THE UNIVERSITY OF MANITOBA

A STUDY OF THE CELLULAR INTERACTIONS AND GENETIC RESTRICTIONS IN THE INDUCTION OF ANTISUPPRESSION

BY

© SHASHI UNIYAL

A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

Department of Immunology
Winnipeg, Manitoba

December, 1988

ISBN 0-315-47994-9

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ACKNOWLEDGEMENTS

I am sincerely grateful to my advisor Dr. Frixos Paraskevas for his guidance, support and encouragement throughout the course of my training. I am indebted to him for affording me the opportunity to accompany him during his sabbatical year at Harvard Medical School. He has constantly challenged my scientific curiosity with provocative and critical discussions. In addition, I am appreciative of the help and friendship of two important members of this laboratory, Dr. Tom Lee and Ms. Jeanette Maeba.

Part of this thesis research was carried out in Dr. Man-Sun Sy's laboratory at Harvard Medical School and I am grateful to him for providing some of the reagents.

I would like to thank the Members of my Advisory Committee, Dr. A. Froese, Dr. E. Sabbadini and Dr. J. Wilkins for their helpful comments.

Last but not least, I would like to thank Ms. G. Fulton for diligently antisuppressing her way through the typing of this thesis.

The financial assistance of a University of Manitoba Graduate Fellowship and a Manitoba Health Research Council Studentship is gratefully acknowledged.

ABSTRACT

Antisuppression is an immunoregulatory T cell pathway activated within 6 hours after antigenic stimulation which enhances immune responses through the inhibition of suppressor cell function. The antisuppressor mediator, present in the serum of mice at 6 hours (6HS), contains a unique form of processed antigen which represents complexes of immunoglobulin (Ig) and antigen. The formation of these complexes is mediated by an inducer $Ly1^+$ $L3T4^+$ T cell while their function depends on binding to and activating an $Ly2^+$ effector T cell. Results from this study have shown that the initial interaction between macrophages and T cells for the *in vitro* production of this mediator requires antigen processing and presentation in association with major histocompatibility complex (MHC) class II molecules to an $Ly1^+$, $L3T4^+$ inducer T cell. Inhibition of antigen uptake and its subsequent degradation abolished the production of this mediator. Its generation was also blocked in the absence of cell contact between the macrophage and T cell by both anti-L3T4 and anti-class II antibodies.

Lethally irradiated mice required reconstitution with syngeneic T cells in order to generate the antisuppressor mediator. When Igh congenic T cells were used for reconstitution the antisuppressor mediator was lacking. However, the addition of IgG from the same Igh haplotype as the T cell donor generated antisuppressor complexes that enhanced IgG antibody response in suppressed cultures. The Igh restriction between the inducer T cell and IgG was observed in several

Igh congenic strains. The inducer T cell was unable to express an altered Igh restriction when exposed to an Igh distinct environment for several days. By using two Igh recombinant strains it was found that the formation of this mediator was regulated by genes of the IghV locus. In addition, it was observed that genes of the IghC locus were not involved in the formation and functional expression of antisuppression. In the absence of the Fc portion of IgG, the divalent $F(ab')_2$ fragment participated in the formation of functional complexes. The effector stage of antisuppression was found to be MHC and Igh nonrestricted.

ABBREVIATIONS

ABA	azobenzenearsonate
APC	antigen-presenting cell
BSA	bovine serum albumin
CFA	complete Freund's adjuvant
CTL	cytotoxic T lymphocyte
GAT	L-glutamic acid-L-alanine-L-tyrosine
GLϕ	L-glutamic acid-L-lysine-L-phenylalanine
GPC	guinea pig complement
HBSS	Hank's balanced salt solution
IACF	immunoglobulin and antigen complexing factor
Ig	immunoglobulin
Igh	immunoglobulin heavy chain
IghV	immunoglobulin heavy chain variable region
IghC	immunoglobulin heavy chain constant region
IL	interleukin
i.p.	intraperitoneal
i.v.	intravenous
mAb	monoclonal antibody
MHC	major histocompatibility complex
NMS	normal mouse serum
NP	4-hydroxy-3-nitrophenyl acetyl
NWC	nylon wool column
PBS	phosphate-buffered saline

PEC	peritoneal exudate cell
PFC	plaque forming cell
RαmIg	rabbit anti-mouse immunoglobulin
RICA	reverse immune cytoadherence
SRBC	sheep red blood cell
SUP	supernatant
TCR	T cell receptor
Tcs	T contrasuppressor cell
TcsF	contrasuppressor factor
Th	T helper cell
Ts	T suppressor cell
TsF	suppressor factor
Vv	*Vicia villosa*
6HS	6 hour serum

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INTRODUCTION

Antigen-specific suppressor T cells (Ts) and their soluble factors (TsF) are important regulatory elements of humoral and cell-mediated immune responses (reviewed by Dorf and Benacerraf, 1984). One model of an immunoregulatory T-cell circuit involved in enhancement of immune responses through the inhibition of suppression has been described by Gershon and colleagues (reviewed by Green *et al.*, 1983). Another model, which bears functional similarity with contrasuppression and has been the focus of studies in this laboratory, is a pathway called antisuppression (reviewed by Paraskevas *et al.*, 1984).

The antisuppressor pathway is distinct in that it is activated within 3 to 6 h after antigenic stimulation and its mediator is a unique form of 'complexes' containing immunoglobulin (Ig), antigen and Ia antigen (Paraskevas and Lee, 1979; Paraskevas *et al.*, 1979). The formation of this complex involves a factor released from the inducer $Ly1^+$ T cells while its function depends on its binding to and activating an effector $Ly2^+$, Ia^+, FcR^+ T cell (Lee and Paraskevas, 1981; Paraskevas *et al.*, 1979; 1984; 1985; Lee *et al.*, 1985). The presence of the complexes has been detected by a highly sensitive and reproducible method called reverse immune cytoadherence (RICA) assay (Paraskevas *et al.*, 1971) while the ability of the complexes to interfere with Ts cell function has been demonstrated in both humoral and cell-mediated immune responses (Lee and Paraskevas, 1981;

Al-Maghazachi *et al.*, 1983; Lee *et al.*, 1985; Paraskevas *et al.*, 1985; 1985a).

Previous studies have demonstrated that 6h complexes are involved in the *in vitro* enhancement of anti-SRBC antibody response in suppressed cultures (Lee *et al.*, 1985). More recently, an *in vitro* method for the production of SRBC-specific antisuppressor mediator has been established in this laboratory (Maeba *et al.*, 1988). Therefore, the present investigation has focused on the role of antisuppression in the regulation of the humoral anti-SRBC antibody response. The main objective of this study was two-fold: (1) to examine the cellular interactions in the stepwise assembly of the antisuppressor mediator, and (2) to determine the nature of the genetic restrictions imposed during the induction as well as the effector stage of antisuppression.

Production of the antisuppressor mediator *in vitro* comprises two stages, first the culture of syngeneic T cells with antigen-pulsed macrophages, and second, the addition of syngeneic IgG to the cell-free culture supernatant in order to detect antisuppressor activity (Maeba *et al.*, 1988). Therefore, it was apparent that an 'intermediate complex' was produced as a result of the interaction between T cells and antigen-pulsed macrophages and syngeneic IgG was required for the generation of antisuppressor activity. Experimental evidence from this laboratory suggests that the early induction of the antisuppressor pathway ensures the development of an immune response by preventing the

early induction and/or function of Ts cells (Al-Maghazachi *et al.*, 1983; Paraskevas *et al.*, 1984; 1985; Lee *et al.*, 1985). Therefore, it was of obvious importance to examine whether there are any striking differences in the macrophage-T cell interactions which result in the production of the antisuppressor mediator as compared to those which lead to T cell activation for other effector functions.

It is now widely accepted that T cells recognize foreign antigen in association with self-MHC molecules on the surface of an antigen-presenting cell (APC). Previous studies have indicated that Ia antigen is one of the components of 6h complexes (Paraskevas and Lee, 1981). In the present study it was of relevance to examine not only the role of Ia molecules in the generation of the 'intermediate complex' but also the requirement for antigen processing. Recent experimental evidence suggests that the T cell surface molecules CD4 and CD8 may be receptors for nonpolymorphic determinants of MHC class II and class I molecules, respectively (Bierer and Burakoff, 1988). Moreover, it appears that CD4 and CD8 molecules participate in cell adhesion between lymphocytes and APC and may have a role in the generation of effector T cells from precursors. Therefore, the involvement of CD4 and CD8 molecules in the macrophage-T cell interaction in the production of the 'intermediate complex' was also examined.

Genes located within the MHC as well as genes in or linked with

the Ig heavy chain (Igh) locus regulate both humoral and cell-mediated immune responses. It has been shown that activation *in vivo* of the anti-suppressor pathway is regulated by genes in the I-A subregion of the MHC (Paraskevas *et al.*, 1985). While the phenomenon of Igh restriction, i.e., a requirement for functional identity between responding lymphocyte populations at the Igh locus is not clearly understood, nevertheless, there is substantial evidence demonstrating Igh restricted cell interactions in both the suppressor (Sy and Benacerraf, 1988) as well as the contrasuppressor circuit (Green and Gershon, 1984). In elucidating the cellular interactions in the stepwise assembly of the antisuppressor mediator it was critical to establish whether genes of the Igh locus are involved in the induction of antisuppression.

In earlier studies (Lee and Paraskevas, 1972; Paraskevas *et al.*, 1985a) it has been established that lethally irradiated mice are unable to generate the 6h complexes unless reconstituted with syngeneic T cells. In the present study, lethally irradiated mice were reconstituted with Igh congenic T cells, i.e., T cells from donor mice that differ from the recipients in the Igh haplotype. In addition, the availability of two Igh recombinant strains, i.e., BAB-14 ($IghV^{a}$-C^{b}) and CBAL ($IghV^{d}$-C^{b}) provided the opportunity to examine whether the induction of antisuppression was restricted by IghV- or IghC-linked genes. Furthermore, the *in vitro* assay for the production of the antisuppressor mediator was utilized in order to examine the structural

constraints, if any, that were imposed upon the IgG molecule during the formation of this mediator. The divalent $F(ab')_2$ fragment prepared by enzymatic cleavage was evaluated for its ability to generate the antisuppressor activity.

It has been demonstrated that the Ts cell repertoire is dependent not only upon the genetic background of the specific T cells, but is also influenced by Igh-linked determinants present when the T cell clones are selected during an immune response (HayGlass *et al.*, 1985). Therefore, it was of interest to examine whether the Igh distinct B cells of the host could influence the pattern of Igh restriction during the *in vivo* generation of the antisuppressor mediator. Two approaches were used, first Igh congenic T cells were 'parked' in an Igh distinct environment for several days before the production of 6h complexes, and second Igh congenic T cells were transferred into athymic mice.

Previous studies (Paraskevas and Lee, 1981) have shown that in the absence of T cells, e.g., in athymic mice, the effector function of the 6h complexes is MHC restricted while in the presence of T cells the complexes enhance anti-SRBC antibody response across MHC barriers. By using Igh congenic strains it was possible to determine what role, if any, the genes linked to the Igh locus have in mediating the effector function of antisuppression.

LITERATURE REVIEW

I. INITIATION OF THE IMMUNE RESPONSE

Processing of antigen

The central role played by the APC in the generation of the T-cell immune response has been well documented (reviewed by Unanue, 1981; 1984). In general, protein antigens need to be physically altered or 'processed' by the APC prior to being recognized by the T cell (Ellner and Rosenthal, 1975; Ellner *et al.*, 1977). Ziegler and Unanue (1981; 1982) were the first to directly demonstrate the requirement for antigen processing. First, fixation of APC's with paraformaldehyde was shown to block antigen internalization, catabolism and presentation to T cells. Second, treatment of APC's with chloroquine or ammonium chloride which inhibits lysosomal proteinases (Poole *et al.*, 1980) blocked degradation of antigen and presentation to T cells. However, it was observed that APC's fixed after exposure to antigen could still serve as presenting cells. Therefore, from these initial studies (Ziegler and Unanue 1981; 1982) it was inferred that antigen had to be internalized into an acid vesicular compartment and an immunogenic determinant, not denatured by fixation, displayed on the cell surface. The lysosomes are the major site of antigen catabolism (Lloyd, 1980) although there is evidence that proteinases in the plasma membrane of macrophages (Lavie *et al.*, 1978) can also produce immunologically relevant peptides (Buus and Werdelin, 1986). Experiments using prefixed APC's by Shimonkevitz *et al.* (1983) and others (Shimonkevitz

et al., 1984; Allen *et al.*, 1984; Kovac and Schwartz, 1985) have shown that peptide fragments of native antigen can be presented to antigen-specific T cell clones. Furthermore, Watts *et al.* (1984) demonstrated that planar membranes containing immunoaffinity-purified Ia antigen and defined peptide antigen was indeed sufficient for T cell recognition. These observations suggested that degradation of an antigen *in vitro* could substitute for whatever processing event(s) occur within an APC.

While processing of antigen has been widely accepted as an early event in the initiation of the T-cell immune response there is however, a minority view (Klein and Walden, 1986) whose proponents claim that degradation of antigen into peptides inside or outside the cell, is not necessary for T cell recognition of most antigenic determinants. Klein and coworkers (1986) believe that antigen binds to certain molecules on the APC and via membrane currents is brought into close physical proximity of the MHC molecule for recognition by the T cell. In their view, antigen catabolism and the physical association of peptide with MHC molecules are not considered critical for an immune response (Walden *et al.*, 1985, 1986).

Presentation of antigen in association with Ia molecules

It has long been accepted that T helper (Th) cells recognize foreign antigen in association with Ia molecules on the surface of an APC (Kindred and Shreffler, 1972; Rosenthal and Shevach, 1973; Katz and Benacerraf, 1975). Until recently, there was no unequivocal evidence

for the existence of Ia-antigen complexes. In some earlier studies (Erb and Feldmann, 1975; Puri and Lonai, 1980; Friedman *et al.*, 1983) it was observed that the supernatant from macrophages cultured in the presence of antigen apparently contained a soluble complex of Ia and antigen. Direct biochemical evidence for the existence of such complexes was first provided by Babbitt *et al.* (1985). Using equilibrium dialysis they and others (Buus *et al.*, 1986) have demonstrated that affinity purified Ia molecules were indeed capable of binding immunogenic peptide antigen. Moreover, the binding of peptide to purified Ia was shown to be immunologically relevant by two different approaches (Buus *et al.*, 1987). Competitive inhibition studies were performed using relevant peptides to inhibit the presentation of antigen by prefixed APCs. In addition, it was shown that Ia-peptide complexes incorporated into planar membranes could also stimulate antigen-specific MHC restricted T cell hybridomas (Buus *et al.*, 1987). The sequence of events which result in an antigen-Ia complex and where this occurs in the macrophage are not clear. One model that has been proposed (Unanue and Allen, 1987) suggests that the antigen enters a prelysosomal compartment where it is subjected to partial fragmentation, encounters nascent Ia molecules and upon binding is transported to the plasma membrane.

The basic function of the Ia molecule

Clearly, the Ia molecules are critical in the initiation of the T-cell immune response. Rosenthal (1978) and Benacerraf (1978)

postulated that control of the T-cell responses is imposed at the level of the APC. In their view, which is referred to as 'the determinant-selection hypothesis', the Ia molecules select determinants on a foreign antigen that are to be presented to T cells. This hypothesis has been challenged experimentally (Ishii *et al.*, 1981; 1982; Clark and Shevach, 1982) yet recent studies have provided evidence that Ia antigens indeed have a higher intrinsic affinity for some peptides compared to others. However, experimental evidence indicates that binding of peptide to Ia is not sufficient to elicit an immune response, e.g., λ repressor peptide (P12-26) binds strongly to I-E^d molecule while it is restricted by I-A^d (Guillet *et al.*, 1987). In view of the recent understanding of peptide-Ia interactions (Guillet *et al.*, 1987; Buus *et al.*, 1987a), it has been suggested that Ia molecules function as a carrier system that protects peptides from catabolism and transports them to the cell surface (Unanue and Allen, 1987; Allen *et al.*, 1987). Experimental evidence exists that autologous peptides compete with immunogenic peptides for binding to the same MHC molecule (Lorenz and Allen, 1988; Adorini *et al.*, 1988) thereby indicating that Ia molecules lack the fine specificity to discriminate between self and non-self. Gefter and colleagues (Guillet *et al.*, 1986) have postulated that the Ia antigen is a receptor containing a single binding site for peptides and its function is to 'uniquely' position the antigen for T cell recognition. In their model it is the binding of self or foreign antigen in the polymorphic region of the Ia molecule which imparts unique qualities to both the peptide

binding site and the self recognition site(s).

Macrophage regulation of the immune response

It is hardly surprising that the synthesis and expression of Ia molecules is highly regulated. The capacity of macrophages to synthesize and express Ia antigens on their surface is not constitutive (Unanue, 1984). The percentage of macrophages bearing detectable Ia determinants has been shown to vary depending on the tissue from which they are isolated (Cowing *et al.*, 1978), e.g. ~50% Ia^+ macrophages are present in spleen and only 10-20% in the peritoneal cavity. The expression of membrane Ia is transient, on the order of 12-48 h, and cells in culture eventually become Ia^- (Beller and Unanue, 1981). Only those cells that display Ia can function as accessory cells for T cell activation (Yamashita and Shevach, 1977). Substantial evidence has accumulated demonstrating the role of the Th cell in regulating Ia expression on the macrophage surface (Beller *et al.*, 1980; Scher *et al.*, 1980; Steeg *et al.*, 1980). It appears that after recognition of the Ia-antigen complex, the T cell secretes interferon-γ (IFN-γ), which interacts with a specific receptor on the macrophage to upregulate the expression of Ia molecules (Steeg *et al.*, 1982; Basham and Merigan, 1983; Celada *et al.*, 1984) thereby enhancing the macrophage's capacity to present antigen (Walker *et al.*, 1982; Beller, 1984).

Macrophages release interleukin (IL)-1, a polypeptide that has been shown to be critical for T cell activation (Larsson *et al.*, 1980;

Gillis and Mizel, 1981). It has been shown that IL-1 acts on T cells in two ways: it induces receptors for IL-2 thus allowing T cells to proliferate (Smith *et al.*, 1980; Kaye *et al.*, 1984), and it also stimulates IL-2 production (Williams *et al.*, 1985; Lowenthal *et al.*, 1986). Unanue and coworkers (Kurt-Jones *et al.*, 1985; Weaver and Unanue, 1986) have suggested a role for a membrane-bound form of IL-1 in antigen presentation by fixed macrophages. However, it is not clear whether all T cells regardless of their state of activation require IL-1. Antigen-specific T cell hybridomas have been shown to respond to peptide-Ia complexes incorporated in planar membranes in the absence of exogenous IL-1 (Watts *et al.*, 1984).

T cell antigen receptor

Our current understanding of antigen recognition by T cells has evolved from a substantial body of work from many laboratories (reviewed by Davis and Bjorkman, 1988). It is now apparent that the T cell antigen receptor complex (TCR-CD3) comprises the clonally variable receptors made up of two different disulfide-linked heterodimers, α:β and γ:δ (Allison *et al.*, 1982; Meuer *et al.*, 1983; Haskins *et al.*, 1983; Saito *et al.*, 1984; Brenner *et al.*, 1986) in non-covalent association with an array of invariant proteins collectively referred to as the CD3 complex (Oettgen and Terhorst, 1987). The more common α:β receptor, expressed on the majority of peripheral T cells in mice, is involved in antigen recognition (Yagüe *et al.*, 1985; Dembic *et al.*, 1986; Saito *et al.*, 1987) while the function of the γ:δ receptor is

unknown. These TCR polypeptides are very similar to immunoglobulins in primary sequence, gene organization, and modes of rearrangement (Hedrick *et al.*, 1984; Yanagi *et al.*, 1984; Saito *et al.*, 1984). The TCR exists only on the cell surface and in contradistinction to immunoglobulin genes shows no evidence of somatic hypermutation (Patten *et al.*, 1984; Behlke *et al.*, 1985). Hence, the repertoire of functional T cells cannot be modified by changes in receptor sequence after exposure to antigen. The TCR associated CD3 complex comprises at least five integral membrane proteins (Minami *et al.*, 1987) which may function in transducing an activation signal upon ligand binding (Oettgen and Terhorst, 1987).

T cell accessory molecules

Although antigen-specific activation of the T cell is mediated by the TCR-CD3 complex, a number of other surface molecules participate in the immune response and appear to be important in T cell adhesion to APC and target cells (Bierer and Burakoff, 1988). Identification of subset-specific cell surface antigens (Cantor and Boyse, 1975; Reinherz and Schlossman, 1980; Dialynas *et al.*, 1983) allowed division of T cells into two classes: the helper/inducer T cell (Th/i) bearing Ly 1 and L3T4 antigens (also known as CD5 and CD4, respectively), and the cytotoxic/suppressor T cells (Tc/s) bearing Ly 2 antigen (known as CD8). The CD4 and CD8 molecules are expressed on mutually exclusive populations of T cells, i.e., about 65% of peripheral T cells are $CD4^+$ and 25-35% are $CD8^+$. It appears that there is a stringent correlation

between the CD4/CD8 phenotype and the class of MHC determinants that these T cells recognize (Meuer *et al.*, 1982; Wilde *et al.*, 1983). Independent of their effector function $CD4^+$ and $CD8^+$ T cells recognize nonpolymorphic determinants of MHC class II and class I molecules, respectively (Swain, 1981). Therefore, it has been suggested that the interaction of CD4 or CD8 antigen with MHC molecules may contribute to the avidity of the T cell for its APC or target cell (Swain, 1981; Marrack *et al.*, 1983). Evidence supporting this hypothesis has been obtained from studies using monoclonal antibodies directed against CD4/CD8 or class II/I determinants (Meuer *et al.*, 1982; Swain, 1983), as well as gene transfer experiments (Dembic *et al.*, 1987; Sleckman *et al.*, 1987; Gay *et al.*, 1987; Gabert *et al.*, 1987). The earlier observations coupled with the recent demonstration of direct binding of CD4 to MHC class II molecules (Doyle and Strominger, 1987; Gay *et al.*, 1988) further support the idea that these accessory molecules strengthen the interaction of the TCR with its ligand. Recent studies have also suggested that CD4 and CD8 molecules may enhance antigen responsiveness by transducing a signal directly or in concert with the TCR-CD3 complex (Janeway *et al.*, 1988; Ledbetter *et al.*, 1988).

Functional heterogeneity of $CD4^+$ T cells

Antigenic stimulation of T cells results in clonal expansion, the generation of T-memory cells and maturation into cells with regulatory or effector function. One aspect of T cell regulation of immune responses is through the release of antigen non-specific lymphokines

that alter the behavior of various target cells. Recent studies with cloned T cell lines have revealed that murine $CD4^+$ T cells could be divided into two subsets, T_H1 and T_H2, a classification that is based on lymphokine secretion and functional analysis (Mosmann *et al.*, 1986; Mosmann and Coffman, 1987; Bottomly, 1988). Both subsets synthesize IL-3 and granulocyte-monocyte colony-stimulating factor (GM-CSF), but only T_H1 clones produce IL-2, IFN-γ and lymphotoxin (LT) while T_H2 clones produce IL-4 and IL-5 (Mosmann and Coffman, 1987; Cherwinski *et al.*, 1987). Furthermore, *in vitro* and *in vivo* studies have demonstrated that IL-4 induces secretion of IgG1 and IgE (Vitetta *et al.*, 1985; Coffman *et al.*, 1986), IL-5 enhances the secretion of IgA (Coffman *et al.*, 1987; Bond *et al.*, 1987) and IFN-γ enhances secretion of IgG2a (Snapper and Paul, 1987). Functional analysis of these subsets indicate that T_H2 is a potent activator of B cells while T_H1 can kill appropriate targets, suppress antibody responses and mediate macrophage activation (Bottomly, 1988).

Phenotypic heterogeneity within the $CD4^+$ T cells has been observed in the human (Morimoto *et al.*, 1985; Smith *et al.*, 1986), rat (Spickett *et al.*, 1983; Arthur and Mason, 1986) and mouse (Bottomly, 1988). In all three species, two functional subsets have been distinguished on the basis of expression of CD45 molecules (T200, leukocyte-common antigen). Cells which express low density of CD45 produce IL-4 and provide effective help to B cells while the CD45 high density subset produce IL-2 and provide poor help to B cells in a polyclonal antibody

assay (Janeway *et al.*, 1988). Experimental evidence strongly supports the existence of functionally distinct CD4 T cells in normal T cell populations as well as in cloned T cell lines. However, it is not clear whether these two subsets represent different developmental stages of the same cell (Akbar *et al.*, 1988) or differ in their activation requirements (Tite *et al.*, 1987).

II. REGULATION OF THE IMMUNE RESPONSE BY SUPPRESSOR T CELLS

General introduction

An important subset of T cells involved in the regulation of the immune response is the suppressor T cell (Ts) (reviewed by Dorf and Benacerraf, 1984; Paraskevas, 1984; Asherson *et al.*, 1986). The existence of antigen-specific Ts cells originally demonstrated by Gershon and Kondo (1970) has recently been viewed with scepticism (Möller, 1988). Nevertheless, the regulation of both humoral (Rohrer *et al.*, 1979; Braley-Mullen, 1980; Abbas *et al.*, 1982; Suemura *et al.*, 1983) and cell-mediated immunity (Bach *et al.*, 1978; Sherr *et al.*, 1979; Weinberger *et al.*, 1979; Tada and Okumura, 1979) by Ts cells has been extensively studied using a number of model antigen systems in an effort to define the molecular basis of antigen-specific suppression.

Induction of Ts cells

The activation of Ts cells *in vivo* has been achieved by a variety of procedures including: (1) hyperimmunization (Gershon and Kondo, 1971), (2) deaggregated antigen (Zan-Bar *et al.*, 1975), (3) anti-idiotypic antibody (Eichmann, 1975), (4) hapten-conjugated syngeneic cells (Bach *et al.*, 1978) and (5) immunization of some non-responder strains (Kapp *et al.*, 1974; 1976). In contrast, it has been reported that the *in vitro* activation of Ts cells can be achieved with either high (Kontiainen and Feldmann, 1976) or low doses of antigen (Ishizaka and Adachi, 1976; Eardley and Gershon, 1976). Some

investigators have considered macrophages unnecessary for the induction of Ts cells (Ishizaka and Adachi, 1976; Feldmann and Kontiainen, 1976) while others have suggested a requirement for Ia^+ APC (Sherr *et al.*, 1980) or I-J^+ APC (Lowy *et al.*, 1983; Nakamura *et al.*, 1986). In general, suppressor cells are often I-J^+ and, when they are genetically restricted, the restriction maps to I-J. However, examples of I-E restricted suppression have been documented (Baxevanis *et al.*, 1982; Waltenbaugh *et al.*, 1986). Strains of mice which did not express the E molecule were shown to respond to the enzyme lactate dehydrogenase B (LDH_B) and mice that were nonresponders to LDH_B became responsive after treatment with monoclonal anti-I-E antibody (Baxevanis *et al.*, 1982). This would suggest that some antigens when presented in association with I-E molecules may induce Ts cells. Treatment of mice with monoclonal anti-I-A antibody has also been shown to induce Ts cells (Rosenbaum *et al.*, 1981; Perry and Greene, 1982). From these data it can be inferred that induction of Ts cells appears to be quite different from that of Th cells or cytotoxic T cells (Tc).

Phenotype of Ts cells

For several model antigens it has been demonstrated that the Ts cells which are first activated as a result of initial contact with antigen or tolerogen are not the cells which actually mediate suppression of the response. Rather a cascade of Ts cells is established (reviewed by Germain and Benacerraf, 1981), i.e., Ts1, Ts2 and Ts3 cells which act sequentially and are interconnected by their

respective suppressor factors. It is agreed that the effector Ts cell belongs to the $CD8^+$ subset while the inducer cell belongs to the $CD4^+$ subset. In two well studied haptenic systems, 4-hydroxy-3-nitrophenyl acetyl (NP) and azobenzenearsonate (ABA), the Ts cells have been characterized: the inducer suppressor cell, Ts1 is id^+, $I\text{-}J^+$, $Ly1^+$; a transducer cell, Ts2 is id-specific, $I\text{-}J^+$, $Ly1,2^+$ and the effector suppressor cell, Ts3 is id^+, $I\text{-}J^+$, $Ly2^+$ (Dorf and Benacerraf, 1984).

Attempts to elucidate the nature of the antigen receptor on cells of the Ts lineage have been unsuccessful. Earlier studies on murine Ts hybridomas indicated that the TCR β-chain genes were either deleted or in the germline configuration (Hedrick *et al.*, 1985; Kronenberg *et al.*, 1985; Blanckmeister *et al.*, 1985). Repeated observations that Ts cells, like B cells, appear to bind antigen directly (Germain *et al.*, 1979; Yamauchi *et al.*, 1982; Nakanishi *et al.*, 1982; Taniguchi *et al.*, 1984) and bear I-J encoded determinants (Tada and Okumura, 1979) suggested that these cells may use a distinct set of genes for their receptors. However, recent studies in the human (Modlin *et al.*, 1987) and mouse (Weiner *et al.*, 1988) have identified $CD8^+$ Ts cells which express the clonally unique α:β TCR-CD3 complex.

The controversy and ambiguity surrounding the existence of Ts cells (Möller, 1988; Janeway, 1988; Tada, 1988; Pereira *et al.*, 1988) stems from the fact that two subsets of cells with distinct effector function, i.e., cytotoxic and suppressor, reside within the $CD8^+$

population. It is likely that Ts cells belong to a separate differentiation lineage from $CD8^+$ cytolytic precursor cells. Experimental evidence in studies of human lymphocyte populations by Schlossman and associates suggests that the precursors of Ts and Tc cells are phenotypically similar. By using a monoclonal antibody that detects a novel epitope of lymphocyte function-associated antigen (LFA)-1 they have demonstrated that the effector Tc cells may be phenotypically distinguished from the effector Ts cells (Morimoto *et al.*, 1987). Alternatively, it has been postulated that these two cell populations may arise as a consequence of differential activation (Pereira *et al.*, 1985; 1986). Accumulating experimental evidence suggests that this may indeed be the case. Again, *in vitro* studies of the human indicate that only $CD4^+$ T cells expressing high density of CD45 molecules (identified by monoclonal anti-2H4 antibody) were essential in the induction of CD8 suppressor cell function in both a pokeweed mitogen-stimulated IgG synthesis (Morimoto *et al.*, 1985a; 1988) and an antigen-specific antibody synthesis system (Morimoto *et al.*, 1986).

Structure of antigen-specific suppressor factors

In nearly all the model systems examined to date it has been demonstrated that the cells in the suppressor circuit communicate with each other and their targets via soluble factors (TsF's). Considerable information exists regarding the serological properties and physicochemical characteristics of TsFs isolated from hybridomas or

cell lines (reviewed by Webb *et al.*, 1983). In general, antigen-specific TsF comprise two-chains, an antigen-binding chain and an I-J bearing nonantigen-binding chain. Studies using the synthetic polypeptide L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) have described both a single chain TsF1 and a two-chain TsF2 (Sorensen and Pierce, 1982). Evidence in the SRBC system suggests that TsF1 activity can result from complementation between separate antigen-binding chain and the I-J chain made by cells of different phenotypes (Yamauchi *et al.*, 1982). Thus, the two chains may not be disulfide-bonded and not necessarily the product of a single cell, however, they need to be present simultaneously for suppression to occur.

Antigen-specific Ts cell hybridomas have been used in order to isolate the messenger RNA (mRNA) coding for GAT-TsF1 (Wieder *et al.*, 1982), GAT-TsF2 (Funckes-Shippy *et al.*, 1987) and keyhole limpet hemocyanin (KLH) specific TsF2 (Taniguchi *et al.*, 1982). The *in vitro* translation products have been shown to be biologically active, yet little is known regarding the structure and arrangement of genes specifying these factors. The GAT-specific Ts2 hybridoma failed to constitutively secrete a biologically active suppressor factor unless it was induced with the suppressor inducer factor (GAT-TsF1) and GAT (Funckes-Shippy *et al.*, 1987). Furthermore, mRNA coding for the I-J^{+} chain was detected 8 h after induction, whereas the mRNA coding for the antigen-binding chain was detected 16 h after induction. Therefore, it appears that GAT-TsF2 is the product of two genes and the I-J^{+} chain

may exert a regulatory effect on the expression of the antigen-binding chain.

Genetic restrictions in the suppressor circuit

It has been observed that suppressor factors are often I-J$^+$ and, when they are genetically restricted, the restriction maps to I-J (Murphy *et al.*, 1976; Tada *et al.*, 1976). This suggests that the I-J restricted Ts cell is signalled by I-J$^+$ factors using an I-J/receptor for I-J interaction. Several serological and biochemical studies have confirmed the existence of I-J determinants on Ts cells and their factors (Habu *et al.*, 1981; Yamauchi *et al.*, 1982) although molecular biologists have shown that the I-J locus does not map within the MHC region (Steinmetz *et al.*, 1982). It has been suggested that I-J is a marker on a molecule that is a receptor for I-E (Waltenbaugh *et al.*, 1986; Flood *et al.*, 1986). Recent studies by Tada and associates indicate that I-J epitopes are adaptively acquired by T cells differentiated in radiation bone marrow chimeras according to the environmental MHC phenotype (Uracz *et al.*, 1985). Although the molecular nature of I-J is still unknown, it has now been postulated that I-J may be a new isomorphic receptor inducible on functional T cells distinct from the α:β TCR-CD3 complex (Nakayama *et al.*, 1988).

An additional genetic restriction that has been documented in the suppressor circuit is attributed to Igh-linked gene products. In the simple hapten systems such as ABA and NP, the inducer Ts1 cells and

their factors, TsF1 and the effector Ts3 cells were shown to express idiotypic determinants that were crossreactive with those found on antibodies of the same nominal specificity (Bach *et al.*, 1979; Weinberger *et al.*, 1979). Furthermore, the action of TsF1 was shown to be functionally restricted by genes linked to the Igh locus, i.e., TsF1 produced in Balb/c (Igh^a) mice could suppress delayed-type hypersensitivity (DTH) reaction and *in vitro* generation of cytotoxic T cell (CTL) response in Balb/c mice but not Cal.20 (Igh^d) mice (Sy *et al.*, 1984). It is well documented that the Ig idiotopes on Ts cells are not the result of the expression of Igh variable region (V_H) genes in these cells (Nakanishi *et al.*, 1982; Kronenberg *et al.*, 1983). In fact, experimental evidence supports the postulate that Ig idiotypes of B cells influence some T cell subsets during T-cell differentiation and/or in the course of T-cell-specific responses. It was shown that ABA-specific TsF1 prepared from Balb/c mice that had been treated with anti-IgM (μ) antibody from birth, to eliminate their B cells, exhibited an altered Igh restriction, i.e., it could now suppress DTH responses in Cal.20 mice (Sy *et al.*, 1984). Moreover, the ABA-TsF1 prepared from anti-μ treated animals did not express the major crossreactive idiotypic determinants normally present in TsF1 from untreated animals (Sy *et al.*, 1985). Subsequent studies (HayGlass *et al.*, 1985) showed that 'parking' of T cells in an Igh congenic recipient for 12-15 days resulted in ABA-TsF1 having dual functional specificity, i.e., it was restricted by the Igh haplotype of both the donor and recipient mice. From these studies it has been inferred that Ts cells, if induced in an

Igh distinct environment, can undergo a form of adaptive differentiation (Sy and Benacerraf, 1988).

The phenomenon of Igh restriction has also been documented in studies of KLH-specific suppressor factor (Tokuhisa and Taniguchi, 1982) and SRBC-specific suppressor inducer factor, TsiF (Yamauchi *et al.*, 1982). In fact, the SRBC-TsiF is composed of two chains: an antigen-binding chain which confers the specificity and an I-J$^+$ chain which determines the intact factor's Igh-V linked restriction. It was shown that the antigen-specific TsiF secreted by the Lyl$^+$ inducer cell can activate an Ly2$^+$ transducer (or acceptor) cell, to express suppressive activity, only if the two cells are from mice with the same Igh V haplotype. Therefore, it has been inferred that a binding site or recognition structure for the I-J$^+$ chain must exist on the transducer cell. Earlier studies (Owen *et al.*, 1979; 1981; Owen, 1980) have provided serological evidence that Igh-linked allotypic determinants are expressed on different functional T cell subsets. Alloantibodies were raised by immunizing Balb/c (H-2^d, Igh^a) mice with mitogen-induced blastic T cells of Igh congenic Cal.20 (H-2^d, Igh^d) strain that reacted specifically with Ly2$^+$ Ts cells of the Igh^d haplotype (Owen, 1980). From these and other studies (Tokuhisa and Taniguchi, 1982; 1982a; Karasuyama *et al.*, 1984) it has been inferred that Igh-linked allotypic determinants are associated with the functioning recognition structures on some subsets of mature T cells. Immunochemical characterization of these unique structures on Ts cells

has been difficult (Owen, 1983). However, in view of our current understanding of the TCR-CD3 complex it is likely that these Igh-linked structures are distinct from the α:β TCR. While the molecular basis of Igh restriction is still not clear, experimental evidence clearly shows that a mode of selective communication, involving Igh-restricted cell interactions, exists within the suppressor pathway.

III. CONTRASUPPRESSION: AN IMMUNOREGULATORY PATHWAY

General introduction

In 1974 Gershon postulated that active suppression of an immune response was countered by an opposing immunoregulatory activity which he called 'contrasuppression'. The end function of contrasuppressor T cells (Tcs) was to interfere with the inhibitory function of Ts cells without otherwise augmenting immune responses (Green and Gershon, 1984). Contrasuppression, like the suppressor circuit, has been shown to be mediated by a pathway consisting of three T cell types (Gershon *et al.*, 1981; Green *et al.*, 1981). Recent studies from several laboratories have established a role for Tcs cells in the regulation of humoral and cell-mediated immunity. The function(s) of Tcs cells has been described in a number of experimental systems: (1) in enhancement of *in vitro* anti-SRBC antibody responses (Gershon *et al.*, 1981; Yamauchi *et al.*, 1981; Green *et al.*, 1981; Green and St. Martin, 1983); (2) in adoptive transfer of contact sensitivity to haptens (Iverson *et al.*, 1983; Ptak *et al.*, 1984); (3) in studies of the regulation of B cell responses to type III pneumococcoccal polysaccharide (S3) (Braley-Mullen, 1984; 1986); (4) in IgA synthesis by MOPC 315 myeloma cells (Rohrer and Kemp, 1988); (5) in a model of spontaneous autoimmune interstitial nephritis (Kelly and Neilson, 1988); and (6) in the regulation of isotype specific responses (Kiyono *et al.*, 1988). In studies of human lymphocyte response to streptococcal antigen (SA), Lehner (1982), first identified a subset of $CD8^+$ T cells with contrasuppressor activity.

Induction of Tcs cells

The priming requirements of antigen-specific Tcs cells have varied depending upon the model antigen. In studying the antibody response to xenogeneic red cells, the contrasuppressor T-cell circuit has been activated by preculture of T cells with antigen (Gershon *et al.*, 1981), by repeated *in vivo* exposure to antigen (Green and St. Martin, 1983), or by the culture of spleen cells from very young (Green *et al.*, 1981) or very old animals (Green *et al.*, 1983). Activation of the contrasuppressor circuit in contact sensitivity responses to haptens such as trinitrophenyl (TNP) or picryl chloride (PCl) requires conjugation to specific carriers, e.g., Ig (Green and Ptak, 1986), Langerhans' cells (Ptak *et al.*, 1986), splenic dendritic cells or peritoneal exudate cells induced with complete Freund's adjuvant (CFA) (Britz *et al.*, 1982). Low doses of S3 or S3-coupled spleen cells (S3-SC) have been shown to induce antigen-specific Tcs cells (Braley-Mullen, 1986a). Oral immunization of mice with SRBC for up to 4 weeks has also been reported to induce Tcs cells in Peyer's patches (Kiyono *et al.*, 1988). Studies examining the role of contrasuppression in tumor regression have demonstrated that adjuvants for tumor therapy such as poly (A:U) and *Corynebacterium parvum* can directly activate Tcs cells (Flood *et al.*, 1988). In some cases, activation of Tcs cells seems to be dependent upon B cells and/or their products (Braley-Mullen, 1986). In several studies it has been found that Tcs cells cannot be activated in mice depleted of B cells by treatment from birth with anti-μ antibody (Green and St. Martin, 1983; Green and Ptak,

1986) or in immune defective (xid) mice which lack the $Lyb5^+$ subset of B cells (Braley-Mullen, 1986a; 1988). Recently, activation of Tcs cells has also been achieved by neonatal administration of monoclonal anti-TNP antibody carrying a recurrent idiotype (Andrighetto and Zöller, 1987).

Phenotype of Tcs cells

According to Gershon and colleagues (1981) the contrasuppressor pathway consists of two independent stages, an induction stage followed by an effector stage. The contrasuppressor inducer cell (Tcsi) is an immune $I\text{-}J^+/Ly2$ T cell which produces a factor (TcsiF) that bears I-J determinants and binds antigen. Activation of contrasuppression requires an interaction between the Tcsi cell or its factor and an $I\text{-}J^+/Ly1,2$ transducer cell. The final effector cell (Tcs) is an $I\text{-}J^+/Ly1$ T cell which requires the presence of an Ly2 Ts cell in order to produce its effect.

From recent studies it is apparent that considerable heterogeneity exists within the subset of T cells having contrasuppressor activity. There is no single phenotypic marker which allows one to definitively identify Tcs cells with the apparent exception of their ability to adhere to *Vicia villosa* (Vv) lectin (Green and Ptak, 1986; Lehner, 1986; Braley-Mullen, 1986). Studies examining the antibody response to S3 indicated that there are two distinct subsets of Tcs cells, i.e., those induced by S3 are $CD4^-$ while those induced by S3-SC are $CD4^+$

(Braley-Mullen, 1986; 1988). Induction of Tcs cells by oral or systemic immunization has also shown phenotypic heterogeneity. The effector Tcs cells isolated from spleens of orally immunized mice are $CD4^-$ (Kitamura *et al.*, 1987). In contrast, when mice were primed intravenously (i.v.) the phenotype of Tcs cells was dependent upon the dose of antigen, e.g., low dose of antigen (0.01-1% SRBC) induced $CD4^-$ Tcs cells whereas high dose of antigen (10% SRBC) induced $CD4^-$ and $CD4^+$ Tcs cells (Kiyono *et al.*, 1988). In several studies it has been observed that the effector Tcs cells fail to express either CD4 or CD8 molecules, nevertheless, they function in an antigen-specific manner, thereby suggesting that these cells use a functional TCR. By using an anti-CD3 antibody Kiyono *et al.* (1988) have demonstrated that $CD4^-$, $CD8^-$ Tcs cells in fact express the TCR-CD3 complex and abrogate unresponsiveness in orally tolerized mice. Only a minor population of peripheral T cells in mice are double negative and these express the $\gamma:\delta$ form of the TCR (Brenner *et al.*, 1986; Lew *et al.*, 1986). It is not clear whether the effector Tcs cells in fact belong to the $\gamma:\delta$ bearing $CD3^+$ subset which has been suggested to represent a separate T cell lineage (Bluestone *et al.*, 1987). In humans a $CD8^+$, Vv adherent T cell capable of binding antigen has been shown to mediate Tcs cell function (Lehner and Jones, 1984; Brines and Lehner, 1988).

Contrasuppressor factors

Substantial functional evidence for active contrasuppression exists in many model systems, yet biochemical characterization of the

factors that mediate this effect is lacking. Biologically active factors from the various Tcs cell populations have been obtained by culturing the cells for 48 h in the absence of additional antigenic stimulation (Yamauchi *et al.*, 1981; Ptak *et al.*, 1988) and also by rapid freeze-thawing (Braley-Mullen, 1986). In some aspects these factors show similarities with TsF, i.e., they bind antigen and bear I-J determinants. Gershon *et al.*, (1981) had demonstrated that the SRBC-specific TcsiF could be removed by absorption with heterologous erythrocytes such as horse (HRBC) and burro (BRBC) suggesting that it has a broad specificity. In contrast, the SRBC-specific TsF could only be removed by absorption with specific antigen.

Studies of adoptive transfer of contact sensitivity responses to PCl indicated that two distinct cell populations were required to reconstitute the TcsF (Ptak *et al.*, 1988). The antigen-binding factor was isolated from PCl-primed Vv^- spleen cells while the $I\text{-}J^+$, antigen-nonbinding factor was isolated from normal $I\text{-}J^+$, Lyl^+, Vv^+ T cells. The activity of Ts cells in the adoptive transfer of DTH to PCl was overcome by the addition of both components of TcsF. In studies of a TNP-specific tumor rejection model, Flood *et al.* (1988) have generated a hybridoma producing TcsF. The monoclonal TcsF has been characterized to be functionally and serologically identical to spleen-cell derived TcsF, i.e., it binds specifically to TNP, bears I-J determinants and functions by inducing release of an antigen-nonspecific intermediate contrasuppressor factor. The human

$CD8^+$, Vv^+ Tcs cells have also been shown to mediate their function via release of a nonspecific mediator (Brines and Lehner, 1988).

Target cells for Tcs cells

Contrasuppression was first described as a regulatory activity which counteracted suppressive influences on Th cells and thereby promoted antibody responses (Gershon *et al.*, 1981; Yamauchi *et al.*, 1981; Green *et al.*, 1981). The mechanism by which target Th cells were rendered resistant to subsequent Ts cell signals has not been elucidated. However, it was observed that Th cells treated with nonspecific contrasuppressor factor lost their ability to bind suppressor factor (Green and Ptak, 1986). Subsequent studies have demonstrated that several cell types other than Th cells are targets of contrasuppression, e.g., T cells mediating contact sensitivity (Iverson *et al.*, 1983), B cells in the T-independent response to S3 (Braley-Mullen, 1984; 1988) and MOPC-315 myeloma cells (Kemp and Rohrer, 1984; Rohrer and Kemp, 1988). In studies of the antibody response to S3 it has been suggested that Tcs cells may interact directly with Ts cells and neutralize their activity or Tcs cells somehow make S3-specific B cells resistant to Ts cell signals (Braley-Mullen, 1984). In agreement with the murine studies, the activity of human $CD8^+$, Vv^+ Tcs cells has also been reported to be directed not at the $CD8^+$ Vv^- Ts cells but at one of the $CD4^+$ T cell, B cell or monocyte components in the culture system (Brines and Lehner, 1988). In several model systems experimental evidence supports the

hypothesis that contrasuppression is manifested only in the presence of Ts cells and is unable to reverse an ongoing suppressed response (Green and Gershon, 1984; Braley-Mullen, 1986; Green and Ptak, 1986). Given the apparent phenotypic heterogeneity of Tcs cells, it has been demonstrated that at the functional level these cells interact with distinct subsets of Ts cells, e.g., $CD4^+$ Tcs cells induced by S3-SC are unable to reverse suppression mediated by S3-specific Ts cells (Braley-Mullen, 1984).

Genetic restriction in contrasuppression

Genes linked to the Igh locus have been shown to regulate both the induction and the effector stage of contrasuppression. Braley-Mullen (1986) demonstrated by adoptive transfer experiments that Tcs cells could not abrogate the effects of Ts cells from Igh-incompatible mice. In addition, the restriction was found to be Igh-V linked since Tcs cells from BAB-14 (Igh V^a-C^b) mice were unable to interfere with the suppressor activity of CB.20 (Igh V^b-C^b) Ts cells. Furthermore, contrasuppression was observed only when the Tcs cells were Igh compatible with both the Ts cells and the responding B cells.

The SRBC-specific TcsiF comprises two chains, one binds antigen and the other bears I-J determinants and is antigen-nonbinding (Yamauchi *et al.*, 1981). Green *et al.* (1988) showed that the action of TcsiF was not restricted by genes of the MHC locus since TcsiF produced in B10 ($H\text{-}2^b$) mice abolished suppression in several B10 MHC congenic

strains, i.e., H-$2^{a,d,k}$. By using Igh congenic strains for the production of TcsiF, Green *et al.* (1988) demonstrated that the factor and the target cell, an Ly1,2 transducer cell, required identity at the Igh-V locus. The role of each chain in controlling the Igh restriction was assessed by affinity isolating the I-J^+ chain and preparing a hybrid TcsiF, i.e., an antigen-binding chain from Igh^a haplotype and I-J^+ chain from Igh^b haplotype. From these results it was concluded that the I-J^+ antigen-nonbinding chain imparts the Igh restriction of TcsiF.

IV. ANTISUPPRESSION: AN IMMUNOREGULATORY PATHWAY

General introduction

An immunoregulatory T cell pathway termed antisuppression has been extensively studied by Paraskevas and colleagues (Paraskevas *et al.*, 1984; 1984a). Many similarities have been noted between the antisuppressor pathway and Gershon's contrasuppressor circuit, nevertheless, several unique features which distinguish these two pathways have also been documented (Paraskevas *et al.*, 1985; Lee *et al.*, 1985; Maeba *et al.*, 1988). By definition, the antisuppressor mediator, i.e., the '6 h complexes', act by interfering with the inhibitory function of Ts cells without otherwise augmenting the immune response (Al-Maghazachi *et al.*, 1983; Paraskevas *et al.*, 1984; 1985; Lee *et al.*, 1985). Antisuppression has been shown to regulate both humoral and cell-mediated immune responses. Paraskevas and associates have examined the function(s) of the antisuppressor mediator in several model systems: (1) in the *in vivo* enhancement of antibody responses against soluble and particulate antigens at subimmunogenic dose (Lee and Paraskevas, 1981; Paraskevas and Lee, 1981), (2) in the enhancement of *in vitro* anti-SRBC antibody response (Lee *et al.*, 1985), (3) the *in vivo* enhancement of CTL responses (Al-Maghazachi *et al.*, 1983), (4) in a model of tumor progression (Harrop and Paraskevas, unpublished data), (5) in studies of unresponsiveness to the synthetic polypeptide L-glutamic acid^{56}-L-lysine^{35}-L-phenylalanine^{9} (GLϕ) (Anhalt, 1987), (6) in the regulation of isotype-specific antibody responses (Paraskevas *et al.*, 1988a; Ernst *et al.*, 1988), and (7) in studies of the

immunosuppression in graft-versus-host (GVH) reaction (Paraskevas *et al.*, 1988).

Terminology

The terms 6HS, 6 h complexes or antisuppressor mediator all refer to a soluble mediator which consists of 3 components: (1) an immunogenic epitope of foreign antigen in association with MHC class II molecules, (2) a factor released from Lyl^+, $L3T4^+$ T cells, and (3) normal immunoglobulin from B cells. The T cell factor, the nature of which has not been identified at present, has been termed immunoglobulin and antigen complexing factor (IACF). All 3 components need to be present in order to detect a functionally active mediator with antisuppressor activity.

Complete complexes are assembled with the contribution of 3 components derived from appropriate cells as shown below:

Macrophage	Ia-Ag
Lyl^+, $L3T4^+$ T cell	Factor 'X' or IACF
B cell	Immunoglobulin

Induction of antisuppression

One feature that readily distinguishes the anti-suppressor pathway from contrasuppression is its mode of activation. The antisuppressor pathway is activated within the first 3-6 h after primary immunization (Lee and Paraskevas, 1981; Paraskevas and Lee, 1981; Al-Maghazachi *et*

al., 1983; Paraskevas *et al.*, 1985) whereas contrasuppression is detected late and requires repeated antigenic stimulation. Earlier studies had indicated that the antisuppressor mediator present in the serum of mice within 6 h after immunization comprised a unique form of complex between Ig, antigen and a T-cell-derived factor (Orr and Paraskevas, 1973; Paraskevas *et al.*, 1979). These observations have recently been substantiated with an *in vitro* method for the production of the antisuppressor mediator (Maeba *et al.*, 1988). Several immunogenic stimuli have been shown capable of activating this pathway, e.g., soluble protein antigens in CFA, synthetic polypeptides of at least three amino acids in CFA, particulate antigens, allogeneic cells and syngeneic tumor cells (Paraskevas *et al.*, 1984). In addition, it has been noted that self antigens are incapable of activating antisuppression. Chronic administration of anti-μ antibody to mice from birth disrupts the antisuppressor pathway (Gordon *et al.*, 1985). It has been observed that in the absence of B cells, anti-μ treated mice lacked both the antisuppressor inducer as well as the effector T cells.

Phenotype of the cells of the antisuppressor pathway

The antisuppressor pathway consists of two stages: an induction stage and an effector stage. Experimental evidence has indicated that T cells are crucial in the induction of the antisuppressor mediator since nude mice or thymectomized, lethally irradiated and bone marrow reconstituted (TxXBM) mice do not produce 6h complexes (Lee and

Paraskevas, 1972; Paraskevas *et al.*, 1979; 1984). It has been suggested that the *in vivo* formation of the complexes requires the participation of two T cells both of which bear the Ly1 phenotype (Paraskevas *et al.*, 1985a). The two $Ly1^+$ T cells have been distinguished on the basis of their sensitivity to antilymphocyte serum and dependence on the presence of the thymus (Paraskevas *et al.*, 1985a). Furthermore, experimental evidence indicates that the inducer antisuppressor T cell also bears Ia antigens and is FcR^+ (Paraskevas *et al.*, 1985a). Production of the antisuppressor mediator *in vitro* has also demonstrated a requirement for an $Ly1^+$ inducer T cell (Maeba *et al.*, 1988). It should be noted that the antisuppressor inducer T cell is phenotypically distinct from the $I\text{-}J^+$, $Ly2^+$ inducer T cell of Gershon's contrasuppressor circuit (Gershon *et al.*, 1981).

Expression of the antisuppressor effect involves an interaction between two T cells, one which bears the $Ly2^+$ phenotype and is the immediate target of the 6h complexes, while the other bears the $Ly123^+$ phenotype (Lee *et al.*, 1985). Previous studies have reported that the 6h complexes are strongly cytophilic for T cells (Orr and Paraskevas, 1973) and in particular for an $Ly2^+$, FcR^+, Ia^+ subset (Paraskevas and Lee, 1979; Paraskevas *et al.*, 1984). It has been postulated that uptake of the 6h complexes by an $Ly2^+$ T cell and its subsequent interaction with an $Ly123^+$ T cell result in a potent antisuppressor effect (Lee *et al.*, 1985; Paraskevas *et al.*, 1985a). Therefore, antisuppression is mediated by an $Ly2^+$ effector cell which requires an

$Lyl23^+$ transducer cell, whereas contrasuppression is mediated by an $I\text{-}J^+$, Lyl^+ effector cell which also needs a phenotypically similar transducer cell (Gershon *et al.*, 1981).

Nature of the antisuppressor mediator

In several model systems, Paraskevas and associates have provided evidence that the formation of the 6h complexes (also referred to as the antisuppressor mediator) is critical in the events leading to an immune response. Several approaches have been used in an effort to define the individual components of the 6h complexes. In 1973, Orr and Paraskevas, first provided evidence that within 6h after immunization the serum of mice contained a unique complex of Ig and foreign antigen. Using radioactively labelled antigen, bovine serum albumin (BSA), it was found that the serum of mice contained some form of processed antigen in association with Ig (Orr and Paraskevas, 1973). The appearance of the complexes in the serum within 6h suggests that they do not represent conventional antigen-antibody complexes. Moreover, the formation of these complexes depends on a factor which can be induced *in vivo* (Orr and Paraskevas, 1974; Paraskevas *et al.*, 1984), and *in vitro* (Paraskevas *et al.*, 1979) from an Lyl^+ T cell (Paraskevas *et al.*, 1984; 1985a; Maeba *et al.*, 1988). In earlier studies, this factor known as Ig and antigen complexing factor (IACF), has been characterized by serological methods. The production of IACF depends on a macrophage-T cell interaction (Paraskevas *et al.*, 1979) and the IACF molecule(s) comprises Ia antigens and idiotype (Paraskevas *et al.*,

1984; 1984a). At present, it is not known whether IACF is a single chain or two-chain factor, however, affinity chromatography with anti-Ia antibody removes the 6h complexes (Paraskevas *et al.* 1981; Maeba *et al.* 1988). The idiotype component of the IACF has been demonstrated with an anti-idiotypic antibody against an anti-ABA antibody and subsequent removal of an ABA-specific IACF using an anti-idiotypic immunoadsorbent column (Paraskevas *et al.*, 1984). Therefore, based on these observations it was postulated that the Ig in the 6h complexes represents a naturally occurring anti-idiotypic antibody (Paraskevas *et al.*, 1984; 1984a). Recent studies by Gordon and Abikar (1988) have in fact shown that antigen-specific T cell antisuppressor factors (IACF), prepared against antigens such as GAT, PC1, phosphorylcholine (PC) and dextran, carry an idiotype which can interact with the respective monoclonal anti-idiotypic antibody. Experimental evidence indicates that the 6h complexes are taken up by an $Ly2^+$ T cell which bears Fc receptors and Ia antigens (Paraskevas and Lee, 1979; Paraskevas *et al.*, 1984). In contrast to conventional antibody-antigen complexes which bind to T cells through the Fc receptor, the binding of the 6h complexes has been suggested to involve Ia antigens (Paraskevas and Lee, 1979). Production of the antisuppressor mediator *in vitro* in cocultures of antigen-pulsed macrophages with normal $Ly1^+$ T cells and addition of Ig (Maeba *et al.*, 1988), has further verified the earlier observations on the nature of these complexes. By using affinity columns it has again been observed that the antisuppressor mediator produced *in vitro* also consists of

Ig, foreign antigen and Ia antigens (Maeba *et al.*, 1988). The manner in which these components are assembled to produce the antisuppressor effect is not known at present, however, it has been demonstrated that removal of any one component results in loss of antisuppressor function (Maeba *et al.*, 1988).

Target cells for antisuppression

Although the cells and mediators of antisuppression and contrasuppression are distinct, it is apparent that functionally these two pathways are similar. Experimental evidence indicates that B cells as well as T cells are the targets of antisuppression. Evaluation of the role of 6h complexes in the *in vivo* humoral response to subimmunogenic dose of SRBC showed that they enhanced the IgG anti-SRBC response in athymic (nu/nu) mice (Lee and Paraskevas, 1981). The exact mechanism by which this occurs remains to be investigated, however, it has been suggested that in the absence of T cells these complexes activate B cells directly or through macrophages (Lee and Paraskevas, 1981; Paraskevas and Lee, 1981). Considerable experimental evidence exists indicating that 6h complexes interfere with the suppressor cell pathway (Al-Maghazachi *et al.*, 1983; Paraskevas *et al.*, 1985; Lee *et al.*, 1985; Maeba *et al.*, 1988). The role of antisuppression was first documented in studies of CTL responses (Al-Maghazachi *et al.*, 1983) wherein it was observed that 6h complexes induced by allogeneic stimulation enhanced the *in vivo* CTL response. Adoptive transfer of Ts cells was shown to be capable of suppressing the *in vivo* CTL response

and the suppressive effect was abolished by injecting Ts cells together with the 6h complexes. Therefore, it was inferred that the complexes blocked the function of Ts cells (Al-Maghazachi *et al.*, 1983; Paraskevas *et al.*, 1984).

In studies of the enhancement *in vitro* of the anti-SRBC antibody response it has been demonstrated that 6h complexes exert no augmenting effect on antibody formation in the absence of Ts cells (Lee *et al.*, 1985). The addition of either the 6h complexes or activated $Ly2^+$, Ia^+, FcR^+ antisuppressor effector cells restores the anti-SRBC antibody response of only suppressed spleen cell cultures (Lee *et al.*, 1985). It has been suggested that the antisuppressor cell function is dominant over the Ts cell function since elimination of the former, by adherence to Vv lectin, allowed recovery of fully functional Ts cells (Lee *et al.*, 1985). Studies of the DTH response to 1509a fibrosarcoma tumor cells have provided yet another model where the antisuppressor function of 6h complexes has been documented. By adoptive transfer of Ts cells the DTH response to tumor cells was suppressed and suppression was abolished only when tumor-specific 6h complexes were injected together with Ts cells (Harrop and Paraskevas, unpublished data).

Recent studies by Paraskevas and colleagues have provided a role for 6h complexes in the regulation of isotype-specific antibody response (Paraskevas *et al.*, 1988a; Ernst *et al.*, 1988). The antisuppressor mediator was prepared *in vitro* by the addition of a

single Ig isotype, e.g., normal IgG2a, and it was found that this mediator enhanced only IgG2a anti-SRBC antibody when added to suppressed spleen cell cultures (Paraskevas *et al.*, 1988a). From these observations it has been inferred that more than one IACF exists which can selectively interact with Ig of only one isotype. Experimental evidence provided by the studies of Ernst *et al.* (1988) has in fact shown that antisuppressor inducer and effector cells isolated from Peyer's patches selectively enhance the production of IgA antibody in spleen cell cultures. In contrast, the antisuppressor inducer and effector cells isolated from the spleen enhanced IgG antibody over IgA. These observations suggest that besides interfering with Ts cell function the antisuppressor mediator may in fact exert a positive influence on the humoral immune response. At present it is not known whether the positive influence reflects direct activation of B cells by the antisuppressor mediator as postulated in earlier studies (Lee and Paraskevas, 1981). Alternatively, it may be a consequence of selective T cell activation, i.e., cells analogous to T_H1 or T_H2, which have been shown to regulate the isotype of antibody produced by B cells via appropriate lymphokines (Mossman and Coffman, 1987).

Genetic restriction in antisuppression

The genetic restrictions imposed at the effector stage of antisuppression were first examined in studies of the *in vivo* enhancement of anti-SRBC antibody response at subimmunogenic dose of antigen (Paraskevas and Lee, 1981). The 6h complexes were unable to

enhance *in vivo* antibody formation across MHC barriers in mice lacking T cells, i.e.,TxXBM mice, thereby indicating that the interaction between the complexes and B cells (or macrophages) is MHC restricted (Paraskevas and Lee, 1981). In the presence of T cells, however, the complexes enhanced antibody formation across MHC barriers suggesting that the uptake of the complexes and subsequent activation of the effector cells is MHC nonrestricted. More recently, it has been demonstrated that treatment of mice with monoclonal anti-I-A antibody blocks the induction of the complexes (Paraskevas *et al.*, 1985), thereby abolishing the enhancing effect on the *in vivo* IgG antibody response. Furthermore, the *in vitro* production of the antisuppressor mediator using H-2 congenic strains of mice has shown that the induction of antisuppression is MHC restricted and is, in fact, regulated by genes of the I-A subregion (Paraskevas *et al.*, manuscript in preparation).

Functional significance of antisuppression

Jerne (1974) postulated a universal form of suppression in his network hypothesis: in the nonimmune state, cells of the immune system communicate with each other via interactions of their specific receptors and thus form a self-suppressive network. Paraskevas and associates have postulated that antigenic stimulation results in the early activation of the antisuppressor pathway, thereby allowing the immune system a means of overcoming the normal self-suppressive state (Paraskevas *et al.*, 1984; 1984a). A basic premise of this hypothesis

states that antisuppression is dominant over suppression. Furthermore, it has been postulated that the fundamental cause of suppressor cell-mediated unresponsiveness may be due to an inability in the induction of antisuppression or interference with its effector mechanism(s) (Paraskevas *et al.*, 1984a). In several model systems where abnormal suppressor cell function has been established, Paraskevas and colleagues have now documented accompanying abnormalities in the antisuppressor pathway (Paraskevas *et al.*, 1988, 1988a; Anhalt, 1987).

In a murine model of acute GVH reaction, which results in profound suppression of humoral and cell-mediated immunity (Lapp and Möller, 1969; Lapp *et al.*, 1985), both the induction and effector stage of the antisuppressor pathway are compromised (Paraskevas *et al.*, 1988). It was found that within 3 weeks of the induction of GVH reaction the antisuppressor inducer cells lost their ability to generate the antisuppressor mediator. In addition, the $Ly2^+$ effector cells collected 2 weeks after the induction of GVH reaction also lost their ability to be activated by the antisuppressor mediator produced in normal mice.

Studies of immune responsiveness to synthetic polypeptide antigens, such as GAT, have suggested that unresponsiveness in nonresponder strains of mice may be attributed to the induction of Ts cells (Kapp *et al.*, 1974a). The unresponsiveness to the synthetic polypeptide GLϕ has been examined in two nonresponder strains, C3H and

C57BL/6 mice (Anhalt, 1987). In C57BL/6 mice there was a defect in the uptake of antisuppressor mediator by the $Ly2^+$ T cells, whereas C3H mice showed normal uptake but were unable to activate the antisuppressor effector cell. In this model, it has been documented that competent $Ly2^+$ effector cells are in fact present in C3H mice and it is the antisuppressor mediator that is functionally inactive.

Mice bearing Ig-secreting plasmacytomas have been reported to develop isotype-specific Ts cells (Hoover and Lynch, 1980; Mathur and Lynch, 1986). The functional integrity of the antisuppressor pathway has been studied in mice bearing IgA and IgG2a-secreting plasmacytomas (Paraskevas *et al.*, 1988a). The Lyl^+ T cells isolated from spleens of tumor bearing mice were unable to generate an isotype-specific antisuppressor mediator *in vitro*, thereby suggesting that the function of the antisuppressor inducer cell has been compromised.

The experimental evidence accumulated to date clearly demonstrates that a functionally intact antisuppressor pathway is crucial in the expression of immunity.

MATERIALS AND METHODS

1. Buffers

The composition of routinely used buffers in this study are:

(a) Phosphate-buffered saline (PBS): 0.01M sodium phosphate, 0.13M NaCl, pH 7.4 and pH 8.0;

(b) Acetate buffer: 0.1M sodium acetate/acetic acid, pH 4.5;

(c) Citrate buffer: 0.1M sodium citrate/citric acid, pH 3.5;

(d) Phosphate buffer: 0.02M sodium phosphate, pH 6.6;

(e) Tris Buffer: 1M Tris - (hydroxymethyl) aminomethane/HCl, pH 9.0;

(f) Tris-NH_4Cl: 1 part 0.17M Tris/HCl, pH 7.6 + 9 parts 0.15M NH_4Cl.

2. Tissue culture medium

Cells were cultured in Eagle's minimum essential medium (MEM) or RPMI 1640 (Gibco, Grand Island, NY). The medium was prepared according to the manufacturer's instructions and supplemented with: 50 units/ml penicillin, 50 μg/ml streptomycin, 1 mM sodium pyruvate, 3 mM HEPES, 0.3 mM non-essential amino acids and 6 mM L-glutamine. The medium was sterilized by filtration through a 0.22 μm filter (Millipore, Bedford, MA) and stored at 4°C. Prior to use the medium was supplemented with 10% fetal calf serum (FCS; Gibco, Grand Island, NY).

3. Animals

Mice used in this study were generally 6-8 weeks old. All animals

were maintained in the Animal Care Facility of the University of Manitoba. Balb/c mice were obtained from Canadian Breeding (Montreal, Canada). The following strains were obtained from the Jackson Laboratory (Bar Harbor, ME): C57BL/6 (abbreviated B6), DBA/2, AKR/J, (C57BL/6 x DBA/2) F_1-hybrid (abbreviated BDF_1) and Balb/c (nu/nu). The athymic (nu/nu) mice were maintained under aseptic conditions in a restricted access room. The colony of Igh congenic and recombinant strains was established from breeding pairs kindly provided as indicated: CB.20 and Balb/b, Dr. D.B. Murphy, Yale University; Cal.20, Dr. J. Gordon, McGill University; BAB-14, Dr. K.T. HayGlass, Harvard Medical School; CBAL, Dr. R. Riblet, Institute for Cancer Research, Philadelphia. The H-2 and Igh haplotypes of these mice are listed in Table I.

4. Antigen

Sheep red blood cells (SRBC) were obtained weekly from National Biological Laboratories (Winnipeg, Canada). Prior to use the SRBC were washed three times (1000 x g, 5 min) in sterile Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY).

5. Antisera

Anti-Thy 1.2 antiserum was prepared according to established procedures (Reif and Allen, 1964). Adult AKR/J mice were injected intraperitoneally (i.p.) at weekly intervals with C3H/HeJ thymocytes ($5x10^7$ cells/mouse) for 6 weeks. The serum was collected by cardiac

Table 1

H-2 and Igh haplotypes of mice used in this study

	Haplotype	
Strain	H-2	Igh
Balb/b	b	a
C57BL/6	b	b
Balb/c	d	a
CB.20	d	b
Cal.20	d	d
BAB-14	d	v^a-c^b
CBAL	d	v^a-c^b
DBA/2	d	c
AKR/J	k	d
BDF_1	b/d	-

puncture 10 days after the last injection. It was heat inactivated (56°C, 30 min) and then absorbed with packed red blood cells from C3H/HeJ and Balb/c mice (4°C, 1 hr). The titre of this antiserum was established by the microcytotoxicity assay (Mittal *et al.*, 1968). Thymocytes and spleen cells from Balb/c mice were used as target cells (10^6 cells/0.1 ml) together with serial dilutions of the antiserum in the presence of Hemo-Lo guinea pig complement (1/4 final dilution; Cedarlane Laboratories, Hornby, Ontario) and incubated at 37°C for 45 min. The cytotoxicity titre was 1:256 by trypan blue exclusion. Aliquots of this antiserum were stored at -70°C until further use.

Rabbit anti-mouse IgG (RαmIgG) for developing indirect plaque-forming cells (PFC) was prepared according to previously described methods (Paraskevas and Lee, 1976). Briefly, rabbits were immunized by multiple injections of purified mouse IgG emulsified in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI). The immunoglobulins were isolated from the rabbit serum by an anion-exchange column (DEAE Sephadex A50; Pharmacia Fine Chemicals, Uppsala, Sweden). The immunoglobulins carry a predominantly positive charge at pH 6.6 and therefore pass through the column. The concentration of protein was determined by measuring the absorbance at 280 nm (A_{280}). Further purification was achieved by gel filtration on a Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (bed height 94 cm; flow rate 2.6 ml/min).

6. Monoclonal antibodies

Monoclonal antibodies (mAb) specific for T cell surface markers Ly-1.2 and Ly-2.2 were purchased from New England Nuclear (Boston, MA). The hybridoma cell line GK1.5 secreting the rat mAb specific for the murine T cell surface antigen L3T4 (Wilde *et al.*, 1983) was obtained from the American Type Culture Collection (ATCC; Rockville, MD). All hybridoma cell lines were cultured according to the recommended instructions.

Hybridoma cell lines secreting mAbs with specificities against determinants encoded by the I-A or I-E subregions of the MHC were also obtained from the ATCC: 25-9-3s, anti-I-A^b (Ozato and Sachs, 1981); MKD6, anti-I-A^d (Kappler *et al.*, 1981); 10.2.16, anti-I-$A^{k,r,f,s}$ (Oi *et al.*, 1978) and 14.4.4s, anti-I-$E^{k,d,p,r}$ (Ozato *et al.*, 1980). The mAbs were prepared either as the cell-free supernatant of the hybridoma cultures grown to 10^6 cells/ml or as the ascitic fluid of pristane (2,6,10,14-tetramethylpentadecane; Aldrich, Milwaukee, WI) primed mice carrying the hybridoma tumor. A minimum of 10^6 viable cells was inoculated i.p. between 7 to 10 days after pristane priming.

7. Purification of monoclonal antibodies

Antibodies were isolated from the cell-free supernatant using Protein A-Sepharose CL-4B affinity column (Pharmacia Fine Chemicals, Uppsala, Sweden) according to established procedures (Ey *et al.*, 1978). Large volumes (2-3 liters) of cell-free supernatant were concentrated 100 to 200-fold by membrane ultrafiltration (YM 30, nominal cut-off

30,000 daltons; Amicon, Lexington, MA) and the pH adjusted to 8.0. The sample was applied to a Protein A-Sepharose CL-4B column which was pre-equilibrated with 0.14 M PBS, pH 8.0 at 4°C. The column was washed extensively with PBS, pH 8.0 and the bound material eluted with 0.1 M citrate buffer, pH 3.5 in 3 ml fractions in tubes containing 0.5 ml 1 M Tris buffer, pH 9.0. The A_{280} was measured and the appropriate fractions were pooled and concentrated. All mAbs were dialysed overnight against PBS, pH 7.4 at 4°C and sterilized by filtration through a 0.22 μm filter. The protein concentration was determined using an extinction coefficient, $_{280}E^{1\%}$ (HGG) = 14.3.

Monoclonal antibodies from ascites were first partially purified by precipitation with ammonium sulfate at 40% saturation (4°C, overnight with stirring). The precipitated proteins were removed by centrifugation (15,000 x g, 30 min, 4°C), dissolved in PBS, pH 7.4 and dialysed extensively to remove all traces of ammonium ions. Further purification was achieved by using the protein A-Sepharose column. All mAbs were stored at -70°C until further use.

8. Isolation of normal IgG from mouse serum

Serum was collected from each of the mouse strains used in this study. Normal mouse IgG was purified by elution from Protein A-Sepharose column as described in sec. 7. The eluate was concentrated in order to approximate the IgG concentration of normal mouse serum (NMS). The purity of the IgG preparation was verified by Ouchterlony (1958) immunodiffusion using rabbit anti-mouse antisera specific for

γ-chain, μ-chain and α-chain (Miles Scientific, Naperville, IL).

9. Preparation of F(ab')2 by pepsin degradation

The divalent $F(ab')_2$ fragment of normal mouse IgG was prepared according to the method of Lamoyi and Nisonoff (1983). Mouse IgG at a concentration of 20 mg/ml was dialysed overnight against 0.1 M acetate buffer, pH 4.5 at 4° C. The pH was lowered to 4.2 with 2 M acetic acid and sufficient pepsin (2x crystallized; Calbiochem-Behring, La Jolla, CA) was added to give an enzyme: globulin weight ration of 1:60. The mixture was incubated at 37°C for 6 h. The digestion was stopped by raising the pH to 8.0 with 0.5 N NaOH. The $F(ab')_2$ fragments were separated from the intact IgG and smaller peptides by gel filtration on a Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (bed height 90 cm; flow rate 0.25 ml/min). The column was calibrated using molecular weight markers and the fractions corresponding to a relative molecular weight of 100,000, a value which agrees well with the expected molecular weight of $F(ab')_2$ fragments, were collected. The preparation was subsequently passed through a protein A-Sepharose column in order to ensure complete removal of all Fc components. The purity of the preparation was determined by Ouchterlony immunodiffusion using an Fc-specific goat anti-mouse antiserum (Miles Scientific, Naperville, IL).

10. Antigen presenting cell lines

The cell line A.20, a Balb/c B cell lymphoma was obtained from Dr.

D. Rayner, Department of Pathology, University of Manitoba. This is an Ia^+ B cell line capable of presenting both alloantigens and protein antigens to T lymphocytes in an MHC restricted manner (Kim *et al.*, 1979). The cells were cultured in RPMI + 10% FCS.

$P388D_1$ cells were obtained from Dr. P.K. Maiti, Department of Immunology, University of Manitoba. This is an Ia^- macrophage-like cell line. It was originally isolated from a methylcholanthrene-induced lymphoid neoplasm of a DBA/2 mouse (Koren *et al.*, 1975). These cells were cultured in RPMI + 10% FCS.

11. Collection of macrophages

Mice were sacrificed by cervical dislocation. The abdomen was liberally wetted with 70% alcohol and 7 ml RPMI was injected (21 G 1½ needle) into the peritoneal cavity of each mouse. Resident macrophages were collected by gently massaging the peritoneum and withdrawing the medium back into the syringe. The pooled medium was centrifuged (200 x g, 10 min) and the cells washed once with RPMI. The peritoneal cells (10^7 cell/ml) were depleted of $Thy1.2^+$ cells by treatment with anti-Thy1.2 antiserum (1/10 final dilution) and guinea pig complement (1/4 final dilution) for 45 min at 37°C. The treated cells were washed twice with RPMI and resuspended in RPMI + 10% FCS at a final concentration of $5x10^5$ cells/ml. The cells were cultured at 37°C for 2 h in Falcon petri dishes (35x10 mm; Becton Dickinson, Lincoln Park, *NJ)* in a 5% CO_2 atmosphere. The cells were washed with warm medium to remove loosely adherent cells.

12. Preparation of spleen cell suspension

Spleens were removed aseptically from mice and placed in a sterile petri dish containing MEM medium. A single cell suspension was made by teasing the spleen with forceps. The suspension was filtered through a fine mesh screen to remove any large clumps of cells or tissue and centrifuged (150 x g, 7 min). The pelleted cells were resuspended in 5 ml of Tris-NH_4Cl buffer (5 min, 37°C) to lyse the red cells. The splenocytes were washed twice, counted ($1x10^8$ cells/spleen, 98% trypan-blue impermeable), resuspended in MEM + 10% FCS and stored on ice.

13. Nylon wool enrichment of T cells from the spleen

T cells were obtained by passing normal spleen cells through a nylon wool column (NWC) according to the method of Julius *et al.* (1973). Before use the scrubbed nylon fiber (3 denier, 3.81 cm, type 200; Fenwal Laboratories, Deerfield, IL) was acid treated (boiled for 30 min in 0.2 N HCl) and washed with distilled, deionized water (ddH_2O) for 5 consecutive days. The clean nylon wool was dried at 37°C and ready for use. Nylon wool (0.6 gm/spleen) was teased in ddH_2O to remove air bubbles, packed into a 20 cc syringe fitted with a stop-cock and autoclaved. The NWC was first flushed with 50 ml RPMI + 10% FCS followed by 50 ml Dulbecco's phosphate-buffered saline (DPBS; Gibco, Grand Island, NY) + 10% FCS and incubated at 37°C for 1 h. The spleen cells resuspended in 2-3 ml of DPBS + 10% FCS were layered on top of

the NWC and allowed to percolate about 1/4 into the column. The column was left at 37°C for 1 h and the non-adherent cells (referred to as NW T cells) were eluted with warm (37°C) DPBS + 10% FCS. The cells were washed twice (150 x g, 7 min) then resuspended in MEM + 10% FCS. The NW T cells (98% trypan-blue impermeable) contained 80-85% Thy1.2$^+$ and 2-4% sIg$^+$ cells as assessed by membrane immunofluorescence using the fluorescence activated cell sorter (EPICS, Coulter Electronics Inc., Hialeah, FL). The cell recovery was 20-30% of the starting population.

14. Enrichment of T cell subpopulations

The concentration of NW T cells was adjusted to 10^7 cells/ml in cytotoxicity medium (RPMI containing 0.3% bovine serum albumin). In order to deplete the Ly-1.2$^+$ cells anti-Ly1.2 mAb was added (1/20 final dilution) and the cells incubated at 4°C for 1 h. The cells were centrifuged, the supernatant discarded and the pellet resuspended in Low-Tox-M rabbit complement (1/10 final dilution; Cedarlane, Hornby, Ontario) and the cells incubated at 37°C for 1 h. The cells were washed three times with MEM and resuspended in MEM + 10% FCS.

15. Induction and preparation of antigen-specific Ts cells

Antigen-specific Ts cells were induced as previously described (Perry and Greene, 1982). Balb/c mice were injected i.v. with anti-I-A^d mAb (20 μg/mouse). Three days later the mice were immunized with 10^8 SRBC/0.2 ml (i.p.). Spleen cells were collected 4 days after antigenic stimulation and enriched for T cells by NW filtration as

described previously. The cells remaining after depletion of the Ly-1.2$^+$ cells (sec. 14) were used as antigen-specific Ts.

16. Induction of antisuppressor mediator *in vivo*

Mice were immunized with 5x10^8 SRBC (i.p.) and the serum collected 6 h (6HS) later. Serum collected from mice in the absence of antigenic stimulation (NMS) served as control. The 6HS has been shown to contain the antisuppressor mediator (Paraskevas *et al.*, 1985; Lee *et al.*, 1985). Two assays (described below) have been used primarily, first an assay that detects the presence of 6h complexes and second, an assay for the function of these complexes.

17. Detection of antisuppressor mediator

The reverse immune cytoadherence (RICA) technique has been used for detecting the presence of 6h complexes which are present in the serum shortly after antigenic stimulation (Paraskevas *et al.*, 1971). When normal spleen cells are incubated with 6HS the complexes are passively adsorbed by a subpopulation of T cells thereby rendering them sIg$^+$. Briefly, the method utilizes a 5S hybrid antibody with one combining site specific for Ig while the other site is specific for bovine serum albumin (BSA). The hybrid antibody binds to an Ig$^+$ cell with one combining site and to BSA-coated SRBC (Paraskevas *et al.*, 1971) with the other, thereby forming a rosette. The number of rosette forming cells (also referred to as Ig$^+$ cells) were calculated by counting 500-1000 lymphocytes. This assay detects ~25-30% sIg$^+$ B cells

in the spleen.

18. Function of antisuppressor mediator

The *in vitro* assay for the function of the complexes has been described previously (Lee *et al.*, 1985) and involves: (a) a read-out system necessary to measure the level of immune responsiveness, (b) suppression of antibody formation upon addition of antigen-specific Ts cells, and (c) restoration of immunologic responsiveness of suppressed cultures upon addition of the antisuppressor mediator. The assay involves culturing spleen cells in Marbrook flasks using the modified Diener and Armstrong method (1967).

Briefly, the antibody response was elicited by culturing $2x10^7$ spleen cells in 1 ml MEM + 10% FCS and stimulating them with $5-8x10^6$ SRBC/0.1 ml. The immune response was suppressed by the addition of $2-3x10^6$ Ts/0.1ml which were prepared as described in sec. 15. Serum collected 6 h after antigenic stimulation, containing the antisuppressor mediator, was added to the suppressed cultures at a final concentration of 1%. The Marbrook flasks were incubated for 5 days at 37°C in 5% CO_2 atmosphere. The number of plaque forming cells (PFC) were enumerated as described below.

19. Plaque forming cell assay

The PFC assay was a modification of the Cunningham assay (Cunningham and Szenberg, 1968). Briefly, precleaned microscope slides (Gold Seal; Becton Dickinson, Lincoln Park, NJ) were separated from

each other by doubled sided tape in such a manner that 2 microchambers are formed between the 2 slides.

The lymphoid cells were collected from the Marbrook culture flasks and washed once with HBSS. The cell pellet was resuspended in 1 ml HBSS and stored on ice. A suspension of the indicator cells, i.e., SRBC ($2.5x10^9$/ml) was prepared. Guinea pig complement (GPC) was used at 1/4 dilution. The RαmIgG (sec. 5) was added in order to develop the IgG plaques. The spleen cells (SC), SRBC and complement were mixed as follows:

IgM PFC	(IgM + IgG) PFC
30 μl SRBC	30 μl SRBC
30 μl SC	30 μl SC
30 μl HBSS	30 μl RαmIgG
30 μg GPC	30 μl GPC

The Cunningham slides were filled with the cell suspensions, sealed with melted paraffin wax, and incubated at 37°C for 45 min. The number of PFC were enumerated using a Zeiss microscope at low magnification (30X).

20. Preparation of antisuppressor mediator *in vitro*

The method for the *in vitro* production of the antisuppressor mediator has been previously described (Maeba *et al.*, 1988). Briefly, resident peritoneal macrophages were isolated as described in sec. 11. The adherent cells were pulsed with $5x10^6$ SRBC resuspended in 1 ml RPMI + 10% FCS for 4 h at 37°C in 5% CO_2 atmosphere. Subsequently, the

adherent cells were washed 5-6 times in warm medium (37°C) to remove excess SRBC and $2x10^7$ NW T cells in 1.5 ml RPMI + 10% FCS were added to each petri dish. The macrophage-T cell cultures were incubated overnight at 37°C. The cell free supernatant was collected and used in both the RICA assay as well as the functional assay. In these assays, the macrophage-T cell supernatant was added together with NMS or IgG such that the final concentration of the supernatant was 5% and that of the NMS was 1%. Instead of the intact IgG, the divalent $F(ab')_2$ fragment (sec. 9) was also used together with the macrophage-T cell supernatant.

21. Macrophage-T cell culture: treatment of macrophages

Resident macrophages were isolated from the peritoneal cavity and split into 2 groups. One group of adherent cells was treated: (a) before exposure to antigen, (b) after exposure to antigen, or (c) continuously during the entire antigen pulsing period. The treated and untreated cells were pulsed with antigen for 4h, washed and subsequently exposed to NW T cells overnight. The macrophage-T cell supernatant was evaluated for antisuppressor activity as described above (sec. 17 and 18).

(i) Fixation: The macrophage monolayers were fixed in 1% paraformaldehyde (20', 37°C) according to established procedures (Ziegler and Unanue, 1982). The cells were fixed both before and after exposure to intact SRBC. In addition, sonicated SRBC were also used for pulsing fixed and unfixed macrophages. Packed SRBC were sonicated

and used at a final concentration of 10% for pulsing macrophages.

(ii) Lysosomotropic agents: The macrophage monolayers were cultured with medium containing either 10 mM NH_4Cl (Fisher Scientific, Fairlawn, NJ) or 0.1 mM chloroquine (Sigma Chemical Co., St. Louis, MO) according to established methods (Ziegler and Unanue, 1982). These agents were present continuously during the 4 h antigen-pulsing period.

(iii) Anti-I-A^d + complement: The macrophages were treated with anti-I-A^d mAb (100 μg/10^6 cells, 4°C, 1 h). The cells were resuspended in GPC (1/4 final dilution) and incubated at 37°C, 1 h. The cells were washed three times and then cultured ($5x10^6$ cells/ml) in the presence of SRBC.

(iv) Blocking with anti-class II mAbs: The macrophages were pretreated with mAbs that have specificity against determinants encoded by the I-A or I-E subregion (100 μg mAb/10^6 cells, 45 min, 4°C) and subsequently exposed to SRBC in medium containing these antibodies (100 μg/ml). Anti-class II mAbs which did not cross-react with the H-2 haplotype of the macrophages provided specificity control.

22. Macrophage-T cell culture: treatment of T cells

Normal NW T cells were treated (as indicated below) before culturing overnight with SRBC-pulsed macrophages. The supernatant from the macrophage-T cell interaction was evaluated for antisuppressor activity as described earlier (sec. 17 and 18).

(i) Anti-L3T4 + complement: The NW T cells were depleted of $L3T4^+$ cells by treatment with anti-L3T4 mAb (100 μl GK 1.5 culture

supernatant/$2x10^6$ cells, 4°C, 1 h) then resuspended in GPC (1/4 final dilution and incubated at 37°C, 1 h. The cells were washed, counted and cultured with SRBC-pulsed macrophages.

(ii) Blocking with anti-L3T4 and anti-Ly-2.2 mAbs: The NW T cells were treated with anti-L3T4 or anti-Ly-2.2 mAbs (50 $\mu g/10^6$ cells, 4°C, 1h). The cells were washed with ice-cold medium and then cultured with SRBC-pulsed macrophages.

23. X-irradiation of mice

Mice were lethally irradiated (850 rads, whole body irradiation) using a Theratron F cobalt 60 unit, Atomic Energy of Canada. The ^{60}Co source was calibrated by the Physics Department of the Manitoba Cancer Treatment and Research Foundation.

24. Reconstitution of mice with NW T cells and collection of 6HS

Twenty-four hours after lethal irradiation mice were injected (i.v.) with $15-20x10^6$ syngeneic or Igh congenic NW T cells. These mice were immunized 24 h later with $5x10^8$ SRBC/0.2 ml (i.p.). Serum was collected from these mice 6 h after antigenic stimulation. The 6HS was evaluated by both the RICA and functional assays (sec. 17 and 18). When Igh congenic T cells were used for reconstitution of irradiated mice, affinity purified IgG from the same Igh haplotype as the T cell donor was added to the serum at a concentration of 1 mg/ml.

RESULTS

1. Production of antisuppressor mediator: *in vivo* and *in vitro*

Earlier studies from this laboratory (Orr and Paraskevas, 1973) have identified in the serum of mice within 3-6 h after immunization a unique form of 'processed' antigen representing complexes of Ig and antigen. More recently, a simple method for the generation of a soluble mediator with functional properties identical to those of the complexes formed *in vivo* (6 h after immunization) has been described (Maeba *et al.*, 1988). In contrast to the *in vivo* method (Fig. 1a) where serum was collected 6 h after antigenic stimulation, the *in vitro* method for the production of the antisuppressor mediator involved 2 stages. During the first stage (Fig. 1b) antigen-pulsed macrophages were co-cultured with NW T cells. In the second stage, normal mouse IgG was added to the cell-free supernatant obtained from the macrophage-T cell interaction.

2. Assays for the 6HS or antisuppressor mediator

2.1 Detection of the complexes

The antisuppressor mediator produced by both *in vivo* as well as *in vitro* methods is cytophilic for a subpopulation of T cells (Orr and Paraskevas, 1973; Maeba *et al.*, 1988). For detection of the cytophilic Ig, Balb/c spleen cells were incubated with the serum and the uptake of Ig by these cells was examined by the previously established RICA method (Paraskevas *et al.*, 1971) (Fig. 2). The number of Ig^+ cells in

Fig. 1 Production of antisuppressor mediator

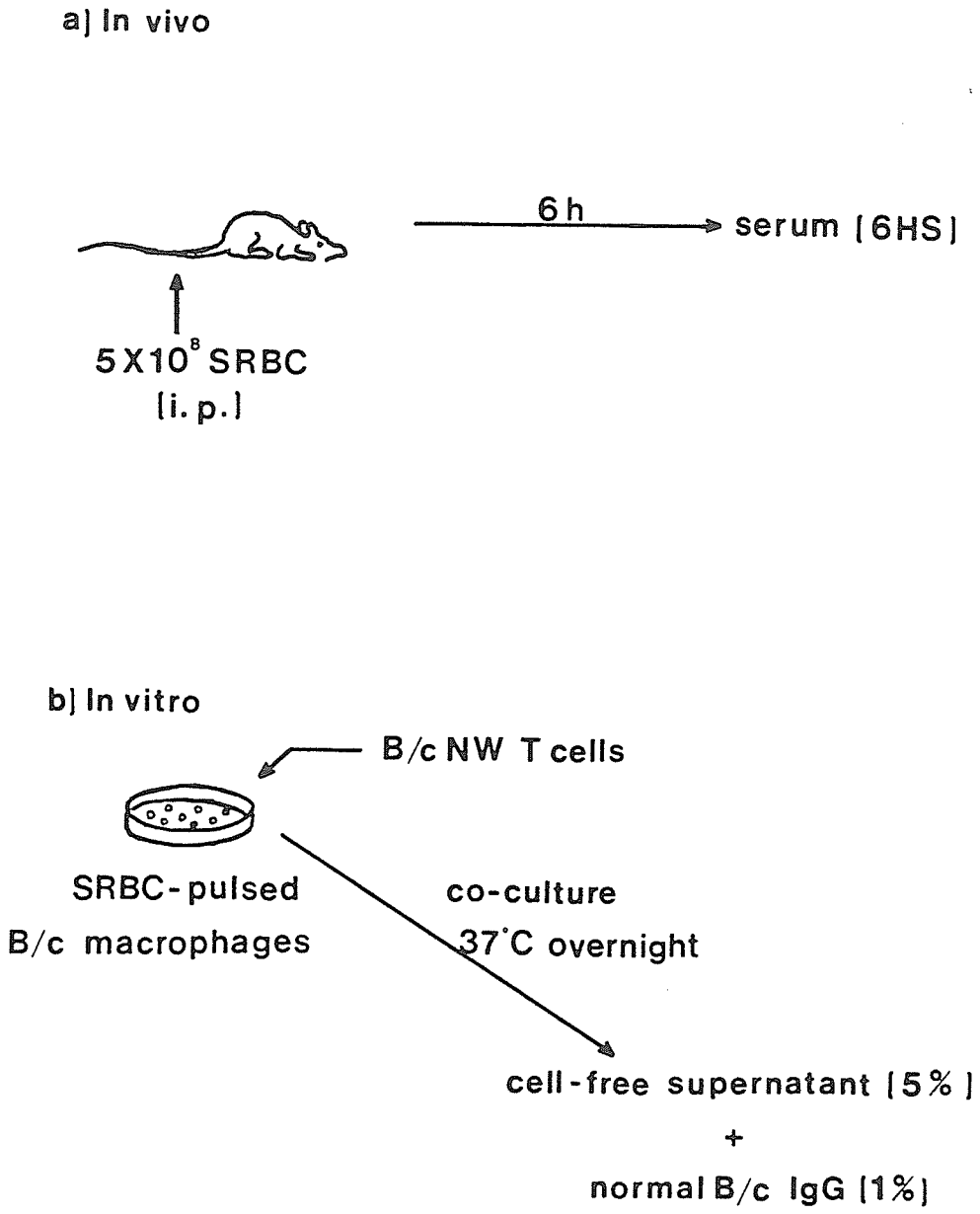
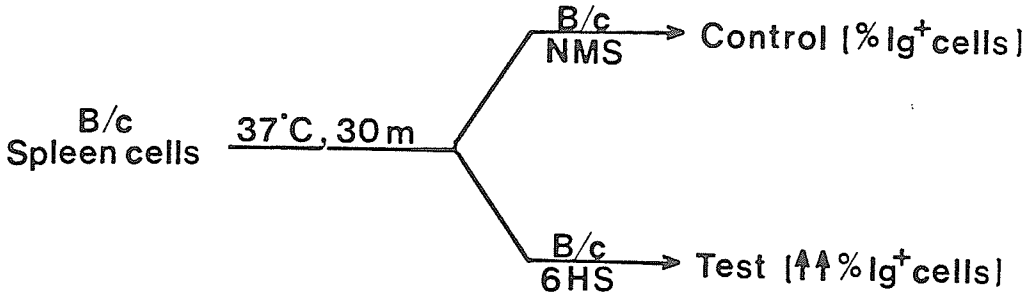
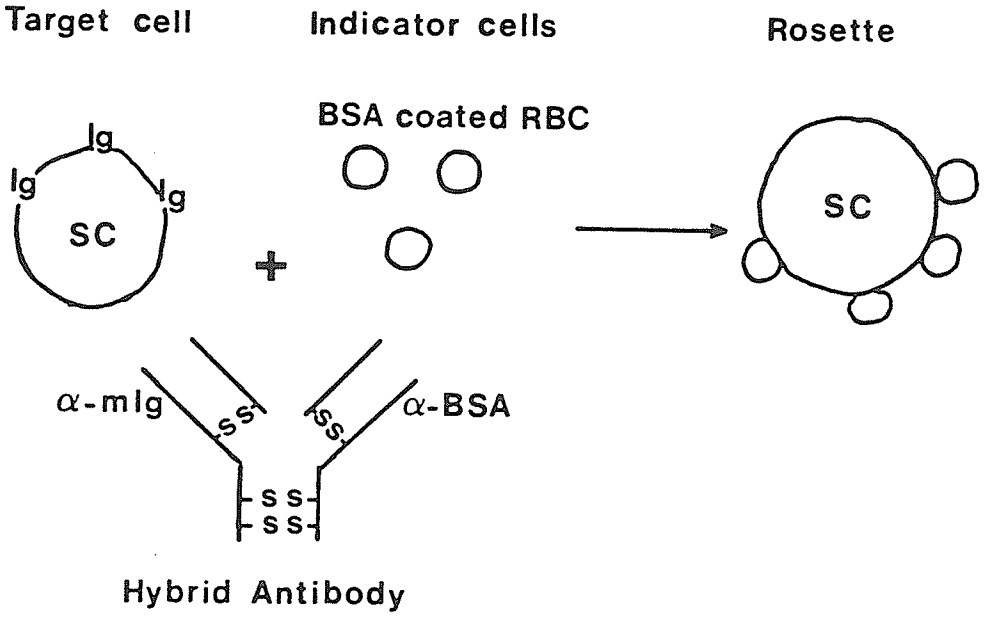


Fig. 2 Detection of antisuppressor mediator





the Balb/c spleen has been shown to vary between 29-33%. When normal spleen cells were incubated with the 6HS the number of Ig^+ cells increased by 5-8% above the control. The results reported in this study represent the mean of two separate counts of Ig^+ cells detected after incubation with NMS or 6HS.

2.2 Functional assay

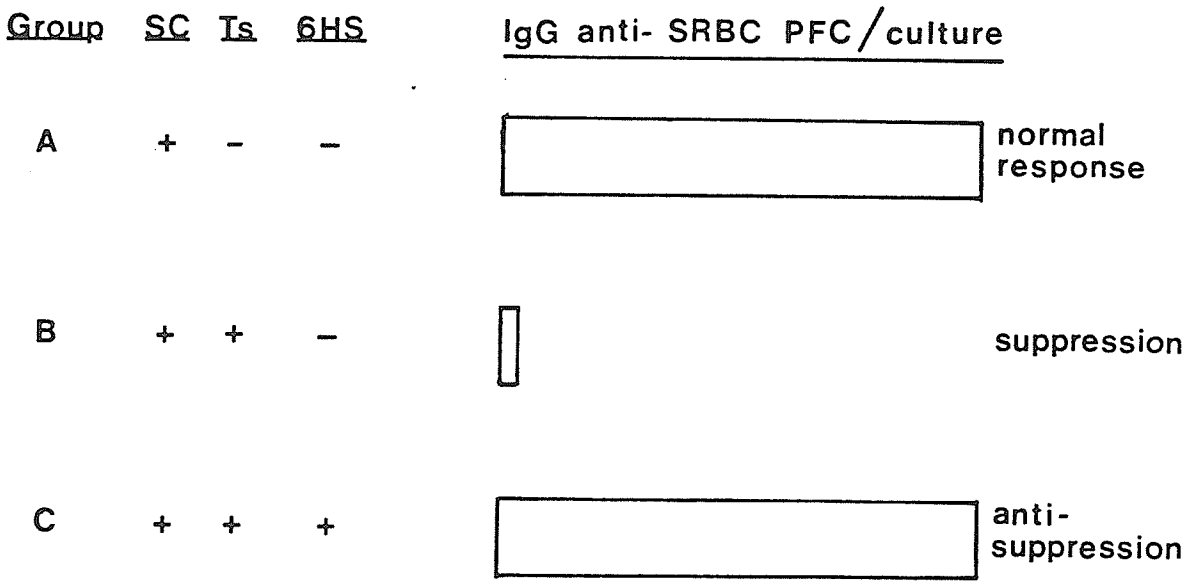
In humoral responses the 6 h complexes have been found to interfere with suppressor T cell function (Lee *et al.*, 1985; Paraskevas *et al.*, 1985) and have, therefore, been termed antisuppressor mediator. The previously described (Lee *et al.*, 1985) Marbrook-Diener culture system (Diener and Armstrong, 1967) has been utilized for the antisuppressor assay. Spleen cells from Balb/c mice were stimulated *in vitro* with SRBC and the IgG anti-SRBC response determined (Fig. 3, group A). The response in these cultures was suppressed upon the addition of antigen-specific Ts cells (Fig. 3, group B) induced by the method of Perry and Greene (1982). The subsequent addition of 6HS or the antisuppressor mediator produced *in vitro* was able to restore the anti-SRBC response of such suppressed cultures (Fig. 3, group C). Previous studies have shown that only the IgG antibody response has been regulated by the complexes while the IgM response is not affected (Paraskevas *et al.*, 1985). Similar results have also been obtained in the present study. Both the IgM and IgG antibody responses have been determined but only the IgG response is reported here. The number of PFC/culture is the mean of six to nine cultures ± S.E. from

Fig. 3 Assay for antisuppressor function

1. Cultures: Marbrook culture flasks containing $2x10^7$ spleen cells (SC) and antigen.

2. Ts cells: Induced by anti-I-A treatment followed by antigenic stimulation.

3. Antisuppression: 1% 6HS or the *in vitro* mediator.



two or 3 separate experiments.

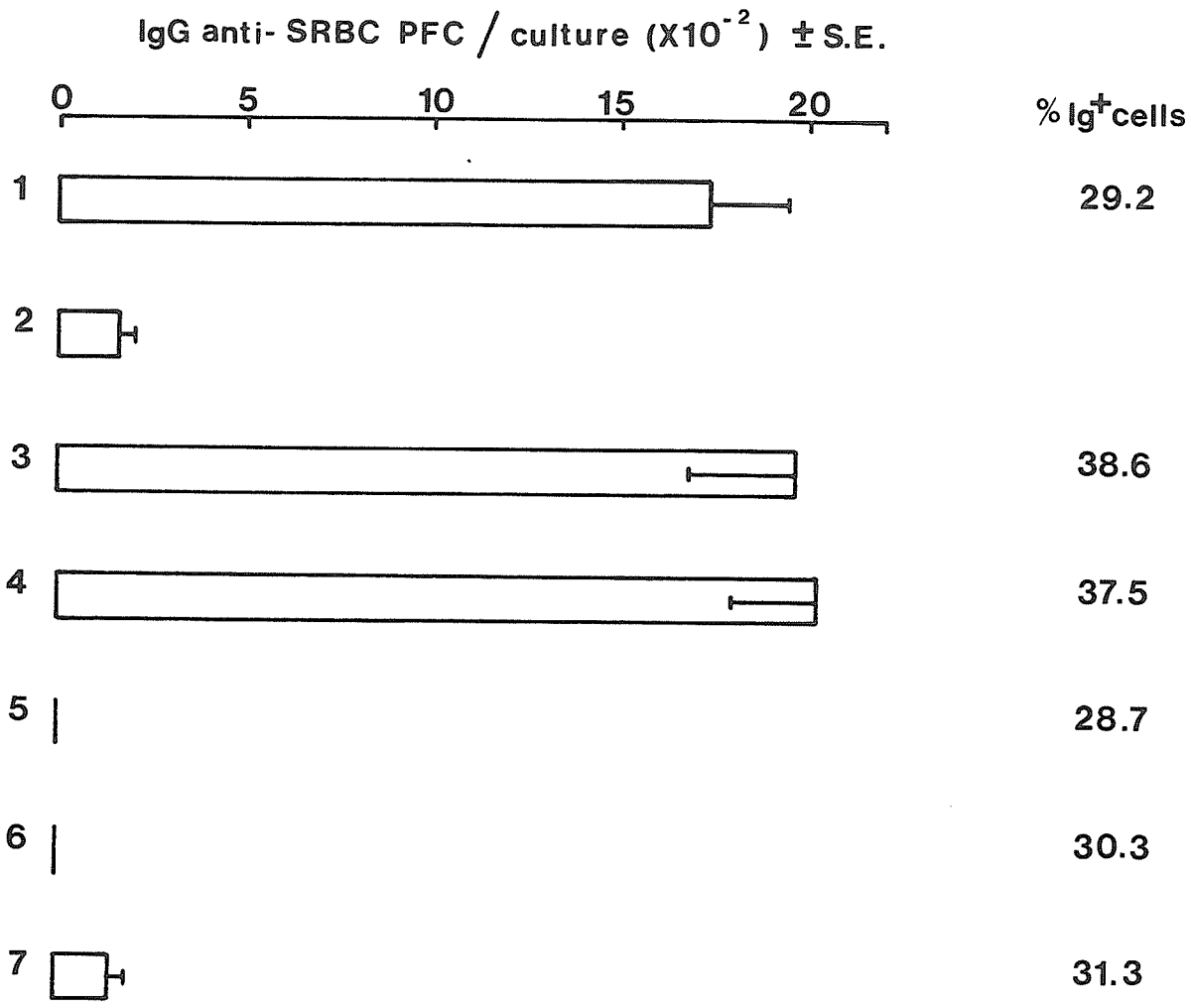
3. Antigen presenting cell lines for generation of antisuppressor mediator

In the present study it was decided to examine whether antigen presenting cell lines could also be used for the production of the antisuppressor mediator. The antisuppressor mediator produced *in vivo* and *in vitro* served as positive controls in the experiment. As shown in Fig. 4 (group 1), stimulation of $2x10^7$ Balb/c spleen cells *in vitro* with SRBC gave an IgG anti-SRBC response of 1700 PFC/culture. The response was suppressed upon the addition of antigen-specific Ts cells (group 2). The immune response was restored when 6HS at 1% final concentration was added to the suppressed cultures (group 3). The presence of the cytophilic Ig was evident by the increase in the number of Ig^+ cells as compared with the control (group 3 vs 1). As expected, the cell-free supernatant from the co-culture of antigen-pulsed macrophages and NW T cells in the presence of Balb/c NMS (final concentrations: 5% supernatant + 1% NMS) was also able to restore the anti-SRBC response of the suppressed cultures (group 4).

The mouse macrophage-like cell line, $P388D_1(H\text{-}2^d)$ (Koren *et al.*, 1975) was used instead of normal peritoneal adherent cells. These cells were pulsed with SRBC, then cultured with (group 6) and without (group 5) Balb/c ($H\text{-}2^d$) NW T cells. In each case the cell-free supernatant in the presence of Balb/c NMS was examined for antisuppressor activity.

Fig. 4 Antigen presenting cell lines for generation of antisuppressor mediator

Group	SC	Ts	(APC-T)SUP	NMS
1	Balb/c	-	-	-
2	"	+	-	-
3	"	+	-	- 6HS
4	"	+	Mϕ-T	Balb/c
5	"	+	$P388D_1$ -	-
6	"	+	$P388D_1$ -T	Balb/c
7	"	+	A.20-T	Balb/c


IgG anti- SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+}cells
1
29.2
2
3
38.6
4
37.5
5
28.7
6
30.3
7
31.3

The $P388D_1$ supernatant (group 6) was found to be lacking in antisuppressor activity by the RICA and PFC assays. The lack of Ia antigens on this phagocytic cell line may provide an explanation for the absence of antisuppressor activity since previous studies (Paraskevas *et al.*, 1984) have demonstrated that one of the components of the complexes was Ia antigens.

The cell line A.20, a Balb/c B cell lymphoma (Kim *et al.*, 1979) was also used for the production of the antisuppressor mediator. After exposure to SRBC the A.20 cells were co-cultured with Balb/c NW T cells. The supernatant from this B cell line which is capable of presenting both alloantigens and protein antigens (Glimcher *et al.*, 1982) had no antisuppressor activity (group 7) when examined by the RICA and PFC assays.

4. Metabolic requirements of macrophages during generation of antisuppressor mediator

4.1 Fixation of macrophages

Fixation of APCs with paraformaldehyde or glutaraldehyde has been extensively used to inhibit antigen processing (Ziegler and Unanue, 1982). In order to demonstrate that internalization and processing of antigen were requisite early steps in the formation of antisuppressor mediator, the macrophages were fixed both before and after exposure to SRBC. The IgG anti-SRBC response is shown in Fig. 5 (group 1). The response was suppressed upon addition of antigen-specific Ts cells

(group 2). The addition of supernatant from untreated macrophages together with Balb/c NMS (group 3) restored the anti-SRBC response of the suppressed cultures. In contrast, the supernatants from macrophages that had been fixed both before and after exposure to SRBC were lacking the antisuppressor mediator when examined by RICA and PFC assays (group 4 and 5).

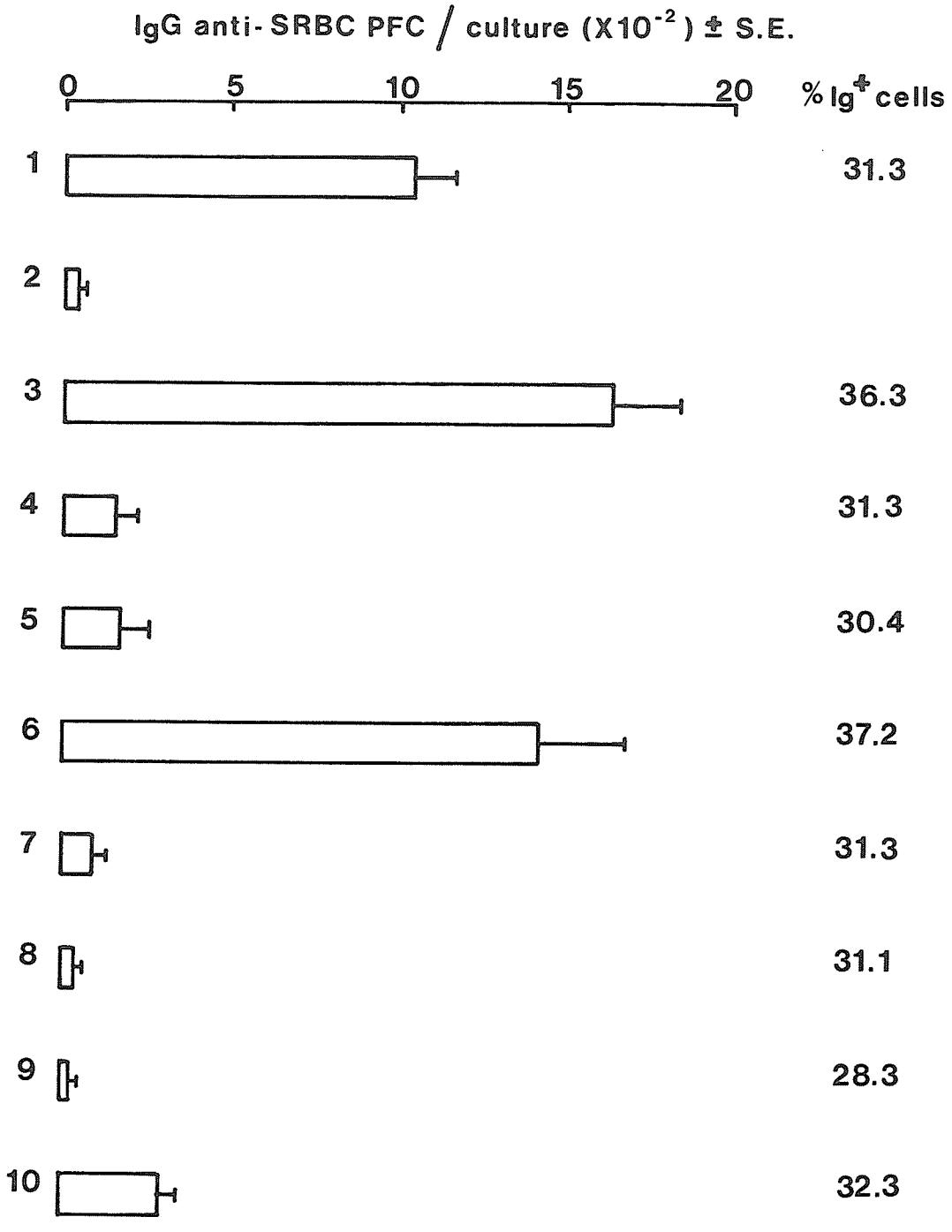
It has been demonstrated that prefixed macrophages while incapable of presenting intact protein antigens can present fragments of the protein (Shimonkevitz *et al.*, 1983). Therefore, SRBC were sonicated and the hemolysate used for pulsing the macrophages. The supernatant from untreated macrophages pulsed with SRBC-hemolysate showed antisuppressor activity (group 6), whereas supernatant from macrophages fixed before and after exposure to the hemolysate lacked antisuppressor activity (group 7 and 8). The requirement for antigen internalization as one of the initial events in the production of an antigen-specific antisuppressor mediator was most likely since fixation of macrophages before exposure to intact SRBC or hemolysate abolished this activity. The fact that macrophages fixed after exposure to antigen were also unable to produce the antisuppressor mediator suggests that some other as yet unidentified signal(s) between the macrophage and T cell may be required.

4.2 Treatment of macrophages with lysosomotropic agents

It was decided to use the lysosomotropic agents chloroquine and

Fig. 5 Metabolic requirement of macrophages during generation of antisuppressor mediator

Group	SC	Ts	(Mϕ-T) SUP Mϕ Treatment	NMS
1	Balb/c	-	-	-
2	"	+	-	-
3	"	+	None	Balb/c
4	"	+	Fixed Pre-Ag	"
5	"	+	Fixed Post-Ag	"
6	"	+	Hemolysate	"
7	"	+	Fixed Pre-Hemolysate	"
8	"	+	Fixed Post-Hemolysate	"
9	"	+	Chloroquine	"
10	"	+	NH_4Cl	"


IgG anti-SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
31.3
2
3
36.3
4
31.3
5
30.4
6
37.2
7
31.3
8
31.1
9
28.3
10
32.3

NH_4Cl to demonstrate that inhibition of antigen catabolism would abolish the production of the antisuppressor mediator. Chloroquine and NH_4Cl are thought to exert their effects via disruption of normal lysosome function, i.e. by raising lysosomal pH with concomitant decrease in the activity of acid hydrolases (Poole *et al.*, 1980). The macrophages were exposed continuously to these agents during the 4 h incubation with SRBC. The adherent cells were subsequently co-cultured with NW T cells. As expected, the cell-free supernatant from these treated macrophages was lacking the antisuppressor mediator (Fig. 5, groups 9 and 10) by RICA and the PFC assay.

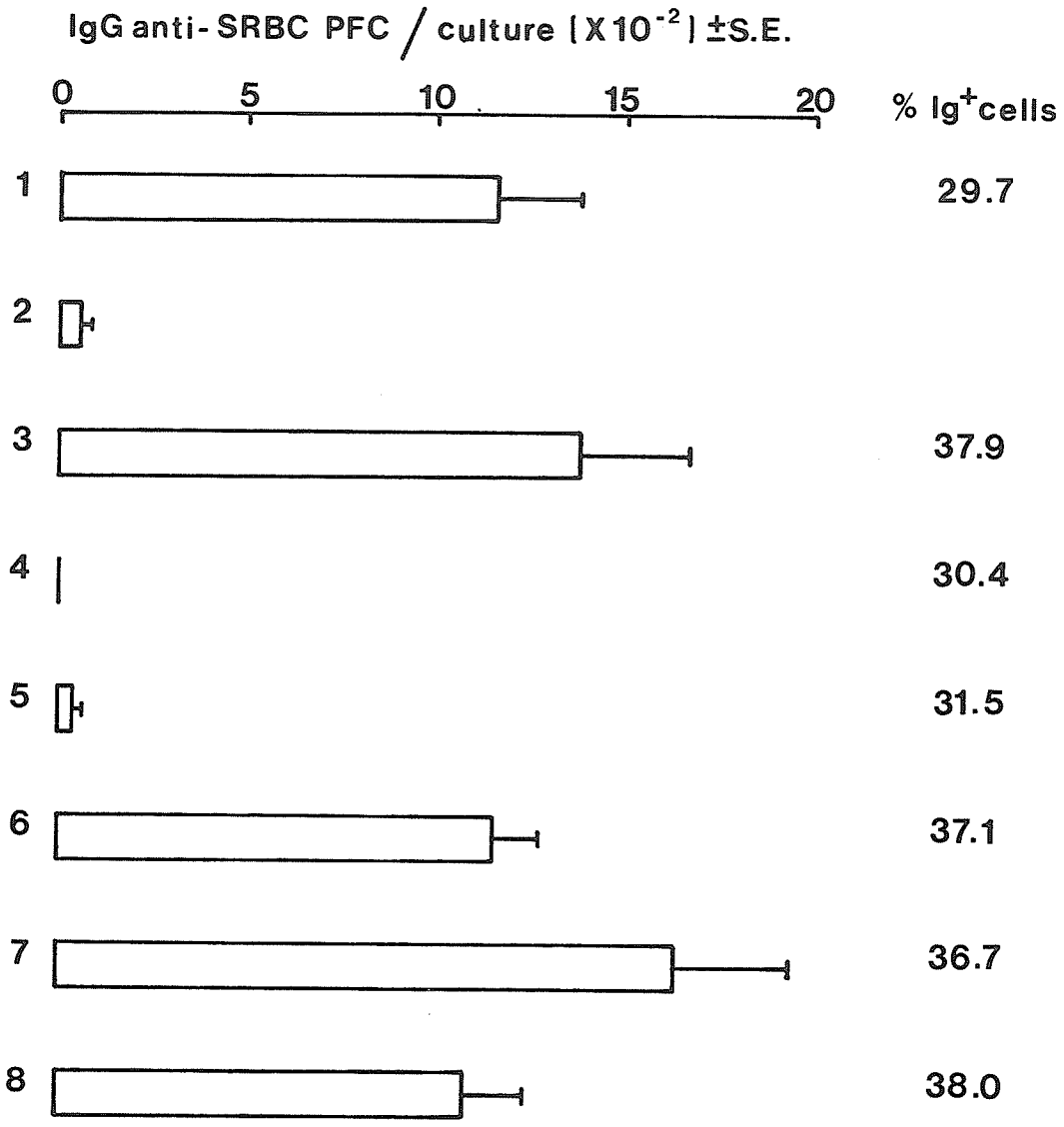
5. Role of MHC class II antigens during generation of antisuppressor mediator

5.1 Elimination of Ia^+ macrophages

Previous studies from this laboratory have demonstrated that Ia antigen was one of the serologically detectable components of the complexes (Paraskevas *et al.*, 1984). In addition, Maeba *et al.* (1988) have provided evidence that the antisuppressor activity was removed from the supernatant of the macrophage-T cell co-culture by adsorption to a specific anti-Ia immunoadsorbent column. Therefore, it was expected that elimination of Ia^+ macrophages from the PECs would also result in a loss of antisuppressor activity. Fig. 6 shows the IgG anti-SRBC response of Balb/c spleen cells *in vitro* (group 1), the suppression of this response (group 2) and the subsequent antisuppression (group 3) when normal macrophages were used for the

Fig. 6 Role of MHC class II antigens during generation of antisuppressor mediator

Group	SC	Ts	(Mϕ-T) SUP Mϕ Treatment	NMS
1	Balb/c	-	-	-
2	"	+	-	-
3	"	+	None	Balb/c
4	"	+	anti-I-A^d+C'	"
5	"	+	anti-I-A^d	"
6	"	+	anti-I-$E^{k/d}$	"
7	"	+	anti-I-A^k	"
8	"	+	anti-I-A^b	"


IgG anti-SRBC PFC / culture [X10^{-2}] ±S.E.
0
5
10
15
20
% Ig^{+}cells
1
29.7
2
3
37.9
4
30.4
5
31.5
6
37.1
7
36.7
8
38.0

production of the antisuppressor mediator. The Ia^+ macrophages were eliminated by treating the PECs with anti-I-A^d mAb plus complement and the remaining Ia^- cells were pulsed with antigen then co-cultured with NW T cells. As shown in Fig. 6 (group 4), the supernatant after elimination of Ia^+ macrophages was indeed lacking the antisuppressor mediator.

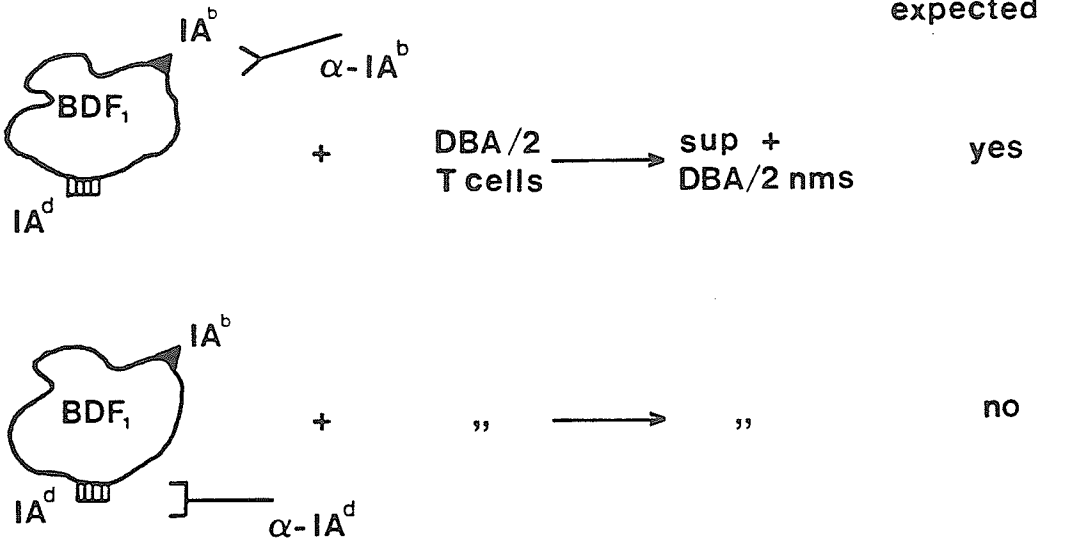
5.2 Blocking with anti-class II mAbs

Monoclonal antibodies with specificities against determinants encoded by the I-A or I-E subregion were used for blocking. As shown in Fig. 6 (group 5) anti-I-A-^{d} mAb was able to block the production of antisuppressor mediator from Balb/c (H-2^d) macrophages whereas anti-I-E^k mAb cross-reacting with H-2^d haplotype was ineffective (group 6). Monoclonal antibodies with specificity against the I-A molecules of H-2^k and H-2^b haplotype were also ineffective (group 7 and 8). While this data suggested that the I-A molecules were involved in the production of the antisuppressor mediator it did not exclude the possibility that the anti-I-A^d mAb may be working by some unknown mechanism.

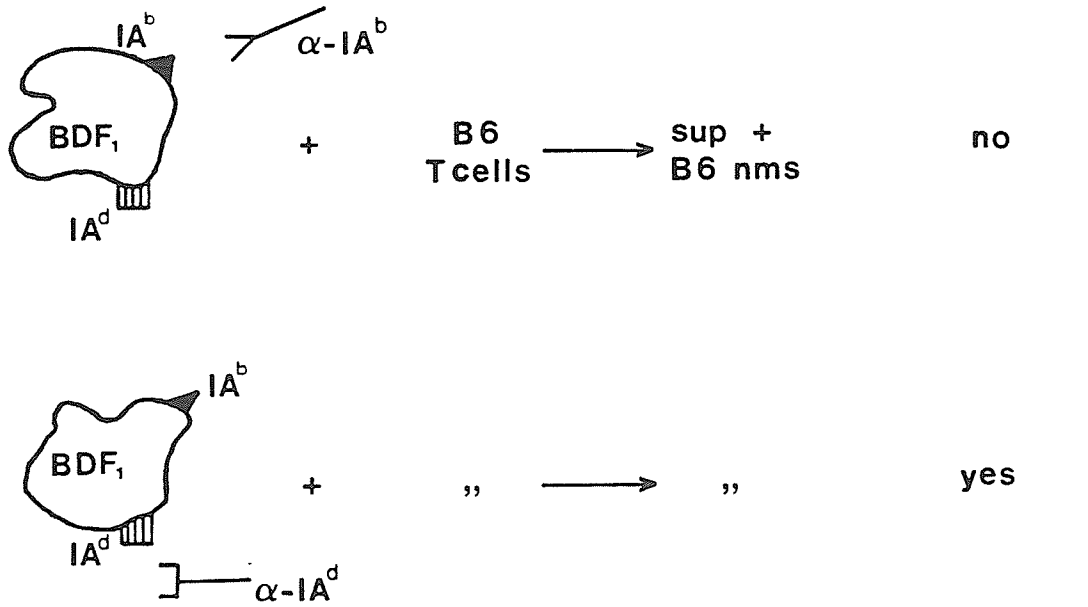
Direct evidence implicating the I-A antigens was provided by using macrophages from BDF_1 (H-$2^{b/d}$) mice. The experimental protocol is in outlined Fig. 7. The BDF_1 macrophages were blocked separately with anti-I-A^b and anti-I-A^d mAbs. These macrophages were subsequently co-cultured with NW T cells, in the first experiment from DBA/2 (H-2^d)

Fig. 7 Blocking MHC class II antigens on BDF_1 macrophages for *in vitro* production of antisuppressor mediator

A) BDF1 Mϕ - DBA/2 NW T cells



B) BDF1 Mϕ - B6 NW T cells



mice (Fig. 7A) and in the second experiment from B6 (H-2^b) mice (Fig.7B). In each case it was expected that blocking the macrophage-T cell interaction with only the specific mAb would abolish the production of the antisuppressor mediator.

The results of the first experiment are shown in Fig. 8. The IgG anti-SRBC response of DBA/2 spleen cells is shown in group 1 and this response was suppressed by antigen-specific Ts cells (group 2). The supernatant from DBA/2 macrophages co-cultured with DBA/2 NW T cells in the presence of DBA/2 NMS had antisuppressor activity (group 3). Substitution of F_1 macrophages in place of DBA/2 macrophages also restored the anti-SRBC response of suppressed cultures (group 4). As expected, blocking of F_1 macrophages with anti-I-A^d mAb and subsequent co-culture with DBA/2 T cells abolished the production of the antisuppressor mediator (group 6) whereas blocking with anti-I-A^b mAb was ineffective (group 5).

The results of the second experiment (Fig. 9) show the IgG anti-SRBC response of B6 spleen cells (group 1) and suppression of the response (group 2). The positive control for antisuppression was supernatant from B6 macrophages co-cultured with B6 NW T cells in the presence of B6 NMS (group 3). Again, using F_1 macrophages instead of B6 macrophages also produced antisuppressor activity (group 4). Blocking F_1 macrophages gave the expected results, i.e., only anti-I-A^b mAb effectively blocked the production of the antisuppressor mediator

Fig. 8 Generation of antisuppressor mediator blocked by anti-I-A^d monoclonal antibody

Group	SC	Ts	(Mϕ-T) SUP Mϕ Treatment	NMS
1	DBA/2	-	-	-
2	"	+	-	-
3	"	+	DBA/2(Mϕ-T)	DBA/2
4	"	+	(BDF_1Mϕ-DBA/2T)	"
5	"	+	(BDF_1Mϕ) anti-I-A^b	"
6	"	+	(BDF_1Mϕ) anti-I-A^d	"

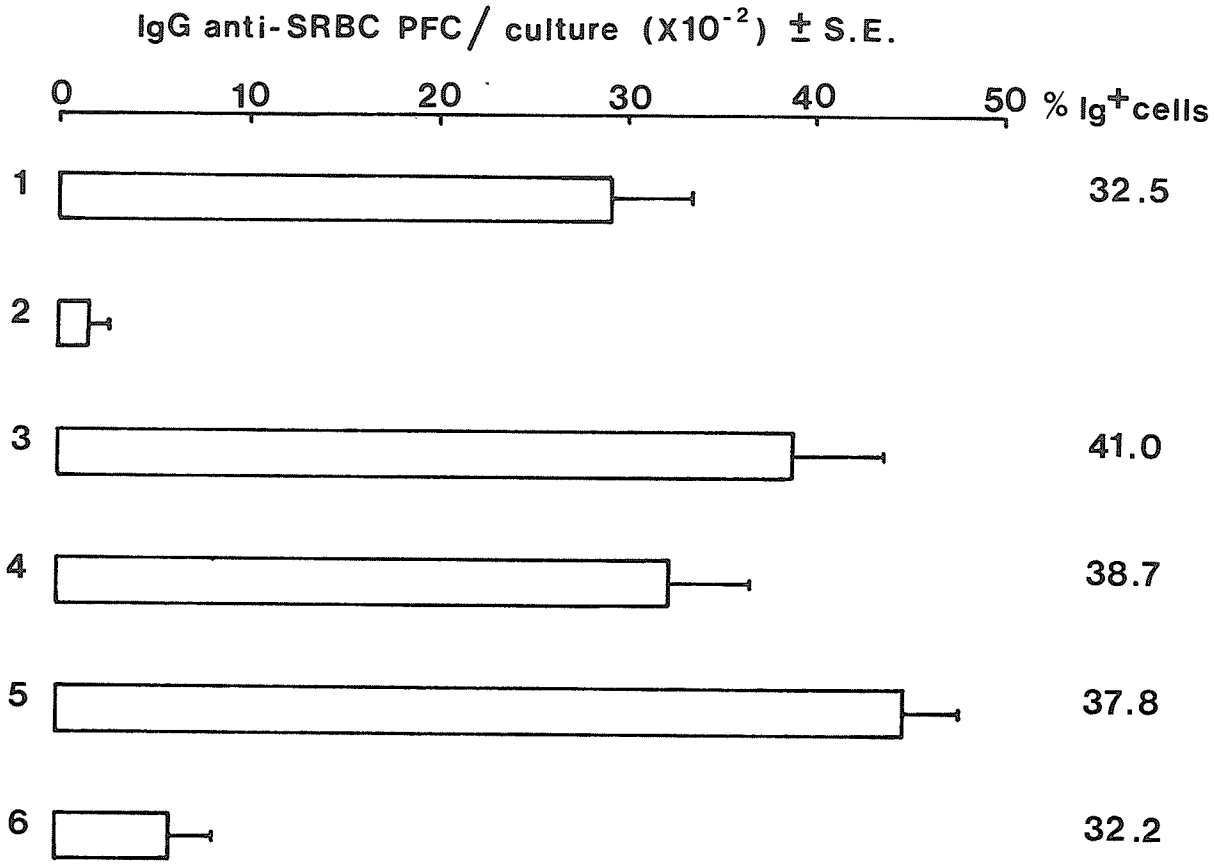
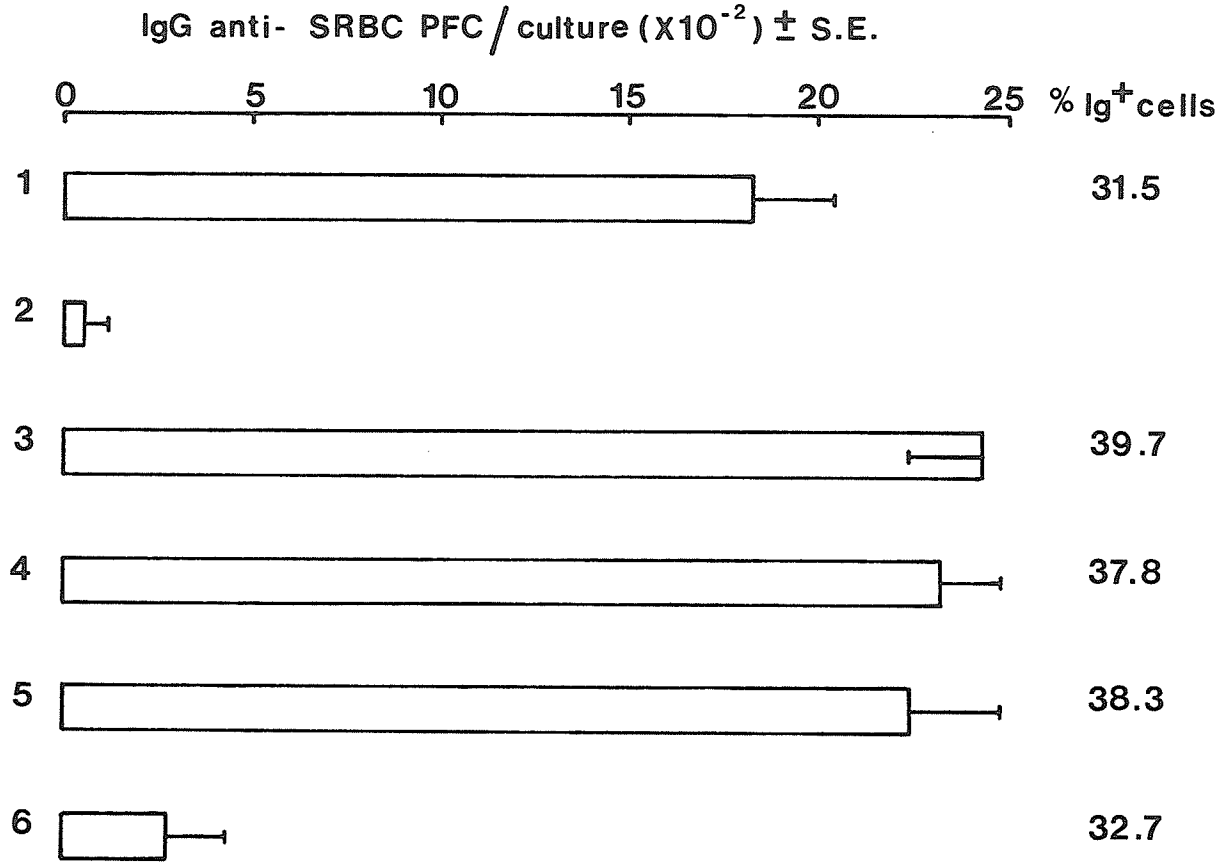

IgG anti-SRBC PFC/ culture (X10^{-2}) ± S.E.
0
10
20
30
40
50
% Ig^{+}cells
1
32.5
2
3
41.0
4
38.7
5
37.8
6
32.2

Fig. 9 Generation of antisuppressor mediator blocked by anti-I-A^b monoclonal antibody

Group	SC	Ts	(Mϕ-T) SUP Mϕ Treatment	NMS
1	B6	-	-	-
2	"	+	-	-
3	"	+	B6(Mϕ-T)	B6
4	"	+	(BDF_1Mϕ-B6T)	"
5	"	+	(BDF_1Mϕ) anti-I-A^d	"
6	"	+	(BDF_1Mϕ) anti-I-A^b	"


IgG anti- SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
25
% Ig^{+} cells
1
31.5
2
3
39.7
4
37.8
5
38.3
6
32.7

(group 6) whereas the anti-I-A^d mAb was ineffective (group 5).

Therefore, these data suggested that the macrophage-T cell interaction involved I-A antigens.

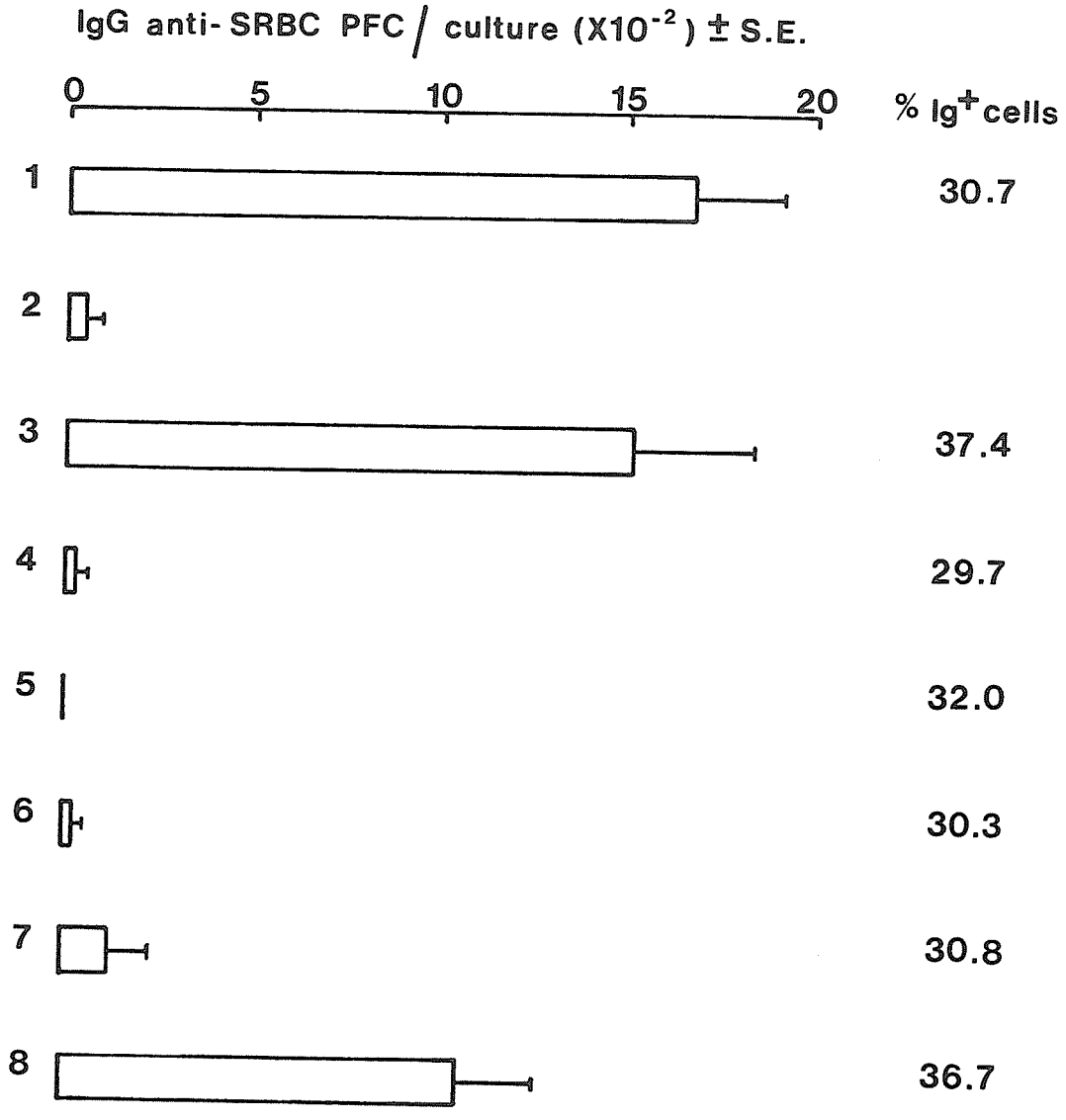
6. Characterization of T cell surface antigens in the generation of antisuppressor mediator

6.1 Role of T cells

In the present study an attempt was made to characterize the T cell surface molecules that may be involved in the macrophage-T cell interaction for the production of the antisuppressor mediator. Fig. 10 shows the IgG anti-SRBC response of Balb/c spleen cells (group 1) and the suppression of this response by Ts cells (group 2). The addition of the cell-free supernatant from the macrophage-T cell co-culture in the presence of Balb/c NMS restored the anti-SRBC response (group 3). However, when supernatant obtained from macrophages cultured in the absence of NW T cells together with Balb/c NMS was used, no antisuppressor activity was observed (group 4). Previous studies have established the requirement for T cells in the production of the anti-suppressor mediator (Paraskevas *et al.*, 1985a; Maeba *et al.*, 1988). In order to demonstrate that cell-cell interactions between macrophages and T cells may be required, the T cells were cultured alone using the cell-free supernatant of SRBC-pulsed macrophages. When the supernatant from these T cells not directly stimulated by APCs was examined in the presence of Balb/c NMS it was found lacking in antisuppressor activity (group 5).

Fig. 10 Characterization of T cell surface antigens in the generation of antisuppressor mediator

Group	SC	Ts	(Mϕ-T) SUP T cell Treatment	NMS
1	Balb/c	-	-	-
2	"	+	-	-
3	"	+	None	Balb/c
4	"	+	No T-cells	"
5	"	+	No Mϕ	"
6	"	+	anti-L3T4 + C'	"
7	"	+	anti-L3T4	"
8	"	+	anti-Ly 2.2	"


IgG anti-SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
30.7
2
3
37.4
4
29.7
5
32.0
6
30.3
7
30.8
8
36.7

6.2 Elimination of $L3T4^+$ cells

The phenotype of the antisuppressor inducer cell has been established as the Lyl^+ T cell from *in vivo* studies (Paraskevas *et al.*, 1985a) and also from the *in vitro* work of Maeba *et al.* (1988). Therefore, it was decided to examine whether the elimination of $L3T4^+$ cells would consequently abolish the production of the antisuppressor mediator. The NW T cells were treated with anti-L3T4 mAb plus complement and the remaining cells co-cultured with SRBC-pulsed macrophages. The cell-free supernatant in the presence of Balb/c NMS was found lacking in antisuppressor activity (group 6).

6.3 Blocking with anti-L3T4 mAb

It is widely accepted that the MHC class II molecules on the surface of APCs interact with the L3T4 antigen on the surface of T cells (Wilde *et al.*, 1983). Previous experiments in this study showed that anti-I-A mAbs could effectively block the production of the antisuppressor mediator. Therefore, the anti-L3T4 mAb was used to saturate the L3T4 molecules on the surface of T cells. These cells were co-cultured with SRBC pulsed macrophages. As expected, the anti-L3T4 mAb effectively blocked the production of the antisuppressor mediator (group 7) whereas the anti-Ly2 mAb was ineffective (group 8).

7. Igh restriction in the generation of antisuppressor mediator

In order to examine the Igh restriction imposed during the assembly of antisuppressor complexes strains of mice which were

identical at the MHC locus but differed only at the Igh locus were used. Previous studies (Paraskevas *et al.*, 1984) had shown that induction of the antisuppressor mediator was abolished in lethally irradiated mice and T cells alone reconstituted its formation. Therefore, the experimental approach (Fig. 11) involved lethal irradiation of mice and subsequent reconstitution 24 h later with: (a) syngeneic NW T cells, or (b) Igh congenic NW T cells. The mice were immunized with $5x10^8$ SRBC and the serum (6HS) collected 6 h later. The antisuppressor activity of the 6HS was examined by the RICA and PFC assays.

7.1 Balb/c ($H\text{-}2^d$, Igh^a) - CB.20 ($H\text{-}2^d$, Igh^b) combination

Fig. 12 (group 1) shows the IgG anti-SRBC response of Balb/c spleen cells and suppression of this response by Ts cells (group 2). The antisuppressor activity was present in the 6HS of irradiated Balb/c mice reconstituted with syngeneic T cells (group 3). In contrast, this activity was lacking in the 6HS of irradiated CB.20 mice reconstituted with Igh congenic Balb/c T cells (group 4). However, addition of Balb/c NMS to CB.20 6HS showed the presence of complexes by the RICA assay and the antisuppressor function in the PFC assay (group 5). When NMS from other strains was added to CB.20 6HS it was observed that only Balb/b (Igh^a) NMS restored antisuppressor activity (group 6), whereas serum from B6 (Igh^b) or DBA/2 (Igh^c) mice (groups 7 and 8, respectively) was ineffective.

Fig. 11 Protocol to examine Igh restriction in the generation of antisuppressor mediator

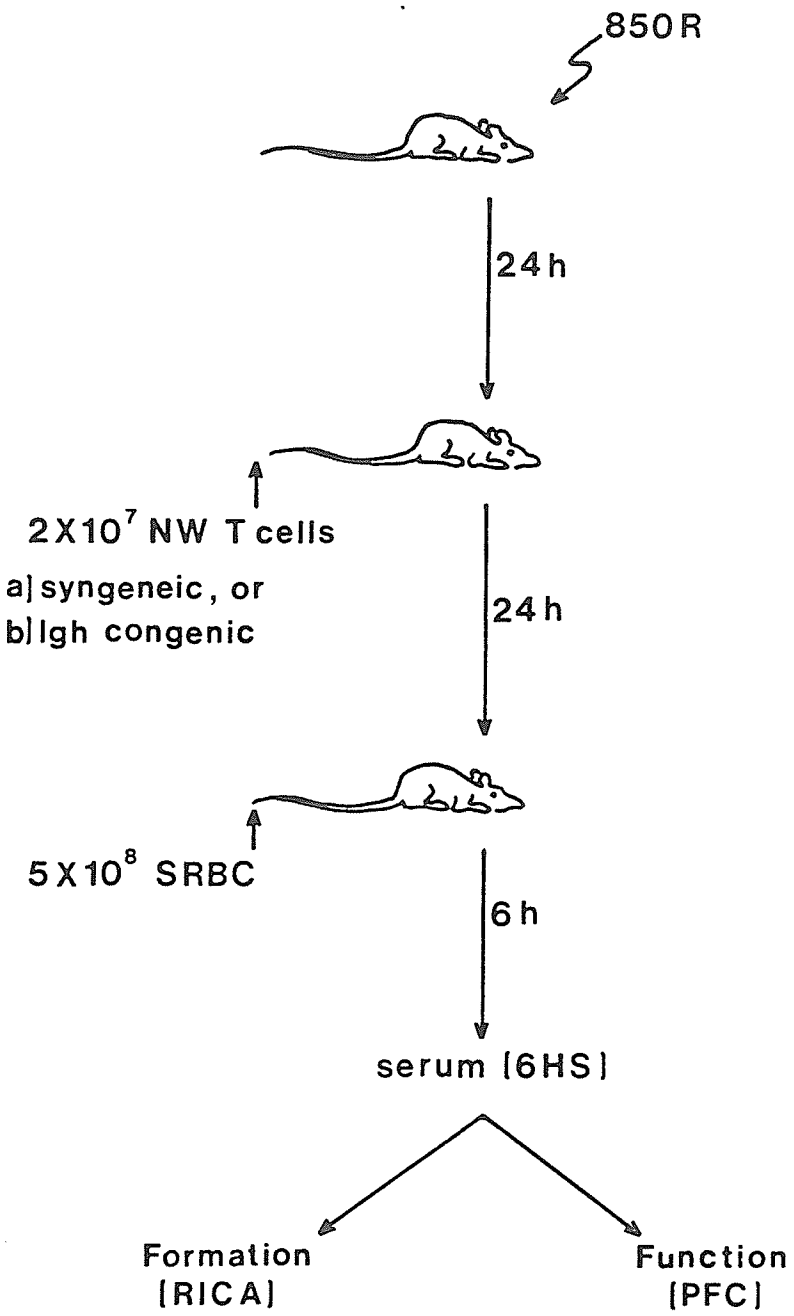
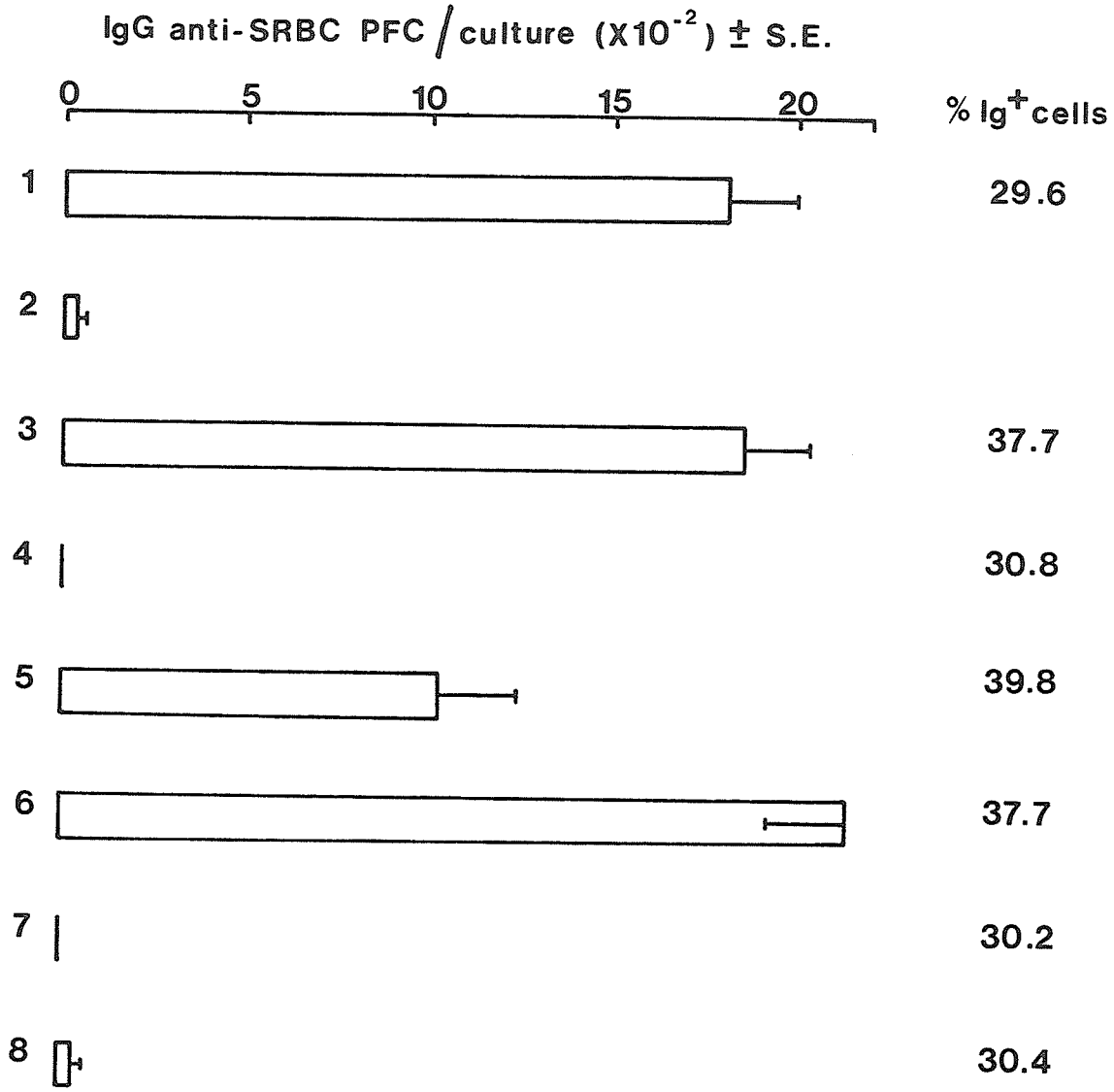


Fig. 12 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	NMS
1	Balb/c	-	-	-	-
2	"	Balb/c	-	-	-
3	"	"	Balb/c	Balb/c	-
4	"	"	Balb/c	CB.20	-
5	"	"	"	"	Balb/c, Igh^a
6	"	"	"	"	Balb/b, Igh^a
7	"	"	"	"	B6, Igh^b
8	"	"	"	"	DBA/2, Igh^c


IgG anti-SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
29.6
2
3
37.7
4
30.8
5
39.8
6
37.7
7
30.2
8
30.4

The results of the reverse experiment are shown in Fig. 13, i.e., Igh congenic CB.20 T cells were used to reconstitute irradiated Balb/c mice. The anti-SRBC response of CB.20 spleen cells is shown (Fig. 13, group 1) and this response was suppressed by Ts cells (group 2). The antisuppressor activity was present in the 6HS of irradiated CB.20 mice reconstituted with syngeneic T cells (group 3). This activity was lacking in the 6HS of irradiated Balb/c mice reconstituted with Igh congenic CB.20 T cells (group 4). As expected, addition of CB.20 NMS to Balb/c 6HS showed the presence of complexes by the RICA assay and the antisuppressor function in the PFC assay (group 5). When NMS from other strains was added to Balb/c 6HS it was noted that only B6 (Igh^b) NMS restored antisuppressor activity (group 7), whereas serum from Balb/b (Igh^a) or DBA/2 (Igh^c) mice (groups 6 and 8, respectively) was ineffective.

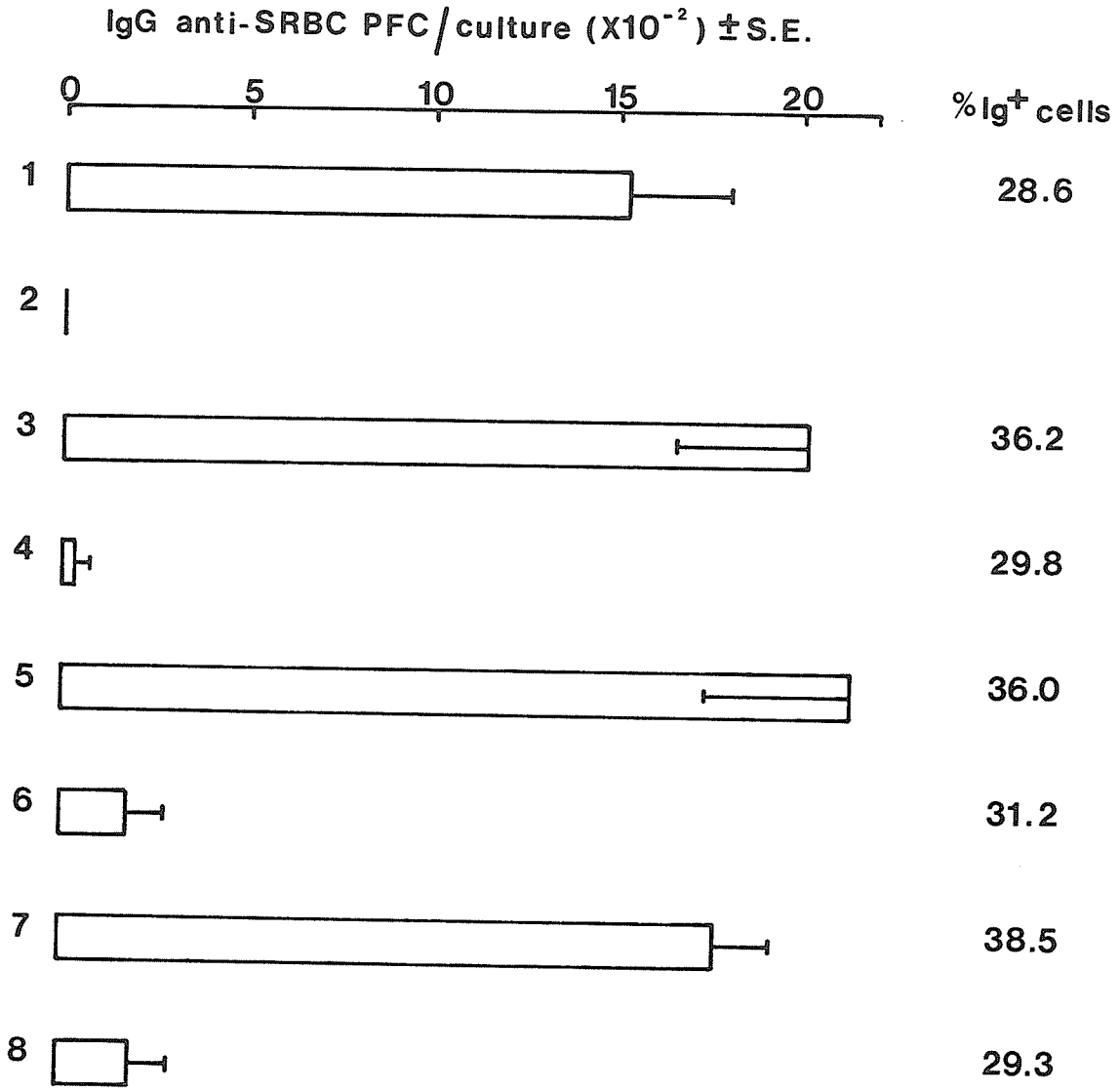
These data suggested the presence of some 'intermediate' component in the 6HS obtained from mice reconstituted with Igh congenic T cells which lacked antisuppressor activity. Furthermore, the addition of NMS from the same Igh haplotype as the T cell donor resulted in complete antisuppressor function.

7.2 Normal mouse IgG is a component of the antisuppressor mediator

Previous studies (Paraskevas *et al.*, 1979) from this laboratory have identified immunoglobulins as one of the components of the 6h complexes. Therefore, normal mouse IgG was purified by protein

Fig. 13 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	NMS
1	CB.20	-	-	-	-
2	"	CB.20	-	-	-
3	"	"	CB.20	CB.20	-
4	"	"	CB.20	Balb/c	-
5	"	"	"	"	CB.20, Igh^b
6	"	"	"	"	Balb/b, Igh^a
7	"	"	"	"	B6, Igh^b
8	"	"	"	"	DBA/2, Igh^c


IgG anti-SRBC PFC/culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig+ cells
1
28.6
2
3
36.2
4
29.8
5
36.0
6
31.2
7
38.5
8
29.3

A-Sepharose affinity chromatography from the serum of each mouse strain used in this study. The experiments described in sec. 7.1 were repeated using purified normal IgG in order to demonstrate that only IgG of the appropriate Igh haplotype was effective.

Fig. 14 shows the IgG anti-SRBC response (group 1) and suppression upon addition of Ts cells (group 2). The suppressed response was antisuppressed by 6HS from irradiated Balb/c mice reconstituted with syngeneic T cells (group 3). Suppression was maintained when 6HS from irradiated CB.20 mice reconstituted with Balb/c T cells was used (group 4). As shown previously, the addition of Balb/c NMS (group 5) or Balb/b NMS (group 8) to CB.20 6HS restored the antisuppressor activity. Addition of the serum components lacking IgG from Balb/c (group 7) or Balb/b (group 10) mice was ineffective whereas the purified IgG (groups 6 and 9) restored complete antisuppressor activity as evident by the RICA and PFC assays.

These findings were verified in the reverse experiment (Fig. 15) when irradiated Balb/c mice were reconstituted with CB.20 T cells. The anti-SRBC response of CB.20 spleen cells is shown (group 1) and the suppression of this response (group 2). The 6HS from irradiated CB.20 mice reconstituted with syngeneic T cells contained antisuppressor activity (group 3), whereas 6HS from Balb/c mice reconstituted with CB.20 T cells was lacking such activity (group 4). The addition of CB.20 NMS (group 5) or B6 NMS (group 8) to Balb/c 6HS restored the

Fig. 14 Normal IgG is required for generation of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	NMS
1	Balb/c	-	-	-	-
2	"	Balb/c	-	-	-
3	"	"	Balb/c	Balb/c	-
4	"	"	Balb/c	CB.20	-
5	"	"	"	"	B/c NMS
6	"	"	"	"	B/c IgG
7	"	"	"	"	B/c (NMS-IgG)
8	"	"	"	"	B/b NMS
9	"	"	"	"	B/b IgG
10	"	"	"	"	B/b (NMS-IgG)

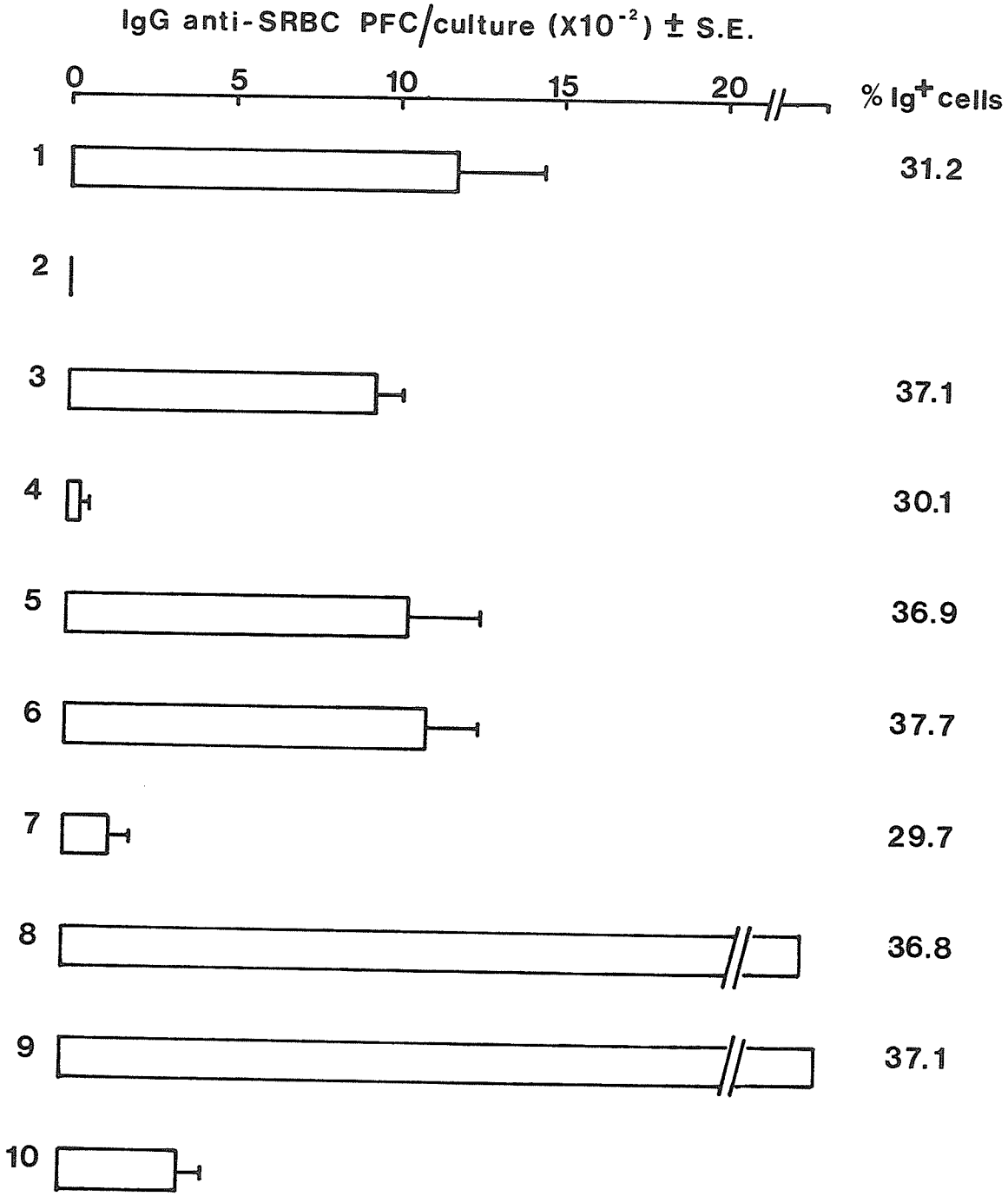
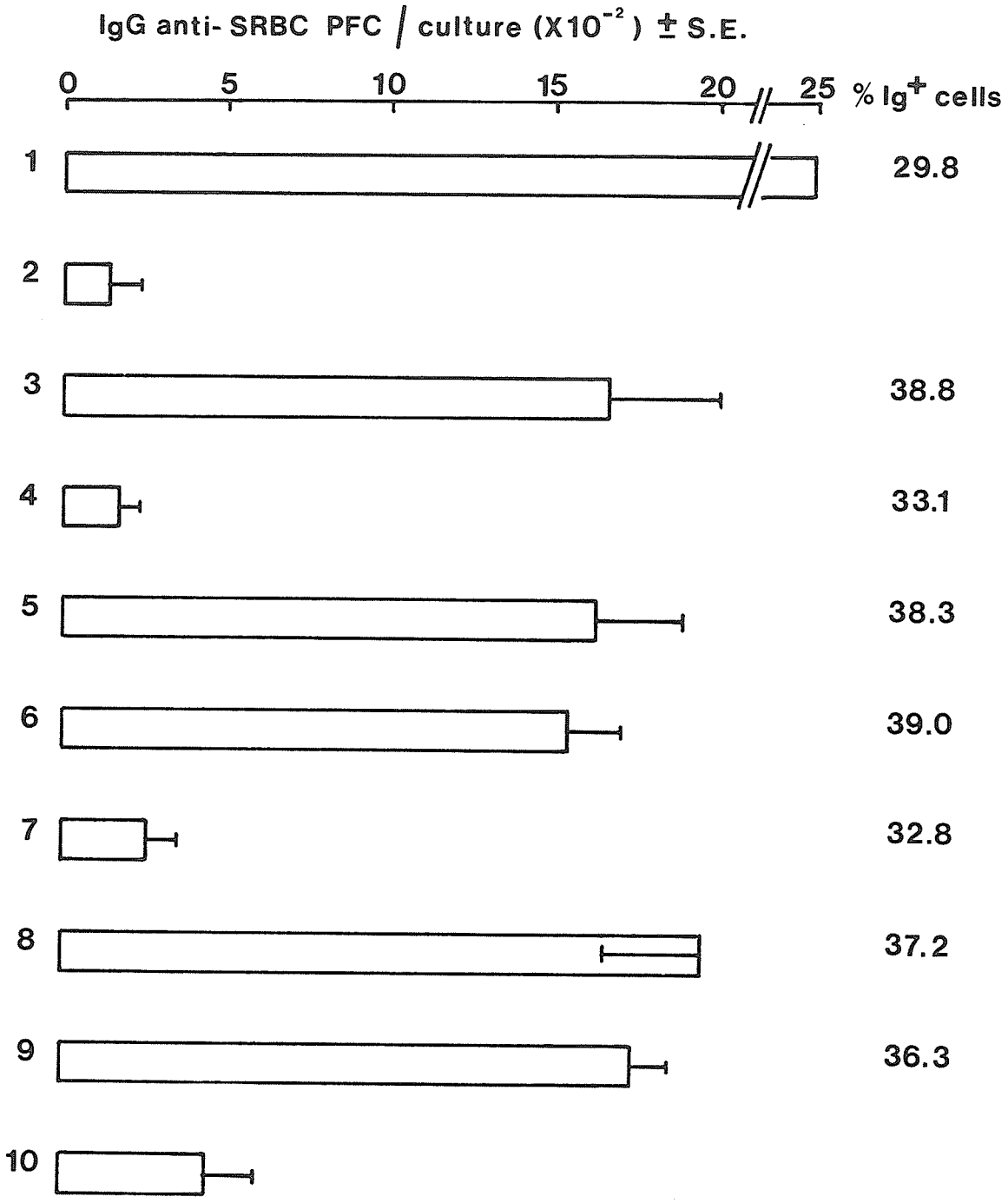

IgG anti-SRBC PFC/culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
31.2
2
3
37.1
4
30.1
5
36.9
6
37.7
7
29.7
8
36.8
9
37.1
10

Fig. 15 Normal IgG is required for generation of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	NMS
1	CB.20	-	-	-	-
2	"	CB.20	-	-	-
3	"	"	CB.20	CB.20	-
4	"	"	CB.20	Balb/c	-
5	"	"	"	"	CB.20 NMS
6	"	"	"	"	CB.20 IgG
7	"	"	"	"	CB.20 (NMS-IgG)
8	"	"	"	"	B6 NMS
9	"	"	"	"	B6 IgG
10	"	"	"	"	B6 (NMS-IgG)


IgG anti- SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
25
% Ig^{+} cells
1
29.8
2
3
38.8
4
33.1
5
38.3
6
39.0
7
32.8
8
37.2
9
36.3
10

antisuppressor activity. Addition of serum components lacking IgG from CB.20 (group 7) or B6 (group 10) mice was ineffective whereas the purified IgG (groups 6 and 9) restored complete antisuppressor activity.

7.3 Cal.20 (H-2^d, Igh^d) - Balb/c (H-2^d, Igh^a) combination

These studies were extended to include other Igh congenic strains. Fig. 16 shows the IgG anti-SRBC response of Cal.20 spleen cells (group 1) and the suppression of this response by Ts cells (group 2). The 6HS from irradiated Cal.20 mice reconstituted with syngeneic T cells showed antisuppressor activity (group 3). However, 6HS from irradiated Balb/c mice reconstituted with Cal.20 T cells lacked antisuppressor activity (group 4). The addition of Cal.20 IgG to the Balb/c 6HS restored antisuppressor activity (group 5). Furthermore, addition of IgG from AKR (Igh^d) mice to Balb/c 6HS was equally effective in restoring antisuppressor function (group 7), whereas Balb/c (Igh^a) IgG was ineffective (group 6).

In the reverse experiment (Fig. 17) irradiated Cal.20 mice were reconstituted with Igh congenic Balb/c T cells. The anti-SRBC response of Balb/c spleen cells is shown in Fig. 17 (group 1) and the suppression of this response by Ts cells (group 2). The 6HS from irradiated Balb/c mice reconstituted with syngeneic T cells showed antisuppressor activity (group 3). This activity was lacking in the

Fig. 16 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	Cal.20	-	-	-	-
2	"	Cal.20	-	-	-
3	"	"	Cal.20	Cal.20	-
4	"	"	Cal.20	Balb/c	-
5	"	"	"	"	Cal.20, Igh^d
6	"	"	"	"	Balb/b, Igh^a
7	"	"	"	"	AKR, Igh^d

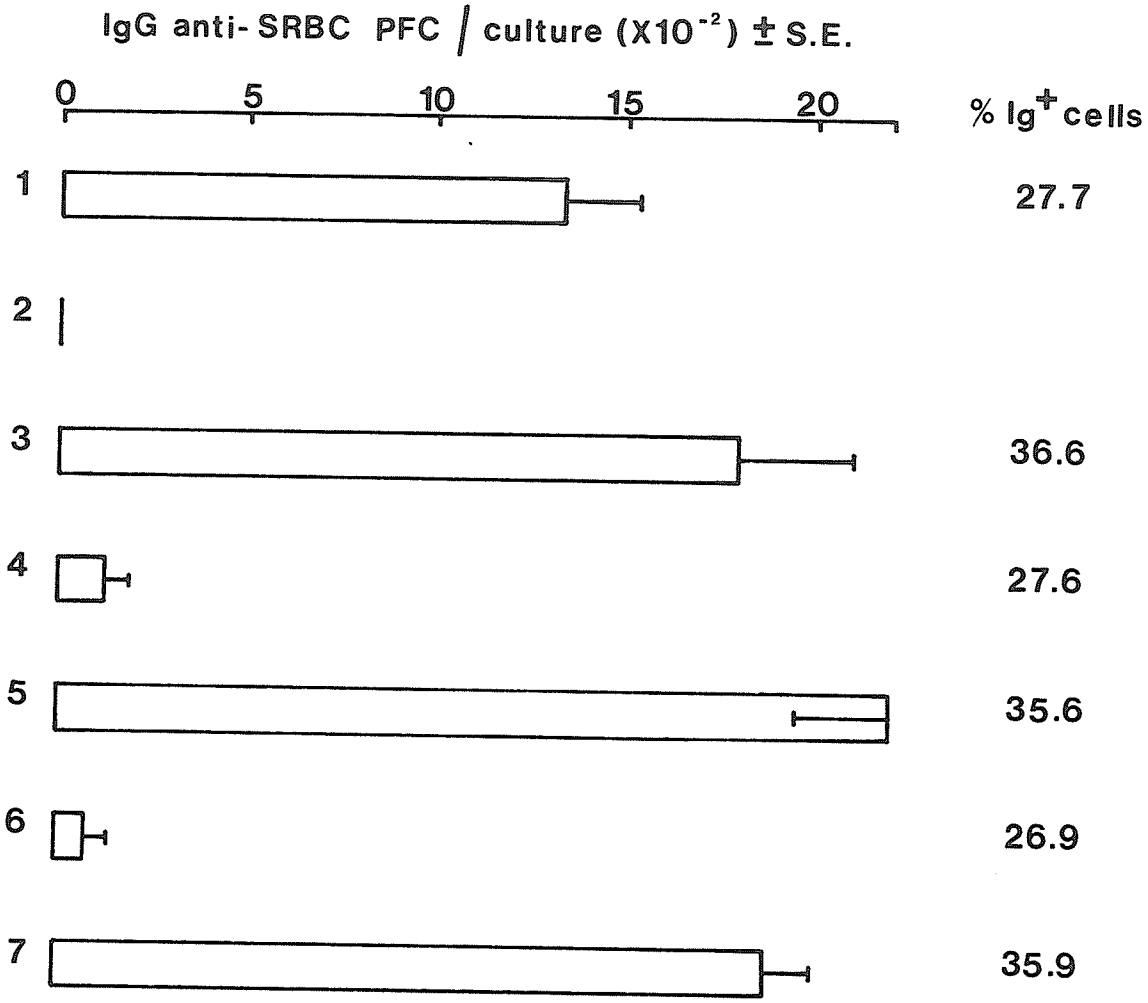
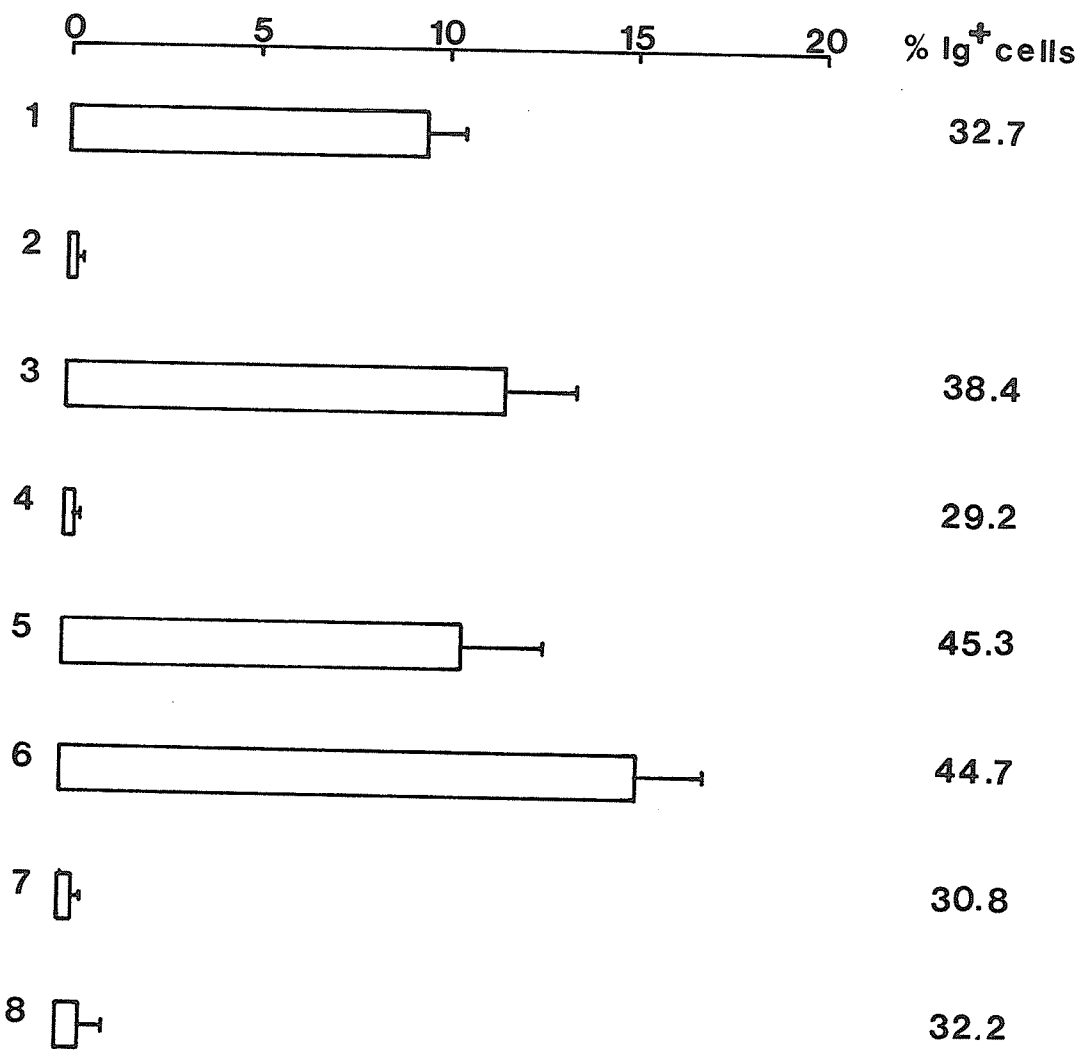

IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig+ cells
1
27.7
2
3
36.6
4
27.6
5
35.6
6
26.9
7
35.9

Fig. 17 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	Balb/c	-	-	-	-
2	"	Balb/c	-	-	-
3	"	"	Balb/c	Balb/c	-
4	"	"	Balb/c	Cal.20	-
5	"	"	"	"	Balb/c Igh^a
6	"	"	"	"	$Balb/^b$ Igh^a
7	"	"	"	"	AKR, Igh^d
8	"	"	"	"	DBA/2, Igh^c


IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig+ cells
1
32.7
2
3
38.4
4
29.2
5
45.3
6
44.7
7
30.8
8
32.2

6HS of irradiated Cal.20 mice reconstituted with Balb/c T cells (group 4) and was restored upon addition of Balb/c IgG to the Cal.20 6HS (group 5). The addition of Balb/b (Igh^a) IgG to the Cal.20 6HS also restored the antisuppressor activity (group 6), whereas AKR (Igh^d) or DBA/2 (Igh^c) IgG (groups 7 and 8, respectively) was ineffective. These findings were not unexpected since the donor T cells in this case were of Igh^a haplotype.

7.4 Cal.20 ($H\text{-}2^d$, Igh^d) - CB.20 ($H\text{-}2^d$, Igh^b) combination

Fig. 18 shows the anti-SRBC response of Cal.20 spleen cells (group 1) and the suppression of this response by Ts cells (group 2). As expected, 6HS from Cal.20 mice reconstituted with syngeneic T cells showed antisuppressor activity (group 3) whereas 6HS from irradiated CB.20 mice reconstituted with Igh congenic Cal.20 T cells lacked this activity (group 4). As the donor T cells were of Igh^d haplotype, therefore addition of Cal. 20 (Igh^d) IgG (group 5) or AKR (Igh^d) IgG (group 6) to CB.20 6HS restored complete antisuppressor activity whereas Balb/b (Igh^a) IgG was ineffective (group 7).

In the reverse experiment (Fig. 19) similar results were obtained. The anti-SRBC response of CB.20 spleen cells is shown (group 1) and was suppressed by Ts cells (group 2). The 6HS from irradiated CB.20 mice reconstituted with syngeneic T cells showed antisuppressor activity (group 3), whereas 6HS from irradiated Cal.20 mice reconstituted with Igh congenic CB.20 T cells lacked this activity (group 4). In this

Fig. 18 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	Cal.20	-	-	-	-
2	"	Cal.20	-	-	-
3	"	"	Cal.20	Cal.20	-
4	"	"	Cal.20	CB.20	-
5	"	"	"	"	Cal.20, Igh^d
6	"	"	"	"	AKR, Igh^d
7	"	"	"	"	Balb/b, Igh^a

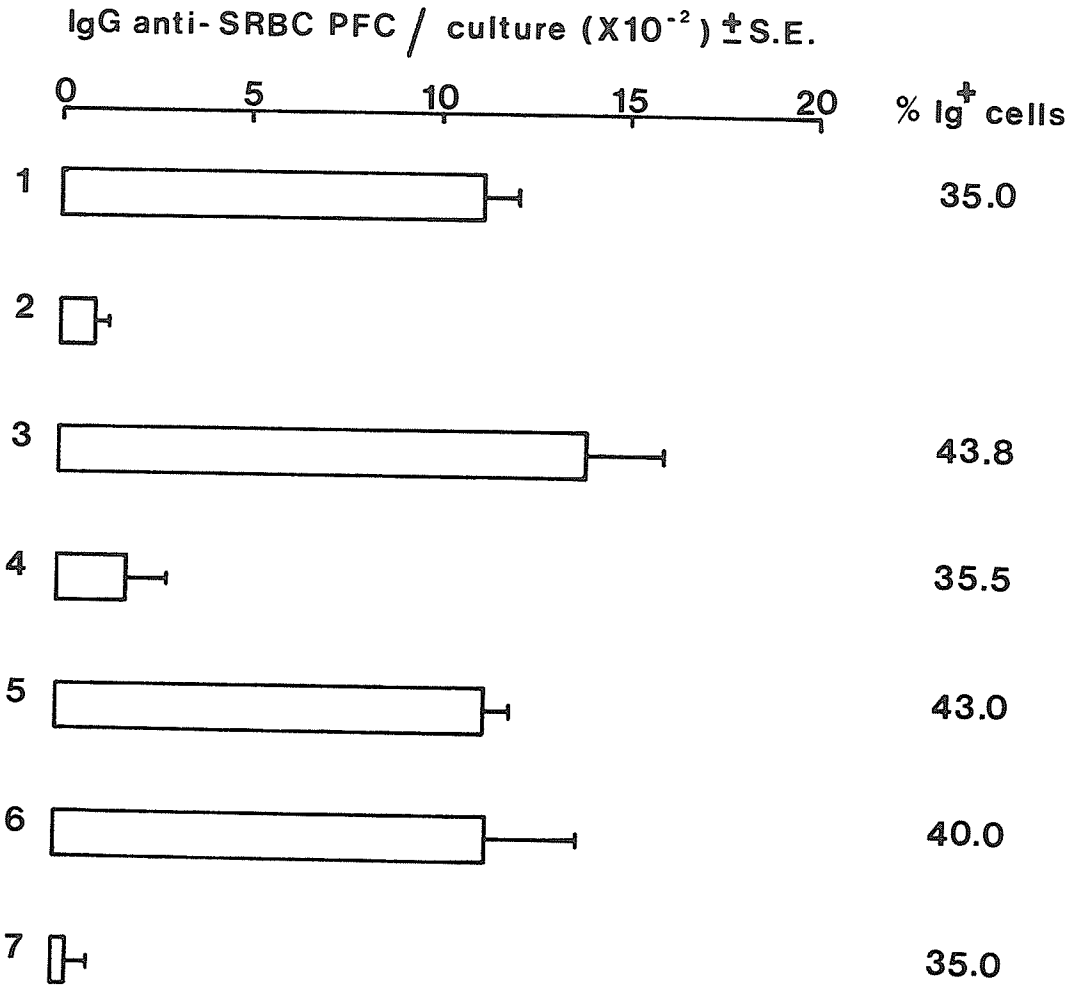
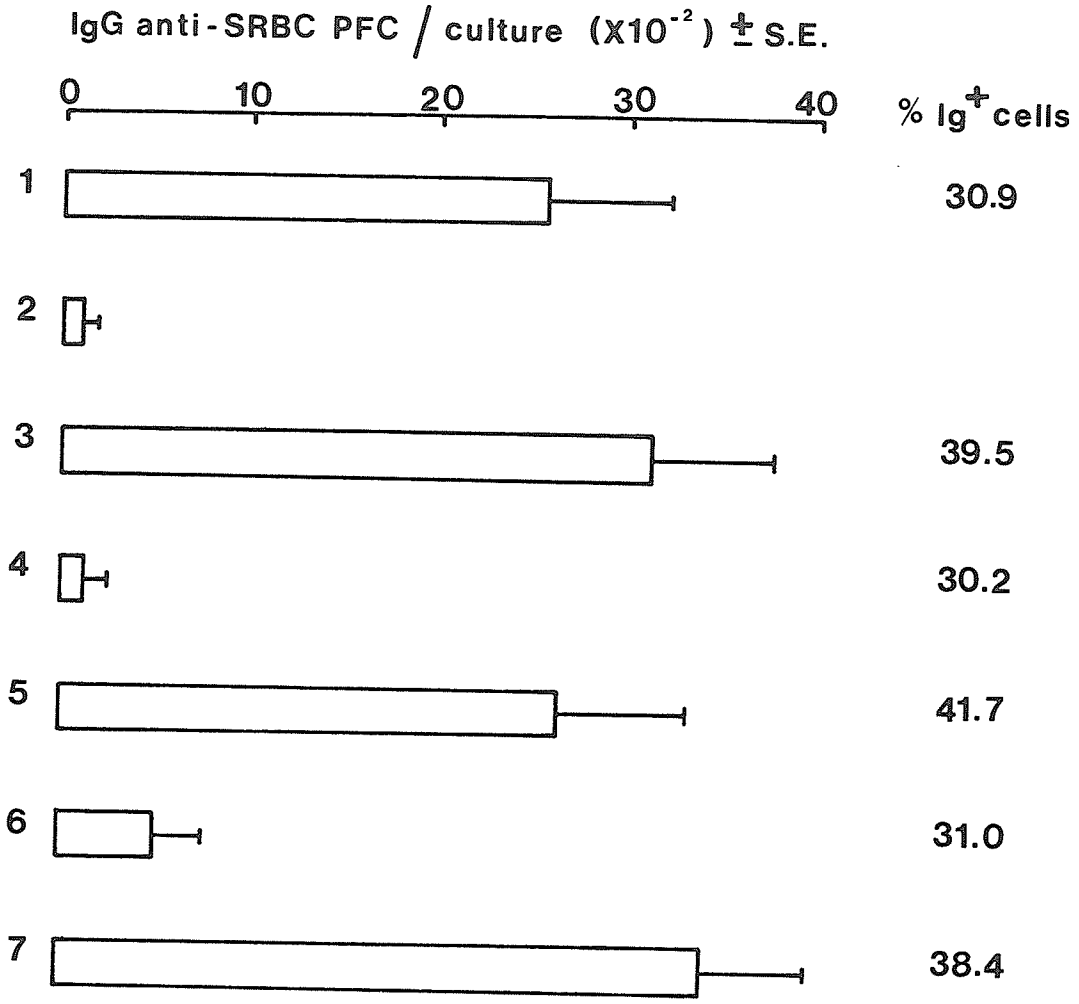

IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig+ cells
1
35.0
2
3
43.8
4
35.5
5
43.0
6
40.0
7
35.0

Fig. 19 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	CB.20	-	-	-	-
2	"	CB.20	-	-	-
3	"	"	CB.20	CB.20	-
4	"	"	CB.20	Cal.20	-
5	"	"	"	"	CB.20, Igh^b
6	"	"	"	"	AKR, Igh^d
7	"	"	"	"	B6, Igh^b


IgG anti-SRBC PFC / culture (X10^{-2}) ± S.E.
0
10
20
30
40
% Ig^{+} cells
1
30.9
2
3
39.5
4
30.2
5
41.7
6
31.0
7
38.4

case, the donor T cells were of Igh^b haplotype, therefore the addition of CB.20 (Igh^b) IgG (group 5) or B6 (Igh^b) IgG (group 7) to Cal.20 6HS restored complete antisuppressor activity whereas AKR (Igh^d) IgG was ineffective (group 6).

7.5 B6 ($H\text{-}2^b$, Igh^b) - Balb/b ($H\text{-}2^b$, Igh^a) combination

It was decided to include one Igh congenic strain combination which was $H\text{-}2^b$ haplotype since the others were $H\text{-}2^d$ haplotype. The results obtained were similar to those shown earlier, i.e., when 6HS from irradiated mice reconstituted with Igh congenic T cells lacked antisuppressor activity and this activity was restored only by the addition of IgG from the same Igh haplotype as the T cell donor.

Fig. 20 (group 1) shows the anti-SRBC response of B6 spleen cells and suppression of this response by Ts cells (group 2). Antisuppressor activity was present in 6HS from irradiated B6 mice reconstituted with syngeneic T cells (group 3), whereas it was lacking in 6HS from irradiated Balb/b mice reconstituted with B6T cells (group 4). The addition of B6 (Igh^b) IgG (group 5) or CB.20 (Igh^b) IgG (group 6) to Balb/b 6HS restored antisuppressor activity, whereas Cal.20 (Igh^d) IgG (group 7) was ineffective.

In the reverse experiment (Fig. 21), the anti-SRBC response of Balb/b spleen cells is shown (group 1) and the suppression of this response by Ts cells (group 2). Again, antisuppressor activity was

Fig. 20 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	B6	-	-	-	-
2	"	B6	-	-	-
3	"	"	B6	B6	-
4	"	"	B6	Balb/b	-
5	"	"	"	"	B6, Igh^b
6	"	"	"	"	CB.20, Igh^b
7	"	"	"	"	Cal.20, Igh^d

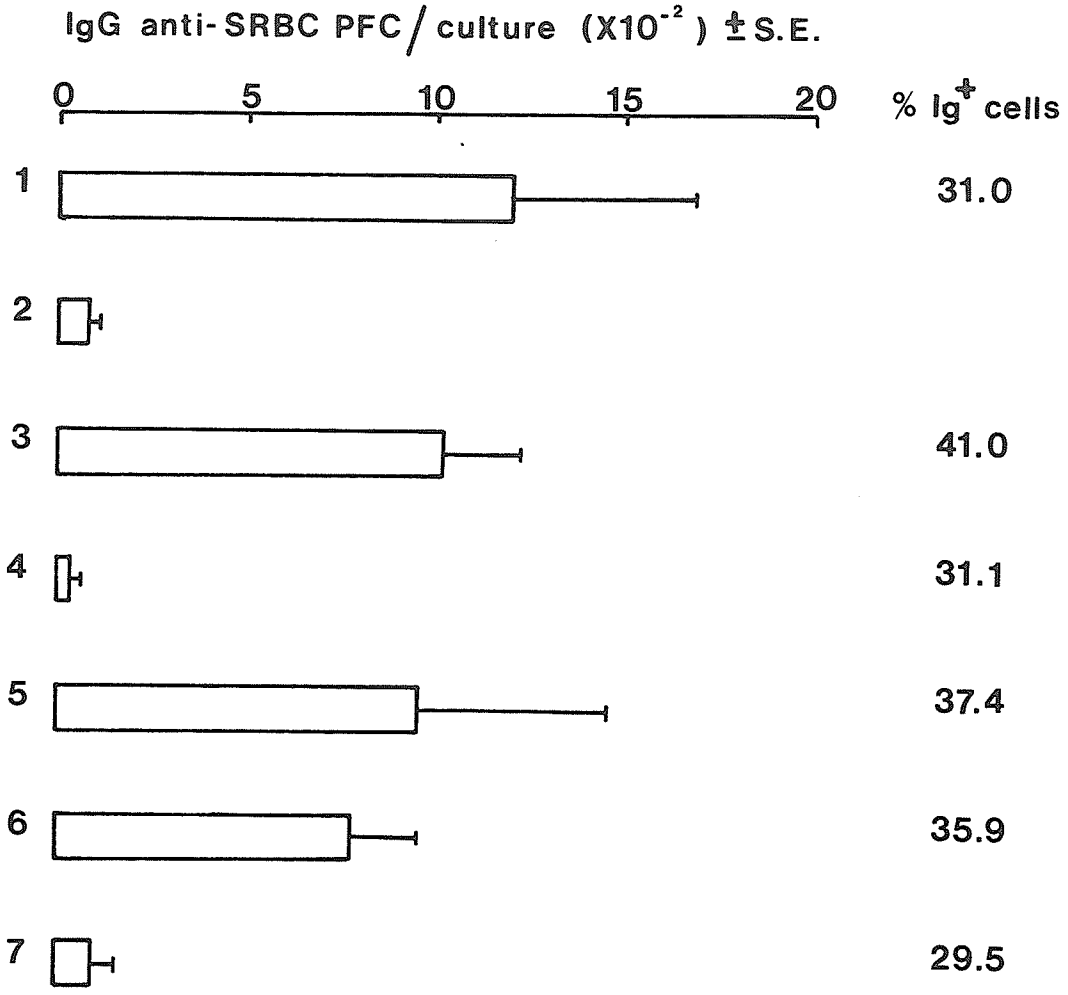
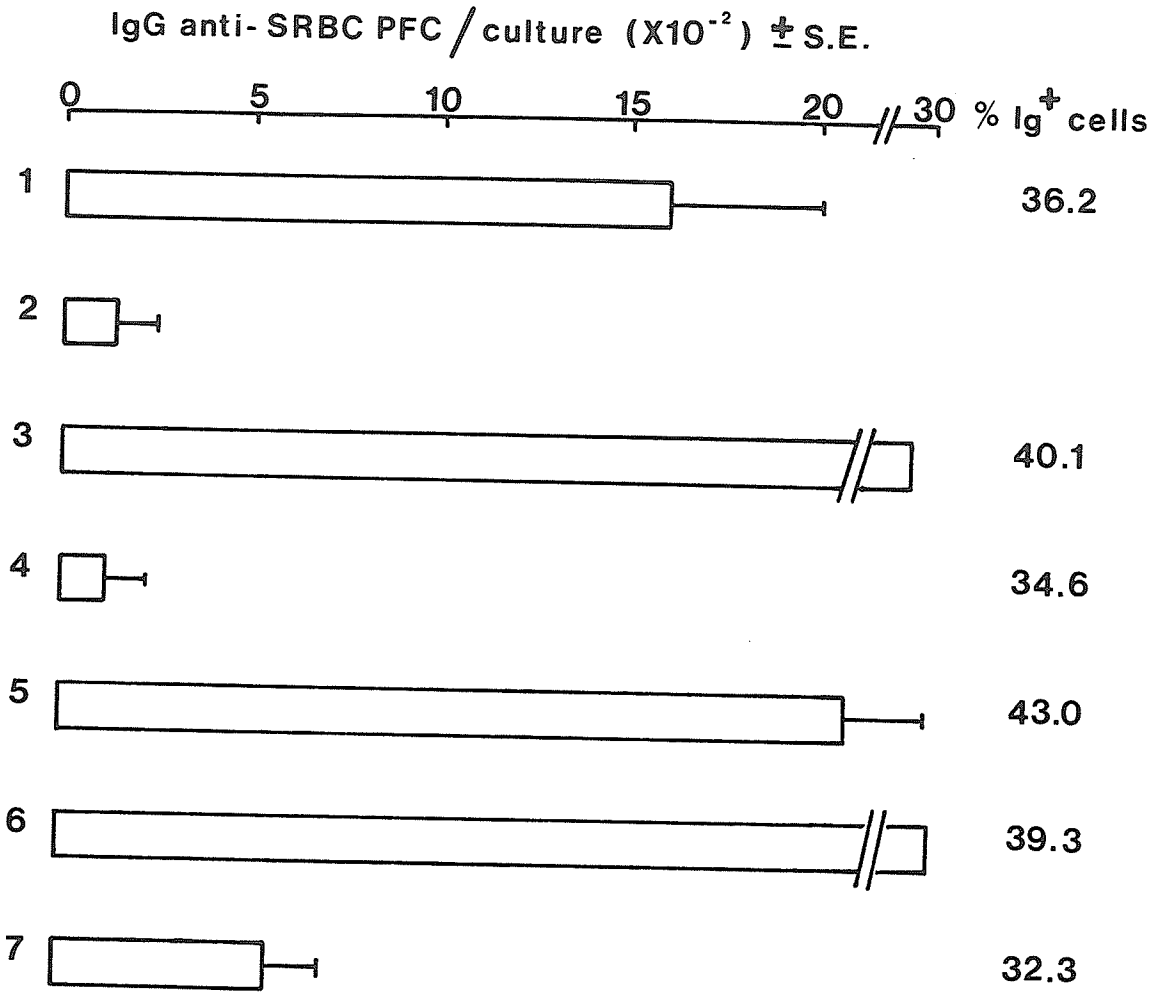

IgG anti-SRBC PFC / culture (X10^{-2}) ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
31.0
2
3
41.0
4
31.1
5
37.4
6
35.9
7
29.5

Fig. 21 Igh restriction in the induction of antisuppressor mediator

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	Balb/b	-	-	-	-
2	"	Balb/b	-	-	-
3	"	"	Balb/b	Balb/b	-
4	"	"	Balb/b	B6	-
5	"	"	"	"	Balb/b, Igh^a
6	"	"	"	"	Balb/c, Igh^a
7	"	"	"	"	Cal.20, Igh^d


IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
30
% Ig+ cells
1
36.2
2
3
40.1
4
34.6
5
43.0
6
39.3
7
32.3

present in 6HS from irradiated Balb/b mice reconstituted with syngeneic T cells (group 3), whereas it was lacking in 6HS from B6 mice reconstituted with Balb/b T cells (group 4). In this case the addition of Balb/b (Igh^a) IgG (group 5) or Balb/c (Igh^a) IgG (group 6) to B6 6HS restored the antisuppressor activity whereas Cal.20 (Igh^d) IgG (group 7) was ineffective.

8. Role of Igh congenic host environment on the genetic restriction of antisuppressor mediator

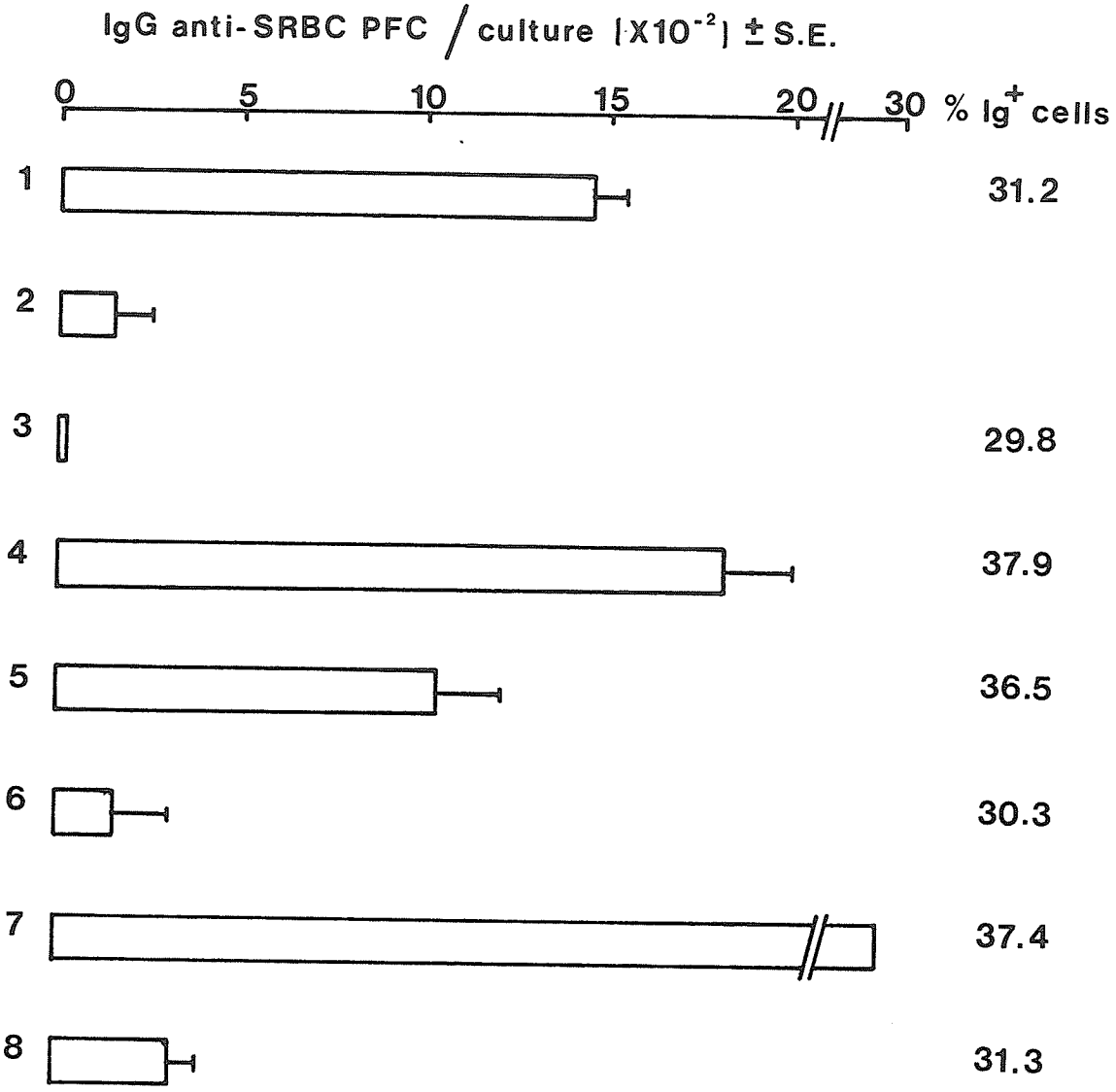
8.1 "Parking" T cells in an Igh congenic host

An attempt was made to examine whether the Igh restriction pattern observed in the induction of the antisuppressor mediator could be altered. In the preceding experiments, irradiated mice were reconstituted with Igh congenic T cells and immunized 24 h later with SRBC. Studies by HayGlass *et al.* (1985) have shown that longer exposure to an Igh distinct environment resulted in an altered pattern of Igh restriction. Therefore, in the present study, irradiated mice were reconstituted with Igh congenic T cells for 5 days. After 5 days the mice were immunized with SRBC and the 6HS examined for antisuppressor activity.

The results of this experiment are shown in Fig. 22 where groups 1-5 were controls. The anti-SRBC response of Balb/c spleen cells (group 1) was suppressed upon addition of Ts cells (group 2). The 6HS collected from irradiated mice reconstituted with syngeneic T cells for

Fig. 22 'Parking' T cells in an Igh congenic host

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
1	Balb/c	-	-	-	-
2	"	Balb/c	-	-	-
3	"	"	-	Balb/c	-
4	"	"	Balb/c	" (d1)	-
5	"	"	"	" (d5)	-
6	"	"	"	CB.20 (d5)	-
7	"	"	"	"	Balb/c, Igh^a
8	"	"	"	"	CB.20, Igh^b


IgG anti-SRBC PFC / culture [X10⁻²] ± S.E.
0
5
10
15
20
30
% Ig+ cells
1
31.2
2
3
29.8
4
37.9
5
36.5
6
30.3
7
37.4
8
31.3

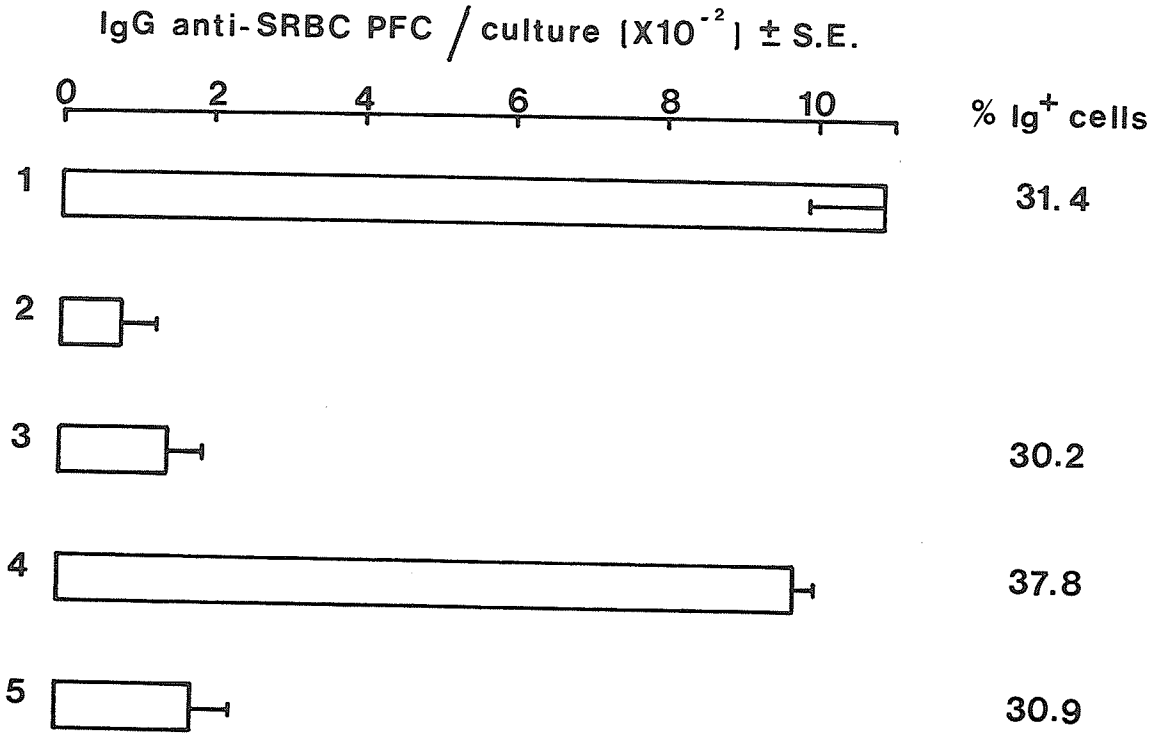
24 h or 5 days (groups 4 and 5, respectively) had antisuppressor activity. In the absence of reconstitution with T cells (group 3) antisuppressor activity was lacking. This data indicated that antisuppressor activity could be induced even 5 days after reconstitution. When the 6HS from irradiated CB.20 mice reconstituted for 5 days with Igh congenic Balb/c T cells was examined antisuppressor activity was not detected (group 6). This suggested that the Igh restriction of Balb/c T cells was not altered since the host IgG (Igh^b) present in the CB.20 6HS was unable to give complete antisuppressor activity. In fact, the addition of Balb/c IgG (Igh^a) to the CB.20 6HS restored antisuppressor activity (group 7).

8.2 Role of B cells in Igh restriction

In order to examine the role, if any, of B cells in altering the pattern of Igh restriction Balb/c athymic mice were reconstituted with Igh congenic CB.20 T cells. The mice were immunized 24 h after reconstitution and the 6HS was examined for antisuppressor activity. Fig. 23 shows the anti-SRBC response of Balb/c spleen cells (group 1) and the suppression of this response upon addition of Ts cells (group 2). Antisuppressor activity was detected in the 6HS of Balb/c (nu/nu) mice reconsituted with Balb/c T cells (group 4), whereas it was lacking in the 6HS from unreconstituted nude mice (group 3). The 6HS from Balb/c (nu/nu) mice reconstituted with Igh congenic CB.20 T cells lacked antisuppressor activity (group 5) indicating that the host B cells or IgG (Igh^a haplotype) was unable to alter the Igh restriction

Fig. 23 Role of Igh congenic B cells in Igh restriction

Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)
1	Balb/c	-	-	-
2	"	Balb/c	-	-
3	"	"	-	Balb/c (nu/nu)
4	"	"	Balb/c	"
5	"	"	CB.20	"


IgG anti-SRBC PFC / culture [X10^{-2}] ± S.E.
0
2
4
6
8
10
% Ig^{+} cells
1
31.4
2
3
30.2
4
37.8
5
30.9

of the donor T cells (Igh^b haplotype).

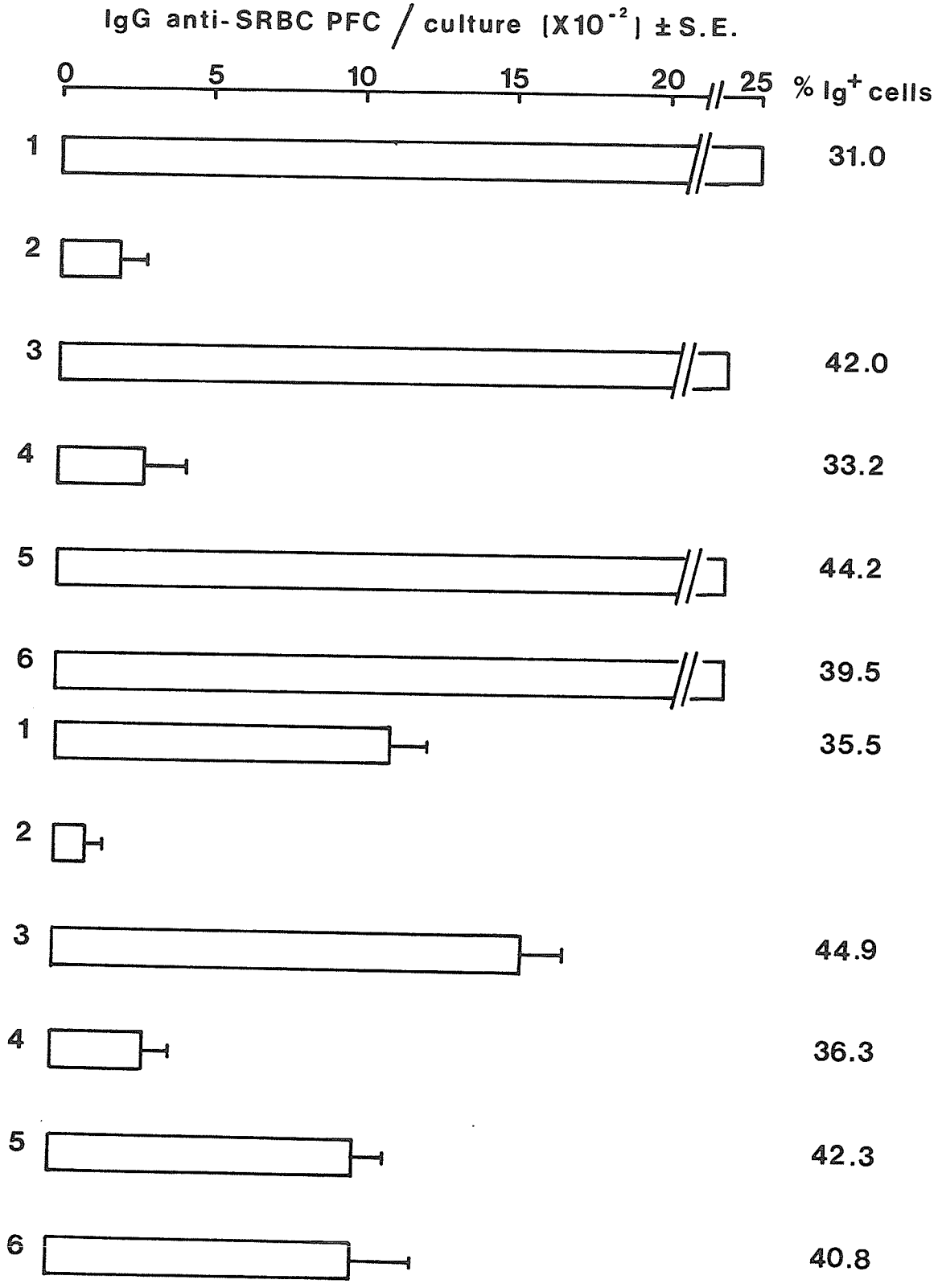
9. Role of IghV and IghC region genes in the generation of antisuppressor mediator

9.1 Regulation by IghV region genes

The data presented earlier showed that antisuppressor activity was generated only when the Igh haplotype of the T cells and IgG was identical. Therefore, it was of interest to examine whether genes of the immunoglobulin heavy chain V-region or C-region were involved in restricting the formation of the antisuppressor mediator. The IgG from the Igh recombinant strains, BAB-14 ($IghV^a$-C^b) and CBAL ($IghV^d$-C^b) was used. The appropriate strain of mice were selected so that the Igh haplotype of the donor T cells and IgG was identical at only the IghV locus. In Fig. 24A, the controls are shown in groups 1-5. The anti-SRBC response of Balb/c spleen cells (group 1); suppression of the response (group 2); antisuppression using 6HS from irradiated Balb/c mice reconstituted with syngeneic T cells (group 3); lack of antisuppressor activity in 6HS of irradiated CB.20 mice reconstituted with Igh congenic Balb/c T cells (group 4); and the antisuppressor activity of CB.20 6HS restored upon the addition of Balb/c IgG (group 5). Furthermore, the addition of BAB-14 IgG ($IghV^a$-C^b) to CB.20 6HS was equally effective (group 6). In Fig. 24B the controls are again shown in group 1-5. The anti-SRBC response of Cal.20 spleen cells (group 1); suppression of the response (group 2); antisuppression using 6HS from irradiated Cal.20 mice reconstituted with syngeneic T cells

Fig. 24 Generation of antisuppressor mediator regulated by IghV region genes

	Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
A)	1	Balb/c	-	-	-	-
	2	"	Balb/c	-	-	-
	3	"	"	Balb/c	Balb/c	-
	4	"	"	Balb/c	CB.20	-
	5	"	"	"	"	Balb/c, $IghV^a$-C^a
	6	"	"	"	"	BAB-14, $IghV^a$-C^b
B)	1	Cal.20	-	-	-	-
	2	"	Cal.20	-	-	-
	3	"	"	Cal.20	Cal.20	-
	4	"	"	Cal.20	CB.20	-
	5	"	"	"	"	Cal.20, $IghV^d$-C^d
	6	"	"	"	"	CBAL, $IghV^d$-C^b


IgG anti-SRBC PFC / culture [X10⁻²] ± S.E.
0
5
10
15
20
25
% Ig+ cells
1
31.0
2
3
42.0
4
33.2
5
44.2
6
39.5
1
35.5
2
3
44.9
4
36.3
5
42.3
6
40.8

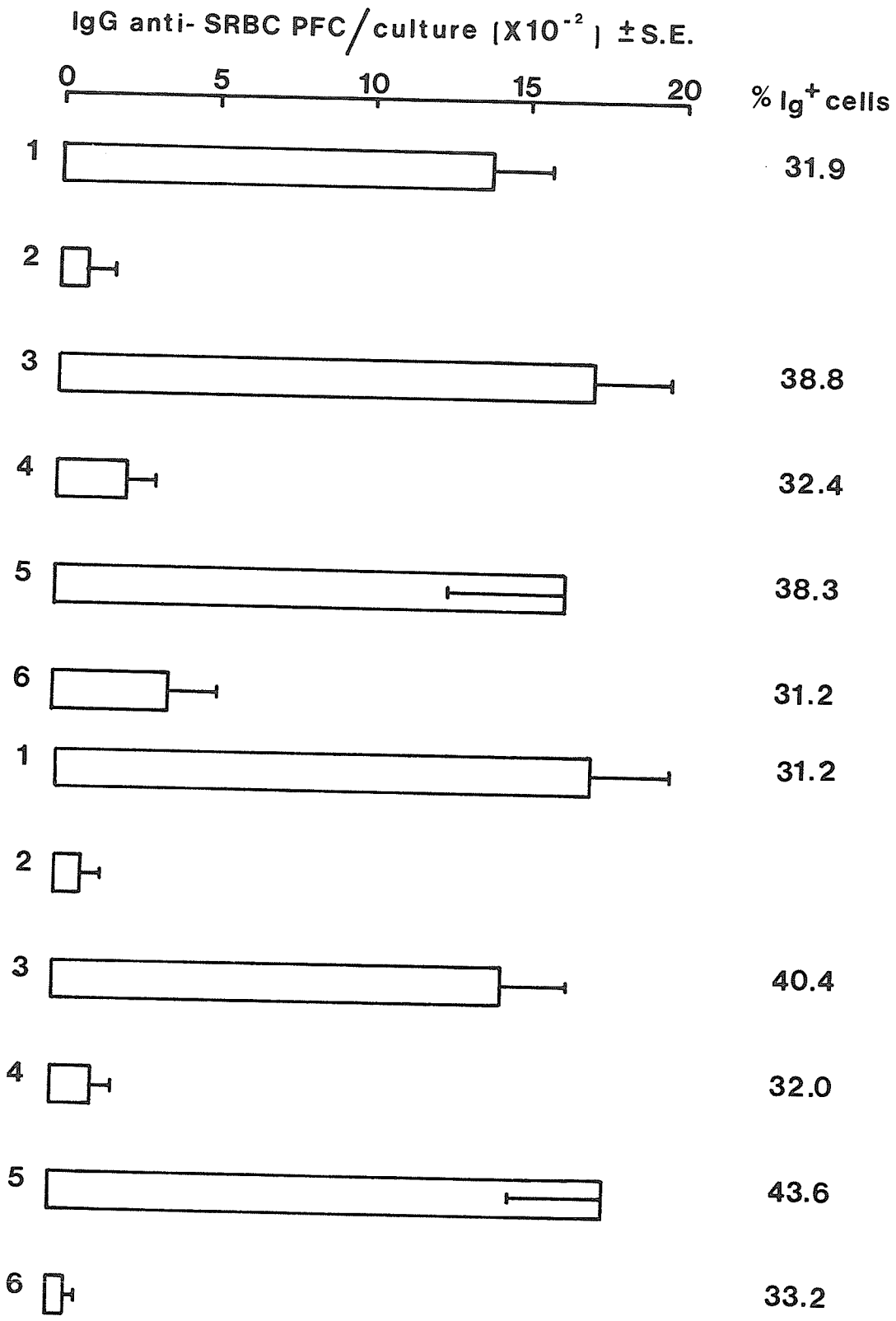
(group 3); lack of antisuppressor activity in 6HS of irradiated CB.20 mice reconstituted with Igh congenic Cal.20 T cells (group 4); and the antisuppressor activity of CB.20 6HS restored upon the addition of Cal.20 IgG (group 5). Moreover, the addition of CBAL IgG ($IghV^d$-C^b) to CB.20 6HS was equally effective (group 6). Therefore, these results showed that when the Igh haplotype of the donor T cells and IgG was identical at the IghV locus antisuppressor activity was generated.

9.2 Induction of antisuppression is independent of Igh C region genes

In order to identify the role of Igh C-region genes the appropriate strains of mice were selected so that the donor T cells and IgG (obtained from the Igh recombinant mice) was identical at only the Igh C locus. In Fig. 25A the controls are shown in groups 1-5. The anti-SRBC response of CB.20 spleen cells (group 1); suppression of the response by Ts cells (group 2); antisuppression using 6HS from irradiated CB.20 mice reconstituted with syngeneic T cells (group 3); lack of antisuppressor activity in the 6HS of irradiated Balb/c mice reconstituted with Igh congenic CB.20 T cells (group 4); the antisuppressor activity detected after the addition of CB.20 IgG to Balb/c 6HS (group 5). However, the addition of BAB-14 ($IghV^a$-C^b) IgG which has the same Igh C haplotype as the donor T cells was unable to restore antisuppressor activity (group 6). In Fig. 25B, the controls are again shown in groups 1-5. In this case, the 6HS from irradiated Cal.20 mice reconstituted with Igh congenic CB.20 T cells was lacking antisuppressor activity (group 4). This activity was restored upon the

Fig. 25 Generation of antisuppressor mediator independent of IghC region genes

	Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
A)	1	CB.20	-	-	-	-
	2	"	CB.20	-	-	-
	3	"	"	CB.20	CB.20	-
	4	"	"	CB.20	Balb/c	-
	5	"	"	"	"	CB.20, $IghV^b$-C^b
	6	"	"	"	"	BAB-14, $IghV^a$-C^b
B)	1	CB.20	-	-	-	-
	2	"	CB.20	-	-	-
	3	"	"	CB.20	CB.20	-
	4	"	"	CB.20	Cal.20	-
	5	"	"	"	"	CB.20, $IghV^b$-C^b
	6	"	"	"	"	CBAL, $IghV^d$-C^b


IgG anti- SRBC PFC/culture (X10⁻²) ±S.E.
0
5
10
15
20
% Ig+ cells
1
31.9
2
3
38.8
4
32.4
5
38.3
6
31.2
1
31.2
2
3
40.4
4
32.0
5
43.6
6
33.2

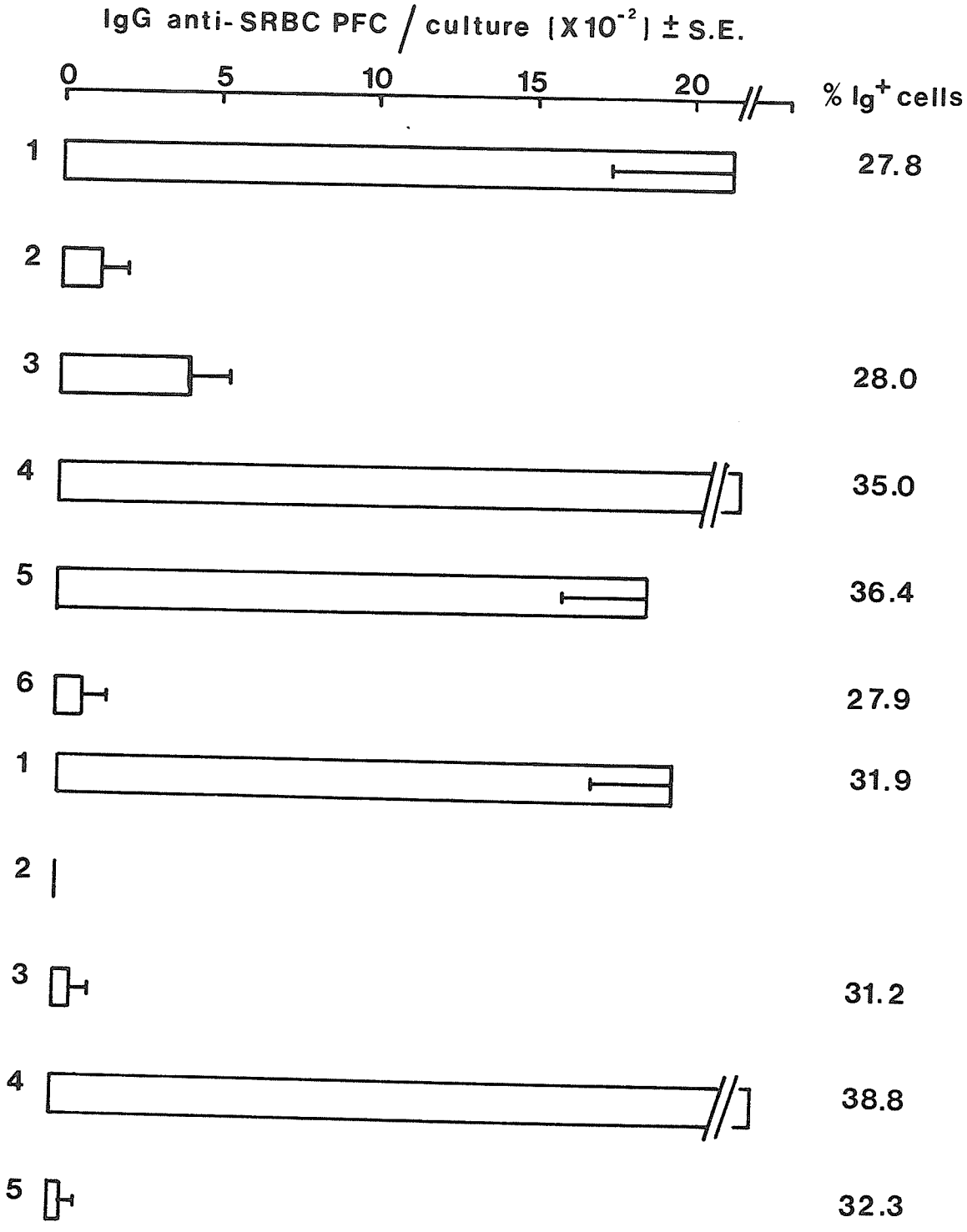
addition of CB.20 ($IghV^b$-C^b) IgG but not CBAL ($IghV^d$-C^b) IgG (groups 5 and 6, respectively). Therefore, these results showed that when the Igh haplotype of the donor T cells and IgG was identical at only the Igh C locus antisuppressor activity was lacking in the 6HS when examined by RICA and PFC assays.

9.3 Igh V restriction in the *in vitro* generation of antisuppressor mediator

The *in vitro* method for the production of the antisuppressor mediator (Maeba *et al.*, 1988) was used to verify the requirement for Igh V identity between the IgG and T cells. In Fig. 26A the anti-SRBC response of BAB-14 spleen cells (group 1) was suppressed upon addition of Ts cells (group 2). The cell-free supernatant from BAB-14 macrophage-T cell cultures lacked antisuppressor activity (group 3). Addition of BAB-14 IgG (group 4) or Balb/c IgG (group 5) restored the antisuppressor activity whereas CB.20 IgG (group 6) was ineffective. In Fig. 26B the anti-SRBC response of CB.20 spleen cells (group 1) was suppressed by Ts cells (group 2). In this case, the cell-free supernatant from CB.20 macrophage-T cell cultures lacked antisuppressor activity (group 3) and this activity was restored upon addition of CB.20 IgG (group 4) but not BAB-14 IgG (group 5). These results were similar to those obtained from the *in vivo* reconstitution experiments.

Fig. 26 IghV restriction in the *in vitro* generation of antisuppressor mediator

	Group	SC	Ts	(Mϕ-T)SUP	IgG
A)	1	BAB-14	-	-	-
	2	"	BAB-14	-	-
	3	"	"	BAB-14	-
	4	"	"	"	BAB-14, $IghV^a$-C^b
	5	"	"	"	Balb/c, $IghV^a$-C^a
	6	"	"	"	CB.20, $IghV^b$-C^b
B)	1	CB.20	-	-	-
	2	"	CB.20	-	-
	3	"	"	CB.20	-
	4	"	"	"	CB.20, $IghV^b$-C^b
	5	"	"	"	BAB-14, $IghV^a$-C^b

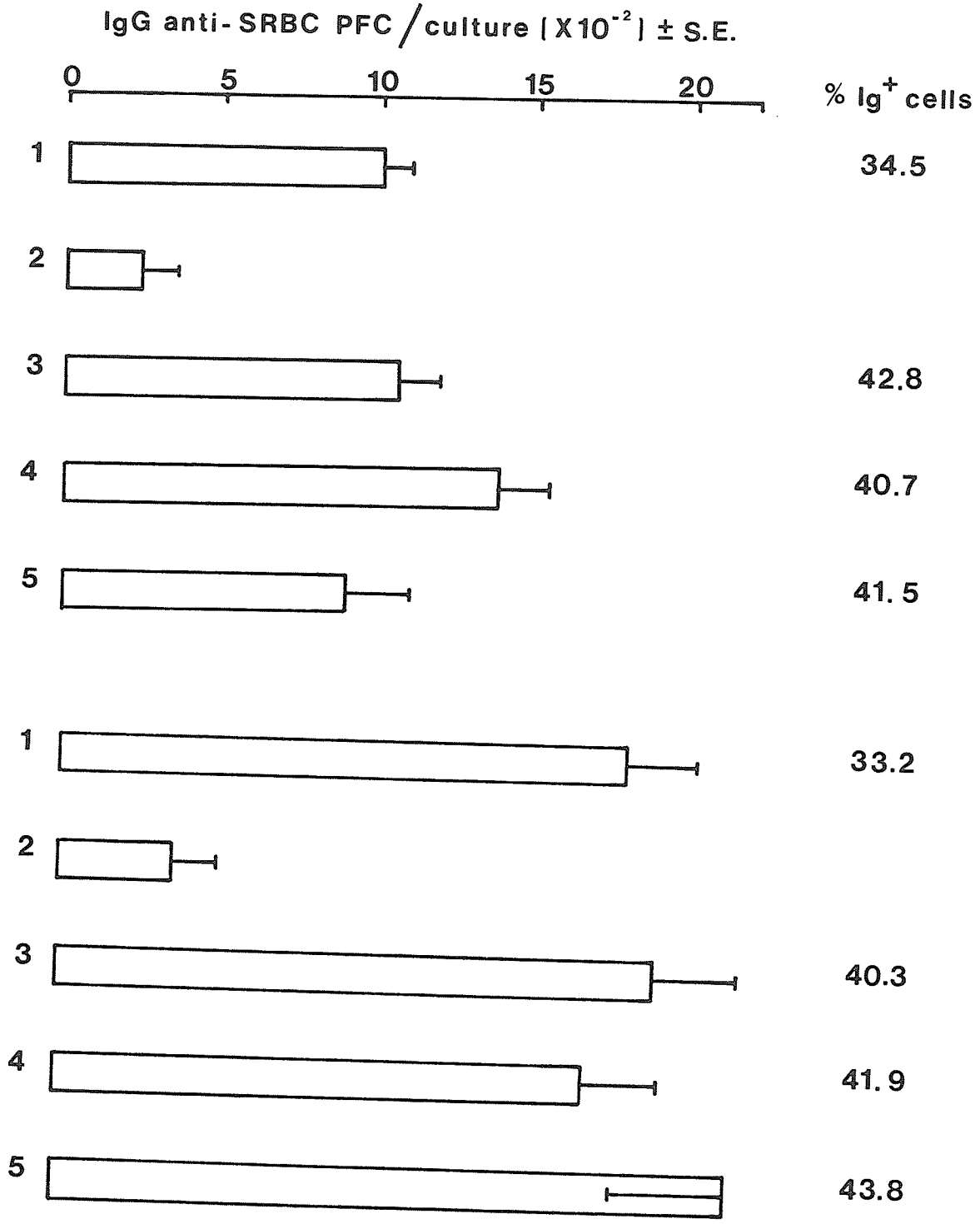

IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig⁺ cells
1
27.8
2
3
28.0
4
35.0
5
36.4
6
27.9
1
31.9
2
3
31.2
4
38.8
5
32.3

9.4 Identity at the Igh V region allows *in vivo* generation of the antisuppressor mediator

Irradiated mice were reconstituted with Igh congenic T cells such that the Igh haplotype of the T cell donor and recipient mice differed at only the Igh C locus. In this case, the 6HS should contain complete antisuppressor activity without the need of exogenous IgG since the host IgG and the donor T cells have the same Igh V haplotype. In Fig. 27A the controls show the anti-SRBC response of Cal.20 spleen cells (group 1); suppression of this response by Ts cells (group 2); and antisuppression using 6HS from irradiated Cal.20 mice reconstituted with syngeneic T cells (group 3). The 6HS from irradiated CBAL (Igh V^d-C^b) mice reconstituted with Cal.20 (Igh V^d-C^b) T cells contained complete antisuppressor activity (group 4). In addition, 6HS from irradiated Cal.20 mice reconstituted with CBAL T cells also contained complete antisuppressor activity (group 5). Therefore, these results showed that the host IgG could be utilized for the production of the antisuppressor mediator provided the Igh V haplotype was identical to that of the donor T cells. The antisuppressor activity of the 6HS was also examined using CBAL cultures and CBAL Ts cells (Fig. 27B). Similar results were obtained thereby indicating that only the generation of the antisuppressor mediator was Igh V restricted.

Fig. 27 Identity at the IghV region allows *in vivo* generation of the antisuppressor mediator

	Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)
A)	1	Cal.20	-	-	-
	2	"	Cal.20	-	-
	3	"	"	Cal.20	Cal.20
	4	"	"	Cal.20	CBAL
	5	"	"	CBAL	Cal.20
B)	1	CBAL	-	-	-
	2	"	CBAL	-	-
	3	"	"	CBAL	CBAL
	4	"	"	CBAL	Cal.20
	5	"	"	Cal.20	CBAL


IgG anti-SRBC PFC / culture [X10^{-2}] ± S.E.
0
5
10
15
20
% Ig^{+} cells
1
34.5
2
3
42.8
4
40.7
5
41.5
1
33.2
2
3
40.3
4
41.9
5
43.8

10. Role of the Fc portion of IgG in the generation of antisuppressor mediator

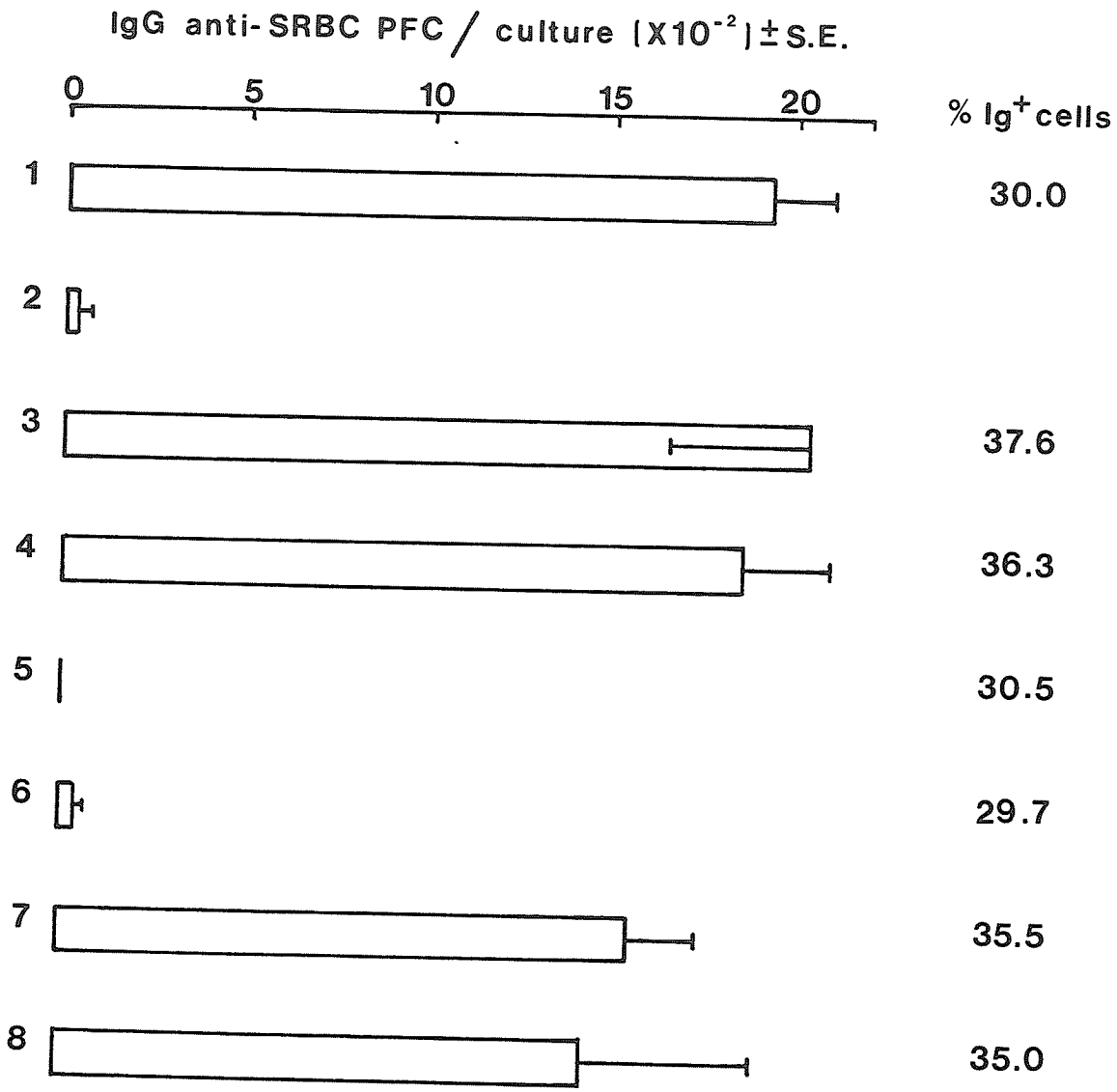
The structural requirements, if any, for the IgG in the 6HS was examined. The Fc portion of the IgG was removed by enzymatic cleavage and the $F(ab')_2$ fragments were examined for their ability to generate the antisuppressor mediator. In this case, the previously established *in vitro* method (Maeba *et al.*, 1988) for the production of the antisuppressor mediator was used. As shown in Fig. 28 the anti-SRBC response of Balb/c spleen cells (group 1) was suppressed upon addition of Ts cells (group 2). The cell-free supernatant from Balb/c macrophage-T cell cultures in the presence of Balb/c IgG had antisuppressor activity (group 3). Antisuppressor activity was also detected in the presence of the $F(ab')_2$ fragment of Balb/c IgG (group 4). The cell-free supernatant from CB.20 macrophage-T cell cultures in the presence of CB.20 IgG also had antisuppressor activity (group 7), whereas it lacked antisuppressor activity in the absence of CB.20 IgG (group 5) and in the presence inappropriate Balb/c (Igh^a) IgG (group 6). Antisuppressor activity was detected when the $F(ab')_2$ fragment of CB.20 IgG was added to the supernatant (group 8). Therefore, these results indicate that the $F(ab')_2$ fragment was equally effective as the intact IgG in producing complete antisuppressor activity.

11. Genetic restriction in the effector stage of antisuppression

It was of interest to examine whether any Igh restriction existed at the effector stage of antisuppression. The MHC and Igh

Fig. 28 Role of the Fc portion of IgG in the generation of antisuppressor mediator

Group	SC	Ts	(Mϕ-T)SUP	IgG
1	Balb/c	-	-	-
2	"	Balb/c	-	-
3	"	"	Balb/c	Balb/c IgG
4	"	"	"	Balb/c $F(ab')_2$
5	"	"	CB.20	-
6	"	"	"	Balb/c IgG
7	"	"	"	CB.20 IgG
8	"	"	"	CB.20 $F(ab')_2$


IgG anti-SRBC PFC / culture (X10⁻²) ± S.E.
0
5
10
15
20
% Ig⁺ cells
1
30.0
2
3
37.6
4
36.3
5
30.5
6
29.7
7
35.5
8
35.0

restriction between the antisuppressor inducer cell and the antisuppressor effector cell was examined by using Balb/c ($H\text{-}2^d$) effector cells and 6HS collected from different strains of mice, i.e., Igh and H-2 distinct. In Fig. 29 the anti-SRBC response of Balb/c mice (group 1) was suppressed by Ts cells (group 2). As expected, Balb/c 6HS had antisuppressor activity (group 3). When CB.20 6HS or Cal.20 6HS (groups 4 and 5, respectively) was examined with Balb/c effector cells antisuppressor activity was detected. In addition, B6 6HS or SJL 6HS (groups 6 and 7, respectively) was also antisuppressive in Balb/c cultures. These results indicated that the antisuppressor mediator present in the 6HS was not Igh or MHC restricted at the effector stage.

In the preceding reconstitution experiments the Igh haplotype of the antisuppressor inducer T cell and the effector T cell had always been identical. Furthermore, the 6HS from irradiated mice reconstituted with Igh congenic T cells lacked antisuppressor activity. It was of interest to see whether using the incomplete 6HS with Igh distinct effector cells would allow completion of antisuppressor activity as this would implicate Igh restriction at the effector stage. In Fig. 30A the anti-SRBC response of CB.20 spleen cells (group 1) was suppressed by Ts cells (group 2). The 6HS from irradiated Balb/c mice reconstituted with syngeneic T cells had antisuppressor activity in CB.20 cultures (group 3). The 6HS from irradiated CB.20 mice

Fig. 29 Antisuppressor mediator produced *in vivo* is MHC and Igh non-restricted at the effector stage

Group	SC	Ts	6HS
1	Balb/c	-	-
2	"	Balb/c	-
3	"	"	Balb/c ($H\text{-}2^d$, Igh^a)
4	"	"	CB.20 ($H\text{-}2^d$, Igh^b)
5	"	"	Cal.20 ($H\text{-}2^d$, Igh^d)
6	"	"	B6 ($H\text{-}2^b$, Igh^b)
7	"	"	SJL ($H\text{-}2^s$, Igh^b)

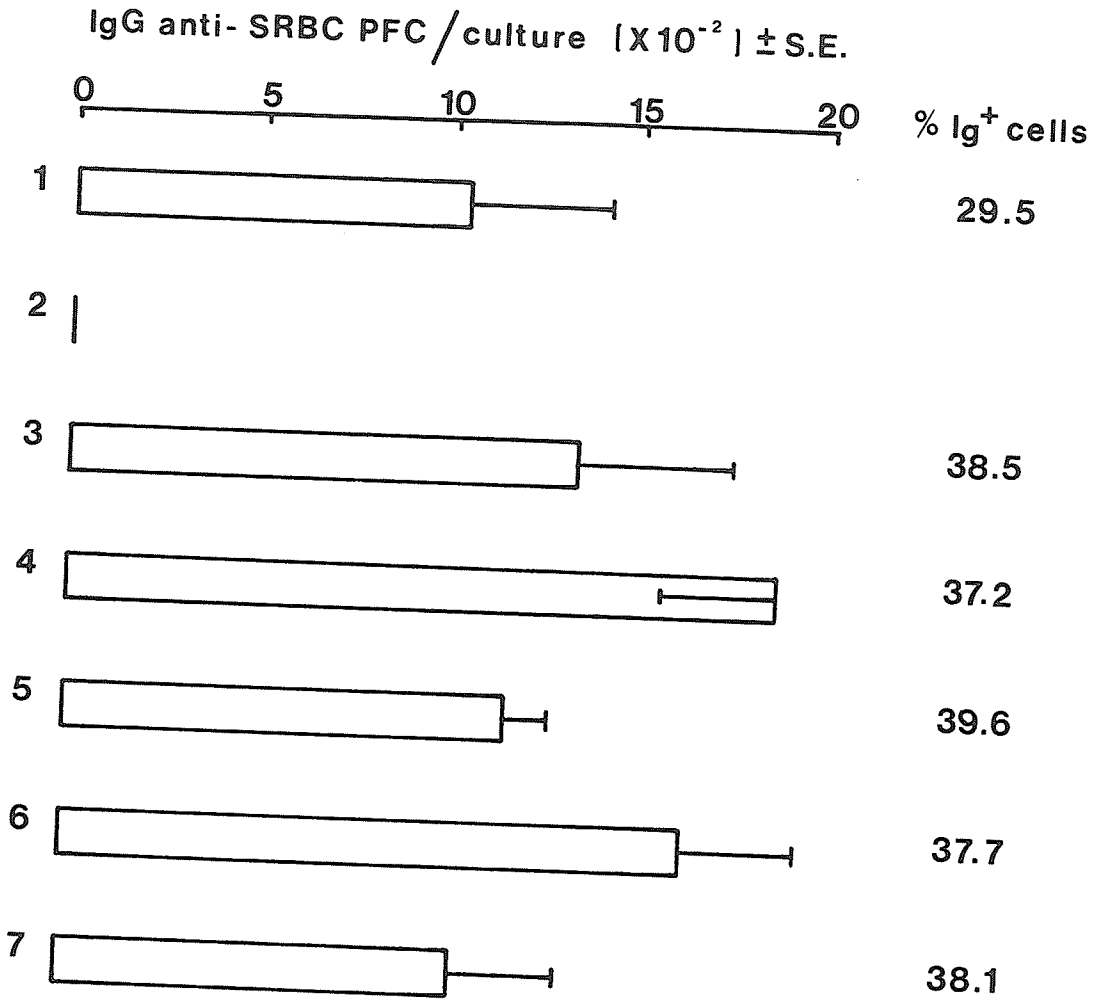
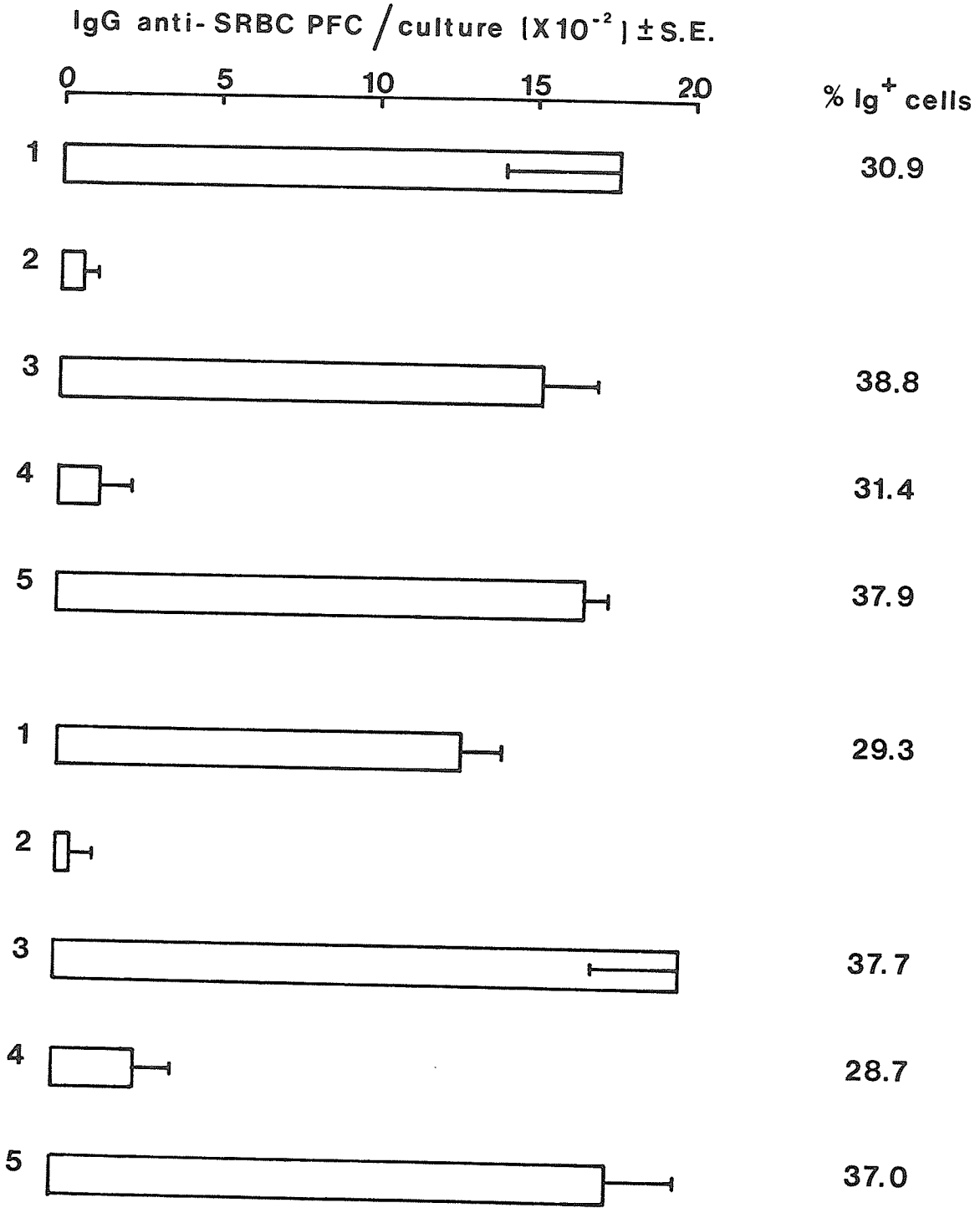

IgG anti- SRBC PFC / culture [X 10⁻²] ± S.E.
0
5
10
15
20
% Ig+ cells
1
29.5
2
3
38.5
4
37.2
5
39.6
6
37.7
7
38.1

Fig. 30 Effector stage of antisuppression is genetically non-restricted

	Group	SC	Ts	T-cell Donor	T-cell Recipient (6HS)	IgG
A)	1	CB.20	-	-	-	-
	2	"	CB.20	-	-	-
	3	"	"	Balb/c	Balb/c	-
	4	"	"	"	CB.20	-
	5	"	"	"	"	Balb/c
B)	1	Balb/c	-	-	-	-
	2	"	Balb/c	-	-	-
	3	"	"	CB.20	CB.20	-
	4	"	"	"	Balb/c	-
	5	"	"	"	"	CB.20


IgG anti-SRBC PFC / culture [X 10⁻²] ± S.E.
0
5
10
15
20
% Ig⁺ cells
1
30.9
2
3
38.8
4
31.4
5
37.9
1
29.3
2
3
37.7
4
28.7
5
37.0

reconstituted with Balb/c T cells lacked antisuppressor activity (group 4) and this activity was restored upon addition of Balb/c IgG (group 5). Similar results were also obtained when the reverse experiment was done (Fig. 30B). These results strongly suggested that the 'incomplete' activity present in CB.20 6HS required the appropriate IgG prior to its interaction with the antisuppressor effector cell.

DISCUSSION

Studies from this laboratory have described the presence of a unique form of processed antigen in the serum of mice 6 h after antigenic stimulation (Orr and Paraskevas, 1973). The components of the 6HS have been partially characterized and include an antigen fragment, Ig and Ia determinants (Paraskevas and Lee, 1981). These complexes enhanced the IgG antibody response *in vivo* to sub-immunogenic dose of antigen (Lee and Paraskevas, 1981). More recently, the 6h complexes were shown to interfere with Ts cell function in humoral (Lee *et al.*, 1985; Paraskevas *et al.*, 1985; 1985a) and cell-mediated responses (Al-Maghazachi *et al.*, 1983) and are therefore considered mediators of an immunoregulatory T cell pathway termed antisuppression.

Blocking of the antisuppressor pathway results in the induction of Ts cells by otherwise strong immunogenic stimuli (Paraskevas *et al.*, 1985). From these observations it is apparent that antisuppression plays an important role in the activation of the immune system. Therefore, the present study was undertaken in an effort to further characterize the cellular interactions leading to the stepwise formation of the antisuppressor mediator. An *in vitro* method recently established in this laboratory (Maeba *et al.*, 1988) was used for the production of the antisuppressor mediator. The first stage in the formation of this mediator involves culture of T cells with antigen-pulsed macrophages, while the second stage requires the

addition of IgG to the cell free supernatant.

It is widely accepted that antigen-specific activation of T cells requires recognition of antigen in association with self MHC molecules on the surface of an APC. The initial step in the production of the antisuppressor mediator also involves a macrophage-T cell interaction (Maeba *et al.*, 1988). Therefore, an attempt was made to examine the role of the macrophage in the generation of the antisuppressor mediator. A cloned B lymphoblastoid cell line, A.20, has been shown capable of presenting protein antigens to MHC restricted, antigen-specific T cells (Chesnut *et al.*, 1982; Glimcher *et al.*, 1982). It was found that the Ia^+, A.20 B-cell line was unable to generate the antisuppressor mediator (Fig. 4, group 7). This may be attributed to the poor capability of these cells to phagocytose and process particulate antigen such as SRBC. It has been demonstrated that effective presentation by B cells requires high concentrations of protein antigen similar to that required by other Ia^+ APC such as macrophages and dendritic cells. The only instance in which B cells have been found to be highly efficient as APC is when they bind antigen via surface Ig receptors (Rock *et al.*, 1984; Abbas *et al.*, 1985). The phagocytic cell line, $P388D_1$ was also unable to replace normal macrophages for the production of the antisuppressor mediator (Fig. 4, group 6). This is most likely due to the lack of expression of Ia antigens on these cells. It has been reported that L cells are incapable of presenting protein antigens but do so upon transfection

with MHC class II genes (Norcross *et al.*, 1984). In the present study it was observed that elimination of Ia^+ macrophages from the PEC by treatment with monoclonal anti-I-A antibody plus complement also abolished the production of the antisuppressor mediator (Fig. 6, group 4). Therefore, these results suggested that the first step leading to the formation of the antisuppressor mediator requires antigen processing and presentation in association with MHC class II antigens.

Additional evidence of the requirement for antigen processing was obtained by: (1) fixing the macrophages with paraformaldehyde to prevent antigen uptake, and (2) by using the lysosomotropic agents chloroquine and ammonium chloride. Fixation of macrophages inhibits all secretory functions of the macrophage and in this case it abolished the generation of antisuppressor activity (Fig. 5, group 4). Inhibition of the intracellular processing step by chloroquine or ammonium chloride also abolished production of the antisuppressor mediator (Fig. 5, groups 9 and 10). For protein antigens it has been observed that fixation of APC after exposure to antigen can still trigger antigen-specific T cell clones or hybridomas for IL-2 production (Allen *et al.*, 1984), thereby suggesting that fixation does not denature the MHC class II molecules or the antigenic determinant. In contrast, the production of the antisuppressor mediator was inhibited when macrophages were fixed after exposure to either intact SRBC or hemolysate (Fig. 5, groups 5 and 8). Unanue and coworkers in their studies with the bacteria *Listeria monocytogenes* (Ziegler and Unanue, 1982) and the protein hen-egg lysozyme (Allen *et al.*, 1984)

have used 'activated' macrophages as well as immune T cells. The *in vitro* production of the antisuppressor mediator however, has involved the culture of resident peritoneal macrophages and normal splenic T lymphocytes. Therefore, it is likely that the kinetics of antigen catabolism and concomitant appearance of immunologically relevant peptide(s) on the cell surface in these two instances is different. Furthermore, experimental evidence favors IL-1 as being an important component in the activation of resting T cells during antigen presentation (Smith *et al.*, 1980; Williams *et al.*, 1985) but not of T cell hybridomas (Watts *et al.*, 1984). It is not clear whether fixation of APC interferes with their ability to secrete IL-1. However, from the data presented here it is apparent that fixation of macrophages after ingestion of SRBC interferes with their ability to generate the antisuppressor mediator. Further studies with well-defined protein antigens are required to determine whether IL-1 and/or some as yet unidentified signal is required during this initial macrophage-T cell interaction in the production of the antisuppressor mediator.

Previous studies have provided evidence that Ia determinants are one of the serologically detectable components present in the 6h complexes (Paraskevas and Lee, 1981). These observations have been verified in a recent study where a specific anti-Ia immunoadsorbent column effectively removed the antisuppressive activity from the macrophage-T cell supernatant (Maeba *et al.*, 1988). In the present study it was observed that a mAb with specificity against determinants

encoded by the I-A subregion blocked the generation of the antisuppressor mediator, whereas the mAb specific for I-E encoded determinants was ineffective (Fig. 6, groups 5 and 6). In earlier studies (Paraskevas *et al.*, 1985), treatment of mice with anti-I-A mAb blocked the induction of the complexes and abolished the enhancing effect these complexes exert on the *in vivo* IgG anti-SRBC antibody response. More recently, studies with H-2 congenic strains have demonstrated that identity at the I-A subregion alone between the macrophage and T cell is sufficient for the production of the antisuppressor mediator (Paraskevas *et al.*, manuscript in preparation). Therefore, taken together these results indicate that in mice of the d haplotype the immunodominant epitopes of a complex antigen such as SRBC are most likely I-A restricted. Studies with protein antigens such as myoglobin (Cease *et al.*, 1986), ovalbumin (Shimonkevitz *et al.*, 1984), lysozyme (Manca *et al.*, 1984) and cytochrome c (Schwartz *et al.*, 1985) have shown that an immunodominant antigenic site exists and is not a single epitope but a cluster of tightly overlapping epitopes seen by different T cells with different receptors (reviewed by Berzofsky, 1988). Furthermore, it appears that immunodominance is not due to the pre-emption of the response by a single predominant clone of T cells, but is due in part to the relative affinity of the processed antigen for either I-A or I-E molecules (Allen *et al.*, 1987; Buus *et al.*, 1987).

Blocking the production of the antisuppressor mediator by treating

the macrophages with anti-I-A antibody did not provide direct evidence that these molecules may be involved in its formation. It is likely that the anti-I-A antibody may be eliciting its effect by some other mechanism such as binding to FcRs on the macrophage surface. Therefore, BDF_1 ($H\text{-}2^{b/d}$) macrophages were used in order to provide direct evidence implicating I-A molecules in the generation of the antisuppressor mediator. Culturing $H\text{-}2^d$ T cells with BDF_1 macrophages pretreated with anti-$I\text{-}A^d$ mAb abolished the generation of the antisuppressor mediator whereas exposure to anti-$I\text{-}A^b$ mAb was ineffective (Fig. 8, groups 6 and 5 respectively). Similarly, when $H\text{-}2^b$ T cells were used it was found that treatment of BDF_1 macrophages with anti-$I\text{-}A^b$ mAb effectively blocked the production of the antisuppressor mediator (Fig. 9, group 6) but not the treatment with anti-$I\text{-}A^d$ mAb (Fig. 9, group 5). These results clearly suggest that Ia antigens are involved in the generation of the antisuppressor mediator.

Previous studies have indicated that an Lyl^+, Ia^+, FcR^+ T cell is required for the *in vivo* production of the antisuppressor mediator (Paraskevas *et al.*, 1985a) as well as its *in vitro* production (Maeba *et al.*, 1988). Substantial experimental evidence exists that the glycoproteins CD4 and CD8, expressed on mutually exclusive populations of T cells, recognize nonpolymorphic determinants of MHC class II and class I antigens respectively on target cells (Swain 1981; Meuer *et al.*, 1982; Marrack *et al.*, 1983). It was found that the inducer antisuppressor T cell belongs to the $CD4^+$ subset since treatment with

anti-CD4 mAb plus complement abolished production of the antisuppressor mediator (Fig. 10, group 6). Furthermore, it was observed that exposure of the T cells to anti-CD4 mAb before culturing with SRBC-pulsed macrophages effectively blocked production of the antisuppressor mediator (Fig. 10, group 7). Antibody inhibition studies (Marrack *et al.*, 1983; Greenstein *et al.*, 1984; Shaw *et al.*, 1985), analysis of spontaneous CD4 loss variants (Gay *et al.*, 1987), as well as gene transfer experiments (Gay *et al.*, 1987; Sleckman *et al.*, 1987) suggest that the expression of CD4 augments T cell responses to specific antigen and that CD4 is particularly important when concentrations of antigen are suboptimal or when the avidity of the T cell for antigen plus MHC is low. Recent studies have suggested an additional role for the CD4 glycoprotein, i.e., it appears to be involved in signal transduction when brought into close physical proximity to the CD3-TCR during MHC class restricted antigen recognition (Ledbetter *et al.*, 1988). It is most probable that in the present case the CD4 molecules participate in strengthening the overall macrophage-T cell interaction since normal resting T cells, instead of cloned cell lines, have been used for the production of the antisuppressor mediator. Therefore, it appears that the initial stage in the generation of the antisuppressor mediator involves conjugate formation between $CD4^+$ T cells and Ia^+ APC. Doyle and Strominger (1987) have reported that cell adhesion mediated by CD4 interaction with class II antigen can occur even in the absence of TCR-antigen interaction and is specifically inhibitable by anti-class II and

anti-CD4 antibodies. The cell-cell contact between the macrophage and T cell appears to be essential since it was observed that T cells cultured alone in supernatant from SRBC-pulsed macrophages were not stimulated for production of the antisuppressor mediator (Fig. 10, group 5).

Two components, which are both required for the functional expression of antisuppression, have been serologically identified in the macrophage-T cell culture supernatant, i.e., antigenic determinant(s) and Ia antigen (Maeba *et al.*, 1988). The presence of Ia-antigen complexes in macrophage supernatants has previously been reported in at least two instances (Erb and Feldmann, 1975; Puri and Lonai, 1980). The difficulty in demonstrating the presence of such complexes in solution may stem from their inability to effectively trigger T cell lines or hybridomas for IL-2 production. More recently, Babbitt *et al.* (1985) and others (Buus *et al.*, 1986) have unequivocally demonstrated the existence of MHC class II molecules in association with immunogenic peptides in solution. The rate of peptide-Ia association is very slow (Buus *et al.*, 1987) and it has been suggested that T cells may promote and stabilize this association (Watts *et al.*, 1986). It is conceivable that although the complexes are formed on the membrane of APC, T-cell-associated proteinases which have been reported to participate in regulation of humoral immune function (Kramer and Simon, 1987) may facilitate their release into solution.

At present the nature of the T cell factor released by the inducer $Ly1^+$, $L3T4^+$ T cell is not known. In previous studies the activity of the *in vivo* induced T cell factor, referred to as IACF, has been found to correspond to a molecular weight of 68,000 (Paraskevas *et al.*, 1984). However, the isolation of this factor from macrophage-T cell cultures in sufficient quantities for biochemical characterization has been difficult. This is not unexpected in view of the fact that biochemical characterization of monoclonal, antigen-specific TsF (Wieder *et al.*, 1982) or TcsF (Flood *et al.*, 1988) has also met with limited success. Unlike antibody-secreting B cell hybridomas, it appears that hybrids which secrete suppressor or contrasuppressor factors in culture do so in limited quantities (Krupen *et al.*, 1982). Moreover, the biologic activity of such factors requires the concerted action of two polypeptide chains which are usually the product of different genes and may be regulated separately (Funckes-Shippy *et al.*, 1987). A SRBC-specific antisuppressor inducer cell line has been cloned recently by Dr. Tom Lee in this laboratory in an effort to facilitate biochemical and molecular characterization of the T cell factor.

The Ig is the most easily detectable structural component of the antisuppressor mediator which is essential for its assembly and its effector function. Genes of the I-A subregion of the MHC regulate the *in vivo* induction of the antisuppressor pathway (Paraskevas *et al.*, 1985). Therefore, it was important to determine whether genes in or

linked to the Igh locus imposed another level of restriction in the induction of this immunoregulatory pathway. In earlier studies it was found that the induction of the 6h complexes is T cell dependent (Lee and Paraskevas, 1972) and 6HS from lethally irradiated mice is incapable of enhancing the *in vivo* IgG anti-SRBC antibody response (Paraskevas *et al.*, 1985a). However, as reported previously (Paraskevas *et al.*, 1985a) and also shown here, 6HS from irradiated mice reconstituted with syngeneic T cells can restore the IgG anti-SRBC antibody response of suppressed cultures (Fig. 12, group 3). In contrast, it was found that 6HS from irradiated CB.20 (H-2^d, Igh^b) mice reconstituted with Igh congenic Balb/c (H-2^d, Igh^a) T cells lacked antisuppressor activity (Fig. 12, group 4). The addition to the CB.20 6HS of NMS from strains of only Igh^a haplotype generated the antisuppressor activity (Fig. 12, groups 5 and 6). Similar results were obtained in the reverse experiment, i.e., 6HS from irradiated Balb/c mice reconstituted with CB.20 T cells lacked antisuppressor activity (Fig. 13, group 4) and this activity was detected upon addition to the Balb/c 6HS of NMS from strains of only Igh^b haplotype (Fig. 13, groups 5 and 7). Furthermore, it was observed that the addition of affinity purified IgG from serum of mice with the same Igh haplotype as the donor T cells generated complete antisuppressor activity. Several Igh congenic strain combinations were used in order to demonstrate that the induction of the antisuppressor mediator requires identity at the Igh locus between the donor T cells and the IgG (Figs. 16-21). The results of these reconstitution experiments are

consistent with the earlier data from the macrophage-T cell cultures since, these data also indicate that a functionally inactive 'intermediate complex' is present in the 6HS of mice reconstituted with Igh congenic T cells. Moreover, the requirement for identity at the Igh locus between the inducer T cell and the IgG implicates Igh genes in exerting the second genetic control regulating the formation of the antisuppressor mediator.

Genes linked to the Igh locus have also been reported to function as restriction elements in both the suppressor (Eardley *et al.*, 1979; Dorf and Benacerraf, 1984) and contrasuppressor circuits (Braley-Mullen 1986; Green *et al.*, 1988). Studies with haptens such as ABA (Bach *et al.*, 1979) and NP (Weinberger *et al.*, 1979), capable of eliciting antibody responses characterized by the expression of dominant crossreactive idiotypes (CRI), have demonstrated the presence of Ig idiotopes on some subsets of T cells involved in immune regulation. In addition, the action of ABA-specific TsF1, for example, was functionally restricted by genes linked to the Igh locus (Bach *et al.*, 1979). Similarly, Braley-Mullen (1986) has demonstrated by adoptive transfer experiments that contrasuppression is elicited only when Tcs cells are Igh compatible with both Ts and responding B cells. It is not known whether the Ig idiotopes on T cells, identified by the anti-CRI reagents, are structural components of the α:β TCR or represent determinants present on some other cell surface molecule. The experimental evidence presented here, on the other hand, clearly

shows that the IgG which is a product of Ig genes is restricted in its interaction with the T cell factor. The finding of a genetic restriction carries two implications: first, that a product of the gene is involved in the phenomenon and second that there must be a binding site or receptor for that product. Based on an earlier study (Paraskevas *et al.*, 1984) it has been postulated that the Ig in the 6h complexes represents a naturally occurring anti-idiotypic antibody while the T cell factor bears the idiotype. Gordon and Abikar (1988) have provided experimental evidence, using antigens such as GAT and PC, that monoclonal anti-idiotypic antibodies can in fact replace the IgG from NMS in the production of the antisuppressor mediator.

It has been reported that the Igh-linked functional restrictions imposed upon the Ts cell repertoire can be partially altered by transfer of peripheral lymph node or splenic T cells into an Igh distinct environment for 12-15 days (HayGlass, *et al.*, 1985). It was observed that the Igh restriction imposed in the induction of the antisuppressor mediator however, was not altered when Balb/c (Igh^a) T cells were allowed to adapt to an Igh congenic CB.20 (Igh^b) environment for 5 days (Fig. 22, group 8). A time interval greater than 5 days was not examined since the lethally irradiated mice became moribund. In some studies it has been suggested that antigen-activated B cells display or produce Ig-associated structures that trigger T cells that have receptors specific for these molecules, thereby clonally expanding the population of Igh restricted T cells (L'age-Stehr, 1980; Nutt *et*

al., 1981). In the present study athymic mice were used since the effect of lethal irradiation on the B cells of the host is not known. It was found that the 6HS from athymic Balb/c mice reconstituted with Balb/c T cells enhanced the IgG anti-SRBC antibody response of suppressed cultures (Fig. 23, group 4). The 6HS produced from athymic Balb/c mice reconstituted with CB.20 T cells was functionally inactive (Fig. 23, group 5), thereby indicating that the factor released by the CB.20 antisuppressor inducer T cell was unable to interact with the host IgG. The *in vivo* activation of antisuppression results in the formation of the antisuppressor mediator within 6 hours (Paraskevas *et al.*, 1985), whereas ABA-specific TsF1 has been isolated from splenic T cells 7 days after the induction of Ts cells (Bach *et al.*, 1979). Due to the difference in the kinetics of induction of these two pathways, i.e., a few hours as opposed to several days, further experiments involving 'parking' of T cells in athymic Igh congenic hosts for an extended time interval are required.

The degree of adaptive differentiation that T cells can undergo in an Igh congenic host in the absence of antigenic stimulation is not known. From the studies of Nutt *et al.* (1981) it appears that priming of Th cells is influenced by the Igh phenotype of the priming environment. Antigen-primed Igh^b T cells were inefficient helpers for Igh^j B cells, but upon activation in an Igh^j host they were capable of helping Igh^j B cells. Therefore, this data suggests that T cells require Igh-linked products to be present at the time of antigenic

stimulation in order to express an altered Igh restriction. Production of a "re-educated" ABA-TsF1 (HayGlass *et al.*, 1985) on the other hand, required an antigen-independent stage where irradiated mice were reconstituted with Igh congenic T cells for 5 days. The Igh chimeric mice were immunized with ABA-conjugated spleen cells and 7 days later ABA-TsF1 was prepared from splenic T cells. The extent of "re-education" that may have occurred during the antigen-independent stage is not clear from this study. Moreover, it is not known whether such Igh-linked alterations reflect a transient adaptation of the T cells or some permanent phenotypic change.

Chronic treatment of mice with anti-μ antibody from birth also altered the Igh restriction of ABA-specific TsF1 (Sy *et al.*, 1984). Similar anti-μ treatment of mice apparently disrupted both the antisuppressor (Gordon *et al.*, 1985) and also the contrasuppressor (Green and Ptak, 1986; Braley-Mullen, 1986a) pathway. It was found that the antisuppressor inducer as well as the effector T cells were lacking in anti-μ treated mice (Gordon *et al.*, 1985). Therefore, from these observations it can be inferred that B cells and/or their products have a significant role in influencing the functional activation and/or differentiation of the subset of T cells mediating antisuppression. In the suppressor cascade however, it appears that in the absence of B cells and/or their products other genes may be capable of influencing the Ts cell repertoire.

It has been postulated that the generation of the 6h complexes occurs as a consequence of an idiotype-anti-idiotype interaction between the T cell derived factor and the IgG (Paraskevas *et al.*, 1984). Therefore, it was expected that genes of the Igh V region may be the restricting element in regulating the formation of the antisuppressor mediator. This was experimentally verified by using IgG isolated from the serum of two Igh recombinant strains, BAB-14 ($IghV^a$-C^b) and CBAL ($IghV^d$-C^b). It was found that the 6HS from irradiated mice reconstituted with Igh congenic Balb/c ($IghV^a$-C^a) T cells was functionally active upon addition of BAB-14 IgG (Fig. 24A, group 6). Similarly, 6HS from irradiated mice reconstituted with Igh congenic Cal.20 ($IghV^d$-C^d) T cells was also functionally active after addition of CBAL IgG (Fig. 24B, group 6). In addition, it was observed that when the Igh haplotype of the IgG and the T cell donor was identical at only the Igh C locus, no antisuppressor activity was generated, e.g., as shown in Fig. 25A (group 6) when BAB-14 IgG ($IghV^a$-C^b) was added to 6HS from mice reconstituted with CB.20 ($IghV^b$-C^b) T cells. Therefore, these results indicate that generation of the antisuppressor mediator is indeed regulated by IghV region genes. Additional verification of this was obtained by using Igh recombinant BAB-14 T cells for the *in vitro* production of the antisuppressor mediator. It was found that only IgG from $IghV^a$ haplotypes interacted with the 'intermediate complex' and enhanced the IgG anti-SRBC antibody response of suppressed cultures (Fig. 26A, groups 4 and 5). Based upon these observations, it was expected that

if two strains of mice having the same IghV haplotype were used for *in vivo* reconstitution experiments, then the host IgG should participate in generating complete antisuppressor activity in the 6HS. Therefore, irradiated CBAL ($IghV^d$-C^b) mice were reconstituted with Cal.20 ($IghV^d$-C^d) T cells and vice versa and the 6HS produced was indeed capable of enhancing the antibody response of suppressed cultures in the absence of exogenous IgG (Fig. 27A, groups 4 and 5). The CBAL and Cal.20 mice differ at only the IghC locus hence, this data clearly indicates that IghV region genes restrict the assembly of a functional antisuppressor mediator. A similar IghV restriction in the induction of contrasuppression has not been reported. Braley-Mullen (1986) has reported an IghV-linked restriction in the effector stage of contrasuppression, while Green *et al.* (1988) have observed such a restriction between the TcsiF and the transducer cell.

An idiotype-anti-idiotype interaction between the T cell factor and the IgG favors the view that the Fc portion of the IgG may not participate in the assembly of the antisuppressor mediator. This was examined by enzymatic cleavage of the Fc fragment and it was found that the divalent $F(ab')_2$ fragment not only formed the complexes, but it was as effective as the intact molecule in eliciting antisuppressor activity in suppressed cultures (Fig. 28, groups 4 and 3 respectively). These results indicate that the product of IghC genes does not appear to participate in the structural assembly and functional expression of antisuppression. Moreover, this data confirms the earlier observation

that the uptake of the 6h complexes by the effector T cells ($Ly2^+$, Ia^+, FcR^+) does not involve FcRs (Paraskevas and Lee, 1979).

In earlier studies (Paraskevas and Lee, 1981) the 6h complexes were reported to enhance *in vivo* antibody formation across MHC barriers, thereby suggesting that the effector stage of antisuppression is MHC nonrestricted. In the present study, 6HS from several strains of mice was examined for its ability to enhance the antibody response of suppressed cultures. It was found that 6HS produced in mice which differed in the Igh and/or MHC haplotype from the assay cells was fully capable of restoring the antibody response in suppressed cultures (Fig. 29). Therefore, these results indicate that the uptake and activation of antisuppressor effector T cells is MHC and Igh nonrestricted.

In order to determine whether Igh restriction was imposed at the effector stage of antisuppression, Igh incompatible inducer and effector T cells were used (Fig. 30A). In this case, the 6HS from irradiated CB.20 mice reconstituted with Balb/c T cells was assayed in Igh distinct CB.20 cultures. The presence of antisuppressor activity in this 6HS (Fig. 30A, group 4), which has been shown to lack functional activity, would indicate that uptake of the 'intermediate complex' allows the effector cell to dictate Igh restriction. However, it was found that only the inducer Balb/c T cell dictates the Igh restriction since addition of Balb/c IgG to this 6HS generated antisuppressor activity (Fig. 30A, group 5).

In conclusion, the results presented in this study have demonstrated that the gene products associated with both the MHC and the Ig gene complex guide a series of cellular interactions leading to the formation and functional expression of a unique mediator linked to immunity. The initial stage in the assembly of the antisuppressor mediator requires antigen processing and presentation in association with MHC class II antigens to an Lyl^+, $L3T4^+$ inducer T cell. Cell contact between the antigen-pulsed macrophages and T cells, mediated by the interaction of L3T4 and MHC class II antigens, is critical for the generation of this mediator. The macrophage-T cell interaction facilitates the release of an 'intermediate complex' which lacks antisuppressor activity and has previously been reported to consist of some form of processed antigen and Ia determinants. The addition of normal IgG to the 'intermediate complex' results in the formation of the antisuppressor complexes which exert their function in the presence of Ts cells by enhancing IgG anti-SRBC antibody.

The *in vivo* reconstitution experiments have indicated that the antisuppressor inducer T cell dictates the Igh restriction imposed during the assembly of this mediator. Formation of the 6h complexes requires IgG of the same Igh haplotype as the inducer T cell. The inducer T cells were incapable of exhibiting an altered pattern of Igh restriction. Furthermore, genes of the IghV region were the only restricting element in the assembly of this mediator. By using the

divalent $F(ab')_2$ fragment of IgG it was demonstrated that the Fc portion of IgG is not involved in the formation and functional expression of the antisuppressor complexes. The effector stage of antisuppression was found to be both MHC and Igh nonrestricted.

Biological significance of antisuppression

According to Jerne's network theory (1974), the immune system maintains a steady state of activity via idiotypic-anti-idiotypic interactions. In line with this hypothesis, the steady state of the immune system has been perceived to be self-suppressive (Eichmann *et al.*, 1983; Coutinho *et al.*, 1984) and response is manifested by release from suppression. Activation of the immunoregulatory antisuppressor pathway could be one mechanism by which release from the self-suppressive state takes place (Paraskevas *et al.*, 1984). Therefore, the detection of the functionally active mediator which inhibits suppressor cell function and is formed within hours after immunization would be of crucial importance to the final outcome of the immune response.

BIBLIOGRAPHY

Abbas, A.K., M. Takaoki and M.I. Greene (1982) J. Exp. Med. 155: 1216.

Abbas, A.K., S. Haber and K.L. Rock (1985) J. Immunol. 135: 1661.

Adorini, L., S. Muller, F. Cardinaux, P.V. Lehmann, F. Falcioni and Z.A. Nagy (1988) Nature 334: 623.

Akbar, A.N., L. Terry, A. Timms, P.C.L. Beverley and G. Janossy (1988) J. Immunol. 140: 2171.

Allen, P.M., D.J. Strydom and E.R. Unanue (1984) Proc. Natl. Acad. Sci. USA 81: 2489.

Allen, P.M., B.P. Babbitt and E.R. Unanue (1987) Immunol. Rev. 98: 171.

Allison, J.P., B.W. McIntyre and D. Bloch (1982) J. Immunol. 129: 2293.

Al-Maghazachi, A., S-T. Lee and F. Paraskevas (1983) Eur. J. Immunol. 13: 799.

Andrighetto, G. and M. Zöller (1987) J. Mol. Cell. Immunol. 3: 199.

Anhalt, C. (1987) M.Sc. Thesis, University of Manitoba, Winnipeg.

Arthur, R.P. and D. Mason (1986) J. Exp. Med. 163: 774.

Asherson, G.L., V. Colizzi and M. Zembala (1986) Ann. Rev. Immunol. 4: 37.

Babbitt, B.P., P.M. Allen, G. Matsueda, E. Haber and E.R. Unanue (1985) Nature 317: 359.

Bach, B.A., L. Sherman, B. Benacerraf and M.I. Greene (1978) J. Immunol. 121: 1460.

Bach, B.A., M.I. Greene, B. Benacceraf and A. Nisonoff (1979) J. Exp. Med. 149: 1084.

Basham, T.Y. and T.C. Merigan (1983) J. Immunol. 130: 1492.

Baxevanis, C.N., N. Ishii, Z.A. Nagy and J. Klein (1982) Scand. J. Immunol. 16: 25.

Behlke, M.A., D.G. Spinella, H.S. Chou, W. Sha, D.L. Hartl and D.Y. Loh (1985) Science 229: 566.

Beller, D.I., J-M. Kiely and E.R. Unanue (1980) J. Immunol. 124: 1426.

Beller, D.I. and E.R. Unanue (1981) J. Immunol. 126: 263.

Beller, D.I. (1984) Eur. J. Immunol. 14: 138.

Benacerraf, B. (1978) J. Immunol. 120: 1809.

Berzofsky, J.A. (1988) Immunol. Letters 18: 83.

Bierer, B.E. and S.J. Burakoff (1988) FASEB J. 2: 2584.

Blanckmeister, C.A., K. Yamamoto, M.M. Davis and G.J. Hämmerling (1985) J. Exp. Med. 162: 851.

Bluestone, J.A., D. Pardoll, S.O. Sharrow and B.J. Fowlkes (1987) Nature 326: 82.

Bond, M.W., B. Shrader, T.R. Mosmann and R.L. Coffman (1987) J. Immunol. 139: 3691.

Bottomly, K. (1988) Immunol. Today 9: 268.

Braley-Mullen H. (1980) J. Immunol. 125: 1849.

Braley-Mullen, H. (1984) J. Exp. Med. 160: 42.

Braley-Mullen, H. (1986) J. Immunol. 137: 2761.

Braley-Mullen, H. (1986a) J. Immunol. 136: 396.

Braley-Mullen, H. (1988) Immunol. Res. 7: 23.

Brenner, M.B., J. McLean, D.P. Dialynas, J.L. Strominger, J.A. Smith, F.L. Owen, J.G. Seidman, S. Ip, F. Rosen and M.S. Krangel (1986) Nature 322: 145.

Brines R. and T. Lehner (1988) Immunology 63: 247.

Britz, J.S., P.W. Askenase, W. Ptak, R.M. Steinman and R.K. Gershon (1982) J. Exp. Med. 155: 1344.

Buus, S. and O. Werdelin (1986) J. Immunol. 136: 459.

Buus, S., A. Sette, S.M. Colon, D.M. Jenis and H.M. Grey (1986) Cell 47: 1071.

Buus, S., A. Sette and H.M. Grey (1987) Immunol. Rev. 98: 115.

Buus, S., A. Sette, S.M. Colon, C. Miles and H.M. Grey (1987a) Science 235: 1353.

Cantor, H. and E.A. Boyse (1975) J. Exp. Med. 141: 1376.

Cease, K.B., I. Berkower, J. York-Jolley and J.A. Berzoksky (1986) J. Exp. Med. 164: 1779.

Celada, A., P.W. Gray, E. Rinderknecht and R.D. Schreiber (1984) J. Exp. Med. 160: 55.

Cherwinski, H.M., J.H. Schumacher, K.D. Brown and T.R. Mosmann (1987) J. Exp. Med. 166: 1229.

Chesnut, R.W., S.M. Colon and H.M. Grey (1982) J. Immunol. 128: 1764.

Clark, R.B. and E.M. Shevach (1982) J. Exp. Med. 155: 635.

Coffman, R.L., J. Ohara, M.W. Bond, J. Carty, A. Zlotnik and W.E. Paul (1986) J. Immunol. 136: 4538.

Coffman, R.L., B. Shrader, J. Carty, T.R. Mosmann and M.W. Bond (1987) J. Immunol. 139: 3685.

Coutinho, A., L. Forni, D. Holmberg, F. Ivars and N. Vaz (1984) Immunol. Rev. 79: 151.

Cowing, C., B.D. Schwartz and H.B. Dickler (1978) J. Immunol. 120: 378.

Cunningham, A.J. and A. Szenberg (1968) Immunology 14: 599.

Davis, M.M. and P.J. Bjorkman (1988) Nature 334: 395.

Dembic, Z., W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer and M. Steinmetz (1986) Nature 320: 232.

Dembic, Z., W. Haas, R. Zamoyska, J. Parnes, M. Steinmetz and H. von Boehmer (1987) Nature 326: 510.

Dialynas, D.P., D.B. Wilde, P. Marrack, A. Pierres, K.A. Wall, W. Havran, G. Otten, M.R. Loken, M. Pierres, J. Kappler and F.W. Fitch (1983) Immunol. Rev. 74: 29.

Diener, E., and W.D. Armstrong (1967) Lancet 2: 1281.

Dorf, M.E. and B. Benacerraf (1984) Ann. Rev. Immunol. 2: 127.

Doyle, C. and J.L. Strominger (1987) Nature 330: 256.

Eardley, D.D. and R.K. Gershon (1976) J. Immunol. 117: 313.

Eardley, D.D., F.W. Shen, H. Cantor and R.K. Gershon (1979) J. Exp. Med. 150: 44.

Eichmann, K. (1975) Eur. J. Immunol. 5: 511.

Eichmann, K., K. Fey, R. Kuppers, I. Melchers, M.M. Simon and H.U. Weltzien (1983) Springer Semin. Immunopathol. 6: 7.

Ellner, J.J. and A.S. Rosenthal (1975) J. Immunol. 114: 1563.

Ellner, J.J., P.E. Lipsky and A.S. Rosenthal (1977) J. Immunol. 118: 2053.

Erb, P. and M. Feldmann (1975) Eur. J. Immunol. 5: 759.

Ernst, P.B., J. Maeba, S-T. Lee and F. Paraskevas (1988) Immunology 65: 59.

Ey, P.L., S.J. Prowse and C.R. Jenkin (1978) Immunochemistry 15: 429.

Feldmann, M. and S. Kontiainen (1976) Eur. J. Immunol. 6: 302.

Flood, P.M., C. Benoist, D. Mathis and D.B. Murphy (1986) Proc. Natl. Acad. Sci. USA 83: 8308.

Flood, P.M., A. Friedman, J. Freedman, B. Horvat, P. Reuter and W. Ptak (1988) Immunol. Res. 7: 12.

Friedman, A., R. Zerubavel, C. Gitler and I.R. Cohen (1983) Immunogenetics 18: 291.

Funckes-Shippy, C.L., C.M. Sorensen, C.W. Pierce and A.D. Levine (1987) Proc. Natl. Acad. Sci. USA 84: 6229.

Gabert, J., C. Langlet, R. Zamoyska, J.R. Parnes, A-M. Schmitt-Verhulst and B. Malissen (1987) Cell 50: 545.

Gay, D., P. Maddon, R. Sekaly, M.A. Talle, M. Godfrey, E. Long, G. Goldstein, L. Chess, R. Axel, J. Kappler and P. Marrack (1987) Nature 328: 626.

Gay, D., S. Buus, J. Pasternak, J. Kappler and P. Marrack (1988) Proc. Natl. Acad. Sci. USA 85: 5629.

Germain, R.N., S-T. Ju, T.J. Kipps, B. Benacerraf and M.E. Dorf (1979) J. Exp. Med. 149: 613.

Germain, R.N. and B. Benacerraf (1981) Scand. J. Immunol. 13: 1.

Gershon, R.K. and K. Kondo (1970) Immunology 18: 723.

Gershon, R.K. and K. Kondo (1971) Immunology 21: 903.

Gershon, R.K. (1974) in "Immunological tolerance: mechansims and potential therapeutic applications" (Ed. by Katz, D.H. and B. Benacerraf), Academic Press, New York, p. 441.

Gershon, R.K., D.D. Eardley, S. Durum, D.R. Green, F-W. Shen, K. Yamauchi, H. Cantor and D.B. Murphy (1981) J. Exp. Med. 153: 1533.

Gillis, S. and S.B. Mizel (1981) Proc. Natl. Acad. Sci. USA 78: 1133.

Glimcher, L.H., K-J. Kim, I. Green and W.E. Paul (1982) J. Exp. Med. 155: 445.

Gordon, J., F. Paraskevas and S-T. Lee (1985) Cell. Immunol. 94: 100.

Gordon, J. and K. Abikar (1988) FASEB J. 2: A1056 (abstract).

Green, D.R., D.D. Eardley, A. Kimura, D.B. Murphy, K. Yamauchi and R.K. Gershon (1981) Eur. J. Immunol. 11: 973.

Green, D.R. and S. St. Martin (1983) Ann. N.Y. Acad. Sci. 409: 284.

Green, D.R., P.M. Flood and R.K. Gershon (1983) Ann. Rev. Immunol. 1: 439.

Green, D.R. and R.K. Gershon (1984) Adv. Cancer Res. 42: 277.

Green, D.R. and W. Ptak (1986) Immunol. Today 7: 81.

Green, D.R., B. Chue and P.M. Flood (1988) Immunol. Res. 7: 82.

Greenstein, J.L., J. Kappler, P. Marrack and S.J. Burakoff (1984) J. Exp. Med. 159: 1213.

Guillet, J-G., M-Z. Lai, T.J. Briner, J.A. Smith and M.L. Gefter (1986) Nature 324: 260.

Guillet, J-G., M-Z. Lai, T.J. Briner, S. Buus, A. Sette, H.M. Grey, J.A. Smith and M.L. Gefter (1987) Science 235: 865.

Habu, S., K. Yamauchi, R.K. Gershon and D.B. Murphy (1981) Immunogenetics 13: 215.

Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler and P. Marrack (1983) J. Exp. Med. 157: 1149.

HayGlass, K.T., S. J. Naides, B. Benacerraf and M-S. Sy (1985) Proc. Natl. Acad. Sci. USA 82: 2133.

Hedrick, S.M., E.A. Nielsen, J. Kavaler, D.I. Cohen and M.M. Davis (1984) Nature 308: 153.

Hedrick, S.M., R.N. Germain, M.J. Bevan, M. Dorf, I. Engel, P. Fink, N. Gascoigne, E. Haber-Katz, J. Kapp, Y. Kaufmann, J. Kaye, F. Melchers, C. Pierce, R.H. Schwartz, C. Sorensen, M. Taniguchi and M.M. Davis (1985) Proc. Natl. Acad. Sci. USA 82: 531.

Hoover, R.G. and R.G. Lynch (1980) J. Immunol. 125: 1280.

Ishii, N., C.N. Baxevanis, Z.A. Nagy and J. Klein (1981) J. Exp. Med. 154: 978.

Ishii, N., Z.A. Nagy and J. Klein (1982) Nature 295: 531.

Ishizaka, K. and T. Adachi (1976) J. Immunol. 117: 40.

Iverson, M., W. Ptak, D.R. Green and R.K. Gershon (1983) J. Exp. Med. 158: 982.

Janeway, C.A., Jr. (1988) Scand. J. Immunol. 27: 621.

Janeway, C.A., Jr., S. Carding, B. Jones, J. Murray, P. Portoles, R. Rasmussen, J. Rojo, K. Saizawa, J. West and K. Bottomly (1988) Immunol. Rev. 101: 39.

Jerne, N.K. (1974) Ann. Immunol. (Inst. Pasteur) 125C: 373.

Julius, M.H., E. Simpson and L.A. Herzenberg (1973) Eur. J. Immunol. 3: 645.

Kapp, J.A., C.W. Pierce and B. Benacerraf (1974) J. Exp. Med. 140: 172.

Kapp, J.A., C.W. Pierce, S. Schlossman and B. Benacerraf (1974a) J. Exp. Med. 140: 648.

Kapp, J.A., C.W. Pierce, F. De La Croix and B. Benacerraf (1976) J. Immunol. 116: 305.

Kappler, J.W., B. Skidmore, J. White and P. Marrack (1981) J. Exp. Med. 153: 1198.

Karasuyama, H., M. Kim, K. Okumura and T. Tada (1984) Eur. J. Immunol. 14: 413.

Katz, D.H. and B. Benacerraf (1975) Transplant. Rev. 22: 175.

Kaye, J., S. Gillis, S.B. Mizel, E.M. Shevach, T.R. Malek, C.A. Dinarello, L.B. Lachman and C.A. Janeway, Jr. (1984) J. Immunol. 133: 1339.

Kelly, C.J. and E. G. Neilson (1988) Immunol. Res. 7: 56.

Kemp, J.D. and J.W. Rohrer (1984) Adv. Cancer Res. 42: 326.

Kim, K.J., C. Kanellopoulos-Langevin, R.M. Merwin, D.H. Sachs and R. Asofsky (1979) J. Immunol. 122: 549.

Kindred, B. and D.C. Shreffler (1972) J. Immunol. 109: 1972.

Kitamura, K., H. Kiyono, K. Fujihashi, J.H. Eldridge, D.R. Green and J.R. McGhee (1987) J. Immunol. 139: 3251.

Kiyono, H., D.R. Green and J.R. McGhee (1988) Immunol. Res. 7: 67.

Klein, J. and P. Walden (1986) Prog. Immunol. 6: 212.

Kontiainen, S. and M. Feldmann (1976) Eur. J. Immunol. 6: 296.

Koren, H.S., B.S. Handwerger and J.R. Wunderlich (1975) J. Immunol. 114: 894.

Kovac, Z. and R.H. Schwartz (1985) J. Immunol. 134: 3233.

Kramer, M.D. and M.M. Simon (1987) Immunol. Today 8: 140.

Kronenberg, M., E. Kraig, G. Siu, J.A. Kapp, J. Kappler, P. Marrack, C.W. Pierce and L. Hood (1983) J. Exp. Med. 158: 210.

Kronenberg, M., J. Goverman, R. Haars, M. Malissen, E. Kraig, L. Phillips, T. Delovitch, N. Suciu-Foca and L. Hood (1985) Nature 313: 647.

Krupen, K., B.A. Araneo, L. Brink, J.A. Kapp, S. Stein, K.J. Wieder and D.R. Webb (1982) Proc. Natl. Acad. Sci. USA 79: 1254.

Kurt-Jones, E.A., H.W. Virgin IV and E. R. Unanue (1985) J. Immunol. 135: 3652.

L'age-Stehr, J. (1980) J. Exp. Med. 153: 1236.

Lamoyi, E. and A. Nisonoff (1983) J. Immunol. Methods 56: 235.

Lapp, W.S. and G. Möller (1969) Immunology 17: 339.

Lapp. W.S., T. Ghayur, M. Mendes, M. Seddik and T.A. Seemayer (1985) Immunol. Rev. 88: 108.

Larsson, E-L., N.N. Iscove and A. Coutinho (1980) Nature 283: 664.

Lavie, G., D. Zucker-Franklin and E.C. Franklin (1978) J. Exp. Med. 148: 1020.

Ledbetter, J.A., C.H. June, P.S. Rabinovitch, A. Grossmann, T.T. Tsu and J.B. Imboden (1988) Eur. J. Immunol. 18: 525.

Lee, S-T. and F. Paraskevas (1972) J. Immunol. 109: 1262.

Lee, S-T. and F. Paraskevas (1979) Cell. Immunol. 48: 1.

Lee, S-T. and F. Paraskevas (1981) Cell. Immunol. 60: 64.

Lee, S-T., F. Paraskevas and J. Maeba (1985) Cell. Immunol. 92: 64.

Lehner, T. (1982) J. Immunol. 129: 1936.

Lehner, T. and T. Jones (1984) Immunology 53: 215.

Lehner, T. (1986) Immunol. Today 7: 87.

Lew, A.M., D.M. Pardoll, W.L. Maloy, B.J. Fowlkes, A. Kruisbeek, S-F. Cheng, R.N. Germain, J.A. Bluestone, R.H. Schwartz and J.E. Coligan (1986) Science 234: 1401.

Lloyd, J.B. (1980) Ciba Found. Symp. 75: 151.

Lorenz, R.G. and P.M. Allen (1988) Proc. Natl. Acad. Sci. USA 85: 5220.

Lowenthal, J.W., J-C. Cerottini and H.R. MacDonald (1986) J. Immunol. 137: 1226.

Lowy, A., A. Tominaga, J.A. Drebin, M. Takaoki, B. Benacerraf and M.I. Greene (1983) J. Exp. Med. 157: 353.

Maeba, J., S-T. Lee and F. Paraskevas (1988) J. Immunol. Methods 106: 7.

Manca, F., J.A. Clarke, A. Miller, E.E. Sercarz and N. Shastri (1984) J. Immunol. 133: 2075.

Marrack, P., R. Endres, R. Shimonkevitz, A. Zlotnik, D. Dialynas, F. Fitch and J. Kappler (1983) J. Exp. Med. 158: 1077.

Mathur, A. and R.G. Lynch (1986) J. Immunol. 136: 521.

Meuer, S.C., S.F. Schlossman and E.L. Reinherz (1982) Proc. Natl. Acad. Sci. USA 79: 4395.

Meuer, S.C., K.A. Fitzgerald, R.E. Hussey, J.C. Hodgdon, S.F. Schlossman and E.L. Reinherz (1983) J. Exp. Med. 157: 705.

Minami, Y., A.M. Weissman, L.E. Samelson and R.D. Klausner (1987) Proc. Natl. Acad. Sci. USA 84: 2688.

Mittal, K.K., M.R. Mickey, D.P. Singal and P.I. Terasaki (1968) Transplantation 6: 913.

Modlin, R.L., M.B. Brenner, M.S. Krangel, A.D. Duby and B.R. Bloom (1987) Nature 329: 541.

Möller, G. (1988) Scand. J. Immunol. 27: 247.

Morimoto, C., N.L. Letvin, A.W. Boyd, M. Hagan, H.M. Brown, M.M. Kornacki and S.F. Schlossman (1985) J. Immunol. 134: 3762.

Morimoto, C., N.L. Letvin, J.A. Distaso, W.R. Aldrich and S.F. Schlossman (1985a) J. Immunol. 134: 1508.

Morimoto, C., N.L. Letvin, J.A. Distaso, H.M. Brown and S.F. Schlossman (1986) Eur. J. Immunol. 16: 198.

Morimoto, C., C.E. Rudd, N.L. Letvin and S.F. Schlossman (1987) Nature 330: 479.

Morimoto, C., T. Matsuyama, C.E. Rudd, A. Forsgren, N.L. Letvin and S.F. Schlossman (1988) Eur. J. Immunol. 18: 731.

Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin and R.L. Coffman (1986) J. Immunol. 136: 2348.

Mosmann, T.R. and R.L. Coffman (1987) Immunol. Today 8: 223.

Murphy, D.B., L.A. Herzenberg, K. Okumura, L.A. Herzenberg and H.O. McDevitt (1976) J. Exp. Med. 144: 699.

Nakamura, R.M., Y. Nakamura, A. Nagayama and T. Tokunaga (1986) Immunol. Res. 5: 106.

Nakanishi, K., K. Sugimura, Y. Yaoita, K. Maeda, S-I. Kashiwamura, T. Honjo and T. Kishimoto (1982) Proc. Natl. Acad. Sci. USA 79: 6984.

Nakayama, T., R.T. Kubo, M. Kubo, I. Fujisawa, H. Kishimoto, Y.Asao and T. Tada (1988) Eur. J. Immunol. 18: 761.

Norcross, M.A., D.M. Bentley, D.H. Margulies and R.N. Germain (1984) J. Exp. Med. 160: 1316.

Nutt, N., J. Haber, H.H. Wortis (1981) J. Exp. Med. 153: 1225.

Oettgen, H.C. and C. Terhorst (1987) CRC Critical Rev. Immunol. 7: 131.

Oi, V.T., P.P. Jones, J.W. Goding, L.A. Herzenberg and L.A. Herzenberg (1978) Curr. Top. Microbiol. Immunol. 81: 115.

Orr, K.B. and F. Paraskevas (1973) J. Immunol. 110: 456.

Orr, K.B. and F. Paraskevas (1974) Can. J. Microbiol. 20: 535.

Ouchterlony, O. (1958) Prog. Allergy 5: 1.

Owen, F.L., A. Finnegan, E.R. Gates and P.D. Gottlieb (1979) Eur. J. Immunol. 9: 948.

Owen, F.L. (1980) J. Immunol. 124: 1411.

Owen, F.L., R.Riblet and B.A. Taylor (1981) J. Exp. Med. 153: 801.

Owen, F.L. (1983) Adv. Immunol. 34: 1.

Ozato, K., N. Mayer and D.H. Sachs (1980) J. Immunol. 124: 533.

Ozato, K. and D.H. Sachs (1981) J. Immunol. 126: 317.

Paraskevas, F., S-T. Lee, K.B. Orr and L.G. Israels (1971) J. Immunol. Methods 1: 1.

Paraskevas, F. and S-T. Lee (1976) Eur. J. Immunol. 6: 862.

Paraskevas, F. and S-T. Lee (1979) Cell. Immunol. 48: 31.

Paraskevas, F., K.B. Orr and S-T. Lee (1979) Cell. Immunol. 48: 15.

Paraskevas, F. and S-T. Lee (1981) Cell. Immunol. 60: 77.

Paraskevas, F. (1984) CRC Critical Rev. Immunol. 5: 95.

Paraskevas, F., S-T. Lee, K.C. Wang and A. Al-Maghazachi (1984) Lymphokines 9: 201.

Paraskevas, F., S-T. Lee and A. Al-Maghazachi (1984a) Surv. Immunol. Res. 3: 115.

Paraskevas, F., S-T. Lee, J. Maeba and C.S. David (1985) Cell. Immunol. 92: 53.

Paraskevas, F., S-T. Lee and J. Maeba (1985a) Cell. Immunol. 92: 74.

Paraskevas, F., J. Gartner, J. Maeba and S-T. Lee (1988) Clin. Immunol. Immunopathol. 47: 270.

Paraskevas, F., J. Teoh and S-T. Lee (1988a) FASEB J. 2: A1061 (abstract).

Patten, P., T. Yokota, J. Rothbard, Y-H. Chien, K-I. Arai and M.M.Davis (1984) Nature 312: 40.

Pereira, P., E-L. Larsson, L. Forni, A. Bandeira and A. Coutinho (1985) Proc. Natl. Acad. Sci. USA 82: 7691.

Pereira, P., L. Forni, E-L. Larsson, M. Cooper, C. Heusser and A. Coutinho (1986) Eur. J. Immunol. 16: 685.

Pereira, P., E-L. Larsson-Sciard, A. Coutinho and A. Bandeira (1988) Scand. J. Immunol. 27: 625.
Perry, L.L. and M.I. Greene (1982) J. Exp. Med. 156: 480.

Poole, B., S. Ohkuma and M. Warburton (1980) Ciba Found. Symp. 75: 189.

Ptak, W., M. Bereta, J. Marcinkiewicz, R.K. Gershon and D.R. Green (1984) J. Immunol. 133: 623.

Ptak, W., M. Ptak and A. Gryglewski (1986) Scand. J. Immunol. 23: 555.

Ptak, W., A. Friedman, M. Bereta, J. Marcinkiewicz, B. Horvat, P. Reuter, D.R. Green and P.M. Flood (1988) Immunol. Res. 7: 1.

Puri, J. and P. Lonai (1980) Eur. J. Immunol. 10: 273.

Reif, A.E. and J.M.V. Allen (1964) J. Exp. Med. 120: 413.

Reinherz, E.L. and S.F. Schlossman (1980) Cell 19: 821.

Rock, K.L., B. Benacerraf and A.K. Abbas (1984) J. Exp. Med. 160: 1102.

Rohrer, J.W., B. Odermatt and R.G. Lynch (1979) J. Immunol. 122: 2011.

Rohrer, J.W. and J.D. Kemp (1988) Immunol. Res. 7: 45.

Rosenbaum, J.T., N.E. Adelman and H.O. McDevitt (1981) J. Exp. Med. 154: 1694.

Rosenthal, A.S. (1978) Immunol. Rev. 40: 136.

Rosenthal, A.S. and E.M. Shevach (1973) J. Exp. Med. 138: 1194.

Saito, H., D.M. Kranz, Y. Takagaki, A.C. Hayday, H.N. Eisen and S. Tonegawa (1984) Nature 309: 757.

Saito, T., A. Weiss, J. Miller, M.A. Norcross and R.N. Germain (1987) Nature 325: 125.

Scher, M.G., D.I. Beller and E.R. Unanue (1980) J. Exp. Med. 152: 1684.

Schwartz, R.H., B.S. Fox, E. Fraga, C. Chen and B. Singh (1985) J. Immunol. 135: 2598.

Shaw, S., G. Goldstein, T.A. Springer and W.E. Biddison (1985) J. Immunol. 134: 3019.

Sherr, D.H., N-K.V. Cheung, K.M. Heghinian, B. Benacerraf and M.E. Dorf (1979) J. Immunol. 122: 1899.

Sherr, D.H., K.M. Heghinian, B. Benacerraf and M.E. Dorf (1980) J. Immunol. 124: 1389.

Shimonkevitz, R., J. Kappler, P. Marrack and H. Grey (1983) J. Exp. Med. 158: 303.

Shimonkevitz, R., S. Colon, J.W. Kappler, P. Marrack and H.M. Grey (1984) J. Immunol. 133: 2067.

Sleckman, B.P., A. Peterson, W.K. Jones, J.A. Foran, J.L. Greenstein, B. Seed and S.J. Burakoff (1987) Nature 328: 351.

Smith, K.A., L.B. Lachman, J.J. Oppenheim and M.F. Favata (1980) J. Exp. Med. 151: 1551.

Smith, S.H., M.H. Brown, D. Rowe, R.E. Callard and P.C.L. Beverley (1986) Immunology 58: 63.

Snapper, C.M. and W.E. Paul 1987) Science 236: 944.

Sorensen, C.M. and C.W. Pierce (1982) J. Exp. Med. 156: 1691.

Spickett, G.P., M.R. Brandon, D.W. Mason, A.F. Williams and G.R. Woollett (1983) J. Exp. Med. 158: 795.

Steeg, P.S., R.N. Moore and J.J. Oppenheim (1980) J. Exp. Med. 152: 1734.

Steeg, P.S., R.N. Moore, H.M. Johnson and J.J. Oppenheim (1982) J. Exp. Med. 156: 1780.

Steinmetz, M., K. Minard, S. Horvath, J. McNicholas, J. Srelinger, C. Wake, E. Long, B. Mach and L. Hood (1982) Nature 300: 35.

Suemura, M., A. Ishizaka, S. Kobatake, K. Sugimura, K. Maeda, K. Nakanishi, S. Kishimoto, Y. Yamamura and T. Kishimoto (1983) J. Immunol. 130: 1056.

Swain, S.L. (1981) Proc. Natl. Acad. Sci. USA 78: 7101.

Swain, S.L. (1983) Immunol. Rev. 74: 129.

Sy, M-S., A. Lowy, K. HayGlass, C.A. Janeway, Jr., M. Gurish, M.I. Greene and B. Benacerraf (1984) Proc. Natl. Acad. Sci. USA 81: 3846.

Sy, M-S., K.T. HayGlass and B. Benacerraf (1985) J. Exp. Med. 161: 1402.

Sy, M-S. and B. Benacerraf (1988) Immunol. Rev. 101: 133.

Tada, T., M. Taniguchi and C.S. David (1976) J. Exp. Med. 144: 713.

Tada, T. and K. Okumura (1979) Adv. Immunol. 28: 1.

Tada, T. (1988) Scand. J. Immunol. 27: 623.

Taniguchi, M., T. Tokuhisa, M. Kanno, Y Yaoita, A. Shimizu and T. Honjo (1982) Nature 298: 172.

Taniguchi, M., T. Tokuhisa, T. Itoh and M. Kanno (1984) J. Exp. Med. 159: 1096.

Tite, J.P., H.G. Foellmer, J.A. Madri and C.A. Janeway, Jr. (1987) J. Immunol. 139: 2892.

Tokuhisa, T. and M. Taniguchi (1982) J. Exp. Med. 155: 126.

Tokuhisa, T. and M. Taniguchi (1982a) Nature 298: 174.

Unanue, E.R. (1981) Adv. Immunol. 31: 1.

Unanaue, E.R. (1984) Ann. Rev. Immunol. 2: 395.

Unanue, E.R. and P.M. Allen (1987) Science 236: 551.

Uracz, W., Y. Asano, R. Abe and T. Tada (1985) Nature 316: 741.

Vitetta, E.S., J. Ohara, C.D. Myers, J.E. Layton, P.H. Krammer and W.E. Paul (1985) J. Exp. Med. 162: 1726.

Walden, P., Z.A. Nagy and J. Klein (1985) Nature 315: 327.

Walden, P., Z.A. Nagy and J. Klein (1986) J. Mol. Cell. Immunol. 2: 191.

Walker, E.B., L.L. Lanier and N.L. Warner (1982) J. Exp. Med. 155: 629.

Waltenbaugh, C., L. Sun and H-Y. Lei (1986) J. Exp. Med. 163: 797.

Watts, T.H., A.A. Brian, J.W. Kappler, P. Marrack and H.M. McConnell (1984) Proc. Natl. Acad. Sci USA 81: 7564.

Watts, T.H., H.E. Gaub and H.M. McConnell (1986) Nature 320: 179.

Weaver, C.T. and E.R. Unanue (1986) J. Immunol. 137: 3868.

Webb, D.R., J.A. Kapp and C.W. Pierce (1983) Ann. Rev. Immunol. 1: 423.

Weinberger, J.Z., R.N. Germain, S-T. Ju, M.I. Greene, B. Benacerraf and M.E. Dorf (1979) J. Exp. Med. 150: 761.

Weiner, D.B., J. Liu, N. Hanna, J.A. Bluestone, J.E. Coligan, W.V. Williams and M.I. Greene (1988) Proc. Natl. Acad. Sci. USA 85: 6077.

Wieder, K., B.A. Araneo, J.A. Kapp and D.R. Webb (1982) Proc. Natl. Acad. Sci. USA 79: 3599.

Wilde, D.B., P. Marrack, J. Kappler, D.P. Dialynas and F.W. Fitch (1983) J. Immunol. 131: 2178.

Williams, J.M., D. Deloria, J.A. Hansen, C.A. Dinarello, R. Loertscher, H.M. Shapiro and T.B. Strom (1985) J. Immunol. 135: 2249.

Yagüe, J., J. White, C. Coleclough, J. Kappler, E. Palmer and P. Marrack (1985) Cell 42: 81.

Yamashita, U. and E.M. Shevach (1977) J. Immunol. 119: 1584.

Yamauchi, K., D.R. Green, D.D. Eardley, D.B. Murphy and R.K. Gershon (1981) J. Exp. Med. 153: 1547.

Yamauchi, K., N. Chao, D.B. Murphy and R.K. Gershon (1982) J. Exp. Med. 155: 655.

Yanagi, Y., Y. Yoshikai, K. Leggett, S.P. Clark, I. Aleksander and T.W. Mak (1984) Nature 308: 145.

Zan-Bar, I., D. Nachtigal and M. Feldman (1975) Cell. Immunol. 17: 202.

Ziegler, K. and E.R. Unanue (1981) J. Immunol. 127: 1869.

Ziegler, K. and E.R. Unanue (1982) Proc. Natl. Acad. Sci. USA 79: 175.