Helper and Suppressor T Cell Factors

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I. Introduction

The emerging view of the immune system is that of a collection of lymphocyte networks, in which the cellular elements are related by a common set of antigen reactivities or the possession of a distinct subset of antigen receptors [14, 28, 59]. Each network involves two separate components, the first responsible for effector function, and the second consisting of the regulatory apparatus for controlling the effector activity. Over the past few years, great strides have been made in identifying the cells involved in immune regulation. It is clear that T lymphocytes play a crucial role in this process, and can be divided generally according to whether their activity is to amplify overall effector function, or to dampen (suppress) immunity. T cell subsets with distinct differentiation (cell surface) markers, tissue localization, drug sensitivities, and lifetimes have been found to mediate these various effects [15, 16, 45, 47]. In addition, there is growing evidence that the regulatory capacity of these T

Abbreviations: ABA azobenzenearsonate; BGG bovine gamma globulin; C complement; CGAT common idiotype of anti-GAT antibodies; C_H constant region of immunoglobulin heavy chain; C_L constant region of immunoglobulin light chain; CRI cross-reactive idiotype of anti-ABA antibodies of A/J mice; CTL cytolytic T lymphocytes; DNP dinitrophenyl; EA egg albumin; GA random copolymer of L-glutamic acid⁶⁰-L-alanine⁴⁰; GAT random copolymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰; GLØ random copolymer of L-glutamic acid⁵⁶-L-lysine³⁵-L-phenylalanine⁹; GT random copolymer of L-glutamic acid⁵⁰-L-tyrosine⁵⁰; H₇G human gamma globulin; KLH keyhole limpet hemocyanin; MBSA methylated bovine serum albumin; MHC major histocompatibility complex; MLR mixed lymphocyte response; MØ macrophage(s); OX oxazalone; PCl picryl chloride = trinitrochlorobenzene; PEC peritoneal exudate cells; PFC plaque forming cells; (Phe, G)-A-L (poly-L-phenylalanine, L-glutamic acid)-poly-DL-alanine-poly-L-lysine; PSA picryl sulfonic acid = TNBS – trinitrobenzene sulfonic acid; T_aF T cell derived augmenting (enhancing) factor; (T, G)-A-L (poly-L-tyrosine, L-glutamic acid)-poly-DL-alanine-poly-L-lysine; (T, G) pro-L (poly-L-tryptine, L-glutamic acid)-poly-L-proline-poly-L-lysine; T_H helper T lymphocyte; T_HF T cell derived helper factor; T_S suppressor T lymphocyte; T_SF T cell derived suppressor factor; T_S, suppressor T lymphocyte induced by T_SF; V_H variable region of the heavy chain of immunoglobulin; X_R X-irradiated;

lymphocytes resides largely in a closely related family of soluble macromolecular factors endowed with the ability to either mediate the activity ascribed to the parental T cell, or in fact, to induce lymphocytes producing such end-stage regulatory molecules [34, 38, 102].

The present review is an attempt to summarize current information on one particular type of T cell regulatory macromolecule, namely the family of factors bearing determinants coded for by major histocompatibility complex (MHC) linked genes. This is done, not to diminish the importance of the myriad of non-MHC coded lymphokines, but to restrict the discussion to a manageable size, in the hope that a meaningful analysis of at least this small area of immunologic research can be made. Toward this end, we will review, in a succinct manner, the data available on T derived helper and suppressor factors in the various model systems being studied, and then attempt to integrate the seemingly disparate findings into a scheme which can serve as a basis for hypotheses testing. Finally, we will speculate briefly on the *in vivo* significance of these T cell factors and the immune pathways in which they are involved.

II. Helper Factors

A. Acting on B Cell Responses

1. In vivo Studies on Helper Factors Specific for Branched Chain Synthetic Polypeptides. Synthetic polypeptide antigens such as (T, G)-A-L [116] have played a major role in studies on soluble helper factors. Immune responses to these compounds in animals of responder Ir genotype are characterized by both IgM and IgG antibody production and T cell immunity. Ir low responder mice make either no antibody or IgM only, depending on the batch of antigen used, and these animals never show classic *in vivo* T cell immunity [82, 83, 120].

Using (T, G)-A-L, Taussig first reported in 1974 on the existence of soluble, T cell derived materials from responder strain mice able to replace specific helper function *in vivo* for antibody responses [139]. The basic experimental model used for this and subsequent studies on *in vivo* active (T, G)-A-L T_HF involved priming thymocytes in lethally X-irradiated (XR) hosts in the presence of (T, G)-A-L, then removing these "activated T cells" and culturing them for 6-8 h *in vitro* at high cell density in the presence of antigen. The supernatant of these cells was then assayed for helper function by admixture with unprimed bone marrow cells plus antigen, followed by adoptive transfer to an XR syngeneic recipient. Either the specific plaque response or serum antibody was assayed 12 or more days after transfer. Taussig demonstrated in his initial experiment that while bone marrow ("B" cells) plus antigen gave few anti-(T, G)-A-L IgM plaque forming cells (PFC), the addition of the supernatant T cell factor led to the same level of PFC as did addition of unprimed T cells. This factor appeared to be specific in that it did not augment anti-sheep red blood cell responses under the same conditions, and it would only be released from (T, G)-A-L primed cells recultured with (T, G)-A-L. This factor could, however, help responses to the related antigens (Phe, G)-A-L and (H, G)-A-L, but not (T, G)-pro-L, thus distinguishing the functional specificity of this factor from the fine specificity of Ir gene control of responsiveness to these polymers [56].

Immunochemical analysis of this factor involved affinity chromatography of the soluble material on antigen or antibody conjugated gel beads, followed by bioassay of the material passing through the adsorbent, and in most later studies, of the material eluted from the adsorbent. Since the factor passed through adsorbents specific for immunoglobulin determinants, the active principle did not seem to be conventional antibody [140]. Nonetheless, the factor appeared to possess an antigen-combining site, since it bound to (T, G)-A-L conjugated beads [96]; the actual antigen specificity of this T_HF appears to be G-A-L, since (T, G)-A-L, (Phe,G)-A-L, (H,G)-A-L, and G-A-L, but not A-L immunoadsorbents bind (T, G)-A-L T_HF [56]. These binding data are consistent with the functional specificity described above. Perhaps the most intriguing data concerned the effects of passing (T, G)-A-L T_HF over immunoadsorbents prepared from anti-H-2 alloantisera. Such studies showed complete removal of helper activity from the factor preparation when the allo-antiserum used was directed against the H-2 haplotype of the factor producing strain [95, 140, 142]. Further experiments provided evidence suggesting that it was the anti-Ia and not anti-K or D antibodies which were important in removing the T_HF . These data were obtained by absorbing B10.A anti-B10.D2 serum with B10.D2 erythrocytes to remove selectively anti-K end activity and leave anti-Ia activity. Such absorbed serum bound to gel still removed (T, G)-A-L T_HF derived from H-2^d mice. Similarly A.TH anti-A.TL (anti-Ia^k) antiserum removed activity from H-2^k derived (T, G)-A-L T_HF [142]. Thus, these studies established for the first time that a T cell derived product active in regulating the immune response possessed both antigen binding activity and H-2 (I) coded determinants. Later studies on (T, G)-pro-L T_HF confirmed that this class of T cell factor bears I-A subregion coded determinants [57]. Gel filtration indicated that the active moiety in the factor preparation was approximately 50 000 daltons [95], consistent with the immunoadsorbent studies indicating that the T_HF was not a conventional immunoglobulin.

More recently, Mozes and Haimovich [94] have reported that (T, G)-A-L T_HF does share idiotypic determinants with antibodies of the same specificity. They produced anti-idiotypic antibodies to (T, G)-A-L antibody from two strains with different heavy chain linkage groups, and showed that these anti-idiotypic reagents were restricted to binding anti-(T, G)-A-L antibodies from the original donor strain or Ig C_H matched strains. After conjugation to Sepharose, these anti-idiotypic antibodies bound (T, G)-A-L T_HF only from strains with the appropriate allotype markers.

Taussig and collaborators also employed T_HF to explore the basis for Ir gene control of (T, G)-A-L humoral responses. After demonstrating that helper factors of this type acted across H-2 barriers by using (Phe, G)-A-L specific material derived from a series of B10 congenic strains [142], they tested putative T_HF from cells of mice of several H-2 haplotypes on responder strain bone marrow cells. Conversely, known active T_HF was tested using bone marrow cells from a variety of non-responder strains. These studies showed that non-responders fall into three broad categories: (a) those *unable* to produce T_HF , but able to respond to it, (b) those producing T_HF , but *not* responding to it, and (c) those with both defects [55, 93, 96, 141]. These data are consistent with earlier limiting dilution studies showing both T and B cell Ir gene defects for these polypeptide antigens [91, 92]. Based on

these results, Munro and Taussig proposed a two gene model for Ir gene action in which one gene coded for or regulated the production of T_HF by T cells, while another gene coded for or regulated the expression of a T_HF acceptor on the B cell [96]. They also demonstrated the ability of anti- θ treated spleen ("B" cells) to absorb T_HF , in accord with this hypotheses. Both genes mapped in the I-A subregion for (T, G)-A-L. The T_HF had been shown to be antigen specific by direct binding studies (see above). Munro and Taussig postulated that the acceptor site appeared antigen specific but in fact reflected the inclusion of the tested T_HF in a certain "family" of factors using one of the few acceptor molecules possessed by that strain. In their paper, Munro and Taussig reported that the predicted intergeneic complementation between (T_HF^+ , acceptor $^-$) and a (T_HF^- , acceptor $^+$) strains existed in the (B10.BR $\times$ B10.M)F $_1$, a finding that could not be reproduced, though, by these same workers [97].

At the time the complementation data were first reported, the issue was raised whether the two gene control of immune responses to other antigens, particularly the well studied random terpolymer of glutamic acid-lysine-phenylalanine (GL $\emptyset$), might in fact reflect this type of regulation of T cell help and B cell triggering. However, experiments clearly showed that *both* α and β genes of the GL $\emptyset$ system needed to be possessed by *both* the immune T and the immune B cell donors for successful antibody production [72], in disagreement with the predictions based on the (T, G)-A-L system. In later studies, Schwartz and coworkers [115] demonstrated the necessity for both the α and β GL $\emptyset$ Ir genes in a single cell, the responder macrophage. In light of these data and the recent question concerning the (T, G)-A-L complementation studies, it is difficult to draw any firm conclusions concerning the manner in which Ir gene defects in this system can be complemented for humoral responses in terms of factor production and activity, but this model has served to stimulate extensive work on T cell derived MHC coded factors.

2. In vitro Studies on T_HF Specific for Synthetic Polypeptides. Howie, Feldmann, and coworkers have also studied soluble helper factors specific for polypeptide antigens, but have carried out these experiments totally in vitro. Spleen cells are cultured in Marbrook-Diener flasks together with low concentrations of antigen (about 1 μ g/ml) for four days, washed, and then recultured in fresh medium, again with 1 μ g/ml antigen. The cell-free supernatant is recovered and assessed for activity by addition to another culture containing fresh unprimed spleen cells or B cells plus polypeptide or hapten-polypeptide conjugate as antigen. IgM PFC are assayed four days later and compared to responses in cultures without any T_HF . Under these conditions, a soluble material is elaborated which specifically augments the PFC response when the antigen (or its haptened derivative) used for T_HF elicitation is in the assay culture [29, 33, 52, 73]. This factor comes from T lymphocytes, and for (T, G)-A-L T_HF bears determinants coded for by I region genes, in agreement with data of Taussig et al. Further, this (T, G)-A-L T_HF acts directly on T depleted spleen cells, as for the in vivo factor. In contrast to the in vivo (T, G)-A-L T_HF , the in vitro factor binds to (T, G)-A-L but *not* (Phe, G)-A-L immunoadsorbents. The reason for this discrepancy, which also applies to the functional activity of the two factors, is unclear at present. This T_HF lacks Ig determinants detected by

mammalian xenoantisera, but it is bound by chicken anti-mouse Ig antibody immunoadsorbents.

Most intriguing are the data concerning the genetics and mode of action of (T, G)-A-L $T_H F$ in this in vitro model. Howie and Feldmann [52] find that the production of (T, G)-A-L $T_H F$ is under H-2 genetic control and that the haplotypes able to produce this mediator correspond precisely to those reported as $T_H F$ producers by Taussig et al. using in vivo methods. These data confirm the hypothesis that at least one site of Ir gene function for (T, G)-A-L is the control of (T, G)-A-L $T_H F$ production by T cells. These data do not allow one, however, to determine whether the absence of $T_H F$ production by T cells of a given haplotype reflects an inherent T cell deficit or is the result of Ir gene activity affecting the triggering of $T_H F$ -producing T cells by macrophages. The data of Singer et al. [118] and Marrack and Kappler [81] on macrophage localized Ir gene defects in (T, G)-A-L responses support the latter possibility. However, these studies showed H-2^k macrophages to be deficient, while the $T_H F$ data show H-2^k strains to be factor producers. Taken together, these results may indicate that the observed macrophage defect in H-2^k mice for (T, G)-A-L responses does not reflect a failure of T_H activation for $T_H F$ production, but rather an inability of these macrophages to mediate a subsequent step in the humoral response. Recently, Howie and Feldmann [53] have reported that this postulated macrophage defect may in fact represent an inability of H-2^k macrophages to absorb (T, G)-A-L $T_H F$ and/or transmit the $T_H F$ signal to the B cell, i. e., the Ir gene defect in H-2^k mice affects macrophage-B cell interaction for $T_H F$ activity. Clearly, further work is necessary in this area to clarify the actual site of the Ir gene defect and to distinguish between effects on macrophage antigen presentation to T cells versus macrophage presentation of T cell factors to B cells.

B. Helper Factors Enhancing T Dependent B Cell Responses

Tada and his coworkers [127, 149] have reported on the properties of a soluble factor able to enhance in vitro hapten-carrier humoral responses, which differs somewhat from the material described in the preceding sections. This "augmenting" factor increases the magnitude of secondary anti-hapten PFC responses. It is found in cell-free sonicates of T cells from mice given two injections of soluble carrier protein two weeks apart according to a protocol originally designed to yield potent T_S responses. In contrast to the (T, G)-A-L $T_H F$ this $T_a F$ requires carrier primed T cells for its function, and will not activate B cells plus antigen alone. Furthermore, this material does not act across allogeneic (I region) barriers, in contrast to (T, G)-A-L $T_H F$. In physiochemical respects, the $T_a F$ resembles $T_H F$, being 30–60 000 in mol. wt., having antigen binding activity, lacking conventional Ig determinants, and bearing I-A subregion coded determinants. These latter specificities may be distinct from those expressed on B cells, since absorption of the anti-I-A alloantisera with B cells prior to conjugation to gel leads to the loss of B cell lytic activity without a reduction in $T_a F$ adsorptive capacity. Conversely, adsorption of the anti-I-A antiserum with purified T cells removes all factor binding activity without decreasing the B cell lytic titer. The source of the $T_a F$ is $Ly\ 1^+$, 23^- T cells, in agreement with other studies showing cells of the $Ly\ 1^+$ lineage to function

primarily as helpers cells. Further study is necessary to determine the precise relationship between T_HF and T_HF .

III. Suppressor Factors (T_SF)

A. Studies on Protein Specific T_SF Active in Suppressing Humoral Responses

1. In vivo Experiments on T_SF from Primed Mice. The earliest studies on soluble T suppressor factors are those of Tada and colleagues which investigated the role of cell free extracts in the regulation of IgE antibody production in rats. These experiments showed that T_S -containing thymus and spleen cell populations from rats hyperimmunized to carrier protein yielded a soluble cell free material, following freeze-thaw treatment and centrifugation, which could suppress an ongoing IgE response to the same antigen [100, 124]. This soluble material bound specifically to the antigen used to prime the T_S , and lacked conventional immunoglobulin determinants.

More detailed studies on protein specific T_SF involved murine responses. Tada and coworkers developed a model in which splenic T cells or thymus cells from mice given two injections of soluble carrier protein [usually keyhole limpet hemocyanin (KLH), bovine gamma globulin (BGG), or egg albumin (EA)], on days 0 and 14, could be shown to be suppressive upon transfer in vivo on day 28 to syngeneic unprimed recipients challenged with a DNP conjugate of the same carrier protein [130]. This suppressor cell effect, which decreased high affinity IgG responses preferentially compared to IgM or low affinity IgG, could also be mediated by a cell free extract prepared from the same cell population by sonication, then centrifugation [125].

The in vivo studies [131] revealed that the T_SF was antigen specific in its action, failing to suppress DNP-BGG responses when prepared from KLH primed cells and vice versa. Furthermore, addition of the appropriate carrier antigen KLH to KLH- T_SF , together with DNP-BGG as challenge antigen, also failed to cause suppression of the DNP response in vivo, suggesting that antigen plus T_SF did not generate an antigen nonspecific suppressor signal (but see below on in vitro studies). Finally, the T_SF could be specifically adsorbed by antigen-conjugated Sepharose beads.

Further in vivo studies revealed that the T_SF was not bound to immunoadsorbents specific for a variety of immunoglobulin determinants, and gel filtration suggested a size for T_SF of 40–60 000 daltons. Also, the factor could be adsorbed on columns made from alloantisera specific for the H-2 region of the T_SF donor. In addition to this evidence for H-2 coded determinants on the T_SF itself, it was found that the suppressive activity of a given T_SF was seen only when the factor was tested in the syngeneic or an H-2 compatible strain. Thus, BALB/c (H-2^d) KLH- T_SF worked in BALB/c and DBA/2 (H-2^d) mice, but not in C57BL/6 (H-2^b) mice, although C57BL/6 KLH- T_SF was active in syngeneic animals. These data were the first to indicate that the activity of soluble T cell factors might be restricted by the same genetic region (H-2) containing the genes that coded for the determinants they carry [131].

2. *In Vitro Studies on T_SF from Primed Mice.* Further characterization of the nature and mode of action of protein-specific T_SF was carried out in vitro using secondary DNP PFC responses in Marbrook cultures to DNP-protein conjugates. The spleen cells were obtained from mice primed one month previously with the relevant hapten-carrier conjugate and pertussis adjuvant. Under these conditions, it is the IgG response which is predominantly suppressed by the T_SF. Early experiments confirmed the results obtained in vivo, and provided several new findings [133]: (1) The T_SF could not be recovered from the supernatant of cultures of KLH T_S and KLH, but was still present in the sonicates of such T_S after the culture period. This is different from the results with the T_HF described by Taussig [139], which could be released from in vivo primed cells during in vitro culture. It also differs from the data of several other groups on both T_HF (see above) and T_SF (see below). (2) The T_SF acted on T cells, not B cells. This was shown by adding either EA-T_SF or KLH-T_SF to DNP-EA primed spleen cells together with DNP-EA and DNP-KLH. DNP-B cells should bind both DNP compounds, permitting either or both T_SF materials to affect these cells. However, under such conditions, only the EA-T_SF able to act on the primed EA T_H showed significant suppressor function. (3) The H-2 determinants present on the T_SF were coded for by a "new" I subregion termed I-J [126], first defined at that time by Murphy et al. [98].

The experiments demonstrating the importance of this new subregion were based on the fact that KLH T_SF failed to act across H-2 barriers. By studying a series of congenic mice with recombinant I regions, it was possible to map the location of the genes controlling this restriction. One of the critical tests involved B10.A(5R) mice as factor donors. The data showed that 5R KLH-T_SF was not suppressive for C57BL/6 or BALB/c mice, although H-2 maps available at that time designated the 5R I region as a recombinant containing solely H-2^b and H-2^d genes, and at least one of these assay strains should have possessed the appropriate I region target for the T_SF. Conversely, the same 5R T_SF did suppress CBA (H-2^k) responses, although this would seem to violate the H-2 restriction rule. Because the 5R H-2 derives from a (b × a) recombination, and (a) itself is considered a natural (k × d) recombinant, one possible interpretation of the above data was that the 5R I region included an I^k derived gene located between I-B and I-E/C which was the crucial genetic region for T_SF function. This putative new subregion, termed I-J, was also consistent with the data on antisera absorption of T_SF. B10.A(3R) anti-5R antiserum should have had no anti-Ia activity according to then current data, yet this antiserum did adsorb the KLH-T_SF. Tests of a panel of other sera showed that in all cases, the adsorption of KLH-T_SF correlated with the postulated I-J subregion intercalated between I-B and I-E/C. As will be discussed later, most other antigen-specific T_SF have also been found to bear I-J coded determinants.

The relevant determinants coded for on T_SF by the I-J subregion are not expressed in detectable quantity on B lymphocytes, since large numbers of these cells cannot adsorb the T_SF binding activity from anti-I-J sera. Small numbers of T cells, however, efficiently adsorb the anti-T_SF antibody [123]. Recently, the presence of I-J determinants on T_S has been shown by direct cytotoxicity tests on T_S enriched by antigen binding procedures [101, 135], as well as on a Ly 1⁺ T_H subclass adherent to nylon wool [128]. Adsorption of anti-I-J sera with these distinct I-J⁺ cell types indicates a minimum of two distinct I-J determinants distinguishing the

two T cell subsets, and antibodies to these determinants are present in varying proportion in different anti-I-J sera, complicating the use of such reagents in studies on immunoregulatory T cells. Anti-I-J antisera can also block the action of $T_S F$ [123].

Treatment of T_S with anti-Ly 1 serum plus complement (C), followed by removal of dead cells and debris, left a population of cells that still yielded $T_S F$ upon sonication. Similar treatment with anti-Ly 2 serum + C removed the cells containing the $T_S F$. Thus, $T_S F$ producing cells, at the time $T_S F$ is extracted, are Ly $1^-, 2^+$. Whole spleen cells but not anti-Thy 1.2 + C treated spleen cells of the same H-2 haplotype as the $T_S F$ donor could adsorb the $T_S F$ activity from a sonicate; however, attempts to adsorb $T_S F$ with nylon purified T cells were unsuccessful. The nylon adherent cells proved able to bind $T_S F$, indicating that the target of $T_S F$ was a nylon adherent T cell. This cell was then studied. DNP-primed B cells plus nylon purified KLH T cells were cultured with or without added nylon adherent cells, in the presence or absence of KLH- $T_S F$. Only when the nylon adherent T cells were present did the $T_S F$ suppress the PFC response to DNP-KLH. Treatment of the nylon adherent cells with either anti-Ly 1 or anti-Ly 2 + C removed their activity, and since Ly $1^+ +$ Ly 2^+ cells added together did not reconstitute suppressor function, it appears that the $T_S F$ target is an Ly 123^+ cell. Furthermore, these Ly 123^+ cells must come from mice primed with the same antigen as the $T_S F$ donors and must be I-J compatible with the $T_S F$ for it to act. It is the uptake of $T_S F$ by these Ly 123^+ antigen activated cells that is blocked by anti-I-J antiserum. The effect of $T_S F$ on the Ly 123^+ cell appears to be a triggering of this cell, in the presence of antigen, to either differentiation into an active Ly $2, 3^+ T_S$ or to aid an Ly $2, 3^+$ cell to become an active T_S , i.e., the $T_S F$ induces suppressor T cells. Finally, the suppressor cell induced by $T_S F$ plus antigen via the Ly 123^+ cell can suppress in an antigen nonspecific manner, and, apparently, act across I-J (H-2) barriers. The target of this induced suppressor cell appears to be an Ly 1^+ , I-J $^-$ carrier-specific T_H cell. Recently, it has been found that the same KLH- $T_S F$ extract, though not necessarily the same suppressor molecule, can have a direct suppressive effect on a different Ly $1^+ T_H$, which bears I-J subregion coded determinants [129].

In addition, Taniguchi et al. have derived T cell hybridomas from KLH antigen binding T_S from B6 mice [138] which produce KLH- $T_S F$. Investigation of the nature of this material has revealed it to be virtually identical to the T cell extracts, i.e., about 60 000 daltons in mol. wt. and bearing I-J subregion coded determinants. It also reacts with an anti- V_H , but not anti- V_K or V_L antiserum specific for framework determinants on these chains [102]. Finally, the I-J determinants and antigen combining site seem to be on distinct noncovalently linked chains, since the filtrates of an anti-I-J b column and of a KLH column, each loaded with the hybridoma KLH- $T_S F$ and each devoid of suppressive activity by itself, can, when combined, show potent suppression [132].

3. Other $T_S F$ Specific for Carrier Proteins in Humoral Responses. Several other $T_S F$ specific for conventional protein antigens and active on humoral immune responses have been reported. One system involves $T_S F$ derived from T_S specific for human gamma globulin (H γ G). These T_S are induced using deaggregated protein to tolerate the recipient and induce T_S [6, 60, 135]. Such T_S have been characterized as Ly 2^+ , I-

J cells [135], and yield, upon disruption or by secretion, a T_S F which is strikingly similar to the KLH- T_S F detailed above [18, 136, 137]. The HyG T_S F is 40–50 000 daltons, nonimmunoglobulin, antigen-binding, and bears I region coded determinants. No data is available on the activity of this material in H-2 (I-J) incompatible animals, nor on the ability of HyG T_S F to induce T_S .

The other major experimental system for studying T_S F to protein antigens has been reported by Kontiainen and coworkers. This model makes use of the in vitro humoral response system employed by Feldmann [31, 34]. Spleen cells are cultured for four days in the presence of high concentrations (100 μ g/ml) of antigen (KLH), washed, and recultured with a low concentration (1 μ g/ml) of antigen to permit T_S F secretion. Under these conditions, a soluble supernatant material is released by cells via an energy-requiring process, which can suppress DNP-PFC responses to the same carrier antigen as that used for T_S F production [75]. This suppression occurs using either in vitro or in vivo primed T_H , which are needed for PFC responses in these cultures. No H-2 restrictions on the action of this T_S F have been observed to date. Nonetheless, this T_S F closely resembles in physiochemical terms the Tada-type T_S F it is about 50 000 daltons in mol. wt., has specific antigen binding activity, bears I region coded determinants, lacks Ig determinants recognized by mammalian antisera, and is a protein. This T_S F also differs from the inducing T_S F in that its target is an $Ly\ 1^+2^-T_H$ cell, not an $Ly\ 123^+$ cell, and no evidence exists to indicate that it is capable of inducing $Ly\ 2^+T_S$ [76]. Thus, despite a close physical resemblance, there are at least two distinct T_S F molecules coded for by same I subregion, each with a distinct functional activity. Hybridomas producing this in vitro type of KLH- T_S F have been reported [77].

B. Studies on T_S F Specific for Polypeptides Antigens Under Ir Gene Control

1. General Features of the GAT/GT Models. The use of antigens, the response to which is under Ir gene control, has aided in dissecting the suppressor T cell pathways and the role of T_S F in suppressor cell activity. The best studied of these antigens are the random polypeptides L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) and L-glutamic acid⁵⁰-L-tyrosine⁵⁰ (GT). GAT responses are under dominant H-2 linked Ir gene control mapping to the I-A subregion [24]. The nonresponder haplotypes are H-2^{b, q, s}. Mice bearing these haplotypes fail to give either humoral or cell mediated immune responses to GAT, but will respond with anti-GAT antibody when administered GAT electrostatically complexed with the carrier methylated bovine serum albumin (GAT-MBSA). GAT and GAT-MBSA primary in vitro responses are characterized by IgG PFC under the usual conditions of assay [67].

Gershon et al. [44] first proposed that GAT nonresponder mice did show in vivo lymphoid responsiveness to GAT administration and suggested the possibility that this reflected T_S activation. A series of studies by Kapp and Benacerraf documented in detail the occurrence and T cell nature of the suppressor cells induced in nonresponder mice by GAT. The standard assay for these T_S became their ability to decrease PFC responses to GAT-MBSA in vivo or in vitro [7, 9, 68]. Very recently, the ability of GAT- T_S to suppress nonresponder T cell proliferative responses to GAT after GAT-MBSA priming has also been used for studying suppression in this

model [66]. Similar experiments were carried out using GT. This antigen does not lead to responses in any tested inbred mouse strain, although it is immunogenic in an outbred line. The inbred strains can be divided into two broad groups, however, those whose GT-MBSA responses are suppressed by prior GT administration ($H-2^{d,k,s}$), and those whose responses are not suppressed by such treatment ($H-2^{a,b,q}$) [20, 21]. The work of Tada et al. [131] led to attempts to produce GAT and GT- $T_S F$ by sonication of spleen and thymus cells containing active T_S . Such extracts did suppress GAT-MBSA or GT-MBSA responses of syngeneic mice, and were also active in vitro, permitting easy assay [69, 70, 147, 150]. These polypeptide specific $T_S F$ were subjected to a similar immunochemical and functional analysis to that employed previously for (T, G)-A-L $T_H F$ and simultaneously by Tada for KLH $T_S F$. The results showed that in many respects GAT- $T_S F$ and GT- $T_S F$ are similar to KLH- $T_S F$: (1) They are proteins of 40–50 000 mol. wt.; (2) They bear I region, more specifically, I-J, coded determinants; (3) they have antigen binding specificity, and can, in fact, discriminate between the two closely related polypeptides; and (4) they lack constant region immunoglobulin determinants [145, 146]. In contrast to KLH- $T_S F$ of the Tada type, GAT- $T_S F$ is associated with some serologically active antigen. This was demonstrated by showing that GAT- $T_S F$ activity bound to a rabbit anti-GAT immunoadsorbent. This binding was not seen when the GAT- $T_S F$ had first been purified by passage over insolubilized GAT bound to Sepharose, indicating that this fragment of antigen was lost during the purification process [145]. The functional importance of this antigen fragment will be discussed below.

2. $T_S F$ Stimulates T_{S_2} . These polypeptide specific $T_S F$ differ from the $T_S F$ described by Tada in that they act very efficiently across all allogeneic, including H-2, barriers. Thus $H-2^q$ GAT- $T_S F$ acts on $H-2^s$ target spleen cells with the same activity it shows on syngeneic $H-2^q$ cells [65]. Similar data was obtained for GT- $T_S F$ in the same genetic combinations ($H-2^d$, $H-2^k$) showing strict I-J restrictions for KLH- $T_S F$ [150]. This lack of requirement for I-J homology between $T_S F$ and target cell is not due to a difference in the apparent mode of action of these factors, however. Thus, the I-J restriction for KLH- $T_S F$ applies to its ability to induce $Ly\ 123^+$ T cells to generate active $Ly\ 23^+$ T_S [123]. As noted above, the KLH- $T_S F$ studied by Kontiainen which shows no I-J restriction acts directly on $Ly\ 1^+$ T_H without T_S induction [76]. Waltenbaugh et al. [151, 152] first demonstrated the ability of polypeptide specific $T_S F$ to induce T_S (termed T_{S_2}). They found that in vivo administration of either GT- $T_S F$ or GAT- $T_S F$ gave rise within seven days to active suppressor T cells in the spleens of treated mice. In contrast to the KLH system, the target T cells did not need to be antigen primed, nor did exogenous antigen need to be added to the $T_S F$ for induction to occur. Further, this induction of T_{S_2} occurred across I-J barriers, since BALB/c ($H-2^d$, I-J^d) GT- $T_S F$ was active in producing T_{S_2} in A/J ($H-2^a$, I-J^k) mice.

This induction pathway has been studied in detail in this laboratory. Early experiments demonstrated that T_{S_2} induction could occur in as short a time as two days under in vitro conditions [39]. These T_{S_2} were fully active either in vivo or in vitro in syngeneic mice. If GAT- $T_S F$ purified over GAT-immunoadsorbents was used for T_{S_2} induction instead of the crude extract, no active suppressors appeared. This purified material, which was known to lack the fragment of GAT usually

associated with crude GAT-T_SF, was nonetheless fully active when used to directly suppress GAT-MBSA responses in culture. These data suggested a crucial role for antigen in T_S₂ induction. Addition of nonsuppressive (nanogram) amounts of GAT to the purified GAT-T_SF leads to efficient T_S₂ induction. Thus, although not revealed by the initial in vivo experiments, GAT-T_SF shares with KLH-T_SF a requirement for both an I region coded product and antigen for T_S₂ triggering.

3. *Ir Gene Status and T_S₂ Induction.* The presence of genetic restrictions for T_SF induction of T_S₂ was restudied in both the GAT and GT models in vitro. In both cases, T_SF efficiently activated T_S₂ using allogeneic, I-J incompatible spleen cells ([40] and unpublished observations). Experiments also looked at the existence of Ir gene barriers to GAT-T_SF activity. Early work showed that GAT-T_SF, while active in all nonresponder strains, did not suppress responder mice or (responder × nonresponder)F₁ mice, even when GAT-MBSA rather than GAT was used as antigen [70]. This raised the question as to whether in this system, I-A coded Ir genes, as opposed to I-J subregion genes, restricted GAT-T_SF activity. However, both in vivo and in vitro studies ruled out this interpretation [41]. Administration of GAT-T_SF in vivo to responder mice one week prior to antigen challenge suppressed subsequent GAT PFC responses. This indicated that nonresponder derived GAT-T_SF could work in responder mice, presumably by inducing T_S₂. This was also directly demonstrated in vitro. Responder (BALB/c-H-2^d) spleen cells were incubated in DBA/1 (H-2^a) GAT-T_SF for two days, washed, and transferred into BALB/c mice, or BALB/c spleen cell cultures. Such GAT-T_SF cultured responder cells significantly reduced GAT PFC responses. The active cells were sensitive to anti-Thy 1.2 + C, indicating they were T cells. Thus, Ir genes do not restrict T_SF activity for T_S₂ induction. These experiments do not rule out, Ir gene mediated restrictions on T_SF action at other sites.

These findings in the GAT and GT models have been used to provide information on the target of T_SF action and genes controlling both T_SF production and T_S₂ activity. Because GT-T_SF acts across I-J barriers, it was possible to ask if induced T_S₂ bear I-J determinants and if, in fact, these determinants are passively acquired or actively produced. This was accomplished by treating A/J (I-J^k) T_S₂ induced using BALB/c (I-J^d) GT-T_SF with an anti-I-J^k antiserum prepared so as not to crossreact with I-J^d determinants [(B10.A(3R) × DBA/2 anti-B10.A(5R)]. The elimination of A/J GT-T_S₂ function by treatment with this antiserum + C indicated that T_S₂ are I-J⁺ and that these I-J determinants are derived actively from the T_S₂ precursor itself [40].

4. *Ly Phenotype.* Experiments conducted in collaboration with Dr. Harvey Cantor have provided preliminary evidence that the precursor of the T_S₂ has the same Ly phenotype as the KLH-T_SF nylon wool adherent target cell. (B6D1)F₁ spleen cells were treated with anti-Ly 1, anti-Ly 2, or NMS + C and cultured in vitro with GAT-T_SF for two or three days. Other cells were similarly cultured without pretreatment, then exposed to anti-Ly antisera plus C after T_S₂ induction. These various cell types were then titrated into fresh cultures of syngeneic spleen cells stimulated with GAT. Pretreatment with either anti-Ly 1 or anti-Ly 2 + C prevented T_S₂ induction and cultures containing Ly 1⁺ and Ly 2⁺ cells mixed together did not show a reconstitution of T_S₂ induction. These data indicate an Ly 123⁺ cell is involved in T_S₂

generation. The treatment of already induced T_{S_2} showed that only anti-Ly 2 + C, or anti-I-J + C, but not anti-Ly 1 + C, eliminated T_{S_2} mediated suppression. Thus, the mature T_{S_2} is Ly 1⁻, 2⁺, I-J⁺. Whether this indicates a change in differentiation antigens from Ly 123⁺ to Ly 23⁺ by a single cell type, or whether Ly 123⁺ cells are required for T_S F to trigger Ly 23⁺ cells is not yet known. At this point, these data are in full agreement with those of Tada [123], apart from the I-J restriction phenomenon.

5. MHC Control of T_S F Production and T_{S_2} Induction. We have also looked at the role of H-2 linked genes in the regulation of T_S F production and T_{S_2} induction. Prior investigations revealed that suppression to GT was under the control of complementing genes (termed immune suppression or Is genes) located in the I-A and I-C subregions [8, 22]. Thus two genes, in addition to I-J, are involved in GT specific suppression. Waltenbaugh et al. had demonstrated that A/J mice (non-suppressor animals) fail to produce detectable GT- T_S F, but nevertheless could be suppressed by active GT- T_S F, at least in part due to T_{S_2} induction [152]. Since F_1 mice between A/J and another nonsuppressor strain, B6, led to complementation for the full suppressor pathway, it was postulated that the B6 might provide the ability to produce GT- T_S F which in turn would act due to the ability of the A/J genome to yield T_{S_2} . This hypothesis was tested by preparing putative B6 GT- T_S F and assaying this extract for function both directly and for T_{S_2} induction in B6, and other strains of mice. B6 mice do produce GT- T_S F, which fails to act in B6 itself, but which directly suppresses A/J, (B6A) F_1 , and other strains, and yields T_{S_2} as well. Thus, it appears that A/J mice fail to make GT- T_S F, but respond to active factor with T_{S_2} activity, while B6 mice produce T_S F, but cannot yield T_{S_2} able to suppress syngeneically. These results are reminiscent of the T_H F studies on (T, G)-A - L T_H F, except that they involve two T cell functions, instead of T and B cells. Similar findings were also reported for KLH- T_S F [134]. In this case, A/J also failed to produce T_S F and all B10 background mice failed to respond to the factor. Two crucial observations in these latter studies were that the genes controlling these effects were *not* H-2 linked and that it was the B10, *not* the B6 background, that possessed the defect. Because of these findings the GT model was examined for H-2 control of T_S F production and activity. In contrast to the situation for KLH- T_S F, changing the H-2 type of the animals changes the defect in the GT- T_S pathway. Most telling are the experiments with B10 and B10.A recipients of GT- T_S F. B10 (H-2^b) spleen cells are not suppressed by either B6 or A.BY (H-2^b) GT- T_S F, while B10.A (H-2^a) cells are. Conversely, B10.A mice, in contrast to B6 mice, fail to yield active GT- T_S F, while A.BY (H-2^b) mice, congenic with A strain, now generate GT- T_S F in response to GT challenge. Furthermore, the defect in B6 mice is antigen specific, in that GAT- T_S F can induce GAT specific T_{S_2} under conditions identical to those in which GT- T_S F fails to give GT- T_S . One striking possibility raised by these data is that activation of T_{S_1} by GT requires antigen presentation by cells possessing a surface Ia structure produced by interaction of I-A and I-C Is gene products, in analogy with I-A _{β} :I-E _{α} generation of macrophage Ia for "presentation" of the terpolymer glutamic acid-lysine-phenylalanine [19, 115]. Failure of T_S F to act might also involve similar defects in presentation by Ia molecules, perhaps in this case of the idiotypic determinants of the T_S F combining site [43, 122 and see below].

6. *Idiotypic Analysis of T_SF*. The possible presence of shared idiotypic determinants on GAT specific antibodies and GAT-T_SF has also been examined. A common (cross-reactive) idiootype exists on the majority of anti-GAT antibodies of all studied inbred strains of mice [61–64]. This idiootype, termed CGAT, is also found on anti-GAT antibodies of certain rat strains [62] and may be present in guinea pigs [148] as well. It is associated with binding specificity for GAT or GT-type determinants, being found also in sera to GLT, (T, G)-A – L, and GT, but absent from anti-L-glutamic acid⁶⁰-L-alanine⁴⁰ (GA) sera. Because of its broad representation in mice (and other species) this idiootype is an excellent candidate for a marker for a germ line V_H gene, and as such, was thought likely to occur also on GAT-T_SF if such T cell derived material made use of the Ig V_H gene pool for its antigen combining site.

Anti-CGAT antiserum was raised in guinea pigs using as immunogen anti-GAT affinity purified D1.LP (H-2^b) antibodies which had been passed through an anti-H-2^b immunoadsorbent column to remove any MHC coded T cell products which might have copurified with the antibodies. This anti-CGAT or control guinea pig serum was coupled to Sepharose and DBA/1 and SJL nonresponder GAT-T_SF passed over the gel. The filtrates of these columns, as well as the acid eluates, were tested for both suppressive activity on nonresponder spleen cells and for T_S₂ induction using responder cells [42].

Such anti-CGAT immunoadsorbents totally remove the suppressive activity of purified GAT-T_SF preparations, and the active GAT-T_SF can be recovered in the acid eluate. Furthermore, the eluted T_SF still bears I-J determinants. As a control, it was shown that GA-T_SF did not detectably bind to anti-CGAT adsorbents, in accord with the absence of CGAT determinants on anti-GA antibodies. These data provide presumptive evidence of sharing of V_H structures on antibodies and I region coded T cell products. The possibility that these idiotypic structures may play a direct role in T_S activity beyond antigen binding will be dealt with in a later section.

7. *GAT-T_SF in Responder Strains*. The data described above showing that H-2 controlled defects in T_SF production exist in the GT model, and that nonresponder derived GAT-T_SF could activate T_S₂ in responder mice raised the question of whether GAT responder mice lacked the ability to produce their own T_SF, thus accounting in part for their responder status. This issue was investigated in vitro. Evidence pointing to Ir gene function at the level of macrophage (M ϕ)-T_H interaction [10, 85, 119], together with studies suggesting that antigens which bypassed M ϕ were efficient at T_S induction [6, 58, 60], led to attempts to produce GAT-T_S with responder spleen cells depleted of antigen presenting cells (M ϕ) [104]. Such cells generated active, GAT specific T_S when exposed even to low (5–10 μ g/ml) concentrations of GAT for two days in vitro. Higher concentrations of GAT (50 μ g/ml) could induce T_S even when M ϕ were not removed from the spleen cell preparation. The T_S induced under M ϕ -depleted conditions were then washed and recultured for 24 h in the absence of additional antigen. The cell free supernatant of such T_S cultures was found to mediate suppression in vitro GAT responses. The material responsible for this suppression, which is GAT specific and acts across H-2 barriers, bears I-J subregion coded determinants and has antigen-binding activity. Further, these T_SF containing supernatants also induce T_S₂ under the same conditions as nonresponder T_SF derived by sonication. Thus, it appears that GAT

responder mice can produce GAT- T_S F which shares virtually all of the features of the GAT- T_S F obtained from in vivo stimulated nonresponder T_S [105]. This responder T_S F has the additional property of directly suppressing responder GAT PFC responses, while nonresponder GAT- T_S F must first induce T_{S_2} to affect responder immunity. Whether this difference reflects the different methods of T_S F preparation (in vitro versus in vivo) or a more fundamental difference between T_S F derived from responder and nonresponder mice has not been ascertained.

Kontinen et al. have recently reported on their investigations concerning polypeptide specific T_S F. Using the same in vitro model as employed to study KLH specific T_S F, these workers showed, in agreement with the data just discussed, that responder and nonresponder spleen cells cultured with high concentrations of GAT or (T, G)-A-L (100 μ g/ml) generated T_S [51], which released specific T_S F into the culture supernatant [78]. The action of these GAT and (T, G)-A-L T_S F appears to be mediated at least in part by inhibition of T_H function, since they suppress both polypeptide and DNP-polypeptide PFC responses. No information is available on the question of whether these factors act directly on T_H , or via T_{S_2} induction, or both. It is clear, however, from the data of Kontinen et al. and ourselves, that both responder and nonresponder animals can produce T_S F to antigens under H-2 linked Ir gene control. Future studies will undoubtedly resolve the question of whether responder T_S F differs from nonresponder T_S F, and the issue of the existence of one or more specific, I-J⁺ T_S F in these suppressor materials, each with a distinct mode of action.

C. T_S F Active on Cell-Mediated Immune Responses

Another important area of research on suppressor cell function involves contact hypersensitivity or delayed type hypersensitivity to chemically reactive small molecules ("haptens") or derivatized syngeneic cells. These experimental systems have been particularly revealing insofar as suppressor factors are concerned.

1. TNP and DNP T_S F. The earliest work in this area was carried out by Asherson and Zembala. They demonstrated that lymph node cells from mice given picryl sulfonic acid (PSA) [trinitrobenzenesulfonic acid (TNBS)] iv. contained suppressor T cells which when cotransferred with lymphocytes from mice primed with picryl chloride (PC1) diminished the adoptive transfer of contact sensitivity [1, 154]. If T_S containing lymph node cells were obtained on day 6 from mice receiving PSA iv on day 0 and a cutaneous challenge with PC1 on day 5, these T_S released in culture over the next 48 h a soluble material which could also depress the adoptive transfer of responsiveness to PC1 [155]. This soluble material was not released by cells from mice receiving only PSA iv or PC1 cutaneously—both steps were necessary. The PC1-induced T_S F did not interfere with transfer of immunity to oxazalone (OX), a distinct immunogen, demonstrating the functional antigen-specificity of this factor. This specificity was consistent with later experiments showing that the active material in the supernatant possessed specific binding affinity for TNP but not oxazalone conjugated beads [3]. It was also shown that the T_S F had a mol. wt. of about 50 000, was relatively insensitive to trypsin or heating as compared with other T_S F, contained carbohydrate (based on binding to Con A-Sepharose), and also possessed H-2 coded determinants [3, 156].

Studies on the mechanism of action of TNP-T_SF revealed two apparently distinct modes of action. If PC1 immune lymph node cells were directly incubated in TNP-T_SF, the response to PC1 was decreased upon transfer. Incubation of OX-immune cells in TNP-T_SF had no effect on transfer, whether or not the challenge included PC1 as well as OX. This fine specificity was not observed, however, if peritoneal exudate cells (PEC) were first incubated in TNP-T_SF, and these PEC then cotransferred with the PC1 or OX-immune cells. Under these conditions, PC1 and not OX immunity was decreased when only PC1 or OX, but not both, were used for challenge. However, if both PC1 and OX were used challenge OX immune cells, the response was suppressed by the TNP-T_SF treated PEC. Thus, Zembala and Asherson concluded that after "arming" by TNP-T_SF, exposure of such PEC to antigen led to the activation of an antigen-nonspecific suppressor mechanism mediated by the macrophages in the PEC population [2]. These data are consistent with recent reports from Ptak et al. showing a role for M ϕ binding of "suppressor signals" in vitro [106], and the demonstration of a low molecular weight (10–30,000) material released from "armed" M ϕ which nonspecifically suppresses contact sensitivity responses [107]. They also are similar to the results of Tada [123], insofar as the nonspecific suppression evoked by simultaneous exposure of T_SF treated cells to the antigen recognized by the T_SF and the antigen being used as the immunogen.

Moorhead adopted the general protocol of Asherson and Zembala in studying T_SF specific for DNP [87–90]. This T_SF is produced by T cells from mice given dinitrophenyl sulfonic acid iv and challenged with dinitrofluorobenzene prior to culture. It is assayed by its ability to decrease the transfer of contact sensitivity to DNFB by immune lymph node cells incubated in the factor. Affinity chromatography studies demonstrated that this T_SF is specific for DNP and does not recognize the closely related compound TNP. Further, the T_SF lacks Ig determinants, but possesses H-2 coded determinants. Of great interest are very recent data showing that this DNP-T_SF lacks I-J determinants, but appears to possess I-C subregion coded alloantigenic determinants [90]. DNP suppressor factor does not seem to be associated with antigen, as is GAT-T_SF, since DNP-T_SF is not bound by anti-DNP antibody absorbents [87]. However, the possibility that the T_SF is associated with univalent DNP moieties cannot be ruled out by this method. An intriguing finding in Moorhead's studies is that DNP-T_SF is H-2 restricted in its activity [88]. Exposure of DNFB-immune cells to DNP-T_SF results in suppression of immunity only if the treated cells share H-2K or H-2D identity with the T_SF donor. Furthermore, although DNP-T_SF can be adsorbed by DNP-KLH columns in the absence of added H-2 products, it only can be adsorbed by DNP-conjugated cells sharing H-2K and/or H-2D genes with the DNP-T_SF donor [89]. These data raise important questions concerning the interpretation of the binding to DNP-KLH and the nature of combining site(s) of the T_SF. These data are also consistent with the results of Tada and coworkers showing a requirement for H-2 compatibility between T_SF donor and target cell for KLH-T_SF [126], but differ with respect to the H-2 region involved (K/D for DNP-T_SF and I-J for KLH-T_SF). Miller et al. [84] have reported that transfer of DNP-specific T_S stimulated by DNP cells leads to suppression only if the recipient matches the T_S donor at H-2D, confirming in part the results of Moorhead using T_SF. It may be of some importance that many studies

have documented the role of H-2K and D products as target or restricting antigens for cytolytic T lymphocytes stimulated with haptenated (TNP, DNP, etc.) syngeneic cells [119]. This may indicate that the K/D molecules are particularly important for Ly 2,3⁺ effector function involving these haptens, either as CTL or T_SF producing T_SF. These data, and those to be presented below concerning a T_SF for mixed lymphocyte reactions, clearly demonstrate that although H-2 genes are important in T_SF function, no one subregion is unique in this regard, and in some cases, no restriction for T_SF activity can be seen (e. g., GAT-T_SF).

A third series of experiments on hapten specific T_SF has been carried out by Greene, Benacerraf, and colleagues. In an early study Greene et al. described a TNP-T_SF produced by the Asherson-Zembala method which was active when administered in vivo to animals concomitant with PC1 priming, in contrast to the previous studies employing suppression of the activity of already primed cells in a transfer model. Using this procedure, these authors showed that the TNP-T_SF responsible for the observed effects possessed I-J subregion coded determinants [49]. This contrasts with Moorhead's results for DNP-T_SF, assayed by inhibition of adoptive immunity [90]. It does not appear, however, that the assay accounts for this difference, since a TNP-T_SF assayed in the same manner as the DNP-T_SF was found to bear I-J coded determinants [90], as in the study by Greene et al.

2. Suppression of Azobenzenearsonate Specific DTH by T_SF. More recently, Bach et al. have described the occurrence of suppressor cells and T_SF specific for the hapten azobenzenearsonate (ABA). In this experimental model, immunity is induced by sc injection of hapten-derivatized syngeneic spleen cells, and measured as the 24 h DTH response to challenge with either haptenated cells or free reactive hapten [4]. Suppressor factor is tested by assessing the decrease in immunity that results from T_SF administration at the time of, and for two days following priming [50]. The particular interest in this hapten derives from the investigations of Nisonoff and coworkers, which showed that A/J antibodies to ABA possess a cross-reactive idiotype (CRI) on approximately 20–70% of the anti-ABA molecules [99]. The presence of this idiotype on anti-ABA antibodies is regulated by V_H (-linked) genes. It was thus of great interest to determine if an MHC coded, T cell derived product, ABA-T_SF, shared this CRI with immunoglobulins.

Passage of ABA-T_SF derived from A/J mice over an affinity column prepared from purified rabbit anti-Id leads to the partial removal of suppressor activity from the T_SF preparation, and this activity can be recovered in the eluate from this column. Control experiments demonstrated that these results truly reflected an idiotype-specific binding of the ABA-T_SF. These data thus confirm the studies on GAT-T_SF, demonstrating shared idiotype between antibodies and T cell derived, MHC coded suppressor factors. Bach et al. provided an additional piece of evidence, not available in the GAT model, that the idiotypic cross-reaction between anti-ABA antibodies and ABA-T_SF was the result of the action of Ig (linked) genes. Thus, ABA-T_SF derived from B10.A mice, which share H-2 with A/J mice but differ at background genes including those linked to Ig allotype, fails to bind to the rabbit anti-Id absorbent. Furthermore, ABA-T_SF derived from BALB/c (Ig-1^{a+}) mice is idiotype-negative, while ABA-T_SF from the congeneric partner strain C.AL-20 (Ig-1^{d/c}), which shares much of its Ig genetic region with A/J, is CRI positive [5].

These findings have important implications for the structure of $T_S F$, and the involvement of Ig genes in the construction of T cell-derived antigen binding molecules.

Additional studies on ABA- $T_S F$ have revealed several interesting facts. Sy et al. [121] in these laboratories have recently shown, by the use of PL/J mice which lack the V_K region usually associated with CRI^+ anti-ABA antibodies, that CRI^+ ABA- $T_S F$ does not apparently utilize such a light chain product. In addition, ABA- $T_S F$ has been shown to induce T_{S_2} , as reported earlier for the GAT, GT, and KLH models. However, although these T_{S_2} are part of an ABA specific suppressor pathway, they cannot be lysed by anti-idiotypic and C, in contrast to the T_{S_1} cells producing ABA- $T_S F$, which are CRI^+ . In studying the reason for the absence of detectable CRI^+ molecules on T_{S_2} it was found that T_{S_2} could be bound to plastic dishes coated with CRI^+ antibody, and not to ABA-BSA coated plates. The opposite was true for ABA specific, $T_S F$ -producing T_{S_1} [122]. Finally, ABA- $T_S F$ could be shown to be able to suppress across an H-2 but *not* across an allotype barrier [23]. Thus, it would seem that CRI^+ ABA- $T_S F$ acts by triggering a population of anti-idiotypic T_{S_2} . These data have fundamental importance in understanding the role of $T_S F$ in suppressor activity.

D. $T_S F$ in Tumor Immunity

Greene and coworkers have studied $T_S F$ in another cell mediated system. Fujimoto et al. demonstrated that A/J mice bearing the syngeneic tumor S1509a possess T_S which specifically enhance tumor growth when adoptively transferred to A/J mice hyperimmune to S1509a and bearing a tumor graft destined to be rejected [36, 37]. Thus, in this system, T_S interfere with already established tumor immunity. Greene et al. found that disruption of lymphoid cell populations containing S1509a-specific T_S yielded a soluble material with the ability to enhance tumor growth under the same conditions as T_S themselves [35]. This $T_S F$ was a protein of about 50,000 mol. wt. which bore H-2 coded determinants and could be specifically adsorbed by 1509a tumor cells [48]. Recently, Perry et al. have found that this $T_S F$ bears I-J subregion coded determinants [103]. In this system also, administration of 10×10^6 cell equivalents of $T_S F$ per day for four days to normal A/J mice stimulates the appearance of 1509a specific T_{S_2} in the spleens of the treated mice, demonstrating in a potentially clinically relevant model the same T_S pathway described in more basic model systems.

E. $T_S F$ in Mixed Lymphocyte Responses (MLR)

A final type of $T_S F$ active in diminishing a cell mediated type of immune response is the MLR suppressor factor described by Rich and Rich. Although this material differs from all the above in not showing antigen specificity, the similarities of its means of elicitation, immunochemical properties, and genetics argue for its inclusion here. Suppressor T cells are prepared by immunizing a mouse with allogeneic lymphoid cells administered in the hind footpads. Four days later, the spleen cells from such a mouse will suppress the response of syngeneic lymphoid cells to any allogeneic stimulator [108]. If these T_S containing spleen cells are cultured for 24h in the presence of allogeneic cells of the same H-2 type as the original

immunogen, a soluble factor is released into the culture supernatant which suppresses any MLR of responder cells syngeneic with the T_S [109]. This soluble material does not bind to cells bearing the H-2 antigens of the immunogen responsible for the $T_S F$ production. MLR $T_S F$ does have three interesting properties: (1) it only suppresses MLR of responding cells sharing the I-C subregion with the $T_S F$ producing cells, (2) it can be adsorbed by I-C compatible cells when these cells are either Con-A activated thymocytes or alloantigen activated spleen cells, but not if the cells are unactivated — this activation can occur within 4 h of exposure to alloantigen, and (3) the factor bears determinants coded by the I-C, not I-J, subregion [110, 111, 113].

The lack of antigen specificity of this $T_S F$ may truly reflect the absence of an antigen binding site, making this $T_S F$ of great interest for structural comparison to the other antigen-binding T cell factor, or may reflect a different (perhaps anti-idiotypic) specificity from the conventional anti-antigen specificity of other T_S . Lastly, Rich and Rich have documented that even though this $T_S F$ bears I-C subregion coded determinants, genes outside of H-2 play an important role in $T_S F$ production [112]. Thus, B6 mice fail to give MLR- $T_S F$ under standard conditions, but nonetheless are suppressed by $T_S F$ from other H-2^b strains. (B6 × BALB/c) F_1 mice produce $T_S F$ active on both H-2^b and H-2^d strains, however, indicating that B6 mice can produce $T_S F$ (i. e., possess the structural gene for the I-C restricted factor) but lack an effective non-H-2 coded regulatory gene affecting $T_S F$ production. This situation is similar to that described by Taniguchi et al. [134] for KLH- $T_S F$ production by B10 congenics, and may indicate a general phenomenon of non-H-2 gene regulation of H-2 gene activity.

VIII. Conclusion

A. Molecular Characteristics of Antigen-Specific T Cell Factors

The data presented above point out the many similarities in structure and antigenic determinants of various T cell factors active in immune regulation. The known features of these materials can be divided into four main categories: (1) size, (2) presence of MHC determinants, (3) presence of Ig C_H and V_H determinants, and (4) antigen binding capacity and specificity (see summary in Table 1).

All antigen-specific MHC coded T cell factors studied to date have nominal molecular weights in the 40 000–60 000 dalton range. The lack of precision in the actual mol. wt. probably derives from several sources. At present, most sizing data are based on gel filtration of crude or partially purified factors, followed by bioassay of the fractions from the column. This method is adequate only to give a rough estimate of true molecular size. It further suffers from the fact that many factors tested this way may be associated with antigen fragments [145], which contribute to the apparent mol. wt. and apparent heterogeneity in size of the true T cell product. A final source of difficulty in this area is the fact that none of the mol. wt. determinations on T cell factors has been carried out on material isolated under conditions preventing potential proteolysis. Binz and Wigzell, in their studies on idiotype bearing T cell receptors for alloantigens, found that their T cell material

Table 1. Molecular characteristics of T_HF and T_SF

Factor type	Antigen binding specificity	Size	I-region determinants	C _H , C _L	V _H (Id)
(T, G)-A-L-T _H F (in vivo)	G-A-L	~50 000	I-A	-, -	+
(T, G)-A-L-T _H F (in vitro)	(T, G)-A-L	~50 000	I	-, -	NT
(T, G)-Pro-L-T _H F (in vivo)	(T, G)-Pro-L	NT	I-A	-, -	NT
KLH-T _a F (in vivo)	KLH	30-50 000	I-A	-, -	NT
GAT-T _S F (in vivo)	GAT > > GT	40-50 000	I-J	-, -	+(CGAT
GT-T _S F (in vivo)	GT > > GAT	~50 000	I-J	-, -	NT
KLH-T _S F (in vivo)	KLH	35-60 000	I-J	-, -	+(V _H framework)
H ₇ G-T _S F (in vivo)	H ₇ G	30-55 000	I	-, -	NT
TNP-T _S F (in vivo)	TNP	~50 000	I-J	-, -	NT
DNP-T _S F (in vivo)	DNP	NT	I-C	-, -	NT
ABA-T _S F (in vivo)	ABA	38-68 000	I	-, -	+ A/J CRI
S1509a-T _S F (in vivo)	TAA of S1509a	<70 000	I-J	-, -	NT
MLR-T _S F (in vivo)	None known	NT	I-C	NT	NT

was rapidly degraded from 75 000 to 30-40 000 dalton pieces by protease in serum [11]. These results strongly suggest that T cell factors, derived from cell sonicates or produced in vitro with high cell density or in serum, may also be partially degraded before they are analyzed, perhaps leading to a falsely low estimate of mol. wt. However, it must be noted that insofar as factors are concerned, since the sizing is based on functional assays, both the antigen binding and regulatory properties are retained in a molecular structure of no more than about 50 000 daltons. The actual size of T cell factors will probably best be determined by analytic biochemical techniques such as SDS-PAGE, using purified (antigen free) materials derived in the presence of protease inhibitors from either T cells or T cell hybridomas. One study of T_SF by this approach has been published [143, 144]. A single hybridoma was derived which produced a sheep erythrocyte specific T_SF active in vitro on PFC responses. The hybridoma cells were grown in radioactive amino acids and SRBC binding material from these labeled cells was isolated and analyzed on SDS-PAGE gels. The results indicated a molecule composed of two noncovalently associated chains, a larger chain of 85 000 daltons with antigen-binding activity, and a smaller chain of 25 000 daltons devoid of anti-SRBC activity, but bearing H-2 coded determinants.

All the factors discussed in this chapter bear determinants coded for by genes in the I-region of the H-2 complex. At least three distinct genes have been implicated in construction of T cell factors: one in the I-A subregion coding for determinants on T_aF [149] and T_HF [52, 57], and one each in the I-J and I-C subregions coding for determinants on T_SF [49, 90, 113, 126, 137, 146]. The role of these MHC gene products in the regulatory function of the factors is unknown at present. However, the association of the I-A subregion with helper function and the I-J/I-C subregions with suppressor activity, regardless of the antigen specificity of the factor involved, indicates that these MHC products are probably important in the biologic effector function of these factors, possibly in a manner analogous to the various effector

functions of the F_c portions of immunoglobulins of different isotypes. This analogy would also apply to the observation of restricted interactions of certain factors with their target cells, just as different cells have F_c receptors with affinity for one or another isotype. These MHC restricted interactions will be discussed further below in relation to the mechanism of factor activity. An important point to keep in mind, however, is that the I subregion definitions rely on the detection and preservation of what at present must be considered random recombination events. Thus, if there is more than one gene in each subregion that can participate in factor construction, for certain antigens, one may find "I-J" determinants on a helper factor [54] or, as for the MLC and DNP factors, "I-C" determinants on a suppressor factor, simply because the relevant functional gene is to the right (or left) of the crossover event defining the various subregions. In other words, although in general regulatory function will be associated with subregion assignment, exceptions should be expected for some antigens, as the number of factors studied increases. It will require more precise antisera (perhaps from hybridomas) defined on a functional rather than genetic basis, or detailed structural studies on the MHC portion of T cell factors, to determine how products with similar function but different genetic origins may actually be related.

Two final issues concerning the involvement of I region gene products in factor structure also need to be discussed. First, although it is generally believed that the structures bearing I region coded determinants in factor molecules are polypeptide chains, some experiments have been interpreted as suggesting that the serologically detectable Ia antigens on factors may be carbohydrate, not protein, in nature [54]. Second, as recently pointed out by Schrader [114], one cannot rule out on the basis of available data, that T cell factors do *not* contain I region gene products, but rather, that the alloantisera used to bind the factors contain anti-idiotypic antibodies which are detecting binding sites on the factor molecules with *anti-self* Ia specificity. It should be kept in mind, however, that if this hypothesis was true, it would be necessary to postulate that such anti-self Ia receptor(s) had their idiotypic structures dictated solely by the H-2 I region of the factor producer, independently of its V_H gene cluster, since the binding of factors to anti-Ia immunoadsorbents is strictly determined by H-2I and *not* background (including allotype-linked) genes. Also, such a view is difficult to reconcile with the findings that most factors act with undiminished efficiency across H-2 barriers [65, 76, 150], while this theory suggests the likelihood of strict H-2 restrictions, such as those seen for KLH- $T_S F$ by Tada et al. [126]. Studies using hybridoma reagents and chimeras will aid in testing the validity of this intriguing suggestion.

The issue of Ig determinants on T cell factors is also a complicated one. Early reports on T cell helper factor by Feldmann and Basten suggested a molecule resembling 7S IgM, and possessing certain constant region determinants of normal immunoglobulins [30]. More recently, all laboratories studying T cell-derived materials bearing MHC determinants have demonstrated an absence of C_H and C_L markers on these factors, when mammalian heteroantisera (e. g., rabbit or goat anti-mouse Ig) are used. A few reports that chicken anti-mouse Ig can bind T cell factors [33] have not been confirmed, and the issue of recognition of carbohydrate, rather than protein, determinants by such sera is not yet resolved. Thus, at present, it seems reasonable to conclude that the MHC determinant-bearing T cell factors lack

constant region products of either H or L type. The opposite conclusion has been reached concerning V_H associated structures. In agreement with direct studies of cell bound receptor material [12, 13, 80], several groups have documented idiotypic cross-reaction between T cell factors and antibodies directed toward the same antigen [5, 42, 47, 94]. The existence of these idiotypic relationships raises the possibility that in addition to MHC gene products, T cell regulatory factors possess V_H gene products, presumably as their antigen binding portions. If this is the case, several models of the structure of these factors can be imagined. The simplest structure would consist of a single chain, composed of a V region using the same V region pool as immunoglobulins and a covalently (peptide bond) linked constant region responsible for biologic effector function and coded for by MHC genes. This model is, however, rather complex genetically, as it requires the integration into a single polypeptide chain of gene products from distinct chromosomes. A second model would divide the functions of the molecule in the same fashion, but involve either a disulfide or noncovalent association of two polypeptide chains, one bearing a V_H associated structure (with or without a T cell specific constant region), the other the MHC coded product. A variant of this model would include a third chain, associated with the V_H -structure and contributing to antigen specificity, i. e., a light chain homologue. This possibility is based on recent observations of heteroclitic behavior of T cells and their products in mice of Ig-1^b allotype immunized with NP compounds [79, 153]. The presence of heteroclitic binding by anti-NP antibodies to NIP requires the participation of a λ light chain [71], and thus, a similar structure may be involved in the T cell combining site. Finally, a single chain containing the V_H region shared with Ig, to which a carbohydrate side chain possessing the Ia determinant is attached, is a possibility.

Certain caveats must be mentioned in evaluating these suggestions. At present, the possible involvement of V_H genes in factor construction is based largely on idiotypic cross-reactions. Only one study showing genetic linkage of factor idio type with Ig genes has been reported [5]. It is thus still formally possible that there is simply a great deal of structural similarity in the combining portion of antibodies and specific T cell factors, accounting for idiotypic cross-reactions, and that *identical V* genes are not used by both molecules. This issue will be resolved by further genetic study of T cell factors and by direct biochemical (perhaps sequence) comparison of antibody and factor specific for the same antigen.

This question of the genetic origin of the factor combining site is related to studies on factor antigen fine specificity. In several models, the ability of T cell factors to bind to antigen is distinct from that of antibody from the same animals. Thus, anti-GAT antibody is virtually all GT reactive [63], yet only a fraction of GAT-T₈F binds to GT [145], and the suppression mediated by GAT-T₈F clearly distinguishes functionally between GAT and GT [41]. Similarly, antibodies to (T, G)-A-L and (Phe, G)-A-L are distinct, as is the Ir gene pattern, yet the *in vivo* derived (T, G)-A-L T_HF shows no ability to distinguish between these two polymers [56]. It would therefore appear that either T cell factors do not actually use Ig V_H genes, or that at a minimum, only certain V_H genes are used by T cells in contrast to B cells, giving a very different spectrum of fine antigen specificity to the two sets of lymphocyte products. Studies on monoclonal T and B cell products from hybridomas or T cell lines will help approach this question and possibly reveal that

Fig. 2. Suppressor factor pathways for I-J restricted KLH-T_SF [123, 125–134, 138]. The precise mode of activation of the Lyt 2,3⁺ T_SF producing cell is unclear and is merely suggested in this figure. The action of T_SF on two different T_H may represent the action of a single moiety, or the individual activities of two unseparated and very similar factors

[53] or B cells [96] adsorb the factor. If the B cell data were in fact due to MØ in the bone marrow or anti-Thy 1.2+C treated spleen cell preparations used for absorption, this would lead to a single site for T_HF function, the MØ.

The suppressor systems must be dealt with in a more individual manner. Carrier (KLH) specific T_SF, as studied by Tada and colleagues [123, 125–134, 138] (Fig. 2) resembles the factors acting in the GAT, GT, and ABA models in that it has as a major activity the induction of a second set of T_S cells, which appear to act as the ultimate T_S cell in the pathway (albeit perhaps through a final non-T cell effector). However, one feature serves to distinguish the activity of this type of KLH-T_SF from all other factors. This material shows no detectable activity on either the target cells for its inducing activity, or on T_{H2} direct targets, unless these T cells share I-J subregion markers with the factor donor. Despite deliberate attempts to detect such restrictions, not even a partial limitation of T_SF inducing activity by H-2 I region genes has been found for other T_SF.

The second general type of T_SF is that found in mice suppressed by GAT, GT, or ABA-syngeneic cells (Fig. 3). In common with KLH-T_SF, these factors act mainly, if not exclusively by inducing T_{S2}. They differ in acting efficiently across H-2 differences. The most striking feature of these materials, as studied carefully in the ABA model, is that their interaction with pre-T_{S2} cells is mediated by anti-idiotypic recognition of the antigen-combining portion of the factor. These data provide a clear-cut verification of the network theory of immunity [59], and demonstrate that such idiotypic interactions may occur between T cell subsets without the involvement of immunoglobulin. They also point out the extreme potency of at least certain idiootype bearing structures for activating the network, since the amount of T_SF needed to suppress a mouse is in fact below the range easily detectable by even

Fig. 3. Suppressor pathways involving inducing-type factors [5, 7–9, 20–23, 38–43, 50, 65, 68–70, 103–105, 121, 122, 145–147, 150–152]. This diagram integrates the findings from the GAT, GT, and ABA models, although not all points have been established in each system. Direct acting $T_S F$ [31–34, 73–78, 143, 144] may involve the last two steps in the indicated pathway, though perhaps with antigenic not the idiotypic specificity indicated in the figure

standard radioisotopic means. Whether this potency is a result of the biologic properties of a T cell receptor “constant region” perhaps the I-J coded portion of the $T_S F$, is unknown at present. Another important feature of this class of $T_S F$ is the possible role of antigen presentation in the context of Ia molecules for T_{S1} triggering, and of idiotypic-presentation by similar molecules for T_{S2} induction. These issues have been raised by the investigation of the role of Is genes in GT specific suppression, and analysis of this issue will have important implications for understanding the potential anti-self recognition capacities of T_{S1} in analogy to other T cell subsets.

Before leaving this discussion of “inducing” type $T_S F$, it is important to detail recent findings in another model of suppressor T cell function not dealt with above. Eardley, Gershon, Cantor, and colleagues [17, 25–27, 46] have reported (Fig. 4) that during the course of an ongoing T cell dependent immune response, a subset of $Lyt\ 1^+$ cells is activated which initiates a “feedback” suppressor pathway. Careful investigation of this phenomenon, using an *in vitro* anti-SRBC model, has shown that the critical $Lyt\ 1^+$ cells possess both I-J and Qa 1 cell surface markers, and act as inducers of a new set of T cells with distinct differentiation markers [17, 25–27]. This induction appears to be mediated by a soluble factor that is antigen-specific and which bears I-J subregion coded determinants [46]. This material acts on $Lyt\ 123^+$, I-J $^+$, Qa 1 $^+$ cells to either cause them to differentiate into $Lyt\ 2, 3^+$ T_S or to interact with $Lyt\ 2, 3^+$ cells, which in turn secrete a second factor, I-J $^-$ and antigen-specific, which appears to suppress both the original $Lyt\ 1^+$, I-J $^+$ inducer, and $Lyt\ 1^+$, I-J $^-$ helper cells. In agreement with published data showing that V_H linked genes regulate the inducer- $Lyt\ 123^+$ interaction [27], the activity of the $Lyt\ 1^+$ derived $T_S F$ is V_H restricted. The activity of the second factor appears to be limited by H-2 *not* V_H genes, though the subregion important in this effect is not yet known. These extremely interesting findings appear to relate the available data on induction in the KLH-GAT-ABA models, and the H-2 restricted effector suppressor factors seen in

Fig. 4. Suppressor factors in feedback suppression [17, 25–27, 46]. Based largely on detailed studies of the cells involved in feedback suppression, to which has been added recent data on the importance of factors in the T cell subset interactions

the DNP DTH system. The V_H restriction is most likely a reflection of the idiotype-anti-idiotype interactions mentioned for T_{S1} - T_{S2} interaction for ABA- T_S , raising the question of whether the apparent *antigen* specificity of both the inducing and effector factors in the feedback model is due to complexing of idiotype-bearing material to the antigen (SRBC) used for adsorption.

The last general category of $T_S F$ to be discussed concerns effector-type factors (Fig 5), exemplified by protein specific $T_S F$ studied in vitro by Kontiainen and Feldman [73], and hapten specific $T_S F$ studied by Zembala and Asherson [154, 155], and by Moorhead [87–89]. These materials are distinguished from the inducing materials by acting (without time for T_{S2} generation) directly on primed T cell populations. The lack of any requirement for induction is most clearly seen in the data on in vitro KLH- $T_S F$, which acts on target populations containing only $Lyt 1^+$ cells and B cells, which, lacking $Lyt 123^+$ and $2, 3^+$ cells, cannot serve as a source of pre- T_{S2} [76]. Among the critical features of certain of these effector materials, namely those active on DTH responses, are (1) the requirement for active immunization to follow T_S induction in order for $T_S F_E$ to be released, (2) the restriction of DNP- $T_S F$ activity by H-2K/D gene products, and (3) the possible involvement of macrophages as recipients for the $T_S F$, following which they release nonspecific suppressive signals. The first feature may indicate a need to restimulate T_{S1} to release $T_S F_1$ and trigger T_{S2} cells, which once induced need to receive an additional antigenic signal before reaching full activity. The last feature may explain the repeated finding that in certain cases, bystander suppression is seen when both the original suppressing antigen and an irrelevant antigen are administered as a challenge together.

2. Overview of Factor Activity. In the previous section, we have analyzed the salient features of each of the various experimental models, with a particular emphasis on

Fig. 5. Effector suppression by factors. Based primarily on observations in contact sensitivity models [2, 3, 87-90, 106, 107, 155, 156]

the suppressor factors, for which there is much more information available than for the helper factors. We can attempt to ascertain the degree to which these models can be integrated in a single mechanism of regulation of immune responses by suppressor T cells, or, alternatively, whether they constitute parallel and separate regulatory pathways.

The most striking feature of virtually all $T_S F$ models is that at least one factor in each system plays a crucial role in communication between T cell subsets prior to ultimate suppressor activity. In two experimental systems, it appears that this communication is mediated by idiotypic-anti-idiotypic interactions. The degree to which this finding is generalizable to all systems needs to be determined, particularly in the Tada model [123, 125-134, 138] where this issue has not been addressed. In all cases, the result of suppressor factor mediated T-T interaction is the generation of true functional suppressor cells (generally termed T_{S2}). One is then tempted to suggest that this induced T_{S2} is in fact the T_{SE} , or ultimate suppressor T cell as mentioned above.

Among the features which appear, in contrast, to be unique to an individual model, two are of greatest importance. First, in the feedback suppression model, the initiator cell is a T cell which does not bear $Lyt\ 2^+$ markers, in distinction to all other T_S cell typing data. It is possible, however, that such a cell is also the initial T cell involved in the pathways in other systems and the source of inducing factor(s). The factor producing cell in the GAT/GT/ABA system has not been Lyt phenotyped and thus the involvement of an $Lyt\ 1^+$ cell in these or the earliest stages of the Tada model has never been formally ruled out.

A much greater difficulty is the observation of a strict I-J restriction involving factor and target cells in the Tada system, and the absence of such restrictions in all other studies, including those to the same antigen. It must first be noted that the mode of antigen exposure for T_S induction differs in all these other models. It is unlikely that the I-J restriction seen results from the use of primed target cells for the $T_S F$ assay since (1) Tada found H-2 restriction in KLH- $T_S F$ activity tested on primary anti-hapten responses *in vivo* and (2) the use of secondary responses *in vitro* or *in vivo* by others in testing $T_S F$ failed to reveal such restrictions. Therefore, if the I-J restriction is related to a particular aspect of Tada's experimental protocol, it

would appear to be more likely related to the mode of T_S induction, in particular, to the use of multiple immunizations for generating the T_S F producing T cells. Alternatively, it is also possible that the involvement of common (cross-reactive) idiotypes in GAT and ABA responses may somehow obscure I-J restrictions in these latter cases, or that a different dependence upon various subsets of T_H cells in the various models might play a crucial role in the importance of I-J restrictions in each case. Whatever the explanation, one is forced to consider the implications of the I-J restriction when seen. As hypothesized by Tada et al., the most likely reason for the matching requirement is that the "acceptor" site for the T_S F is determined by I-J coded genes. This can be considered to be due to an inherent "like-like" interaction between identical I-J gene products, or to a unidirectional recognition of one I-J product by another. Given the wealth of data on other H-2 restrictions for T cell activities which indicate that such restrictions are determined by education for anti-self reactivity in the thymus [86], it is also reasonable to consider a similar mechanism for KLH- T_S F. This leads to the same conclusion put forth by Schrader [114], i.e., either the T_S F actually has a combining site (V region) capable of recognizing haplotype specific determinants of I-J molecules on target T cells, or conversely, the target T cells have such V regions recognizing the I-J coded determinants on the T_S F. Similar distinctions between these possibilities and genetically preprogrammed I-J: I-J interactions that do not involve V region genes will hopefully be made by experiments using T_S F and target T cells from chimeric mice.

Lastly, one major unresolved issue is whether the so-called inducing factors (produced by T_{S_i} cells) are truly secreted products with mediator (hormone-like) function. In a majority of cases, the products are obtained by disrupting immune cells. In those cases where T_S F is detected in the supernatant of cells kept in culture, the issue of whether this merely reflects receptor shedding and not active secretion has not been adequately investigated. It was attractive to speculate in the early phases of factor investigation that T_S F was a secreted regulatory molecule. However, in the light of more recent indications of the involvement of these products in idiotypic regulatory networks, it is just as plausible that they simply represent shed idiotypic structures to which the immune system is exquisitely sensitive. In either case, however, it would seem that the possession of I-J (C) related determinants, which appear to be unique features of suppressor pathways, renders these materials especially able to evoke subsequent T cell responses.

In summary then, it is apparent that soluble antigen specific products of T lymphocytes exist which play important roles in both cellular and humoral immune regulation. These factors probably are released *in vivo* in sufficient quantity to act at a distance, perhaps via macrophages or directly on the relevant target cell. They consist of an MHC (I region) coded portion probably related to biologic effector function, and an antigen binding portion, possibly coded for by Ig V_H genes, which is responsible for specificity and the activation of idiotypic networks. For both help and suppression, certain factors act as amplifying signals triggering a second set of T cells to provide a more proximal regulatory signal, again in the form of an antigen specific, MHC coded factor. Such complex pathways both provide for exquisite sensitivity, due to the amplification process, and a series of "fail-safe" check points for regulation, to prevent a defect in a single component from imbalancing the entire

immune system. Future studies on T cell factor, particularly homogeneous products of long-term T cell lines or hybridomas, will provide a more precise picture of their biochemical nature and of the molecular basis for their effector activities, thus bridging the gap between this set of specific lymphocyte products, and the current extensive knowledge of that other set, the B cell derived immunoglobulins.

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Note Added in Proof

Recent data obtained in the ABA suppression model reveal that T_{s2} produce a suppressor factor (T_sF₂) which *fails* to act across H-2 differences. Given this finding, it is possible to synthesize the schemes shown in Figs. 2-5 into a general model: $Ag \longrightarrow T_{s1} \longrightarrow T_sF_1 \xrightarrow{I-J^+}$

Thus, the previous data on inducing factors in ABA and GAT models defined T_{s1}-T_{s2} interaction, while data on I-J restricted KLH-T_sF and DNP DTH suppressor factor defined T_{s2}-T_{s3} (T_{aux}) interaction, accounting for the differences observed in MHC restriction phenomena.