Review

Antigen-specific T-cell factors

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Summary. Antigen-specific T-cell factors are mediator molecules which are produced by helper and suppressor T cells and which can perform the function of those cells in an antigen-specific manner. They probably play an important part in immunoregulation. The major histocompatibility complex has a controlling influence on their structure and activity, while their antigen-recognition properties may be conferred by immunoglobulin V regions. Interest in the factors derives from three related areas of research, namely (i) the problem of T-cell recognition of antigen; (ii) the mechanisms of cellular interactions in antibody production and cell-mediated immunity; and (iii) the genetic control of immune responses. This review discusses the literature up to June 1980 on their production, structure, genetic restriction and mechanism of action.

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Table 1. Properties of antigen-specific helper and suppressor T-cell factors

1. Produced by or extracted from antigen-primed helper or suppressor T cells.

2. Help or suppress immune responses (antibody production, cell-mediated immunity) in antigen-specific manner.

3. Bind specifically to the antigen against which they are produced.

4. Lack all constant region determinants of immunoglobulins, but may carry variable (idiotypic or framework) determinants of Ig heavy chain.

5. Carry determinants of the major histocompatibility complex (Ia antigens): in the mouse, the I-A subregion of H-2 characterizes helper factors, while I-J specificities are often found on suppressor factors.

6. Molecular weight often in the range 40,000–80,000. May possess two component chains, one carrying antigen-binding site, the other H-2 derived and determining function.

7. React with target cells which may be T cells, B cells or macrophages and which carry appropriate acceptor sites for the factors (see Table 2).

8. May be genetically restricted in their effect to acting on cells of the same H-2 type or responder type as the producer of the factor. Some act xenogeneically.

9. Missing in some genetic low responder or non-suppressor strains; defect in production is MHC or background determined. Factors may be Ir-gene products.

Introduction

The regulation of the immune response to specific antigens is carried out to a large extent by differentiated populations of antigen-specific T cells endowed with either enhancing or inhibitory properties, helper and suppressor T cells respectively. The effects of these cells can sometimes be replaced by soluble mediator molecules obtained from them, known as *T cell factors* which, like the T cells, may be of the helper or the suppressor variety. Some factors act in a non-antigen-specific manner (non-specific factors), even when produced by stimulation of specific T cells by antigen; other T-cell factors, the subject of this review, have the all-important property of specificity for antigen and are consequently known as antigen-specific helper and suppressor factors. Despite their mutually antagonistic effects on the immune system (help *vs* suppression), the two categories of specific factors have many structural and functional features in common and these general properties are summarized in Table 1. The most important are (a) their ability to bind specifically to antigen, the response to which they influence, while lacking most of the characteristics of the more familiar antigen-recognition system, namely antibodies, and (b) their relationship to the major histocompatibility complex (MHC)*. A second type of molecule which must be defined here is the *acceptor*, the site on the

* Most of the other abbreviations used in the text refer to antigens and keys can be found as footnotes to Tables 3 and 4.

target cell with which the factor interacts. It too has an important relationship to the MHC. The properties of acceptors are described in Table 2.

If one were to summarize the main areas of immunology in which the specific factors and their acceptors have been considered important, they would be: (a) T-cell recognition of antigen: factors considered as a soluble form of the T-cell receptor; (b) mechanisms of cell interaction: factors and acceptors considered as cell interaction molecules, mediators acting between T cells, B cells and macrophages in various combinations; and (c) genetic control of immune response: factors and acceptors as Ir gene products or indicators of Ir gene controlled defects. The factor/acceptor hypothesis which links these topics is, in general terms, that communication between antigen-stimulated T cells and their target cells (other T cells, B cells or macrophages) occurs, at least in part, via a molecular interaction between the MHC-derived antigen-specific T-cell factors and their MHC-derived cell-bound acceptors and that defects in either factors or acceptors may lead to specific defects or anomalies in the immune response.

Antigen-specific T-cell factors have been described in connection with many different antigens, including proteins, haptens, synthetic polypeptides, red cells and tumour cells. Tables 3 and 4 provide a comprehensive guide to the literature up to June 1980. The reader may be interested to know of two other recent reviews, namely by Tada & Okumura (1979) and Germain & Benacerraf (1980).

Table 2. Properties of acceptors for antigen-specific T-cell factors

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1. Acceptors are cell-bound sites with which factors interact. Detected by ability of certain cell types to absorb specific factors.

2. Specific for factor class (helper or suppressor).

3. Generally present on B cells and/or macrophages for helper factors (in antibody production) and on T cells and/or macrophages for suppressor factors. Probably non-clonally distributed.

4. Binding site (for factor) often preserved across species, enabling factor to act xenogeneically.

5. Coded by MHC genes in mouse and man; in the mouse, acceptors carry Ia determinants. I-A subregion genes code for acceptors for helper factors, I-J genes for acceptors for suppressor factors. Background genes also contribute.

6. Missing in some low responder or non-suppressor strains; defect can be MHC or background determined. May be Ir-gene products.

Sources of antigen-specific factors

(a) Helper T cells

Antigen-specific helper factors were first discovered in the culture fluid of mouse T cells which had been primed to specific antigens *in vivo*, so-called activated or educated T cells (Feldmann & Basten, 1972; Taussig, 1974). In this method of raising helper T cells, thymocytes are transferred into lethally irradiated syngeneic recipients which simultaneously receive the antigen in adjuvant. Over the following few days, antigen-reactive T cells proliferate in the spleens of the recipients from which they can be isolated 5–7 days later. Feldmann & Basten (1972) showed that T cells activated in this way to KLH would send a KLH-specific helper stimulus to B cells across a millipore filter, thereby starting the study of soluble antigen-specific T-cell mediators. It was essentially an attempt to show that such factors functioned *in vivo* that led to the discovery of the MHC-related helper factor (Taussig, 1974; Taussig & Munro, 1974). Helper T cells were obtained against the synthetic polypeptide (T,G)-A–L by education of thymocytes *in vivo* as described above. The educated T cells were then restimulated with (T,G)-A–L in serum-free medium in petri dishes and the supernatant collected some 6–8 h later; this contained the (T,G)-A–L-specific helper factor (see Taussig, 1979b, for further methodological details). Factor production was dependent on cells which were sensitive to anti-Thy-1 or anti-Lyt-1 and complement, but not anti-Lyt-2,3; and the producing cells did not adhere to nylon wool (Taussig, Munro & Luzzati, 1976b; M.J.T. unpublished observations). Factors specific for other polypeptide antigens and for red cells were subsequently prepared in an identical fashion (Taussig, Mozes & Isac, 1974; Luzzati, Taussig, Meo & Pernis, 1976; Isac & Mozes, 1977). Extracts of T cells educated in this way also contain helper factor (Shio-zawa, Singh, Rubenstein & Diener, 1977; Shiozawa, Sonik, Singh & Diener, 1980). The relative yields of helper factor obtained by extraction versus release into supernatants do not seem to have ever been compared and, in general, extraction has not been as popular in preparation of helper factors as it has for suppressor factors (see Table 3).

The T cells of normal antigen-primed mice also yield specific helper factors; in this case only extraction has been reported (Tokuhisa, Taniguchi, Okumura & Tada, 1978; Kilburn, Talbot, Teh & Levy, 1978; Sawada, Dauphinée & Talal, 1980). The serum of antigen-stimulated mice has been found to contain a specific helper factor for SRBC, a mere four hours after injection of antigen (Diamantstein & Naher, 1978)!

Priming of mouse T cells *in vitro* is also a successful way of raising factor-releasing helper cells, and the Diener–Marbrook system has proved very useful in this respect for the antigens (T,G)-A–L, GAT, GLPhe and KLH (Howie & Feldmann, 1977; Howie, Parish, David, McKenzie, Maurer & Feldmann, 1979; McDougal & Gordon, 1977a, b; Baltz, Maurer, Merryman & Feldmann, 1978). Spleen cells or thymocytes will give rise to helper T cells in the presence of 'low' antigen doses (e.g. 1 μg protein per ml) over four days; macrophages are essential for helper cell development (Kontiainen & Feldmann, 1973; Erb & Feldmann, 1975; Howie, Feldmann, Mozes & Maurer, 1977; McDougal & Gordon, 1977a). To release factors, the helper T cells are washed and restimulated with antigen in the Marbrook culture vessel (e.g. Howie & Feldmann, 1977). A mixed lymphocyte culture can also be a source of helper T cells which will subsequently release factors specific for alloantigen (Kindred & Corley, 1977).

An important recent development has been the production of helper factors from human peripheral

Table 3. Antigen-specific helper factors

Specificity†	Species	Source	Type of* response	Principal references
(T,G)-A-L	Mouse	Supernatant	IgM	Taussig, 1974, 1978, 1979b; Taussig & Munro, 1974; Munro & Taussig, 1975; Taussig et al., 1974, 1975, 1976b; Taussig & Finch, 1977; Munro et al., 1974, 1978; Mozes, 1976, 1978, 1980; Mozes & Haimovich, 1979; Howie & Feldmann, 1977, 1978; Howie et al., 1979, Feldmann et al., 1978
	Human	Supernatant	IgM	Kantor & Feldmann, 1979; Rees et al., 1979; Woody et al., 1979a,b; Zvaifler et al., 1979
	Mouse	T-hybrid supernatant	IgG	Eshhar et al., 1980
(T,G)-Pro-L	Mouse	Supernatant	IgM	Mozes et al., 1975; Isac & Mozes, 1977; Isac et al., 1976, 1977
(Phe,G)-A-L	Mouse	Supernatant	IgM	Taussig et al., 1975; Isac & Mozes, 1977; Taussig & Finch, 1977
(H,G)-A-L	Mouse	Supernatant	IgM	Isac & Mozes, 1977
GAT	Mouse	Supernatant	IgM	Howie et al., 1979
	Human	Supernatant	IgM	Rees et al., 1979; Zvaifler et al., 1979; Woody et al., 1979
GLPhe	Mouse	Supernatant	IgM	Baltz et al., 1978
KLH	Mouse	Supernatant	IgM	Feldmann & Basten 1972; McDougal & Gordon, 1977b; McDougal et al., 1977b
	Mouse	Extract	IgG	Tokuhisa et al., 1978; Sawada et al., 1980
	Human	Supernatant	IgM	Kantor & Feldmann, 1979; Rees et al., 1979; Woody et al., 1980
OVA	Human	Supernatant	IgM	Kantor & Feldmann, 1979
	Human	Supernatant	IgG (human)	Ballieux et al., 1979; Heijnen et al., 1980
BSA	Mouse	Extract	IgM	Shiozawa et al., 1977
CGG	Mouse	Supernatant	IgM	Feldmann & Basten, 1972; McDougal & Gordon, 1977b
RGG	Mouse	Extract	IgM	Shiozawa et al., 1977
HGG	Mouse	Extract	IgM	Shiozawa et al., 1977
Tetanus Toxoid	Human	Supernatant	IgG (human)	Mudawwar et al., 1978; Geha, 1979; Geha & Mudawwar, 1979
H-2^k alloantigens	Mouse	Supernatant	IgM	Kindred & Corley, 1977

Chicken B locus antigen	Mouse	Extract	IgM	Shiozawa et al., 1980
Monomeric flagellin	Mouse	Supernatant	IgM	Kirov & Parish, 1976
SRBC	Mouse	Supernatant	IgM	Taussig & Finch, 1977; Taussig, 1978
	Mouse	Supernatant	IgM (human)	Taussig et al., 1976b; Luzzati et al., 1976; Luzzati, 1979
	Mouse	Serum	IgM	Diamantstein & Naher, 1978
	Mouse	Con A supernatant	IgM	Bernabé et al., 1979
	Mouse	*in vivo*	Growth of MOPC 315	Lynch et al., 1979
	Rabbit	Supernatant	IgM	Taussig et al., 1976a
	Human	Supernatant	IgM (human)	Ballieux et al., 1979; Heijnen et al., 1980.
HRBC	Mouse	Supernatant	IgM (human)	Taussig et al., 1976b; Luzzati et al., 1976
	Mouse	Serum	IgM	Diamantstein & Naher, 1978
	Mouse	ConA supernatant	IgM	Bernabé et al., 1979
RRBC	Mouse	in vivo	Growth of MOPC 315	Lynch et al., 1979
Strep. Mutans antigen	Mouse	Supernatant	IgM	Lamb et al., 1980
	Rhesus monkey	Supernatant	IgM	Lamb et al., 1980
P815 mastocytoma	Mouse	Extract	T-cell cytotoxicity *in vitro*	Kilburn et al., 1978

* In mouse unless indicated otherwise.

† Abbreviations: BSA, bovine serum albumin; CGG, chicken gamma globulin; GAT, L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GLPhe, L-glutamic acid^{58}-L-lysine^{37}-L-phenylalanine^{5}; (H,G)-A-L, poly-L-(His,Glu)-poly-DL-Ala-poly-L-Lys; HGG, human gammaglobulin; HRBC, horse red blood cells; KLH, keyhole limpet haemocyanin; OVA, ovalbumin; (Phe,G)-A-L, poly-L-(Phe,Glu)-poly-DL-Ala-poly-L-Lys; RGG, rabbit gammaglobulin; SRBC, sheep red blood cells; (T,G)-A-L, poly-L-(Tyr, Glu)-poly-DL-Ala-poly-L-Lys; (T,G)-Pro-L, poly-L-(Tyr,Glu)-poly-L-Pro-poly-LLys.

Table 4 Antigen-specific suppressor factors

Specificity†	Species	Source	Type of response	Principal references
KLH	Mouse	Extract	IgG	Tada et al., 1975, 1976a, 1977, 1978b; Takemori & Tada, 1975; Taniguchi et al., 1976a, b; Taniguchi & Tokuhisa, 1980
	Mouse	Supernatant	IgM	Kontiainen & Feldmann, 1977, 1978
	Mouse	Supernatant	IgG	Feldmann, 1974a, b
	Mouse	T-hybrid supernatant or extract	IgG	Kontiainen et al., 1978; Taniguchi et al., 1979, 1980a, b
	Human	Supernatant	IgM,IgG	Woody et al., 1980
BGG	Mouse	Extract	IgG	Takemori & Tada, 1975
OVA	Mouse	Extract	IgG	Taniguchi et al., 1976a; Taniguchi & Tokuhisa, 1980
	Human	Supernatant	IgG (human)	Ballieux et al., 1979; Heijnen et al., 1980; UytdeHaag et al., 1980
HGG	Mouse	Extract	IgG	Jones & Kaplan, 1977; Taniguchi & Miller, 1978a
	Mouse	Supernatant	IgG	Chaouat, 1978
	Mouse	T-hybrid extract	IgG	Taniguchi & Miller, 1978
Lysozyme	Mouse	Supernatant	DTH	Kojima et al., 1979
Ascaris suum	Rat	Extract	IgE (rat)	Tada et al., 1973; Okumura & Tada, 1974
Strep. mutans c antigen	Mouse	Supernatant	IgM	Lamb et al., 1979
	Rhesus monkey	Supernatant	IgM	Lamb et al., 1979
GAT	Mouse	Extract	IgG	Kapp et al., 1976, 1977; Kapp, 1978; Germain et al., 1978a, 1979; Germain & Benacerraf, 1978; Thèze et al., 1977a, 1978
	Mouse	Supernatant	IgM	Kontiainen et al., 1979
GT	Mouse	Extract	IgG	Waltenbaugh et al., 1977a,b; Waltenbaugh & Benacerraf, 1978; Thèze et al., 1977b,c; Germain et al., 1978b, 1980
GA	Mouse	Extract	IgG	Germain et al., 1979

(T.G)-A-L	Mouse	Supernatant	IgM	Kontiainen et al., 1979
	Human	Supernatant	IgM	Rees et al., 1979
ABA	Mouse	Extract or supernatant	DTH	Bach et al., 1979; Greene et al., 1979; Hirai & Nisonoff, 1980
DNP	Mouse	Supernatant	Contact sensitivity	Moorhead, 1977a, b; 1979
Picryl	Mouse	Supernatant, serum	Contact sensitivity	Asherson & Zembala, 1974; Asherson et al., 1978; Zembala & Asherson, 1974; Zembala et al., 1975, 1977; Ptak et al., 1978; Greene et al., 1977b; Noonan & Halliday, 1980
SRBC	Mouse	Supernatant	DTH	Liew & Chan-Liew, 1978; Liew et al., 1980
	Human	Supernatant	IgM (human)	Ballieux et al., 1979; UytdeHaag et al., 1979a, b, 1980; Heijnen et al., 1980
	Mouse	T-hybrid supernatant	DTH	Hewitt & Liew, 1979
	Mouse	T-hybrid supernatant	IgM, IgG	Taussig et al., 1979a, b, c, 1980a, b, c; Taussig & Holliman, 1979
	Mouse	in vivo	Growth of MOPC 315	Lynch et al., 1979
MCA-sarcomas	Mouse	Extract	Tumour rejection	Greene et al., 1977a; Perry et al., 1978
	Mouse	Serum, T-hybrid supernatant	T cell cytotoxicity, tumour rejection	Nelson et al., 1975, 1980; Nepom et al., 1977; Hellström, 1978
P815 mastocytoma	Mouse	Extract	T-cell cyto-toxicity	Takei et al., 1978
Anti-ABA idiotype	Mouse	Supernatant	Anti-ABA idiotype	Hirai & Nisonoff, 1980
M315 idiotype	Mouse	in vivo	MOPC 315 secretion	Lynch et al., 1979
Ig-1b‡ allotype	Mouse	Supernatant	IgG2a (Ig-1b)	Jacobsen, 1973; Herzenberg et al., 1976
IgE‡	Mouse	Supernatant, T-hybrid	IgE	Suemura et al., 1977, Kishimoto et al., 1978; Watanabe et al., 1978

* Mouse, unless otherwise indicated.
‡ Not antigen-specific, but specific for antibody class.
† Abbreviations: See Table 2. Also: ABA, azobenzenearsonate; DNP, dinitrophenyl; BGG, bovine gammaglobulin; GA, L-glutamic acid^{60}-L-alanine^{40}, GT, L-glutamic acid^{50}-L-tyrosine^{50}; M315, product of MOPC 315; MCA, methylcholanthrene induced.

blood lymphocytes primed *in vitro*. Here again, a Diener–Marbrook tissue culture flask has been used, with the same conditions as for mouse cells, to secure human helper factors specific for (T,G)-A–L, GAT and KLH (Kantor & Feldmann, 1979; Rees, Feldmann, Erb, Woody, Kontiainen, Bodmer, Kantor & Zvaifler, 1979; see also Table 3). Human T cells can also be primed in microcultures for factor production to SRBC or ovalbumin (Ballieux, Heijnen, Uytde-Haag & Zegers, 1979; Heijnen, UytdeHaag & Ballieux, 1980). To avoid having to prime the T cells *in vitro*, it is possible to restimulate T cells of volunteers recently immunized with 'ethical' protein antigens, a method employed to obtain helper factors for tetanus toxoid (Mudawwar, Yunis & Geha, 1978; Geha, 1979; Geha & Mudawwar, 1979).

The specific helper factors are being used to study various aspects of the human immune response, including its genetic control (see also later sections). Rees *et al.* (1979) established that both macrophages and T cells were necessary for the primary generation of human helper factor—and by implication human helper T cells—*in vitro*. Moreover, mixtures of fully allogeneic macrophages and T cells did not co-operate successfully, while the sharing of a single HLA-DR-locus antigen was sufficient and necessary to allow generation of helper activity. This result suggests that naive helper T cells (or their precursors) are HLA-DR restricted before their contact with antigen.

Finally, rhesus monkey helper cells and helper factors have been generated, again with the aid of Diener–Marbrook flasks, to *Streptococcus mutans* cell wall antigen, as reported recently by Lamb, Kontiainen & Lehner (1980).

(b) Suppressor T cells
A variety of methods have been used to produce suppressor T cells *in vivo* and *in vitro* (see e.g. Möller, 1975). Some of these methods can be rationalized by suggesting that, unlike helper cells, suppressor T cells are stimulated by direct contact with antigen which has bypassed macrophages. Thus, suppressor cells are prominent *in vivo* after priming with high doses of antigen (without adjuvant), after tolerizing with particle-free (ultracentrifuged) antigen, and in genetic non-responders; *in vitro*, superimmunogenic levels of antigen induce suppressors and macrophages are not only unnecessary but their removal can facilitate suppressor cell induction (references below).

In their initial discovery of the antigen-specific suppressor T-cell factor, Tada and colleagues obtained suppressor cells from the thymuses and spleens of rats which had been hyperimmunized with *Ascaris suum* antigen (Tada, Okumura & Taniguchi, 1973), and in most of their subsequent work they have used similar cells from mice which are twice-injected with a high dose (100 μg) of KLH in the absence of adjuvant. Such 'KLH-primed' suppressor cells will suppress the primary response of normal mice to DNP-KLH (Tada & Takemori, 1974; Tada, Taniguchi & Takemori, 1975) and their extracts contain the KLH-specific suppressor factor (Takemori & Tada, 1975). Tolerant animals are also a source of suppressor T cells from which suppressor factors can be extracted, e.g. mice tolerized by deaggregated HGG (Jones & Kaplan, 1977; Taniguchi & Miller, 1977; Chaouat, 1978) or made unresponsive to skin-painting with reactive haptens such as DNFB or picryl chloride by intravenous administration of the hapten-sulphonates or haptenated syngeneic cells (Zembala & Asherson, 1974; Asherson, Zembala, Thomas & Perera, 1980; Greene, Pierres, Dorf, & Benacerraf, 1977b; Moorhead, 1977a).Mice which are genetically unresponsive to the synthetic polypeptides GAT or GT may contain specific suppressor T cells a few days after injection of these molecules; such mice are then unable to mount a response to an otherwise immunogenic complex of the specific polypeptide with methylated BSA, and this unresponsiveness can be transferred with the specific suppressor factor obtained from the T cells (Kapp, Pierce, De La Croix & Benacerraf, 1976; Waltenbaugh, Debré, Thèze & Benacerraf, 1977b).

Other *in vivo* sources of suppressor cells include tumour-bearing mice, which have tumour-specific suppressor cells from which tumour-specific factors can be extracted (Greene, Fujimoto & Sehon, 1977a; Takei, Levy & Kilburn, 1978); and allotype- or idiotype-suppressed animals (Herzenberg, Okumura, Cantor, Sato, Shen, Boyse & Herzenberg, 1976; Jacobsen, 1973; Hirai & Nisonoff, 1980).

Suppressor cells can also be induced *in vitro*, in Diener–Marbrook culture flasks using a 100-fold greater antigen concentration than for induction of helper cells. In this way, suppressors have been obtained against KLH, (T,G)-A–L and GAT (Kontiainen & Feldmann, 1976, 1977; Howie, 1977; Kontiainen, Howie, Maurer & Feldmann, 1979). Macrophages are not required in this process, in contrast with helper cell induction (Howie, 1977). The specific factor is obtained by restimulating the washed suppressor cells with antigen. Suppressor T cells against GAT can be induced *in vitro* from responder spleen

cells, and in the presence or absence of macrophages; but the presence of macrophages increases the concentration of antigen required (Pierres & Germain, 1978). Thus the induction of suppressor cells *in vitro* lends some support to the 'macrophage bypass' hypothesis.

Human suppressor cells and factors can be derived from cultures of peripheral blood lymphocytes stimulated with high doses of KLH or (T,G)-A–L in the Diener–Marbrook system as for mouse cells (Woody, Kontiainen, Zvaifler, Rees & Feldmann, 1980; Rees *et al.*, 1979) and against SRBC and ovalbumin with other culture methods (Ballieux *et al.*, 1979; Heijnen *et al.*, 1980; UytdeHaag, Heijnen, Pot & Ballieux, 1979a, b; UytdeHaag, Heijnen & Ballieux, 1980). Rhesus monkey lymphocytes have also yielded suppressor cells and factors against *Streptococcus mutans* c antigen (Lamb, Kontiainen & Lehner, 1979).

Suppressor T cells can be further enriched from primed or tolerant cells by adsorption to and elution from antigen-coupled Sephadex G-200 (Okumura, Tokuhisa, Takemori & Tada, 1978) or antigen-coated petri dishes (Taniguchi & Miller, 1977). This, together with their induction by apparently direct antigen contact (above), suggests that suppressor T cells do not need to encounter antigen in the context of an MHC molecule, unlike helper T cells and other T-cell subclasses.

Specific suppressor factors have been found both in extracts and in the culture supernatants of suppressor T cells, as indicated in Table 4. Tada and colleagues were the first to isolate suppressor factors by extraction from suppressor cells, using sonication or freeze-thawing to disrupt the cells (Tada *et al.*, 1973; Takemori & Tada, 1975). These methods have been adopted by others, and it seems to be the case that specific factors are often more easily detected in cell extracts than in culture fluid. However, several groups have worked with specific factors found in culture supernatants of suppressor T cells, and T-hybrid lines release factors into their supernatants, or ascitic fluid if grown *in vivo* (below). This point is of more than methodological interest, as it has been claimed that factors in extracts and supernatants behave differently with respect to functional properties such as target cell of action and genetic restriction (Kontiainen & Feldmann, 1978; Kontiainen *et al.*, 1979) and to structure (Taniguchi, Takei & Tada, 1980b).

(c) T-hybrid lines
The most desirable form of helper or suppressor T cell would be one that could be kept permanently available in culture as a growing line, capable of being cloned and steadily producing a monoclonal specific factor. Two methods now available to achieve such ends are the growth of specific T cells in conditioned medium containing T-cell growth factor (Gillis & Smith, 1977; Gillis, Baker, Ruscetti & Smith, 1978) and the production of neoplastic hybrid lines by cell fusion. Of these approaches, the latter has been successfully applied to the problem of obtaining stable factor-producing cell lines and several hybrids of interest have now been reported. In this technique, an adaptation of the Köhler–Milstein method for the production of monoclonal antibody-secreting lines, helper or suppressor T cells are essentially immortalized by fusion with a thymoma line, usually the AKR thymoma BW 5147 (Goldsby, Osborne, Simpson & Herzenberg, 1977). After fusion using polyethylene glycol, 'T-hybrid' lines are selected by growth in HAT medium, which kills unhybridized BW 5147 cells, and their supernatants can then be screened for production of specific helper and suppressor factors or any other mediators of interest. The advantages of such permanent culture lines are obvious: they could potentially provide a constant, indeed unlimited, supply of a monoclonal product, the structure and function of which could be thoroughly studied without the complications and frustrations of making tiny amounts of factors from conventional T cells.

A growing number of functional, factor-releasing T-hybrids is being reported and although so far most have been suppressors, there are very recent descriptions of helper lines (references below and Tables 3 and 4). Thus it seems not to be the case, as was at one time suspected, that fusion with BW 5147 would only allow retention of suppressor properties. It is probably an advantage to be able to enrich the T-cell population for specific cells before fusion and the technique noted above of absorption and elution from antigen-coated petri dishes has been employed to increase the proportion of antigen-specific suppressor cells for HGG or KLH before fusion (Taniguchi & Miller, 1978b; Taniguchi, Saito & Tada, 1979). A pre-fusion augmentation of (T,G)-A–L helper cells by culturing for 24 h on monolayers of antigen-pulsed macrophages was used in a fusion which ultimately produced a (T,G)-A–L-specific helper line (Eshhar, Apte, Löwy, Ben-Neriah, Givol & Mozes, 1980). After fusion and growth in HAT medium, hybrids have in most cases been screened by testing supernatants (or extracts) for function, i.e. help or suppression of specific responses. However, it is a definite advantage at this stage to be

able to identify more rapidly the potentially functional hybrids and in this respect cell surface markers are sometimes useful. As a marker for suppressor cells, anti-I-J serum, together with fluorescent anti-mouse Ig, can be used to identify I-J-positive hybrids, which can then be separated on the cell sorter and cloned straight away (Taniguchi & Miller, 1978b; Taniguchi et al., 1979; Taniguchi, Takei, Saito, Hiramatsu & Tada, 1980a). Other surface markers for which screening could be rewarding are antigen-binding and Ig V-region expression (hybrids formed with BW 5147 do not react with conventional anti-mouse Ig reagents). Hämmerling, Lonai and co-workers were able to select a few lines binding specifically to NP-CGG conjugate after a fusion of BW 5147 with T cells primed to NP-CGG, which yielded about 500 lines (Lonai, Puri & Hämmerling, in preparation). Screening for helper antigen-binding hybrids followed a rather indirect path, since helper T cells themselves do not bind free antigen, nor did any of the hybrids. However, some hybrids bound radio-iodinated Ia-NP-CGG complex, following a recently published method (Puri & Lonai, 1980) and could be detected by autoradiography. Some of the positive binding lines were then found to secrete a CGG-specific helper factor, of which one line was studied in detail (Lonai et al., 1980). Eshhar et al. (1980) fused BW 5147 with T cells educated in vivo against (T,G)-A–L and found that 5 out of the 21 lines produced reacted by immunofluorescence with a rabbit anti-V_H serum (specific for the framework of the Ig heavy chain V-region); one of these V_H-positive lines was established as a producer of (T,G)-A–L-specific helper factor. Other surface markers, such as Lyt, may be a less reliable guide to specific function or T-cell origin of the hybrid as they may be inappropriately expressed as a result of the fusion, or the influence of BW genes (Taussig, Wright & Holliman, 1980). For example, a suppressor line specific for KLH was unexpectedly Lyt-1$^+$, 2$^-$ (Kontiainen et al., 1978).

A problem encountered with T-hybrid lines in general seems to be a rather higher degree of instability, due to chromosomal loss, than is commonly found among antibody-producing lines (e.g. Taniguchi & Miller, 1978b). This can be overcome only by vigilance and recloning as necessary.

Among the functional suppressor lines derived to date, antigen specificities include: KLH (Kontiainen et al., 1978; Taniguchi et al., 1979); HGG (Taniguchi & Miller, 1978b); NP (Kontiainen et al., 1980); SRBC (Taussig, Corválan, Binns & Holliman, 1979a; Hewitt & Liew, 1979); and a fibrosarcoma-specific antigen

(Nelson, Cory, Hellström & Hellström, 1980). One suppressor line is specific for IgE antibody, but is non-antigen specific (Watanabe, Kimoto, Maruyama, Kishimoto & Yamamura, 1978). Helper-factor producing lines are specific for (T,G)-A–L (Eshhar et al., 1980) or CGG (Lonai et al., 1980). The properties of some of these hybrid-produced factors are discussed further below.

Assay methods

(a) Helper factors

Reference to Table 3 shows that the effects of antigen-specific helper factors have most commonly been demonstrated on antibody production rather than cell-mediated immunity. The factors will generally replace the requirement for T cells in antibody production in vivo and in vitro, in primary and secondary responses, and in both IgM and IgG production. They are best assayed either on populations which are poor in T cells altogether, such as bone marrow cells, nude mouse spleen cells, anti-Thy-1 treated cells, etc., or simply poor in the relevant antigen-specific T cells, such as unprimed or hapten-primed lymphoid cells. Macrophages are required for their activity (McDougal & Gordon, 1977b; Howie & Feldmann, 1978). In the original test system, (T,G)-A–L-specific helper factor was assayed in vivo by adoptive transfer with normal bone marrow cells and antigen into irradiated recipients; large numbers of IgM plaque-forming cells to (T,G)-A–L developed in the recipients' spleens 12–14 days later, whereas very few were found in controls from which factor was omitted (Taussig, 1974). Thus the (T,G)-A–L factor replaced T helper cells in the primary response to the multivalent antigen against which it was directed; it will do likewise for haptens coupled to (T,G)-A–L, as in the primary response to DNP as DNP-(T,G)-A–L (Howie & Feldmann, 1977), confirming its T-cell like, carrier-specific activity. Another T-cell replacing, in vivo assay is the use of nude mice in their response to alloantigens (Kindred & Corley, 1977).

For obvious reasons, however, helper factors are more commonly assayed in vitro. Hapten-carrier conjugates are popular as test antigens to detect factors directed against the carrier in either primary anti-hapten responses (e.g. Howie & Feldmann, 1977, 1978) or with hapten-primed cells (e.g. McDougal & Gordon, 1977b). An interesting effect found by Shiozawa et al. (1977) was that helper factors against pro-

tein carriers only provided effective help for TNP-pro-
tein conjugates if the latter were polymerized by conju-
gation to Ficoll as a 'molecular backbone'. The
suggestion was that converting monomeric antigens
into a polymeric array enabled sufficient factor to be
bound to trigger the B cell.

An unusual and interesting system is that of Lynch
et al. in which factors and antigen are able to regulate
the growth of myeloma cells (MOPC 315) *in vivo*
(Lynch, Rohrer, Odermatt, Gebel, Autry & Hoover,
1979). MOPC 315 cells bind DNP and TNP groups by
their IgA receptors and will form rosettes and plaque-
forming cells with TNP-SRBC. Despite their neoplas-
tic nature, during their growth *in vivo* they maintain a
differentiative progression similar to that of normal B
cells (though of course not requiring antigen), from
relatively small, lymphocytoid cells which express but
do not secrete antibody, to seceretory plasmacytoid
cells. Lynch *et al.* put MOPC 315 cells together with
TNP-SRBC inside peritoneal diffusion chambers
which were then implanted into mice previously im-
munized with SRBC; the immunization was such as to
raise predominantly helper or suppressor T cells
against SRBC. They found that the growth of the
myeloma cells was enhanced in mice pre-immunized
with 4×10^8 SRBC, as measured by numbers of viable
cells, rosette-forming and plaque-forming cells inside
the diffusion chambers. The helper effect was carrier-
specific (rabbit RBC used as control) and could be
specifically transferred with T cells. The implication is
that in the presence of TNP- SRBC, MOPC 315 cells
can be influenced by a soluble, SRBC-specific T-cell
molecule which passes into the diffusion chamber (the
factor itself was not isolated). Analogous experiments
showed that MOPC 315 growth and differentiation
could be suppressed by using mice primed with lower
numbers of SRBC (4×10^6) to generate suppressor T
cells. The interest of these experiments is that they
show that however abnormal these neoplastic cells
may be in terms of their growth, they can still be
regulated by normal T-cell signals and antigen.

In general, helper factors do not show the strict
genetic restriction of some suppressor factors which
will only suppress MHC-compatible cells (below). In
fact, helper factors can often act xenogeneically, an
ability made use of in assaying factors prepared in
different species. For example, it was possible to pre-
pare SRBC-specific helper supernatants from rabbit
peripheral blood lymphocytes and test them on mouse
cells, avoiding the need to set up a rabbit *in vitro*
system (Taussig, Finch & Kelus, 1976a).

Some studies of the human immune response have
also utilized the xenoreactivity of both human and
mouse helper factors. Three combinations of mouse
and human factors and cells have been used to study
human responses, viz. the stimulation (a) of human
peripheral blood lymphocytes *in vitro* by mouse helper
factors, (b) of mouse spleen cells by human helper
factors, and (c) of human B cells by human factors, as
follows.

(a) The use of mouse factors to stimulate human
peripheral blood lymphocytes was suggested because
of the great difficulty experienced in obtaining a
human response to SRBC *in vitro* (Luzzati *et al.*,
1976). Treatment of human lymphocytes with the
supernatant of mouse T cells educated against SRBC
produced a spectacular improvement in their plaque
response against SRBC (Luzzati *et al.*, 1976; Luzzati,
1979; Taussig *et al.*, 1976b). The effect was antigen-
specific (horse RBC used as reciprocal control) and
apparently mediated by the same factor which helped
mouse bone marrow cells. The development of human
antibody-forming cells was accompanied by impres-
sive cell proliferation, which was far in excess of that
expected of specific clonal proliferation. Thus, it
appeared that addition of specific mouse supernatant
and antigen had triggered an essentially non-specific
mitogenic response in human cells; this was confirmed
by adding both SRBC and HRBC together to a culture
with SRBC-specific factor, whereupon a good re-
sponse to both antigens occurred (Luzzati *et al.*, 1976;
Taussig *et al.*, 1976b). Moreover, SRBC and HRBC
acted synergistically. The cellular mechanisms of trig-
gering human cells by mouse factors still have to be
fully explored and there is also the possibility that the
reaction could be used to demonstrate Ir-gene con-
trolled defects in the human response.

(b) In the reverse of this experiment, human helper
factors can be assayed on mouse cells (Kantor & Feld-
mann, 1979; Rees *et al.*, 1979; Woody, Rees, Zvaifler,
Howie, Ahmed, Strong, Hartzman, Kantor & Feld-
mann, 1979a; Woody, Zvaifler, Rees, Ahmed, Hartz-
man, Strong, Howie, Kantor & Feldmann, 1979b;
Woody *et al.*, 1980; Zvaifler, Feldmann, Howie,
Woody, Ahmed & Hartzman, 1979). Human factors
against KLH, OVA, (T,G)-A–L and GAT have been
demonstrated in this way in Diener–Marbrook cul-
tures, using the proteins or polypeptides as carriers for
DNP or TNP. Non-specific effects of human lympho-
cyte supernatants could be separated from specific
help by titration, or removal and elution of the specific
factor on antigen adsorbents. This system has been

used to study the genetic control of specific factor production in man (Zvaifler et al., 1979) as discussed further below. A Rhesus monkey factor has similarly been tested on mouse spleen cells (Lamb et al., 1980).

(c) Human helper factors can be assayed on human B cells. Thus Mudawwar et al. (1978) measured the specific stimulation of anti-tetanus toxoid antibody by the supernatant of antigen-stimulated human T cells; the factor was again purified on antigen adsorbents to remove nonspecific factors. In this secondary (IgG) response, the factor acted on autologous but not allogeneic B cells (Geha, 1979); xenoreactivity was not tested. A primary human antibody response in vitro to SRBC and OVA can be stimulated with the aid of appropriate specific human factors (Ballieux et al., 1979; Heijnen, UytdeHaag, Dollekamp & Ballieux, 1979; Heijnen et al., 1980); these factors were not HLA-restricted.

In the cell-mediated immune response, transfer factor has some of the functional properties of a specific helper factor (antigen-specific induction of delayed hypersensitivity) but probably belongs to a different class of molecule outside the scope of this review (reviewed by Petersen & Kirkpatrick, 1979). Antigen-specific migration inhibition factor (MIF), on the other hand, is a specific T-cell product which resembles the helper factors described here and indeed was able to co-operate in an anti-hapten response (Amos & Lachmann, 1970; Lowe & Lachmann, 1974). A recent example of specific helper factors in a cell-mediated response occurs in a tumour-specific response, namely the ability of an extract of spleen cells of P815 mastocytoma-bearing mice to enhance specifically the development of cytotoxic T cells against P815 cells in vitro (Kilburn et al., 1978).

A point of general advisability in the assay of factors (suppressor as well as helper) is to carry out titrations of the active supernatant or extract, to determine an accurate end-point of activity. This is often overlooked and can be crucial in evaluating the effects of immunoadsorbents or other procedures on factor activity. The 'all-or-nothing' assessment of factor-mediated responses seen so often in the literature can give quite misleading results. Obviously, an enormous range in titre is to be expected with factors prepared by different methods and assays of different sensitivities; e.g. the (T,G)-A–L factor as originally assayed in vivo had an end-point of dilution of about 1:4, whereas a factor of the same specificity prepared and tested in vitro could be diluted out to $1:10^4$ and still show helper activity (Howie & Feldmann, 1977). There is a real need for a more standardized and rapid factor test, probably based on binding; the measurement of cAMP levels in cells treated with factor and antigen may be a step in this direction (Mozes, 1978).

(b) Suppressor factors

Specific suppressor factors have been assayed in the antibody response in vivo and in vitro, and in cell-mediated immune responses such as delayed hypersensitivity and anti-tumour immunity (Table 4). In the antibody systems, carrier-specific suppression of anti-hapten responses is again widely used as the assay (e.g. Taniguchi, Hayakawa & Tada, 1976a; Kontiainen & Feldmann, 1977, 1978), though with the synthetic polypeptides GAT and GT, suppression of antibody against the inducing molecule itself is generally measured (e.g. Kapp et al., 1976; Waltenbaugh et al., 1977b). Unlike helper factors, however, class specificity is sometimes found for suppressor factors, especially those in cell extracts. Examples include the suppression of the IgE response to Ascaris suum extract in rats (Okumura & Tada, 1974; Tada et al., 1973) or the preferential effect on IgG responses to DNP-KLH and other conjugates (Takemori & Tada, 1975; Taniguchi et al., 1976; Taniguchi & Miller, 1978a). These factors are both antigen and class-specific; the latter may simply reflect a greater T-dependence of some antibody classes or alternatively be due to the existence of class-specific helper cells which would become the targets of the suppressor factor, as indicated in allotype suppression (Herzenberg et al., 1976). The origin of factors, i.e. supernatants vs extracts, seems to have an influence on the class-restriction phenomenon, since KLH-specific factors, and others, in culture supernatants suppressed IgM responses effectively (Kontiainen & Feldmann, 1977; Kontiainen et al., 1979). Similarly, the extract or ascitic fluid of one T-hybrid line is IgG-restricted as well as KLH-specific (Taniguchi et al., 1979) while the supernatants of others suppress IgM and IgG equally well (Kontiainen, Simpson, Bohrer, Beverly, Herzenberg, Fitzpatrick, Vogt, Torano, McKenzie & Feldman, 1978; Taussig et al., 1979a; Taussig, Corvalàn, Binns, Roser & Holliman, 1979b; Taussig, Corvalàn & Holliman, 1979c). The preferential suppression, by a soluble factor, of mouse IgE responses to DNP has been described and the authors believe that their factor has class-specificity but not antigen-specificity, although elicited by antigen from DNP-primed cells (Suemura, Kishimoto, Hirai & Yamamura, 1977; Kishimoto, Hirai, Suemura, Nakanishi & Yamamura, 1978;

Watanabe *et al.*, 1978). Such isotype-specific factors may well be important in physiological regulation of the antibody class 'switch'.

In the delayed-type hypersensitivity response it is, in principle, possible to distinguish effects of factors on the induction of sensitivity (e.g. by giving antigen and factor to unprimed animals) from effects on elicitation of a response by sensitized cells. Asherson, Zembala and colleagues (Asherson & Zembala, 1974; Asherson *et al.*, 1980; Ptak, Zembala & Gershon, 1978; Zembala & Asherson, 1974) and Moorhead (1977a, b, 1979) have found antigen-specific factors which suppress contact sensitivity to picryl chloride and DNFB respectively in mice. They are present in the culture supernatants of lymph node cells of hapten-tolerant mice, and inhibit the passive transfer of sensitivity to normal recipients by hapten-primed cells, i.e. suppress elicitation of contact sensitivity. However, such factors are probably quite heterogeneous, as shown by studies on delayed hypersensitivity to SRBC. Specific factors from primed suppressor T cells suppressed both induction and expression of hypersensitivity (Liew & Chan-Liew, 1978); but when T-hybrid lines were made from SRBC-specific suppressor T cells, the supernatants of individual lines suppressed either induction or elicitation of hypersensitivity, but not both (Hewitt & Liew, 1979). The fact that T-hybrid factors are monoclonal and presumably homogeneous is obviously a great advantage in dissecting such effects.

Tumour-specific immunity can be suppressed by specific factors and effects assayed by enhancement of tumour growth *in vivo* (Greene *et al.*, 1977a; Perry, Benacerraf & Greene, 1978). A definite possibility is that blocking factors detected in the sera of tumour-bearing animals and humans (Hellström & Hellström, 1974) are in fact specific suppressor factors. There are close similarities both in molecular properties and cellular origins. The serum blocking factor specific for a methylcholanthrene-induced sarcoma is a glycoprotein of about 56,000 molecular weight which binds specifically to the sarcoma cells (Nepom, Hellström & Hellström, 1977; Hellström, 1978) and the T-cell dependence of factor production has been demonstrated (Nelson, Pollack & Hellström, 1975). Very recently, a T-hybrid line has been described which produces the specific blocking factor into its culture supernatant (Nelson *et al.*, 1980). The factor interferes directly in the killing of ^{51}Cr-labelled tumour cells by tumour-specific cytotoxic T cells and specifically enhances growth of the relevant sarcoma *in vivo*; the

authors feel that it also affects the development of cytotoxic cells *in vivo*.

Human suppressor factors have, like helper factors, been assayed either directly on responding human peripheral blood lymphocytes (UytdeHaag *et al.*, 1979a, b; 1980) or on mouse spleen cell cultures, made possible through their xenoreactivity (Rees *et al.*, 1979; Woody *et al.*, 1980).

Factor specificity and the antigen-binding site

The specificity of T-cell factors has been demonstrated in two ways: (a) functional specificity, factors help or suppress the response to the antigens against which they are raised and do not influence noncross-reacting antigens, and (b) binding specificity, factors can be removed specifically by insolubilised antigen (or anti-idiotype) and subsequently recovered by elution. Because they possess antigen-binding sites, factors could be regarded as soluble counterparts of T-cell receptors, and any information concerning the structure and genetic origin of their binding sites can directly relate to the long-unsolved problem of antigen recognition by T cells. Recent findings on the factor antigen-binding sites can be summarized in terms of three apparent paradoxes. (i) The factors discriminate between antigens as efficiently as do antibodies; yet they are not immunoglobulins, as that term is generally understood, being much smaller is size (molecular weight $\sim 60,000$) and lacking constant region Ig determinants. (ii) The factor binding site is quite closely related to the antibody binding site, since factors share idiotypic determinants with antibodies reactive with the same antigens, and carry framework determinants of the antibody heavy chain variable domain (V_H). Yet there is no indication of an Ig light chain or light chain variable region (V_L), which would presumably be essential in forming an antibody-like combining site. (iii) Although some factors share idiotypic determinants with antibodies, as defined by anti-idiotypic sera, their fine speicificity for antigen (i.e. pattern of binding to cross-reactive antigens) can be quite different, suggesting that the factor and antibody repertoires are in fact distinct.

Leaving aside those factors which appeared to be 7S IgM molecules (Feldmann & Basten, 1972; Taniguchi & Tada, 1974), the absence of Ig constant region determinants from MHC-related antigen-specific factors has been confirmed in nearly all cases, except for the binding of (T,G)-A–L-specific helper factor by a chicken anti-mouse IgM serum (Howie & Feldmann,

1977) which also stains T cells (Szenberg, Marchalonis & Warner, 1977). Nevertheless, there is growing evidence for shared V-regions between factors and antibodies. Anti-idiotypic sera raised against mouse antibodies to (T,G)-A–L, GAT and ABA (azobenzenearsonate) reacted with the specific T-cell factors against these antigens (Mozes, 1978; Mozes & Haimovich, 1979; Germain, Ju, Kipps, Benacerraf & Dorf, 1979; Bach, Green, Benacerraf & Dorf, 1979). Mozes & Haimovich (1979) raised guinea-pig anti-idiotypic sera to mouse (C3H.SW) (T,G)-A–L antibodies which bound 20–30% of the anti-(T,G)-A–L antibodies in C3H.SW immune serum. Despite the partial reaction with antibody, the anti-idiotypic serum removed all the activity of (T,G)-A–L-specific factor of this strain. Moreover, the factor idiotype, like the antibody idiotype, was coded by Ig heavy chain allotype-linked genes. Similar linkage of the inheritance of a factor idiotype to Ig allotype was established for the ABA-specific suppressor factor (Bach *et al.*, 1979). This factor can also be induced by anti-idiotypic antibodies administered *in vivo* (Hirai & Nisonoff, 1980). Human factors also carry antibody idiotypes: a helper factor specific for tetanus toxoid was bound by anti-idiotype against the donor's anti-tetanus toxoid antibodies (Mudawwar *et al.*, 1978). A complementary approach is to raise antisera directly against factors, and Kontiainen & Feldmann (1979) have produced mouse antisera which seem to react with factor idiotypes. An 'anti-idiotype' made against KLH suppressor factor also reacted with KLH helper factor but not GAT helper factor, suggesting that helper and suppressor factors against the same antigen might share binding sites as do antibodies of different classes.

The other serologically detectable Ig V-region determinants are those of the framework, using rabbit antisera against the V-domains of heavy and light chains, anti-V_H and anti-V_L, which cross-react widely with Igs of different classes (Ben-Neriah, Lonai, Gavish & Givol, 1978a; Ben-Neriah, Wuilmart, Lonai & Givol, 1978b). Anti-V_H serum reacts with the KLH- specific suppressor factor, and probably also with suppressor T cells (Tada & Okumura, 1979); a helper factor for KLH can also be removed by this serum (Feldmann, Erb, Kontiainen, Todd & Woody, 1979). Anti-V_H also reacts with some T-hybrid lines, one of which produces the (T,G)-A–L-specific helper factor and the factor itself is also V_H-positive (Eshhar *et al.*, 1980); so too is the helper factor for CGG produced by another T-hybrid line (Lonai *et al.*, 1980). However, so far there is no evidence for light chain involvement; the

factors lack κ and λ light chain constant regions and until recently the anti-V_L sera were prepared against λ chains only (Ben-Neriah *et al.*, 1978a). The use of an anti-V_κ reagent should make clear whether the factor binding site is truly Ig-like, i.e. made up of both V_H and V_L domains. If V_L is not detectable, the problem arises of how the factor uses its V_H site—perhaps in combination with a new type of light chain (MHC-derived?); or by two V_H domains pairing-off to form a site; or even as a single chain which might have sufficient affinity to bind antigen without a light chain.

Some dissenting results can be noted here. Anti-F_V sera (against the combined V_H and V_L domains) failed to bind the (T,G)-A–L-specific factor from educated T cells (Munro, Taussig, Campbell, Williams & Lawson, 1974) or a T-hybrid suppressor factor for SRBC (Taussig, Holliman & Corvalàn, 1980b); and a rabbit SRBC-specific helper factor lacked the rabbit *a* allotype determinant which is a marker for most rabbit Ig V_H-regions (Taussig *et al.*, 1976a).

Despite the evidence that the factor-binding site is coded by Ig V_H-genes, the fine specificity of their recognition of antigen tends to emphasize differences between factors and antibodies rather than similarities. For example, anti-(T,G)-A–L antibodies are largely, if not totally, cross-reactive with (T,G)-A–L, so that anti-(T,G)-A–L plaque-forming cells can be detected equally well with either antigen coupled to SRBC (e.g. Taussig, Mozes & Isac, 1974). However, there is universal agreement that the (T,G)-A–L-specific helper factor neither binds to (T,G)-Pro–L, nor assists in the antibody response to (T,G)-Pro–L (Taussig & Munro, 1976; Taussig *et al.*, 1976b; Isac & Mozes, 1977; Howie & Feldmann, 1977). Thus the factor, unlike antibody, does not recognize the '(T,G)' determinant, (Tyr-Tyr-Glu-Glu), but according to Isac & Mozes (1977) has specificity for (G)-A–L, a molecule similar to (T,G)-A–L but lacking the terminal tyrosines. A similar situation, though less extreme, occurs for the GAT-specific suppressor factor, which is only partially bound by GT, whereas anti-GAT antibody is totally cross-reactive (Thèze, Kapp & Benacerraf, 1977a; Germain & Benacerraf, 1980). The problem is to reconcile these differences with the sharing of V_H and idiotype between factors and antibodies. There are two solutions, namely (a) that the factor binding sites are a narrow population included within the repertoire of antibody binding sites, but only as a small minority, or (b) that the factor sites are a distinct repertoire not represented among the antibody sites, but nevertheless similar in structure

and coded by a V-gene set linked to the Ig V_H genes. The material with which to solve these problems may soon be to hand in the form of T-hybrid lines producing antigen-specific, idiotype- and V_H- positive factors which can be sequenced both at the protein and the DNA level.

Finally, the fact that specific factors bind to antigen adsorbents and do not require antigen to be presented to them on cell (e.g. macrophage) surfaces, argues against recognition of antigen-Ia complexes by single T-cell receptor sites and hence favours 'dual recognition' at the T-cell surface. However, a counter-argument could be made that binding sites for such complexes might retain sufficient affinity to bind to antigen without the MHC determinant. This point is referred to again in a later section.

The MHC and structure of factors

The discovery that brought antigen-specific factors to widespread interest and attention was their relationship to the MHC, namely the ability of anti-H-2 sera to remove mouse specific helper and suppressor factors (Taussig & Munro, 1974; Takemori & Tada, 1975). Subsequently, the helper factor for (T,G)-A–L was mapped, using antisera, to the I-A subregion of H-2 (Taussig, Munro, Campbell, David & Staines, 1975) and the suppressor factor for KLH to I-J (Tada, Taniguchi & David, 1976a 1977). Indeed, Tada and colleagues first defined the existence of the I-J subregion on the basis of the unexpected ability of certain alloantisera [e.g. B10.A(3R) anti -B10.A(5R)] to remove the KLH-specific factor (Tada *et al.*, 1976a, 1977). The coding of mouse helper factors in the I-A subregion has been reconfirmed for factors specific for (T,G)-A–L (Howie & Feldmann, 1977; Howie *et al.*, 1979), (T,G)-Pro-L (Isac, Dorf & Mozes, 1977), KLH (McDougal, Cort & Gordon, 1977; Tokuhisa *et al.*, 1978), SRBC (Luzzati *et al.*, 1976) and chicken MHC antigens (Shiozawa *et al.*, 1980), and to the I-region for helper factor for P815 mastocytoma cells (Kilburn *et al.*, 1979). In addition, human helper factors have been shown to react with human antisera against HLA-D locus antigens (Mudawwar *et al.*, 1978) or a rabbit anti-human Ia (Rees *et al.*, 1979). Similarly, the I-J coding for suppressor factors has been reconfirmed with factors for KLH from T-hybrids (Kontiainen *et al.*, 1978; Taniguchi *et al.*, 1980a, b) and factors specific for GAT (Thèze *et al.*, 1977a; Germain *et al.*, 1979), GT (Thèze, Waltenbaugh, Dorf & Benacerraf, 1977b), TNP (Zembala, Asherson, Munro & Taegart,

1977; Greene *et al.*, 1977b; Noonan & Halliday, 1980), SRBC (Lièw, Sia, Parish & McKenzie, 1980) and a tumour-antigen specific suppressor factor (Perry *et al.*, 1978). I-J is also present on suppressor T cells themselves (Murphy, Herzenberg, Okumura, Herzenberg & McDevitt, 1976; Okumura, Takemori, Tokuhisa & Tada, 1977) and suppressor T-hybrids (Taniguchi and Miller, 1978b; Taniguchi *et al.*, 1980a). Coding within the H-2 complex has also been demonstrated for the IgE-specific suppressor (Kishimoto *et al.*, 1978), for factors specific for ABA (Greene, Bach & Benacerraf, 1979), DNP (Moorhead, 1979) and for SRBC from a T-hybrid line (Taussig *et al.*, 1979a, b, c). In the last two cases, however, the I sub-region contributing to the factor is to the right of I-J (Moorhead, 1979; Taussig *et al.*, 1979c, 1980a, b). Thus, in short, the I-J and I-E/C subregions contain genes which contribute to the structure of specific suppressor factors, while I-A subregion genes code, in part, for helper factors. A single exception to this generalisation is a helper factor for GAT coded apparently in I-J (Howie *et al.*, 1979).

The Ia determinants present on T-cell helper factors appear to be distinct from those detected predominantly on B cells by alloantisera. For example, anti-I region alloantisera remove the factors of the strain against which they were raised, but not the factors of strains of other haplotypes known to share B-cell determinants in the I-A subregion (Taussig & Munro, 1976). Further, anti-Ia sera can be absorbed with B cells and retain their factor-removing capacity, which they lose on absorption with T cells (Tokuhisa *et al.*, 1978). (This question does not arise for suppressor factors, of course, since I-J is exclusively a T-cell determinant). The factor Ia specificities are generally assumed to be part of an I-region coded polypeptide chain, analogous with B-cell Ia, but in fact there is little evidence for this. I-J molecules, for example, have not been analysed structurally, and two reports suggest that the factor Ia determinants are due to carbohydrate, based on the removal of factors by a rabbit antiserum to 'carbohydrate-defined Ia' (Howie *et al.*, 1979; Liew *et al.*, 1980). It is obviously important to clarify this point.

The factor antigen-binding site, idiotypic determinants (where present) and I-region determinants are all carried on a single molecule, the most rigorous proof of this being that factors eluted from antigen or anti-idiotype adsorbents can subsequently be readsorbed and eluted from an anti-H-2 or anti-I-J adsorbent (Thèze *et al.*, 1977b; Germain *et al.*, 1978a; Germain, Thèze, Waltenbaugh Dorf & Benacerraf, 1978b)

and *vice versa* (Bach *et al.*, 1979). Also, after passing supernatant factors through antigen or anti-H-2 adsorbent columns, the effluents (non-adsorbed fractions) cannot be recombined to give activity (although this has been done for the KLH-suppressor extract of a T-hybrid line below).

In considering the structural possibilities implied by the properties of the factors, it should be borne in mind that the molecular weight of these molecules generally falls in the 40,000–80,000 range as determined by gel filtration. However, the suppressor factor produced by a T-hybrid line specific for SRBC has a molecular weight of about 200,000, and the possibility cannot be ruled out that the size of factors has in some cases been underestimated due to partial proteolysis or inherent instability, particularly where extracts are used as the factor source (Taussig & Holliman, 1979).

The combination of Ia and antigen-binding site in a single molecule suggests at least five possibilities for the structure and genetic origin of specific factors.

(a) The molecules are coded entirely within the MHC. The model proposed by Munro & Taussig (1975) suggested that the H-2 I-region included Ig-like sets of factor V and C-genes. Obviously this is now less likely in view of the evidence (above) that Factor binding sites are coded by Ig V_H-genes, although the possibility remains that the I-region codes for factor light chains with V and C-regions.

(b) The factor consists of an Ig-coded V_H-domain attached to a constant region coded in the MHC, the two forming a single polypeptide chain. Since the genes for Ig heavy chains and the MHC are on different chromosomes, this would involve a novel genetic or translational mechanism.

(c) The factors are composed of two polypeptide chains, one of which is Ig coded and carries the V_H-domain perhaps attached to a 'new' Ig constant region; the second chain is I-region coded. The latter might serve a role analogous to a light chain and contribute to the antigen-binding site (overlap with proposal a), or rather dictate the function of the molecule, i.e. help *vs* suppression.

(d) The polypeptide portion of the factor is entirely Ig coded (V_H plus hitherto undefined constant region) and the Ia determinants are merely carbohydrate groups attached by MHC-coded glycosyltransferases.

(e) The factor is entirely Ig coded, but the specificity of its binding site is directed to antigen in association with an Ia molecule, i.e. the factor recognizes an antigen-Ia complex. The Ia determinants detected on factors merely occupy the binding site and do not play any role in factor structure. Alternatively, Schrader proposes that alloantisera contain anti-idiotypic antibodies which bind the postulated Ia recognition part of the factor binding-site, and that the factor Ia determinants are entirely illusory (Schrader, 1979).

In all these models, the possible contribution of Ig light chain V_L-domains is left open. At the moment, the two-chain model, alternative (c), seems the most likely. Evidence in its favour comes from studies on the factor specific for SRBC present in the supernatant of a T-hybrid line (Taussig and Holliman, 1979; Taussig *et al.*, 1979c). This factor, as already noted, is of higher molecular weight than other specific factors, being about 200,000 by gel filtration. Internal labelling by incorporation of [^{3}H]-leucine, followed by adsorption onto SRBC and SDS-PAGE, indicated the presence of two non-disulphide-linked polypeptide chains of sizes about 85,000 and 25,000, respectively. Both chains were removed if the supernatant was precipitated with anti-H-2 serum before absorbing onto SRBC. NP-40 extracts of the labelled cells analysed in the same way showed only a single labelled band of 85,000 mol. wt which was not precipitable by anti-H-2, but bound specifically to SRBC. It was suggested that the factor consisted of 'heavy' and 'light' chains, of which the former carried the antigen-binding site while the latter carried MHC specificities. Similar suggestions of two factor chains have been made by Mozes (1976), who found chains of 45,000 and 75,000 mol. wt on SDS gels of (T,G)-A-L-specific factor eluted from (T,G)-A-L adsorbents; and by Kontiainen and co-workers in preliminary biochemical studies on a T-hybrid suppressor factor internally labelled with ^{35}S-methionine (Kontiainen, Cecka, Culbert, Simpson & Feldmann, 1980). Taniguchi *et al.* (1980b) found that when an extract of a T-hybrid line containing suppressor factor for KLH was passed over KLH or anti-I-J adsorbent columns, factor activity was removed but could be reconstituted by mixing the effluents of the columns, which should lack the KLH-binding and I-J-positive components respectively. Assuming the adsorbents had not simply been slightly overloaded, the reconstitution implies the presence of two separable chains in the cell extract (reconstitution is not seen if supernatant rather than extract is used). Efforts being made to purify factors from T-cell supernatants by physical methods are achieving a high degree of purification of KLH-specific helper factor (Henriksen, Alvarez, Howie, Frey & Lefkovits, 1980) and should lead to structural advances; the most promising approach, however, continues to be that of the growing number of T-cell

hybrids secreting or expressing antigen-specific molecules.

Self-MHC restriction in factor action

An important feature of some antigen-specific factors is their genetically restricted activity, in which the MHC again features prominently. Experimental findings on genetic restriction of factors can be divided into three categories. (a) Self-MHC restriction: the factor will only help or suppress cells of the same MHC type as those which made the factor. (b) Response restriction: the factor acts on fully allogeneic cells, provided they are of a certain responder or non-responder type, a characteristic usually dictated by immune response (Ir) or immune suppression (Is) genes in the MHC. (c) Genetically unrestricted activity. It will be appreciated that the difference between categories a and b is qualitative and not simply one of degree, while apparently unrestricted factors may show some restrictions when more MHC-types are studied. Response restriction is discussed in a following section (Factors, acceptors and Ir genes).

Self-MHC restriction takes the form of an apparently absolute requirement for MHC compatibility between the factor donor and the recipient or target cell. In most published work, however, relatively few combinations of factor and target haplotypes are used, so that the real degree of restriction is not known. For helper factors, self-MHC restriction is rather uncommon, but there are a few examples. Shiozawa *et al.* (1977) found that RGG-specific helper factor of CBA/J mice would help the primary response to TNP-RGG-Ficoll of syngeneic cells *in vitro*, but would not help BALB/c cells or the closely related CBA/CaJ strain; and this was also true in reciprocal combinations of factors and cells. While BALB/c and CBA differ throughout the H-2 complex ($H2^d$ *vs* $H2^k$), both CBA strains are nominally H-2^k; they are known to differ in the M locus and the H-2 public specificity 8 (H-2K or D locus). It is not known which of these differences is the relevant one. On the other hand, a KLH-specific helper factor was restricted to acting on I-A compatible strains (Tokuhisa *et al.*, 1978), and it will be recalled that the helper factor itself is also I-A coded. Geha (1979) states that the human helper factor for tetanus toxoid would act on autologous cells only. These examples contrast with the majority of helper factors which do not show self-restriction (e.g. Taussig *et al.*, 1975) and indeed examples of xenoreactive helper factors have been quoted above (Luzzati *et al.*, 1976; Kantor & Feldmann, 1979).

Self-MHC restriction for suppressor factors has been much more closely studied, and was first described by Tada and his colleagues with the KLH-specific suppressor factor. This would only suppress H-2 compatible cells and would not suppress the primary or secondary response of fully H-2-different cells (Takemori & Tada, 1975; Taniguchi, Tada & Tokuhisa, 1976b). However, identity between factor donor and target at the whole of the MHC was not required and the use of recombinant strains established that identity in the I-J subregion of H-2 was sufficient (and necessary) for suppression to occur (Tada *et al.*, 1976a, b, 1977). The KLH-suppressor factor produced by a T-hybrid line also shows self-restriction (Taniguchi *et al.*, 1980a).

Another example of restriction was studied by Moorhead (1977a, b, 1979) for the factor which suppresses contact sensitivity to DNP. In this case however, the factor itself is an I-C product, but the required homology mapped to the K and/or D loci of H-2, with separate factors being restricted to acting on K- or D- compatible cells. The IgE class specific suppressor factor also shows self-MHC restriction, which was not mapped (Kishimoto *et al.*, 1978). A human SRBC-specific suppressor factor prepared after 24 h incubation of human T cells with SRBC was restricted to acting on autologous or HLA-compatible cells in culture (UytdeHaag *et al.*, 1979), though OVA-specific factors made after longer periods of incubation (120 h) were unrestricted (Ballieux *et al.*, 1979).

By no means all specific suppressor factors show self-MHC restriction. Kontiainen and colleagues observed no restrictions of the H-2 or background type for KLH-, GAT- or (T,G)-A–L specific factors, perhaps because their factors were derived from supernatants rather than extracts (Kontiainen & Feldmann, 1978; Kontiainen *et al.*, 1979). Their results would be more conclusive, however, if each factor had been fully titrated to its end-point on different strains. Suppressor factors against GAT and GT have also been shown by others to act on fully allogeneic cells (Kapp, 1978; Waltenbaugh, Debré, Thèze Benacerraf, 1977a), and so too has the SRBC-specific suppressor product of a T-hybrid line (Taussig *et al.*, 1979a, c).

Even though self-MHC restriction is seen with only some factors, it is of particular importance because of its similarity to the behaviour of T cells during the induction processes of antibody or cell-mediated responses, and obviously demands an explanation. There are at least three possible mechanisms. (a) The I-region component of the factor reacts with itself on a

target cell and binds by like-like interaction. (b) The factor reacts with an I-region coded acceptor molecule by complementarity—the factor/acceptor model in which the two are products of separate, but closely linked genes. This explanation is favoured by Tada & Okumura (1979) for I-J restricted suppression. In both mechanisms a and b, the I-coded part of the factor serves as an interaction (rather than specific recognition) element, and both can explain why the same I-subregion not only codes for the factor but also specifies restriction. (c) The factor recognizes both antigen and a self-MHC molecule, either as a single complex or as separate entities; hence antigen and the self-MHC determinant must both be recognized on the target cell for the factor to bind. In contrast with a and b, restriction in this case is due to self-recognition, and there is no obvious reason why the restriction subregion of the MHC should be that which also codes for the factor. The results of Moorhead (1977b, 1979) could be explained in this way, for while the factor which suppresses DNP contact sensitivity is an I-C product, its action is restricted by the H-2K or D loci. Moreover, it binds to DNP-primed but not normal cells and the binding can be blocked by anti-DNP or anti-H-2 sera. Hence this suppressor factor appears to recognise antigen and the H-2K or D molecule together and therefore self-recognition seems the best explanation for self-restriction. In general terms, mechanism c would lead to the greatest degree of restriction and b to the least.

Factors, acceptors and Ir genes: a four-gene model

According to the factor/acceptor hypothesis, the antigen-specific factors exert their effects by interacting with acceptor molecules on particular target cells, which may be other T cells, B cells or macrophages, depending on the function of the factor. In responses which are under the control of MHC-linked Ir genes, it is sometimes possible to relate low or non-responsiveness to a lack of expression of specific factors and/or their acceptors. Since both factor and acceptor are I-region coded, they have been candidates for the true Ir-gene products (e.g. Munro & Taussig, 1975). Uncertainty over whether they are really so or only indicators of Ir-gene control has not prevented their application in studies of genetic control, some of which are described below.

A few words are required first on the acceptor molecules, the properties of which are outlined in Table 2. The acceptors for helper factors are expressed on B cells and probably macrophages as well, and carry the serologically detected B-cell Ia antigens of the I-A subregion (Munro & Taussig, 1975; Taussig et al., 1976b; Taussig & Munro, 1976). Anti-Ia sera block the acceptor and genetic evidence (below) confirms the I-A coding. Acceptors can be assayed by their ability to absorb specific factors and this method has been used to show that acceptors on human lymphocytes are coded in the HLA region (Taussig & Finch, 1977; Taussig, 1978, 1979a). Acceptors for suppressor factors are present on T cells which express I-J determinants (Taniguchi et al., 1976b; Taniguchi & Tokuhisa, 1980). The self-MHC restriction of some suppressor factors (above) has shown that the target T cell must be of the same I-J type as the factor, indicating that the T-cell acceptor is I-J coded (Tada, Taniguchi & Takemori, 1976b; Tada & Okumura, 1979). Thus for both helper and suppressor systems, factor and acceptor are coded in the same I-subregion; nevertheless, they are serologically distinguishable (Taussig & Munro, 1976; Tada, Nonaka, Okumura, Taniguchi & Tokuhisa, 1978a).

The relationship between factors, acceptors and Ir genes was first explored in the antibody response to (T,G)-A–L, in studies which have been emulated in several systems. In brief, they showed that antigen-specific Ir-gene control can be reflected in either specific factor production or in factor action (acceptor expression) or both; that Ir genes could be expressed at different cellular loci; and that at least two MHC-linked Ir genes controlled the antibody response. This last point was confirmed by complementation studies and indeed dual gene control seems to be the rule for many antigens (Dorf, 1978; Benacerraf & Germain, 1978). The response to (T,G)-A–L is controlled by Ir genes in the I-A subregion, with H-2^b being a high responder haplotype, H-2^d medium-high, and H-$2^{k,f,q,s}$ and others being low responders. Ir-gene control over both IgM and IgG responses can be demonstrated. In the initial experiments, it was shown that both high responder (H-2^b) and low responder (H-2^k) T cells educated to (T,G)-A–L produce the specific factor, which was assayed in both cases with antigen and high responder bone marrow cells in vivo; low responder (H-2^k) bone marrow cells could not respond to factor of either H-2^k or H-2^b origin (Taussig et al., 1974). This apparent equality of high and some low responder T cells has since been observed independently in factor production and cell co-operation (Howie, 1977; Howie & Feldmann, 1977; Feldmann, Howie, Erb, Maurer, Mozes & Hämmerling, 1978), in cell co-ope-

ration using TNP-(T,G)-A–L (Kappler & Marrack, 1978) and in delayed hypersensitivity to (T,G)-A–L (Strassmann, Eshhar & Mozes, 1980a, b). The inability of H-2^k bone marrow cells to respond to the combination of (T,G)-A–L and its specific helper factor was traced to B cells: purified H-2^k B cells failed specifically to absorb the (T,G)-A–L factor, whereas responder B cells absorbed successfully. The correlation between lack of acceptor and low or non-responsiveness was confirmed for several strains (C3H, A/J, B10.BR, B10.A, SJL,DBA/1, I/St) (Munro & Taussig, 1975). This by no means rules out a simultaneous defect in macrophages (e.g. Howie & Feldmann, 1978); but the fact that B cells show Ir-gene determined absence of an acceptor site must constitute strong evidence that the acceptor is an Ir gene product.

However, not all low responders could produce (T,G)-A–L-specific helper factor: B10.M (H-2^f) and A.SW (H-2^s) had a reciprocal defect from H-2^k in that they failed to produce the factor, but their B cells absorbed and responded to factor produced in other strains (Munro & Taussig, 1975, and M.J.T. unpublished observations). The T cell defect in H-2^f has likewise been reconfirmed independently in both antibody production and delayed hypersensitivity (Howie & Feldmann, 1977; Feldmann *et al.*, 1978; Marrack & Kappler, 1980; Strassman *et al.*, 1980). Thus, there are distinct loci, in T and B cells respectively, for expression of Ir genes, and their occurrence in H-2 congenic strains on the same background (e.g. B10, B10.BR, B10.M) indicates their control by the MHC. Finally, a third phenotype was also found, namely the SJL strain (H-2^s) which fails either to make or respond to the (T,G)-A–L factor (Mozes, Isac & Taussig, 1975). (The difference between the H-2^s strains A.SW and SJL is discussed below).

Since some strains showed reciprocal defects in either factor production or acceptor expression, it was possible to test the hypothesis that the (T,G)-A–L response was under the control of two genes by mating selected low responder strains of complementary type. Three combinations predicted from the above studies showed successful *in vivo* complementation, in that the F$_1$ hybrids between low responders were high responders to (T,G)-A–L namely (B10.M × B10.BR)F$_1$ and (B10.M × I/St)F$_1$ (Munro & Taussig, 1975) and (A.SW × B10.BR)F$_1$ (M.J.T., unpublished observation), proving the two-gene hypothesis to be correct. Complementation restored both the IgM response (Munro & Taussig, 1975) and IgG response (M.J.T. unpublished observations). Particular importance has

been attached to the (B10.M × B10.BR)F$_1$ because, as a hybrid of congenic strains, it is an excellent demonstration that the (T,G)-A–L response is controlled by two MHC genes. These experiments were successful on several separate occasions between 1975 and 1977 (in both Cambridge and Basel), but other groups had difficulty in obtaining the same result with the (B10.M × B10.BR)F$_1$ (e.g. McDevitt, 1976; Deak, Meruelo & McDevitt, 1977). In 1977, difficulties were also experienced in Cambridge with this combination, and this was duly reported (Munro & Taussig, 1977). Since this has attracted widespread attention, a few comments are appropriate here. Firstly, although the response of (B10.M × B10.BR)F$_1$ was acknowledged to be erratic, complementation was not only observed unambiguously several times, but in a double-blind back-cross experiment with progeny of (B10.M × B10.BR)F$_1$ × B10.M, heterozygotes (H-2^k/H-2^f) could be distinguished from homozygotes (H-2^f/H-2^f) on the basis of response to (T,G)-A–L with 90% accuracy (unpublished observations). Secondly, the (B10.M × I/St)F$_1$ hybrids, retested at the same time as (B10.M × B10.BR) in 1977 and on several previous occasions showed reproducible complementation, and with the (A.SW × B10. BR)F$_1$ complementation was successful on both occasions tested (15/16 mice) (M.J.T. unpublished observations). Finally, regardless of the behaviour of (B10.M × B10.BR)F$_1$s on different occasions, the conclusions regarding expression of factor and acceptor in different low responders and the demonstration of distinct types of low responder to (T,G)-A–L are unaffected; indeed, the latter point has received independent confirmation as already noted above.

Although the differences between congenic strains on the same background indicated that dual MHC gene control over the anti-(T,G)-A–L response was expressed in factor and acceptor molecules, background genes were also found to influence both these interaction molecules. Table 5 summarizes evidence for control of the acceptor by a background gene and an MHC-linked gene by comparing mice of the same background but different in H-2 (A.SW and A/J) or the same H-2 type on different backgrounds (A.SW and SJL). In the complementation test, the mating of two low responders which both failed to express the acceptor, A/J × SJL, produced responder, acceptor-positive F$_1$s (Munro, Taussig & Archer, 1978), confirming dual-gene control over the acceptor. Moreover, back-cross analysis of (B10.M × I/St)F$_1$ animals showed clearly that non-MHC genes contribute to the

Table 5. Dual gene control of the acceptor for (T,G)-A-L-specific helper factor in mice

Strain	H-2	Factor	Acceptor	Response (PFC/spleen) to (T,G)-A-L	
				IgM	IgG
A.SW	s	−	+	550	250
A/J	a	+	−	1400	620
SJL	s	−	−	300	830
(A/J × SJL)F₁	a/s	+	+	4700	34,000

Factor and acceptor for the (T,G)-A-L specific helper factor were assayed in the three inbred strains (A.SW, A/J. SJL). Apparently, A/J lack an acceptor H-2 gene, while SJL lack an acceptor background gene. Dual gene control over the acceptor was confirmed by the (A/J × SJL)F₁ which showed complementation for the acceptor and the anti-(T,G)-A-L PFC response. The secondary response to (T,G)-A-L was assayed after injection of 10μg in adjuvant, and a boost of 10μg in saline 1 month later; PFC were measured 7 days after boost (six mice/group). Complementation in both IgM and IgG responses is evident.

expression of both the factor and the acceptor (Munro et al., 1978). Hence, at least four genes seem to be involved in controlling the (T,G)-A-L immune response via the specific factor and its acceptor. As this conclusion seems to have quite wide applicability, it may be expressed as a general four-gene factor/acceptor model, as shown in Fig. 1. From what has gone before, it is quite possible that the background gene for the factor is linked to Ig heavy chain genes.

Similar studies on Ir-gene controlled responses have been carried out with human helper factors and accep-
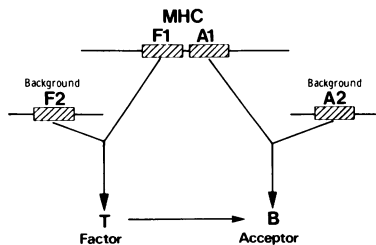
Figure 1. A four-gene factor/acceptor model. The antigen-specific T-cell factor and its appropriate acceptor are coded by closely linked MHC genes (F1, A1 respectively); in addition, the products of background genes (F2, A2) either contribute to their structure or control their production. The background factor gene (F2) may be Ig heavy chain linked. Other MHC genes (not shown) may also control factor and acceptor production.

tors, in order to define human Ir genes without recourse to immunisation. Variation in the ability to produce specific factors for (T,G)-A-L and GAT were indeed readily found among unrelated human donors, though family studies have not yet been carried out (Zvaifler et al., 1979; Rees et al., 1979; Woody et al., 1979a, b). In extensive controlled experiments, Taussig & Finch (1977) showed that human peripheral blood lymphocytes expressed acceptor sites for the mouse factors specific for (T,G)-A-L, (Phe,G)-A-L and SRBC, and that some individuals were unable to absorb the factors against the synthetic polypeptides (also Taussig, 1978, 1979a). For example, lymphocytes of about 65% of unrelated individuals absorbed the (T,G)-A-L-specific mouse factor and 35% failed to absorb under standard test conditions; for the (Phe,G)-A-L factor the ratio was similar, but acceptors for the two factors were expressed independently (as in the mouse). Family studies showed that these polymorphic acceptors were controlled by HLA-linked genes and moreover were separable by recombination. This indicated that there are at least two HLA-linked acceptor loci in man, and on the murine analogy it is possible that these represent human Ir gene loci. The recombination observed favoured the possibility that one acceptor locus would be associated with the HLA-D locus and the other with HLA-A (Taussig & Finch, 1977; Taussig, 1978). The xenoreaction of the helper factors can thus be useful in analysing cellular expressions of Ir genes in different species, including man, and it is hoped that these studies will be extended.

Independent genetic control over production of the specific T-cell factor and its action has also been demonstrated in suppression. Tada and colleagues, as already noted, showed that the KLH-specific suppressor factor and its T-cell acceptor were both I-J products. Background genes influenced the production of factor and expression of its acceptor. Thus, A/J mice are non-producers of the KLH-factor, whereas B10.A (same H-2, different background) are producers (Taniguchi et al., 1976b). Conversely, none of the B10-congenic mice express an acceptor site for this factor, and can neither absorb nor be suppressed by it. The (A/J × B10.A)F₁ hybrid showed complementation of factor and acceptor genes. Interestingly, C57Bl/6 mice (B6), which differ only slightly from B10, do express the acceptor, and this should make it possible to pinpoint the background acceptor gene and perhaps raise an anti-acceptor antibody (B10 anti-B6).

In the suppressor T-cell response to the polypeptide

GT, experiments have been reported which are very similar in concept to those with (T,G)-A–L helper factor described above (Waltenbaugh *et al.*, 1977a, b; Germain, Waltenbaugh & Beneacerraf, 1980). Injection of GT does not lead to an antibody response in any inbred mouse strain, unless it is complexed to a carrier such as methylated (m) BSA. In 'suppressor' strains (haplotypes H-$2^{d,k,s}$), injection of GT gives rise to GT-specific suppressor T cells which prevent a subsequent response to GT-mBSA, while 'nonsuppressor' strains (H-$2^{a,b,q}$) do not generate GT suppression. T cells from suppressor strains produce a GT-specific suppressor factor which is I-J coded (Waltenbaugh *et al.*, 1977a; Thèze *et al.*, 1977b). The suppression characteristic, which is antigen-specific, is controlled by two I-region immune suppression (or Is) genes, which map in I-A and I-C respectively, but not in I-J (Benacerraf & Germain, 1978). At least two cells are involved in generating suppression, namely a 'Ts$_1$ cell' which produces the suppressor factor, and a 'Ts$_2$ cell', a suppressor cell which is induced by the factor. Defects in either production of the suppressor factor by Ts$_1$ cells or in generation of Ts$_2$ cells by the factor could be analysed. A/J mice (H-2^a) were non-producers of GT-factor (i.e. lacked Ts$_1$ cells), but were nevertheless suppressible by factor produced in other strains and could generate Ts$_2$ cells. In contrast, B6 mice (H-2^b) had the reciprocal defect, and were producers of the factor which could not be suppressed. The F$_1$ between these strains, B6A, showed complementation and is a 'complete' suppressor hybrid (Germain *et al.*, 1980). Clearly there is a close analogy with the (T,G)-A–L Ir genes. Note that in GT-suppression, production of the specific factor is under the control of two Is genes, which are both independent of the factor; the latter is therefore an indicator of Is-gene control but is not the Is gene product itself. With the (T,G)-A–L factor, arguments can be advanced either that the factor is a true Ir-gene product or that its production is controlled by a separate I-A coded Ir gene.

Target cells and mechanisms of factor action

Despite the antagonistic effects of helper and suppressor factors, the possible mechanisms of their action are mostly similar in principle. For both, an important step is the focussing of specific factor onto a target cell by a dual binding reaction, namely (a) to antigen (or idiotype) on the cell surface, via the factor's antigen-combining site, and (b) to an acceptor molecule, which binds some part of the factor, probably its MHC component (Munro & Taussig, 1975). When the factor is trapped in this way, the target cell is either stimulated or suppressed. Helper factors presumably always stimulate their target cells, and suppressor factors may do likewise to recruit further suppressor T cells, acting in effect as helpers for suppressor T cells (below). The difference in action, then, between the two factor types may only be in their cellular targets rather than their molecular mechanisms. Directing a factor onto the correct cell type is therefore all-important, and is probably the role of the MHC products on the factor and acceptor: I-A-positive helper factors selectively react with cells carrying I-A-coded acceptors, namely B cells and macrophages, whereas I-J-positive suppressor factors are directed onto I-J-bearing helper or suppressor T cells.

(a) Helper factors

In antibody responses, most specific helper factors described replace T cells and act either on B cells or macrophages; when they function in cell mediated immunity, the target is of course likely to be a T cell (Kilburn *et al.*, 1979). Helper factors for antibody production act *in vivo* on T-cell depleted bone marrow cells (personal observation) and in nude mice (Kindred & Corley, 1977), while *in vitro* they help the response of T-cell depleted spleen cells (McDougal & Gordon, 1977b; Howie & Feldmann, 1977; Shiozawa *et al.*, 1977), but macrophages must be present (McDougal & Gordon, 1977b; Feldmann *et al.*, 1978). Furthermore, helper factor for (T,G)-A–L was absorbed by purified B cells, but not by nylon wool passaged T cells (Taussig *et al.*, 1976b). In one case, T cells were found to be necessary for the action of a KLH-specific helper factor present in cell extracts (Tokuhisa *et al.*, 1978). Though this might have been for purely quantitative reasons (i.e. too little factor in the extract to replace T cells completely), it may indicate that some helper factors actively recruit helper T cells (Tada & Okumura, 1979).

The concept that antigen concentrates factor onto antigen-specific B cells was supported by an experiment in which bone marrow cells were allowed to react *in vitro* with the (T,G)-A–L factor in the presence or absence of antigen, and were then washed and transferred with antigen into irradiated recipients. Only cells which had been exposed to both factor and antigen simultaneously *in vitro* went on to make a response (Munro & Taussig, 1975; Taussig & Munro, 1976). Since B cells absorb factor in the absence of

added antigen (Munro & Taussig, 1975), one possibility is that the role of antigen is to focus factor onto the antigen-specific B cell. It is envisaged that acceptor sites are nonclonally distributed on all B cells and that without such a mechanism factor would not be bound with sufficient affinity, and in sufficient concentration, to trigger the B cell. There are alternative explanations, for example that the B cell must bind antigen before factor, or that the physical apposition of Ig receptor and acceptor by a factor-antigen bridge is the necessary triggering signal. But in all cases, it is assumed that the factor uses its antigen-binding site to ensure specificity of action.

The role of the macrophage as intermediary is speculative. Macrophages carry I-A-coded Ia antigens and by inference have acceptor sites for helper factor (though their ability to absorb helper factors has not been reported). Binding of antigen via factor to macrophages might be a presentation device, or cause the release of non-antigen-specific macrophage mediators. Very few experiments have been reported which explore the possible non-specific helper effects of antigen-specific factors, e.g. 'bystander effects', where a specific factor in the presence of its specific antigen is able to produce help for a non-cross-reacting antigen. Such effects have been noted, however, in the xeno-reaction of mouse SRBC-specific helper factor with human cells in the presence of both SRBC and HRBC (Luzzati *et al.*, 1976; Taussig *et al.*, 1976b).

Rather than act via antigen, specific helper factors might be part of an idiotype-anti-idiotype network (Jerne, 1974). Factors of anti-idiotypic specificity could trigger B cells by binding directly to the Ig receptor idiotype and acceptor, without a requirement for antigen. Experiments of Bernabé *et al.* imply such a possibility (Bernabé, Martinez-Alonso & Coutinho, 1979). They found that the non-antigen-specific helper supernatant generated by stimulating T cells with con-canavalin-A might in fact be a mixture of many antigen-specific factors. A supernatant made by con-A stimulation of T-cell populations depleted of SRBC-reactive cells had reduced helper activity specifically for SRBC, and *vice versa*, T cells from mice primed against SRBC or HRBC produced a con-A supernatant which was restricted to the priming antigen. However, the functionally SRBC-specific factors could not be absorbed out by SRBC, unless the red cells were complexed with antibodies in early bleed mouse anti-SRBC sera. Thus the suggestion is that the factors had anti-idiotypic rather than antigen specificity. As far as mechanism is concerned, idiotype and

antigen would be equivalent targets on the B-cell surface, but the effects would be different: antigen-mediated help is carrier-specific, whereas anti-idiotypic factors would only trigger B cells carrying the relevant idiotype.

(b) Suppressor factors

The simplest way in which antigen-specific suppressor factors might act would be the blocking of antigen recognition by specific T or B cells. In general, this seems unlikely to be a major mechanism; for example, monoclonal factors against proteins or SRBC behave in a carrier-specific rather than a determinant-blocking manner (Taniguchi *et al.*, 1979; Taussig *et al.*, 1979b, c). However, one case where blocking does seem to be involved is the 'specific blocking factor' produced by tumour-specific suppressor T cells and a T-hybrid line (Nelson *et al.*, 1980).

A second mechanism would be the direct action of suppressor factor on specific B cells, in a manner analogous to that described above for helper factors, but with the opposite effect. For the majority of suppressor factors studied, this does not seem to occur, their target being another T cell (below). Nevertheless, there are some examples of specific suppressor factors acting on B cells. One is the class-specific factor for IgE, which is absorbed by DNP-primed B cells (Kishimoto *et al.*, 1978). Another is the SRBC-specific suppressor product of a T-hybrid line (Taussig *et al.*, 1979b). This is absorbed by unprimed B-cell-containing populations, including normal and nude spleen cells, but not by spleen cells from which Ig-bearing cells have been removed, nor by nylon wool passaged T cells or peritoneal exudate macrophages (Taussig *et al.*, 1979b). Genetic and blocking studies show that, for this factor, B cells carry acceptors coded in the I-E/C subregion of H-2, and that an I-A gene is also involved in suppression (Taussig, 1980).

Thirdly, some suppressor factors act directly to inhibit helper T cells. Kontiainen & Feldmann (1978) showed that the KLH suppressor factor in supernatants of suppressor T cells acted on nylon wool non-adherent helper T cells, and could be absorbed completely by such cells but not by B cells. Suppression by the factor did not require nylon wool-adherent T cells, nor Ly-2^+ cells. In contrast, functional studies have indicated that the KLH-specific factor in cell extracts acts on a subpopulation of nylon wool adherent T helper cells (Tada *et al.*, 1978a; Tada, Takemori, Okumura, Nonaka & Tokuhisa, 1978b). According to Tada and colleagues, the helper T cells affected are I-J

positive, probably indicative of an I-J coded acceptor.

Instead of (or perhaps in addition to) a direct effect on helper T cells, an important mode of action of suppressor factors is to induce further suppressor T-cell populations, in short, to act as helper factors for suppressor T cells. The cell population producing the specific factor is termed Ts$_1$ and the induced population Ts$_2$. Tada and colleagues have found that the KLH-specific factor (I-J positive, self-MHC-restricted) induces nylon wool adherent, I-J positive precursor T cells to become mature Ts$_2$ cells, which finally release non-specific suppressive factors on meeting antigen (Taniguchi *et al.*, 1976b; Tada *et al.*, 1978b; Tada & Okumura, 1979; Taniguchi & Tokuhisa, 1980). In support of such a scheme, Taniguchi and Tokuhisa (1980) showed that a mixture of nylon wool non-adherent helper T cells and hapten-primed B cells could not be suppressed *in vitro* by the KLH factor; suppression required the addition of the nylon wool adherent T cells (cf. Kontiainen and Feldmann, above). In the presence of KLH, non-specific suppression of the response to bystander antigens occurred, indicating that the final step in the process is non-antigen specific (in effect, antigenic competition). The Ts$_2$ precursors, like Ts$_1$ and Ts$_2$ cells, are Lyt-1^-,2^+,3^+, but an Lyt-1^+,2^+,3^+ cell is involved as an intermediary in generating Ts$_2$ cells (Tada & Okumura, 1979).

A similar situation exists for GAT and GT suppression. The GT-specific suppressor factor (I-J positive, but not MHC-restricted) was most effective if given to animals a few days, or even weeks, before challenge with GT-MBSA implying that an inductive process was taking place. (Waltenbaugh *et al.*, 1977b). Ts$_2$ cells had apparently been induced by the suppressor factor and could be transferred into normal recipients; they were, however, strictly GT-specific and did not suppress bystander antigens. Similarly, GAT-specific Ts$_2$ cells can be induced *in vivo* and *in vitro* (Germain & Benacerraf, 1978; Germain *et al.*, 1978b). In their recent review, Germain & Benacerraf (1980) suggest that their Ts$_2$ cells produce a second antigen-specific, but self-MHC restricted suppressor factor, and would therefore be equivalent to the Ts$_1$ cells of Tada and co-workers. The specific Ts$_2$ cells would induce a non-specific final suppressor, which would have to be termed Ts$_3$ and be the equivalent of Tada's Ts$_2$ cells. The reader is referred to the reviews by these authors for expositions of their suppressor schemes (Germain & Benacerraf, 1980; Tada & Okumura, 1979).

As hinted at for specific helper factors, suppressor factors may take part in the idiotype network (Jerne, 1974). Factors which share idiotypes with antibodies have been discussed in an earlier section of this review. There is now evidence that, in the absence of antigen, idiotype-positive suppressor factors induce anti-idiotypic suppressor cells, which in turn release anti-idiotype specific factors (Hirai & Nisonoff, 1980; Sy, Dietz, Germain, Benacerraf & Greene, 1980). In the anti-arsonate idiotype system, Hirai & Nisonoff (1980) find that idiotype-suppressed animals are a source of both idiotype-positive and anti-idiotypic factors, and that either factor would specifically suppress the production of that fraction of anti-arsonate antibodies which carry the same idiotype, without affecting the production of other anti-arsonate antibodies. In another network-type experiment, Lynch *et al.* (1979) showed that anti-idiotypic suppressor factor against the MOPC 315 idiotype would inhibit the growth of MOPC 315 cells *in vivo*. These demonstrations of the idiotype network functioning through factors are surely important, but do not detract from the central role of antigen in factor-mediated suppression. Thus, monoclonal specific factors produced by T-hybrids are presumably idiotypically unique but are nevertheless carrier-specific in their effect and can totally suppress the heterogeneous response to complex antigens. Doubtless, both antigen- and idiotype-specific networks exist together.

Finally, there are non-antigen specific suppressor pathways activated by antigen-specific suppressor factors. Besides the example noted above, such mechanisms have been demonstrated in delayed hypersensitivity. Factors which suppress contact sensitivity to picryl chloride or delayed footpad reactions to lysozyme appear to act by 'arming' (i.e. passively sensitising) macrophages, which thereby become endowed with nonspecific suppressor properties if triggered by the specific antigen (Zembala & Asherson, 1974; Asherson & Zembala, 1974; Ptak *et al.*, 1978; Kojima, Tamura & Egashira, 1979; Asherson *et al.*, 1980). The macrophage acceptors are blocked by heat aggregated IgG and may, therefore, be identical with Fc receptors (Ptak *et al.*, 1978). Contact sensitivity can also be suppressed by the direct action of specific factor on effector T cells (Moorhead, 1977a, 1979; Asherson & Zembala, 1974; Asherson *et al.*, 1980).

Probably, the heterogeneity of factors present in supernatants or extracts of suppressor T cells leads to this daunting variety of suppressor mechanisms. No doubt the use of T hybrids will enable dissection of

individual mechanisms, and indeed the unique behaviour of factors from individual lines is already becoming apparent.

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