada). In our hands, injection of B10A.3R anti-B10A.5R (anti-"I-J") serum had no effect on *in vivo* helper function, even though suppression is ef-

fectively abrogated. Once again, this raises the question of whether CD4⁺ clones cultivated *in vitro* share the normal physiological characteristics of their counterparts *in vivo*.

Cellular target of suppression.

We were disappointed that there appears to be so little definitive data relating to this question. Kolsch's BSA model seems to be a unique one involving cytotoxicity of helper cells, a mechanism which cannot account for the findings in our system. Tada suggests that the final effector mechanism is a simple one involving inhibition of helper function, but this is based on his observations with CD4⁺ "suppressor" clones about which we have already expressed our reservations, above.

Herzenberg states her belief that suppression of antibody responses is due to interference with carrier-specific help in the epitope suppression model. However, it seems to us that the data can be interpreted differently. From experience in our own system, the phenomenon of epitope-specific suppression is consistent with the action of carrier-specific suppressor cells which reach their peak activity during the second week after priming (Loblay *et al.*, 1984). At this time the number of carrier-specific B-cell precursors would have

been expanded by prior stimulation, whereas those specific for the epitope would not. Assuming help and suppression act on B cells in the manner discussed in our paper, the observed differences in anti-carrier versus anti-hapten antibody responses after hapten-carrier challenge could be easily explained.

As far as allotype suppression is concerned, the original conclusion that T helper cells were the primary target of action was based on a mathematical model which assumed that interactions between regulatory T cell subsets were "stoichiometric" (Herzenberg *et al.*, 1975). However, the plausibility of this assumption is highly questionable, and we believe a different conclusion would have been reached by considering such cell interactions to be random, stochastic events, more appropriately modelled using the Poisson distribution. Furthermore, the existence of allotype-specific helper cells is unproven, further weakening the argument. To us, the findings described by Benaroch and Bordenave in the present Forum suggest that B cells are directly inhibited at a fairly late stage of differentiation by allotype-specific suppressor cells, a conclusion which is in keeping with the earlier observations of Jacobson in the allotype model (Jacobson, 1978) and of Lynch in the MOPC-315 system (Lynch, 1987).

References.

- HERZENBERG, L.A., OKUMURA, K. & METZLER, C.M. (1975), Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. *Transplant. Rev.*, **27**, 57-83.
- JACOBSON, E.B. (1978), Adoptive transfer of allotype-specific suppressor cells inhibits thymus-independent immunoglobulin production in syngeneic athymic mice. *J. exp. Med.*, **148**, 607-612.
- LOBLAY, R.H., PRITCHARD-BRISCOE, H. & BASTEN, A. (1984), Suppressor T cell memory. Induction and recall of HGG-specific memory suppressor T cells and their role in regulation of antibody production. *Aust. J. exp. Biol. med. Sci.*, **62**, 11-25.
- LYNCH, R.G. (1987), Immunoglobulin-specific suppressor T cells. *Advanc. Immunol.*, **40**, 135-151.

E. Sercarz:

The variety of suppressive phenomena described in this Forum must have impressed each of us with the potential diversity with which evolution has devised ways for controlling self-activity. There are many questions which could be debated vigorously: *e.g.*, do suppressor systems display memory; are Ts-inducing determinants always different from Th-inducing determinants; how is it imagined that I-J on Th is different from I-J on Ts; and others. The contributors to this Forum would doubtless agree that regulation is too important to be left to a single mechanism.

We regulatory immunologists find ourselves in an interesting quandary: the assays that are most believable in immunology are those involving single effectors and single targets (IL-2 assays on IL-2-requiring clones, plaque-forming cell assays by B cells, chromium release by cytotoxic cells, presentation of antigen by a B lymphoma to a Th-cell clone, etc.). Whenever a third cell is involved, such as a T suppressor-inducer cell, the impatient skeptic is unwilling to confront the added complexity. Nevertheless, the beautiful complexities of trans-acting factors, of VDJ junctional diversification, of T-cell tolerance induction, are readily accommodated. We cannot always simplify our multicellular regulatory interactions, although we can try to study them pairwise. I can only add to the comment by Green and Zheng, that the rest of the community should listen, regard and provide some constructive input, aside from just saying "no" while we persevere.

J.L. Teillaud and W.H. Fridman.

It is striking that almost 20 years after Gershon and Kondo first reported the presence of suppressive factors produced by Ts cells which are involved in "infectious tolerance", their existence is still disputed. In fact, the existence of these factors is even considered as a mass hallucination, as pointed out by

D.R. Green and H. Zheng. From the reading of the twelve chapters, several comments can be made.

1) Suppression is a very heterogeneous phenomenon. It is particularly striking, as the manuscripts show that this phenomenon involves disparate molecules and cells ranging from antigen-specific suppressor factors and antigen-specific T cells (Green and Zheng, Herzenberg, Ishizaka, Iwata and Katamura, Kolsch, Krzych, Nanda and Sercarz, Pritchard-Priscoe and Loblay, Tada, Asano and Sano) to commonly used cytokines with subtle suppressive activities on antibody production (Liew, Tada, Asano and Sano, and ourselves).

2) Even though it has proven to be an extremely difficult task to elaborate *in vitro* models on T-cell mediated suppression, suppression occurs *in vivo* in several systems (Benaroch and Bordenave, Brostoff, Ishizaka, Iwata and Katamura, Kolsch, Leclerc, Liew, Pritchard-Priscoe and Loblay).

3) Since all of the models discussed in this Forum are specific (*i.e.*, antigen-, carrier-, allotype- and isotype-specific), particular TCR rearrangements responsible for this specificity can be hypothesized when no other molecule with specific binding properties is defined. As discussed by Green and Zheng, although TCR rearrangements have been reported in a Ts line and in some Ts hybridomas, the role of the expression of these TCR genes in T-cell mediated suppression has not yet been established.

4) One of the major problems encountered in the *in vitro* studies on T-cell-mediated suppression has been the extreme difficulty in cloning suppressor cells. Pritchard-Priscoe and Loblay's suggestion that the production of lymphokine(s) which inhibit the proliferation of nearby cloned target cells is the cause for problems in such cloning is challenged by recent reports of a strong instability of CD3 molecules on the surface of cells from Ts lines, which would account for the loss of the suppressive properties of these cells. However, their conclusion that only abnormal sup-

pressor T cells can consequently be grown *in vitro* is an interesting one and has to be further investigated.

5) Whatever the molecular origin of T-cell-derived suppressor factors (*i.e.*, TCR-derived or originating from other molecules) depending on the suppressor mechanisms involved, it is notable that several different authors share the view that suppressor factors represent cleavage products of molecules usually present on the cell membrane. The elegant experiments reported by Green and Zheng suggest that TCR genes encode some part of the antigen-suppressive factors which would be obtained by modification of TCR chains, either at the RNA or protein levels, to lead to secreted forms. Similarly, the detailed biochemical analyses of the

FcR-related soluble isotypic-specific suppressor immunoglobulin-binding factors (IgG-BF and IgE-BF) suggest that these molecules are encoded by FcR genes whose products are then modified either by differential RNA processing or by proteolytic cleavages, leading to secreted forms. Such observations, also reported for IL2 receptors that can be found in circulating forms, could have more general implications, as the existence of soluble forms of membrane receptors that could play a very important role in the balance of the whole immune system becomes more and more documented. Clearly, current studies on T-cell-mediated suppression must focus on this rather new concept of truncated forms of membrane molecules acting as potent immunoregulatory molecules.

KEY-WORDS: T lymphocyte, Immunosuppression, Immunoregulation; Forum.

ERRATUM

In issue 139-n° 6 (December 1988), the article of Qadri *et al.*, p. 701, should be entitled "Effect of *in vivo* treatment with antiidiotype on the immune response to human chorionic gonadotropin"; the first author should read A. Qadri; p. 702, line 27, should read "P₃W₈₀"; p. 706, figure 3, should read "group of 10 mice"; p. 707, figure 4, should read (●) for (△) and vice versa.