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ANTIGEN SPECIFIC T SUPPRESSOR FACTORS, A PROPER SUBJECT FOR STUDY: THEIR RELATION TO THE T CELL RECEPTOR, THEIR CLASS AND THEIR MODE OF ACTION

GEOFFREY L. ASHERSON

Division of Immunological Medicine, Clinical Research Centre,
Northwick Park Hospital, Harrow, UK

INTRODUCTION

The object of this review is to draw attention to recent work on antigen-specific T suppressor factors (TsF) and the evidence that many of them share epitopes with the α and β chains of the T cell receptor (TCR) and that their production makes use of the standard α/β TCR. It also comments on the mode of action of certain TsF, which are of particular interest to the author. It is not comprehensive and focuses on work based on T cell hybridomas. It does not cover much of the earlier literature, where basic facts about the genetic restriction and mode of action of antigen-specific TsF are summarised (*Dorf* and *Benacerraf*, 1984; *Asherson* et al., 1986).

The introduction shows the way in which the broad concept of suppressor cells has been refined as it became clear that many cells have pleomorphic effects and upregulate certain immune responses while downregulating others. The concluding remarks deal with the biological significance of antigen-specific T suppressor factors and certain unsolved problems, such as the possible selective use of I-E restriction by suppressor cells and the continuing puzzle of I-J.

Historically, the concept of downregulation in the immune response goes back to work in the sixties on specific antibody mediated depression of antibody production and cellular immunity. In the early seventies, two groups described downregulation of the immune response by T cells and the term suppressor cell arose (*Gershon* et al., 1971; *Asherson* et al., 1971). The use of a single term to describe several different types of cells with different modes of action caused confusion. The term was often taken to imply that T suppressor (Ts) cells were "professional", i.e. they were committed to downregulation and had no other function. The use of T cell lines and hybridomas has shown that some suppressor cells have other activities; in other cases a suppressor cell acts (directly or indirectly) though a known cytokine. Some examples are given below.

Downregulation due to cytokines with negative effects:

Certain cytokines have negative effects and are made *inter alia* by T cells. The best example is TGF-β, which depresses T cell proliferation and NK cytotoxicity (*Lotz* et al., 1990). It is also required for the action of a T suppressor cell which downregulates allergic encephalomyelitis (*Karpus* and *Swanborg*, 1991).

The role of lymphokines in the polarisation of the immune response:

The apparent polarisation of the immune response between antibody production and delayed hypersensitivity was noted a quarter of a century ago (*Asherson* and *Stone*, 1965). The recent

work on Th1 and Th2 cells gives an approach to the underlying mechanism. To a first approximation, the Th1 subset makes IFN and IL-2, while the Th2 subset makes IL-4. The former affects cellular immunity, while the later controls antibody production. The two subsets have a reciprocal relationship to each other. In particular, the IFN made by Th1 cells limits the proliferation of Th2 cells and antagonises the effect of IL-4, while IL-10 made by Th2 cells limits cytokine production by Th1 cells (*Fiorentino* et al., 1989, 1991; *Mossmann* et al., 1990). Because the cells and lymphokines involved augment certain responses while downregulating others, they cannot be usefully described as suppressor cells and factors.

Ts-1 and Ts-3 cells which make antigen-specific factors

By definition, Ts-1 (Ts-inducer or Ts-afferent) cells act at the induction stage of a particular immune response, while Ts-3 cells act at the effector stage. They have different modes of action and are made by cells with different properties. In particular, the antigen-specific TsF-1 made by Ts-1 cells induces idiotope directed cells by a process of immunisation. In contrast, the antigen-specific TsF-3 made by Ts-3 has indirect mode of action through the macrophage and the T acceptor cell. Moreover certain Ts-1 cells resemble T helper cells and make IL-2 and IL-4 (*Kuchroo* et al., 1990). In contrast, Ts-3 cells behave like professional suppressor cells, i.e. they produce an antigen-specific TsF and *pro tem* have no other known action.

These antigen-specific T suppressor factors are distinct from cytokines, in being antigen-specific and genetically restricted in their action. In general, they are disulphide bonded heterodimers with an antigen binding and a non antigen binding chain. Evidence is now available that in some cases the antigen binding chain conveys antigen-specificity, while the non antigen binding chain is responsible for the genetic restriction in the action of the factor. It would be rational to designate these two chains as α and β.

Much of the confusion with suppressor cells has arisen from mistaken view that the term implies a single class of suppressor cell. Similarly, study of immunoglobulins was chaotic before classes of immunoglobulin were recognised. Antigen-specific T cell factors in general have many different effects and an important object of current research is to define distinct classes. In fact, TsF-3 has many different actions which are listed in Table I. By analogy with immunoglobulin these different actions of TsF have structural implications for the constant region of the two chains. Hence study of mode of action of TsF

Table 1: Inhibitory effects of T suppressor factor which acts at the efferent stage (TsF-3)

Contact and delayed hypersensitivity in immune mice
Passive transfer of contact sensitivity
*via macrophage which releases macrophage suppressor factor
*via T acceptor cell which releases antigen non-specific mediator
Granuloma formation
Tumour rejection
Phagocytosis by subset of macrophages
Antibody production

*The cell binds TsF, which acts like a mobile receptor for antigen (cf. IgE). Contact with antigen then causes the release of an antigen non-specific inhibitory mediator of the passive transfer of contact sensitivity.

is complementary to structural studies.

The finding of a disulphide bonded heterodimer produced by a T cell, whose function is antigen and MHC restricted invites comparison with the TCR.

Consider the following analogy:

- The Ig receptor for antibody production and the secreted antibody use the same H and L chain gene rearrangements.
- The TCR for TsF-3 production and TsF-3 use the same α and β chain gene rearrangements.

Current evidence is that TsF-3 studied share epitopes with the α and β chains of the TCR. Moreover, studies of the blocking of TsF secretion with antibody to the α and β chain would indicate that the analogy with surface and secreted immunoglobulin is valid. However, not all the data are in yet and there is little molecular biology.

In due course more information will be needed about the control of the production and secretion of these factors. A beginning has been made through studies of the effect of IL-2 on TsF-3 production and on T suppressor cells (*Perrin* et al., 1989; *Madar* et al., 1987).

STRUCTURE

Antigen-specific TsF-3 is a disulphide bonded heterodimer with antigen binding and non antigen binding chains

Most antigen-specific TsF's are disulphide bonded heterodimers, possessing an antigen binding and a non antigen binding chain. In particular this is true for all the TsF-3 that have been examined. Chain structure of TsF is analysed by reduction with or without alkylation. Usually the chains are partially purified by affinity chromatography on antigen or immobilised antibody to give separate antigen binding and non antigen binding chains. The chains are then tested by biological assay using complementation. This is based on the fact that they have no biological activity when tested singly, but cause suppression, e.g. of contact sensitivity or of an *in vitro* model of granuloma formation when tested together.

Complementation between the two chains occurs, using the assay of inhibition of contact sensitivity in immune mice with monoclonal picryl-TsF (unpublished observations). It also occurs when biological activity is assayed by inhibition of the passive transfer of contact sensitivity by chains from picryl-specific and DNP-specific monoclonal TsF (*Fairchild* et al, 1990). The antigen binding and non antigen binding chains also complement each other when they are used to coat the T accep-

Table 2: Properties of T acceptor cell

- Lyt-2^+ I-J^+ T cells found after immunisation with contact sensitiser, but not in unimmunised mice.
- Production prevented by cyclophosphamide and adult thymectomy
- Binds TsF to its surface and can then be panned on antigen
- Releases nsTsF-1 when activated by antigen (haptenised spleen cells) corresponding to TsF. This interaction is I-J restricted.
- Cross-linking of molecules of TsF required for activation.
- The haptenised spleen cell can be replaced by antigen together with a KCl extract of spleen cells of the appropriate genotype.

See *Zembala* et al. (1982a,b)

tor cell (see Table 2), which then release an antigen non-specific inhibitory mediator (nsTsF-1) when activated by antigen. This is seen with chains from conventional TNP-specific and monoclonal cryptococcal-specific TsF. In contrast, separate chains do not complement each other in the inhibition of phagocytosis by a subset of macrophages (*Blackstock* et al., 1991a,b). It is not known whether separate chains complement each other when used to coat the macrophage. [This cell, after coating with intact TsF, releases an antigen non-specific inhibitory mediator when activated by antigen (*Ptak* et al., 1978)].

Sometimes the antigen binding and non antigen binding chains occur separately (*Asherson* et al., 1984a) or only one of the chains is produced. *Taniguchi* and colleagues (1980, 1981) described a hybridoma in which the two separate chains were released by freezing and thawing, but appeared in the supernatant as a disulphide bonded heterodimer. In a more physiological system, the spleen cells of mice, injected with water soluble chemically reactive haptene [e.g. picrylsulphonic acid (trinitrobenzenesulphonic acid), or "oxazolone-thioglycollic acid"] release the antigen binding chain of TsF without further activation. This is biologically inactive alone but can be assayed by complementation with the non antigen binding chain (*Zembala* et al., 1984). However, constitutive liberation of the antigen binding chain alone is not shown by a inducible picryl-specific hybridoma (unpublished observations)

In fact, after activation with the antigen, the spleen cells from mice injected with chemically reactive, water soluble haptene produce "complete" TsF which does not require complementation. This activation can be achieved *in vivo* by applying contact sensitiser to the skin, or by injecting haptenised cells, and *in vitro* by culturing with haptenised cells (*Blackstock* et al., 1991a,b; *Colizzi* et al, 1983). The non antigen binding chain occurs in KCl extracts of normal cells (*Asherson* 1984a). This might be due to the β chains of the TCR present in the extract as it is known that different non antigen binding chains can complement a particular antigen binding chain (*Perrin* et al., 1989b). See next section on "Antigen-specific factors bear α and β chain TCR determinants".

There is some confusion in the literature as to whether the two chains of TsF covalently link, by reformation of disulphide bonds in complementation assays. There may be a real difference between different TsF's and different assay systems (*Taniguchi* et al., 1981; unpublished observations). However technical factors may be important. For instance, the use of strictly oxygen free conditions renders the reduced intra-chain disulphide bonds more susceptible to alkylation. Another factor is the use of acylation by succinic anhydride instead of alkylation by iodoacetamide to prevent reformation of disulphide bonds. Succinylation increases the negative charge on molecules as it effectively replaces positively charged amino groups by negatively charged carboxylic groups (*Perrin* et al., 1989b). Iodoacetamide is more selective for sulphydral groups and does not cause charge reversal. In our studies on monoclonal TNP- and cryptococcal-specific TsF, iodoacetamide was used and the chains complemented each other without reformation of interchain disulphide bonds. However, if alkylation or acylation is omitted, complementation may be due to reformation of the original molecule through oxidation of the interchain reduced disulphide bonds. This might be the case in studies on DNP-specific TsF in which alkylation was not undertaken (*Fairchild* et al, 1990).

There are other antigen-specific T cell factors which are disulphide bonded

heterodimers. Antigen-specific T helper factor, which specifically increases the contact sensitivity reaction to picryl induced by haptenised cells, is an example and its two chains can be assayed by complementation (*Little* et al., 1985).

In contrast to TsF-3, the chain structure of TsF-1 varies. Monoclonal TMA (phenyltrimethylamino)- and GAT-specific monoclonal TsF-1 have a single chain structure (*Jayaraman* and *Bellone*, 1985), while other GAT-, ABA-, NP- and sheep red blood cell-specific TsF have a two chain structure (*Jendriska* et al., 1986).

In summary, antigen-specific TsF which acts at the expression stage of the immune response, has a two chain disulphide-bonded structure, with one antigen binding and one non antigen binding chain, which can be detected in a complementation assay.

Antigen-specific T cell factors bear α and β chain TCR determinants

Analogy with immunoglobulin suggests that antigen-specific T cell factors are made by a cell with the standard TCR-T3 complex using α/β or possibly γ/δ chains and the soluble factors should closely resemble the TCR. However, the finding that only a small minority of T cell hybridomas had evidence of β chain gene rearrangement, while other hybridomas only expressed the V_β2.5

Table 3: α and β chain determinants of the T cell receptor (TCR) on antigen-specific T cell factors and on the cell that makes them

		Determinants on factor					Determinants on hybridoma			
Type of factor		TCR		I-J	Class markers		TCR		Others	
Specificity	Source	α	β		eff.	aff.	α	β		Reference
TsF-3 (efferent)										
TNP	hyb.	α	V_β8	I-J			α	V_β8	I-J	Zembala (unpubl.)
DNP	hyb.	α	V_β8					V_β8		Fairchild (1990)
NP	hyb.	α	-				α	β*	CD3	Dorf (1989) Collins (1990)
OA	hyb.	α	β		+	-		β	CD3	Iwata (1989)
Schisto.	conv.		V_β8	I-J	+					Perrin (1989)
TsF-1 (afferent)										
NP	hyb.	α	β		-	+	α	β		Kuchroo (1990)
ABA	hyb.								CD3	Weiner (1988)
HGG	line	α	-					β		Takata (1990)
KLH	hyb.						α			Koseki (1989)
Poly-18	hyb.	α	-			+		β		Bissonette (1991)
ThF (T helper factor)										
TNP	hyb.		V_β8							Dieli (unpubl.)
FGG	line		V_β8							Guy (1989)
KLH	line		V_β8							Guy (1989)
OA	hyb.	α	β		-	+		β	CD3	Iwata (1989a,b)
H-2D	hyb.		V_β8							Kwong (1987)

*β chain from BW 5147 (*Collins* et al., 1990)

Table 4: Anti-$V_\beta 8$ and anti-I-J monoclonal antibodies inhibit activation of T suppressor hybridoma

Pre-treatment of hybridoma before activation by antigen	Inhibition of contact sensitivity by TsF
None	100%*
anti-$V_\beta 8$ (F23.1)	26%**
anti-I-J^k (Ig8)	31%**
anti-I-E^k (HB32)	100%
anti-I-A^b	100%

P2.2.B4 picryl specific hybridoma was pre-treated for 1 hour with purified Ig, prepared from ascites with Protein A. After washing, antigen was added (picrylated spleen cells). The supernatant at 24 hours contained TsF which was assayed by its ability to inhibit contact sensitivity in actively immunised mice (unpublished observations).

*The data was normalised by setting the suppression caused by the hybridoma untreated with antibody at 100%.

**Highly significant as compared with hybridoma not treated with antibody ($P<0.005$)

chain of the BW 5147 thymoma (*Imai* et al., 1986; *Lee* and *Davis*, 1988) confused the issue (*Kronenberg* et al., 1985; *Hedrick* et al 1985; *Möller*, 1988). This was despite evidence that a virus transformed Ts-1 specific for lysozyme and producing soluble TsF-1 possessed mRNA for both α and β chains (*De Santis* et al., 1985, 1987).

The current weight of evidence suggests that most and perhaps all TsF-3 and those TsF-1 which possess two chains are coded for by the α and β chains of the TCR (Table 3). What is the evidence? First, in the few cases studied, the antigen binding chain has α chain determinants, while the non antigen binding chain has β chain determinants. In some cases the β chain is coded for by the BW 5147 genes. However, this does not pose a conceptual problem as the non antigen binding chain of one hybridoma can complement the α chain of another hybridoma and indeed convey genetic restriction (*Fairchild* et al, 1990; *Perrin* et al., 1989b). In particular, the TCR and soluble TsF can be assembled with the α chain (which conveys antigen-specificity) from the Ts and the β chain from the BW 5147 thymoma line (*Kuchroo* et al., 1990). The recent development of BW 1100, which lacks the genes for the α and β chains, will allow investigators to study hybridoma TsF, which only has chains derived from T suppressor cell (*White* et al., 1989).

There is a further experimental point. A "good" hybridoma yields supernatant active at dilutions of $>10^4$ (unpublished observations). Hence, as emphasised by *Dorf*, a minority of cells may be responsible for producing TsF. For this reason reselection, by adherence to antigen or to anti-CD3, followed by cloning is an important preliminary to critical experiments (*Kuchroo* et al., 1988).

Evidence that the T cell uses the TCR in the activation that leads to liberation of TsF is provided by studies in which antibody against the β chain ($V_\beta 8$) (*Staerz* et al., 1985) is used to inhibit TsF production. Our recent studies, using inducible I-E restricted, TNP-specific hybridoma, illustrate this point. One chain bound to antigen, while the non antigen binding chain was absorbed by and could be eluted from monoclonal antibody to $V_\beta 8$ and I-J determinants. The same antibodies blocked the induction of TsF production when used to pretreat the hybridoma. See Table 4

(unpublished observations). [Note in passing that the antibody to I-J may block TsF production by combining with a molecule distinct from the TCR (*Nakayama* et al., 1989)]. It may be deduced that determinants on the TsF molecule also occur on the T cell and are involved in activation. The implication is that there is important similarity between the non antigen binding chain of the TsF and the β chain of the T cell receptor.

Dorf and his group pinpointed the role of CD3, by showing that hybridomas, reselected by panning for CD3 positivity, had increased TsF-3 production. Using these hybridomas, it was then possible to demonstrate the α chain of TCR on the surface of the hybridoma by immunoprecipitation (*Kuchroo*, 1988).

Similarly, *Weiner* and colleagues (1988) studied an azobenzenearsonate (ABA)-specific TsF1 which inhibited delayed hypersensitivity and antigen-induced production of IL-2. They established $CD3^+$ and $CD3^-$ lines and showed that many but not all $CD3^+$ lines were unstable over 6 weeks. Only the $CD3^+$ cell lines constitutively released TsF. The implication is that the cells which made TsF possessed CD3 and presumably the TCR/CD3 complex.

Some of the data on antigen-specific T helper factors also bear on the similarity between antigen-specific T cell factors and the T cell receptor. *Guy* and colleagues (1989) developed a cloned antigen-specific and MHC restricted Th2 cell line which secreted IL-4 when stimulated with antigen and an antigen-specific factor which augmented the IgG antibody response *in vitro*. The factor had $V_\beta 8$ determinants and biosynthetic labelling showed that the cell and the antigen-specific factor were heterodimeric 85 kDa molecules with components of 40-45 kDa.

Role of antigen binding chain of TsF in determining antigen-specificity and of non antigen binding chain with $V_\beta 8$ determinants in determining genetic restriction

Moorhead's group studied a DNP-specific TsF whose action was class I (K or D) restricted. The antigen binding chain possessed an epitope of the TCR α chain constant region, while the non antigen binding chain expressed an epitope of variable region of the β chain ($V_\beta 8$). Moreover activation of the hybridoma by antigen to produce TsF was blocked by antibody (F23.1) to $V_\beta 8$. Complementation studies, using chains from hybridomas with different class I genetic restrictions, showed that the restriction (K or D) was controlled by the non antigen binding chain. (This was measured by the ability to bind to DNP-immune lymph node cells with accessible K^k and D^k determinants.) The ability of the antigen binding chain to bind to antigen suggested that it conveyed antigenic specificity. However, not all combinations of antigen binding and non antigen binding chains complemented each other (*Fairchild* et al., 1990).

Some of the observations of antigen-specific T helper factor also suggest that the non antigen binding chain determines genetic restriction. Our group studied an antigen-specific T helper factor which augments the induction stage of the contact sensitivity reaction when used to coat the haptenised spleen cells used for immunisation. The ThF was a disulphide bonded heterodimer and absorption with monoclonal antibody showed I-A α and β chain determinants on the nonantigen binding chain. These determinants controlled the antigen-specificity of the factor as shown by studies using T helper factor from F1 mice (*Little* et al., 1987, 1988). Recently, *Dieli* (personal com-

munication) showed that the non antigen binding chain had $V_\beta 8$ determinants but it is not clear whether the I-A determinants were an integral part of the chain or whether the non antigen binding chain formed an non covalent complex with I-A.

Perrin et al. (1988, 1989b) studied schistosomal and PPD specific "conventional" TsF's, which they assayed by their ability to inhibit an *in vitro* model of granuloma formation. The TsF possessed $V_\beta 8$ determinants of the TCR. Complementation studies, using the antigen binding and non binding chains of schistosomal specific TsF of two different genetic specificities, confirmed that the non antigen binding chain conveyed the genetic restriction. Studies using antigen binding chains of schistosomal- and PPD-specificity showed that this chain conveyed antigen-specificity and complemented the non antigen binding chain from TsF of different specificity. This role of the β chain in determining MHC genetic restriction is in keeping with the finding of *Kappler* and colleagues (1987) that a certain V_β gene is strongly associated with reactivity to allogeneic I-E.

Does TsF always have a non antigen binding chain similar to the β chain of the TCR?

It is clear that some TsF-1 lack a separate non antigen binding chain. However, all relevant studies have shown that the antigen binding chain of TsF bears TCR α chain determinants. In contrast, three studies have raised doubts about the involvement of TCR β chain determinants or of the TCR β chain.

For instance, *Imai* and colleagues (1986) described KLH-specific hyridomas. cDNA studies showed an α chain derived from the suppressor cell, but the β chain was of the thymoma line (BW 5147) on Southern blot analysis. As cell surface labelling showed a disulphide bonded heterodimer, they concluded that the α chain might be coupled with a hitherto unknown chain. However, these findings would now be interpreted as indicating that functional molecules of TCR and of TsF could be assembled using the α chain of the Ts cell and the β chain of BW 5147.

Takata and colleagues (1990) studied a class I restricted clone which suppressed antibody production to ovalbumin *in vitro*. The clone was maintained in crude rat Con A supernatant and periodically stimulated with antigen. The TsF was weakly absorbed by antibody to the α chain but not to an antibody specific for the α/β chain or $V_\beta 8$. It is possible that the line only produced

Table 5: Classification of soluble antigen-specific T cell factors

I Antigen-specific T cell factors with negative effects
TsF-1 which only acts when given at the induction stage
TsF-2 (characteristically anti-idiotypic) which activates Ts-3
TsF-3 which only acts when given at the expression stage
TsF which modulates IgE response
II Antigen-specific T cell factors with positive effects
Antigen-specific T helper factor which augments induction of contact sensitivity*
Antigen-specific ThF which augments antibody production*
Antigen-specific ThF which augments tumour rejection*
Antigen-specific T cell factor which causes local oedema in the contact sensitivity reaction*

*It is not clear whether these belong to different classes.

the antigen binding chain of the TsF and that the non antigen binding chain was provided by the other cells in the culture or (in view of the weakness of the absorption by antibody) that technical factors were important.

Finally, *Zheng* and colleagues (1989) studied a peptide-specific Ts-1 hybridoma. Antisense oligodeoxynucleotide of part of the α chain blocked TsF production. However, antisense constructs to part of the β chain had no effect. It is possible that technical factors were important e.g. limited action of the antisense oligodeoxynucleotide, which depends upon the amount of mRNA, or provision of the non antigen binding chain by factors added during the assay.

In summary, most antigen-specific T suppressor factors behave as a soluble form of the TCR, and the apparent exceptions may have special, technical explanations. The liberation of antigen-specific factors similar but not identical to the TCR may be based on differential splicing, as mRNA alternative splicing with the addition of base pairs between Vβ and Cβ has been described (*Behlke* and *Loh*, 1988). However, a study of a cDNA library of the DNP-specific hybridoma showed no evidence of this (*Fairchild* et al., 1990). Alternatively the soluble antigen-specific product may be a post-translational modification or proteolysed form of the α/β TcR.

CLASSES OF SOLUBLE ANTIGEN-SPECIFIC T CELL FACTORS

The classes of immunoglobulins were originally suspected on the basis of different biological properties, such as the ability of some antibodies to fix complement or coat mast cells. It was then confirmed by raising class-specific antibody to epitopes on the constant region and finally explored at a DNA level. At present only the first two have been undertaken for antigen-specific T cell products. Table 5 gives a classification of the main soluble antigen-specific T cell factors.

Serological differences

Monoclonal antibodies exist which distinguish between TsF-1 and TsF-3 even when the antigen-specificities of the factors are identical, and some of these antibodies have been used in ELISA assays. The monoclonal mouse antibody B16G was raised against a TsF-1 which limits rejection of the tumour P815. It also absorbs NP-specific TsF-1, but not TsF-2 (idiotype specific) or NP-specific TsF-3 (*Steele* et al., 1987; *Gallina* et al., 1990). Monoclonal rat antibody 14-30 has similar properties (*Ferguson* and *Iverson*, 1986). *Sorensen* and *Pierce* (1985) also produced monoclonal rat antibodies against TsF-1. In contrast, another rat monoclonal antibody, 14-12, only reacts with TsF-3 (*Ferguson* et al. 1985). In the few cases studied, these determinants are on the non antigen binding chain of the TsF, the same chain as is responsible for the genetic restriction in the action of TsF (*Perrin* et al., 1989b).

Other serological determinants may be indicators of MHC restriction and not class-specific. For instance, many of the TsF-1 and TsF-3 bear I-J determinants. This has been regarded provisionally as a marker of the TCR with I-E genetic restriction, but see *Nakayama* et al. (1989). In keeping with this, TsF-3 of DNP specificity is class I restricted and is I-J negative. However, no correlative study exists using hybridomas against the same determinant with different genetic restrictions.

T helper factor of picryl specificity carries α and β chain I-A determinants

on its non antigen binding chain (*Little* et al., 1985, 1987, 1988). As preliminary data indicate that this also carries $V_\beta 8$ determinants, it is possible that the I-A is bound non covalently by the non antigen binding chain and is not an integral part of the molecule (*Dieli*, personal communication). The I-A serology probably defines a distinctive class of factor, but it is possible that it simply reflects the genetic restriction in the action of the factor.

Some, but not all, TsF carry $V_\beta 8$ determinants. This is also shown by certain TsF-1, TsF-3 and antigen-specific T helper factor (see Table 3). This is clearly an indication of Vβ gene usage and not a class marker.

T suppressor inducer factor (TsF-1)

The Ts-1 is now usually called T suppressor inducer cell and the factor TsF-1 or TsiF. The defining feature of TsF-1 is that it acts only when given at the induction stage of the immune response and not at the effector stage (*Jendrisak* et al., 1986; *Kuchroo* et al., 1990; *Gallina* et al., 1990). TsF-1 form a distinct class which differ biologically in their time and mode of action, serologically and sometimes structurally from TsF-3. The key difference is their time of action. NP- and TMA-specific TsF-1 give rise to an Ts2 which is idiotype specific and makes a corresponding TsF-2. This in turn activates antigen-specific Ts-3 to produce TsF-3. Some of the TsF-1 are disulphide bonded heterodimers like TsF-3, while others are single chain molecules with antigen binding capacity and I-J determinants on a single chain (*Gallina* et al., 1990; *Jendrisak* et al., 1986). They are sometimes IgH restricted in their action. It presumably reflects their role in inducing anti-idiotypic Ts-2 and is related to the effect of IgH allotypes on the idiotopes of antibodies and hence on the idiotopes of the TCR.

The relation of the Ts-1 to helper cells was investigated in a recent study. Two of three Ts-1 made IL-2 or IL-2 and IL-4. Their distinctive feature was the production of antigen-specific TsF. It is unclear whether this is a potential of many helper cells when stimulated appropriately or represents a distinct subset of cells (*Kuchroo* et al., 1990).

T suppressor factor 3 (TsF-3)

There are several lines of evidence suggesting that TNP-, oxazolone-, NP- and cryptococcal-specific TsF-3 belong to the same class. The serological evidence has been summarised above. In addition, all these TsF-3 have a mode of action through the macrophage and the T acceptor cell (unpublished observations; *Blackstock* et al, 1991b). Additional evidence comes from studies on the activation of the T acceptor cell (Table 2). This cell, when coated with TsF, is activated to release its antigen non specific inhibitory mediator (nsTsF-1) by bivalent low molecular weight haptene. This activation, *inter alia*, requires the haptene to crosslink separate molecules of TsF on the surface of the T acceptor cell. Thus lysine with two TNP groups attached causes activation, while lysine with only one TNP group is inactive. "Mixed haptene", i.e. lysine with one TNP and one oxazolone group attached, is also inactive. However when the T acceptor cells are coated with a mixture of TNP- and oxazolone-specific TsF, the mixed haptene causes activation. In other words, crosslinking of TNP-specific to oxazolone-specific TsF on the surface of the T acceptor cell leads to activation. Similar studies show that monoclonal NP- and conventional oxazolone-specific TsF cause activation when crosslinked by appropriate mixed haptene (*Asherson* et al., 1984b,c).

Multiple effects of TsF-3:

These are listed in Table 1. It is likely that all the effects ascribed to these TsF-3 are due to the same molecular species. In particular, the same cryptococcal-specific TsF inhibits phagocytosis by macrophages and coats them for the production of macrophage suppressor factor, as judged by their common monoclonal origin, similar molecular weight (ca. 70-80 kDa), and similar structure (antigen binding site and I-J determinants on the same molecule). In the case of monoclonal TNP-, cryptococcal- and NP-specific TsF, the same hybridoma supernatant acts through the macrophage and the T acceptor cell. Moreover, the same NP-specific hybridoma supernatant (and presumably the same TsF) affects both antibody production and the effector stage of delayed hypersensitivity (*Hausmann* et al, 1985)

Antigen-specific TsF which depresses IgE response:

The monoclonal ovalbumin-specific TsF described by *Iwata* et al (1989a,b, 1990) probably belongs to a separate class. Its distinctive feature is modulation of the IgE antibody response and its glycosylation inhibiting activity (GIF). The unstimulated hybridomas liberated a glycosylation inhibition factor, which lacks an antigen combining site. However activation by antigen leads to the release of an ovalbumin specific molecule which also has glycosylation inhibition factor activity. This antigen-specific factor has α chain determinants of the TCR and was associated with the non specific GIF chain which bears I-J determinants.

Antigen-specific T helper factor (ThF)

The ThF, which augments the induction of contact sensitivity, is I-A restricted in its action and is an afferent acting factor. (*Colizzi* et al, 1985; *Little* et al., 1987, 1988). In contrast the T cell factor which causes local oedema in the contact sensitivity reaction is genetically unrestricted and may be regarded as an efferent acting factor (*Van Loveran* et al, 1984, 1986). However, there is no formal study indicating whether these two factors are different.

In summary, there is good evidence that TsF-1 and TsF-3 belong to different families on serological and biological grounds. The TsF-3 may divide further as some are selective for the IgE response. The antigen-specific helper factors also belong to a different family, but for the moment the evidence is mainly biological. Finally the TsF-3 have several different actions which are due in all probability to the same molecule.

MODES OF ACTION OF TSF-3

Introduction

The availability of hybridomas making TsF allows structural and molecular biological studies and renews interest in the mode of action of TsF. In fact, studies with "conventional" TNP- and oxazolone-specific TsF showed that it had two distinct modes of action: one through the macrophage and the other through the T acceptor cell. See Table 2. In both cases the TsF behaves like a mobile receptor and coats these cells. This mode of action is formally analogous to that of IgE. This is a class of antibody which acts as a mobile receptor and coats the mast cell. The mast cell then releases histamine and other mediators when exposed to antigen. Both systems show the need for crosslinking of the IgE or TsF on the surface of the

cell. However, with TsF, there is an additional need for genetic matching between the TsF and the haptene on the surface of the antigen presenting population.

Biological role:

There are several reasons why evolutionary pressure may have driven the selection of these complexities. The macrophage and the T acceptor cell probably provide amplification so that a limited amount of antigen-specific TsF has a greater effect. Moreover, the production of antigen non specific inhibitory mediators allows antigen-specific TsF to limit the inflammation to the other antigens liberated by an invading micro-organism or parasite. Finally, the complexity of the system provides multiple levels of control by antigen and presumably cytokines so that the inhibitory response is under tight control. An additional feature may be that the genetic restriction in the production and action of TsF causes a variation of the immune response between related animals and hence reduces the likelihood that infection will kill many members of a species. Similar considerations are presumably responsible for the complexity of the complement system.

Inhibition of contact sensitivity skin reaction in immune mice

Dorf's group was the first to describe the inhibition of the delayed sensitivity skin reaction by injecting monoclonal Ts-3 into immunised mice. The assay is sensitive and in our hands cloned TNP-specific hybridoma supernatant (after stimulation with antigen) causes 50% inhibition of contact sensitivity at a dilution of 1/1,000 , when injected shortly before challenge (unpublished observations). The TsF inhibits the early, 2 hour phase of the contact sensitivity reaction. On current views, this early phase is due to an antigen-specific T cell factor which coats mast cells and perhaps other cells. These cells, when exposed to antigen, liberate serotonin and perhaps other mediators (*van Loveren* et al., 1984; *Kops* et al., 1984).

TsF also limits the 24 and 48 hour skin reaction and diminishes the 48 hour reaction even when given at 24 hours - a time at which the reaction is well developed. This indicates that the TsF (directly or indirectly) limits the cytokine production needed for the persistence of the reaction perhaps by limiting the influx of cells. Paradoxically TsF has no effect on local passive transfer. This suggests that TsF is unable to act, if a large number of antigen-specific and other cells are injected into the skin test site. Perhaps its key mode of action is in preventing the entry of cells. Unfortunately, it is difficult to understand the details of the action of TsF in inhibiting contact sensitivity, because of our ignorance of the basis of the inflammation seen in contact sensitivity [see *Piguet* et al. (1991) on the role of TNF-α].

Inhibition of the passive transfer of contact sensitivity

The inhibition of contact sensitivity in immunised mice provides a convenient assay for monoclonal TsF-3. However, a more analytical approach is to study the inhibition of the passive transfer of contact sensitivity. In this system, cells from immune mice are incubated *in vitro* with the factor under study. They are then injected into naive recipients and contact sensitivity assessed. This system has been used to show that TsF-3 has no direct effect on the passive transfer of contact sensitivity, but two indirect effects: one via the macrophage and the other via the T acceptor cell.

Action of TsF through the macrophage:

In its action through the macrophage, TsF-3 coats this cell (Thy-1 negative, adherent peritoneal exudate cells). Exposure to antigen then leads to the re-

lease of an antigen non specific inhibitory mediator which is called macrophage suppressor factor (MSF). It is detected by its ability to inhibit the passive transfer of contact sensitivity (*Ptak* et al., 1978). The activation of the macrophage to release TsF is antigen-specific, and the haptene needs to be on cells which match the TsF at I-J (*Dieli* et al., 1991). This was assessed using B10.A(3R) and B10.A(5R) mice and it is possible that the TsF uses I-E as the restriction element and that the I-J gene modulates this interaction, by influencing the amount of I-E or the cell type which expresses it. MSF has a molecular weight around 10-20 kDa on gel filtration. Its production is not affected by indomethacin which suggests that MSF is not a carrier protein for prostaglandin. It differs from the non specific inhibitory mediator produced by the T acceptor cell in lacking I-J determinants (*Blackstock* et al., 1991b).

The original findings, using "conventional" TNP- and oxazolone-specific TsF-3, have now been extended to monoclonal TNP- and cryptococcal-specific TsF using TNP-modified spleen cells or soluble cryptococcal polysaccharide and spleen cells as a source of antigen presenting cells (unpublished observations; *Blackstock* et al., 1991b). It is interesting that inhibition of antibody production by TsF-3 may be mediated through macrophages but detailed analysis is not available (*Hausman* et al., 1985).

Action of TsF through the T acceptor cell:

Briefly, the T acceptor cell arises following immunisation with contact sensitiser and is not found in unimmunised mice (Table 2). Its antigen-specificity is unimportant in its interaction with TsF. It binds TsF to its surface (*Zembala* et al, 1982a,b,c). The T acceptor cell coated with TsF is activated by antigen and liberates an antigen non specific mediator called the first non specific T suppressor factor or nsTsF-1. Like MSF, it is detected by its ability to inhibit the passive transfer of contact sensitivity. The activation of the T acceptor cell to release its antigen non specific inhibitory mediator, nsTsF-1, is antigen-specific, and the haptene must correspond to that of the TsF and be on cells which match the TsF at I-J. There is no requirement for matching to the antigen used to generate the T acceptor cell or to its genotype. NsTsF-1 has a molecular weight around 50 kDa on gel filtration. It bears I-J determinants in contrast to macrophage suppressor factor (*Zembala* et al., 1982; *Asherson* et al., 1984; *Blackstock* et al., 1991b). In this, it resembles the antigen non specific inhibitor liberated by staphylococcal enterotoxin B (*Taub* et al., 1989).

The finding that "conventional" TNP- and oxazolone- and monoclonal NP-specific TsF act through the T acceptor cell has now been extended to monoclonal TNP- and cryptococcal-specific TsF using TNP-modified spleen cells and soluble cryptococcal polysaccharide (unpublished observations; *Blackstock* et al., 1991a).

It was originally thought that the non specific mediator, nsTsF-1, acted directly on the cell that transfers contact sensitivity. However, experiment showed that it was unable to affect passive transfer by a population depleted of I-A bearing T cells. This suggested that nsTsF-1 inhibited contact sensitivity by a mechanism involving I-A^+ T cells. Further analysis showed that nsTsF-1 is a "permissive factor" which allows a specifically immunised cell to release a second antigen non specific inhibitory mediator, nsTsF-2, when activated by specific antigen (*Zembala* et al., 1986).

The nsTsF-2 bears I-A determinants and is I-A restricted in its production and action. Further experiments indicate that the genetic restriction in the action

of nsTsF-2 is related to the haplotype of the I-A determinants that it carries (*Asherson* et al., 1989). This suggests that there is an interaction between an I-A determinant on the factor and a cell which has a receptor for I-A which may be the TCR.

TsF inhibits phagocytosis by a subset of macrophages

In its action via the macrophage and the T acceptor cell, TsF acts indirectly and antigen is required to release the inhibitory mediators. However, it also has a direct mode of action on a subset of macrophages, which does not appear to require antigen. Moreover there is no evidence that the factor coats the macrophages which then liberate a factor into the medium which suppresses phagocytosis.

Blackstock and colleagues (1989a,b; 1991a,b) showed that conventional and monoclonal cryptococcal-specific TsF inhibited phagocytosis by a subset of macrophages. This subset was I-A^+ and comprised 8-27% of macrophages in the peritoneal exudate. This inhibition of phagocytosis appears to be a general property of antigen-specific TsF-3 which depress contact sensitivity and is shown by TNP- and oxazolone-specific TsF. However there is an important difference between the inhibition of phagocytosis, and the coating of the macrophage which then releases MSF when exposed to antigen. The inhibition of phagocytosis does not require the addition of antigen. Thus TNP-specific conventional TsF still inhibits phagocytosis after careful purification to remove antigen. Similarly, monoclonal cryptococcal-specific TsF inhibits phagocytosis after purification on antigen and subsequent elution. In contrast, antigen is required in order for the macrophage and T acceptor cell coated with TsF-3 to release antigen non specific inhibitory mediators.

Inhibition of the granuloma formation

The pathology in schistosomiasis is mainly due to the granuloma reaction and fibrosis around the eggs. *Perrin* and colleagues (1989b) studied conventional schistosomal-specific TsF-3. The Ts were activated by antigen *in vitro* to release TsF, and the TsF diminished granuloma formation *in vivo* and in an *in vitro* model. IL-2 blocked the production of TsF both *in vivo* and *in vitro*. However it had no effect on the action of TsF-3. The target of the TsF *in vitro* was the immune population used to generate the granuloma; incubating the antigen coated beads (around which the granuloma formed) in TsF had no effect.

Inhibition of antibody production

Monoclonal NP-specific TsF-3 inhibits antibody production. It may act late in the response, by inhibiting the production or response to key lymphokines, as TsF given 4 hours before measuring plaques reduces the response. The TsF acts in the first instance on an adherent cell. However it is not known whether this cell the acts directly on a B cell or a T cell, or via other cells (*Hausman* et al., 1985).

CONCLUDING REMARKS

Biological significance

The biological role of TsF-3 may be to alter the balance between immunopathology and handling of the microorganism. It is relevant that the action of TsF-3 is tightly controlled by antigen and MHC and that the final mediators are antigen non specific (*Zembala* et al.,

1986) Hence, TsF specific for a particular antigen or epitope of the pathogen will limit inflammation caused by other antigens.

I-E is sometimes involved in activating Ts-3, while I-A is involved in the production and action of the second antigen non specific inhibitory mediator (nsTsF-2). Hence alteration of the I-A:I-E ratio may affect the magnitude of the negative control in a complex fashion. In fact, there is polymorphism in the control regions for the human equivalent of I-A and I-E (*Andersen* et al., 1991) and differential expression of these may influence the balance between immunopathology and the handling of the micro-organism.

Ts-1 and Ts-3 cells bind to antigen, and of TsF-3 coats macrophages and T acceptor cells. This may have implications for antigen presentation. *Lanzavecchia* et al. (1990) has shown that B cells present antigen to T cells. They bind intact antigen by their Ig receptor, internalise and process it, and then present it to T cells. The T cells are activated and *inter alia* liberate lymphokines which provide T cell help for antibody production.

By analogy Ts cells, and cells (macrophages and T acceptor) coated with antigen-specific TsF may bind antigen (*Zembala* et al., 1982c) and then process it and present it to T cells. In the case of B cell presentation, the combination of antibody with antigen alters which epitopes are presented (due to selective protection by the antibody, during intravacuolar proteolysis) and serves to change the epitope to which the animal responds. This may also be true for this T cell presentation. This hypothetical mechanism involving Ts and/or TsF would have the effect of turning off the inflammatory response to one epitope, while changing the dominant epitope presented to T cells. This would provide a mechanism for directing the immune response towards relevant epitopes for handling infection.

Unanswered biological problems

I-E genetic restriction of T suppressor cells:

Oliveira and *Mitchison* (1988) postulated that T suppressor cells are characteristically, but not always I-E restricted. This view, if correct, raises the question of the mechanistic basis of the association. For instance, do suppressor cells use the TcR to selectively recognise I-E and is this linked to a particular constant region associated with suppressor function? Alternatively is there a molecule, which resembles CD4 in binding class II but which is selective for I-E?

It may be relevant that the certain superantigens - bacterial and viral products which activate the TCR of certain V_β genes - are often I-E restricted (*Marrack* and *Kappler*, 1990). A good example is Staphylococcal enterotoxin-B, which is a common cause of food poisoning. This activates T cells to liberate an $I\text{-}J^+$ antigen non specific inhibitory mediator from T cells (Taub et al., 1988). Has evolutionary accommodation between host and pathogen led to a reduction of immunopathology through the selective use of I-E by superantigens and hence to a selective activation of suppressor cells?

The continuing puzzle of I-J:

The basis of I-J genetic restriction in the production and action of certain Ts-F's and the epitopes recognised by sera raised across I-J differences are a continuing puzzle (*Murphy*, 1987).

The common feature between the molecules recognised by anti-I-J monoclonal antibodies is unclear. On the one hand, they react with the non antigen binding chain of TsF. Their ability to block the production of TsF by a hybridoma (unpublished observations) might suggest, but does not prove, that

they react with the T cell receptor. In some experiments the I-J phenotype depended on the I-E environment in which the T cells developed, which led to the suggestion that I-J is an idiotype determinant on the TCR influenced by the MHC specificity of the receptor. In particular, the introduction of an $I\text{-}E^k$ transgene into an $I\text{-}J^b$ mouse, changes the phenotype to $I\text{-}J^k$ (*Flood* et al., 1986). However, this evidence is indirect and *Nakayama* and colleagues (1989) have indicated that the I-J determinant may be on a molecule distinct from the TCR.

On the other hand, I-J monoclonal antibody reacts with antigen non specific mediators including the first non specific inhibitory mediator (nsTsF-1) of contact sensitivity, an inhibitor of antibody production induced by superantigen (*Taub* et al., 1989) and glycosylation inhibition factor - a molecule which inhibits phospholipase A2 activity (*Jardieu* et al., 1986). These molecules may be truncated or modified forms of the β chain of the TCR.

Similarly, the nature of I-J genetic restriction is unclear. There is a technical point. The difference between B10.A(3R) and B10.A(5R) used to define I-J genetic restrictions can be interpreted as showing that the I-J direct gene product is the restricting element or I-E is the restricting element which the I-J gene modifies. For instance, I-J may lead to pre- or post-translational modification of I-E or alter its amount or location. Alternatively the I-J gene may affect the T cell receptor and this might select between different post-translational I-E variants. In any event the description of an adaptive molecule which may be distinct from the TCR is clearly important. *Nakayama* and colleagues (1989) provide a helpful summary.

Burnet drew attention to the tension between the biological and the biochemical approaches to immunology and saw the biochemical approach as negative. In fact, both are needed. In the study of antigen-specific T cell factors, the biological approach was needed to discover factors and their mode of action, while the molecular biological approach is critical to clarifying the precise relation between the TCR and these soluble products and defining classes of antigen-specific T cell factors. It should soon be possible to outline the amino acid sequences on T suppressor factors which renders them cytophilic for other cells and enables them to act as mobile antigen-specific receptors.

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NOTE ADDED IN PRESS

Dorf and colleagues (1992) have summarised recent work on T suppressor factor and I-J, while *Asherson* and colleagues (1994) have offered a possible explanation of the I-J phenomenon in terms of an endogenous superantigen.

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