THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH
P.O., ROYAL MELBOURNE HOSPITAL, VICTORIA, 3050
(Director: Prof. G. J. V. NOSSAL)

ANTIGEN PERCEPTION BY T LYMPHOCYTES

Influence of the Major Histocompatibility Complex

JACQUES F. A. P. MILLER

INTRODUCTION

Recently, it has become apparent that many T cell activities are regulated by genes of the major histocompatibility complex (MHC) which, in the mouse, is localized on chromosome 17 (fig. 1) [25]. The complex is made up of 2 closely linked loci, H-2K and H-2D, or K and D for short. They are separated by about 0.5 map units. Each locus is highly polymorphic and determines a wide range of serologically detectable transplantation antigens. In addition to K and D, the MHC genes code for a variety of molecules which are integrally involved in control mechanisms of immune responses. In the middle of the region and to the left of D is the S region which codes for certain complement components and regulates the activities of others. Between S and K lies the I region. Activities associated with this are numerous and include firstly the H-linked immune responsiveness (Ir) genes; secondly, the I associated or Ia antigens expressed selectively on only some lymphocytes and other cells, and implicated in interactions between T and B cells and T cells and macrophages; and thirdly, the genes which code for the capacity of allogeneic lymphocytes to react by proliferation in the mixed lymphocyte reaction.

T CELL FUNCTIONS INFLUENCED BY THE MAJOR HISTOCOMPATIBILITY COMPLEX

What T cell activities are known to be regulated by the MHC? First, helper T cells were shown by KINDRED and SHREFFLER [13] to function effectively only in mice of the same H-2 genotype. This phenomenon was investigated extensively by KATZ and BENACERRAF [12] and their colleagues who demonstrated that cooperative interactions between T and B lymphocytes required identity at the I region of the MHC. Secondly, DOHERTY et al. [8] showed that specific lysis of virus-infected target cells occurred effectively only when both the strains from which the sensitized T cells and the target cells were derived were MHC compatible. The genes required for this interaction mapped in the K or D regions. This phenomenon has now been extended to other lytic systems involving cytotoxic T cells. For example, SHEARER et al. [24] have shown that identity at K or D was necessary for lysis of TNP-modified target cells. Likewise,

Key-words: Cytotoxicity; Delayed type hypersensitivity; Immune responsiveness (Ir) genes; Macrophage; Major histocompatibility complex; T (thymus-derived) lymphocyte.

Received, June 7, 1976.

La Ricerca Clin. Lab. *6*, 285, 1976.

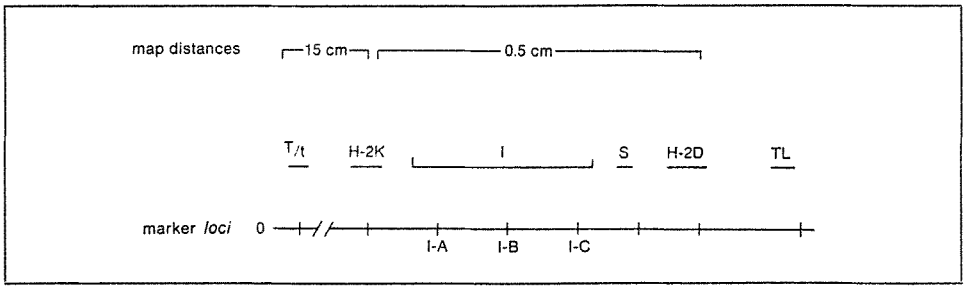

Fig. 1 - The arrangement of some of the genes coding for cell surface structures on chromosome 17 of the mouse. These include the T/t locus, the various genes of the major histocompatibility complex and the TL locus.

BEVAN [5] produced similar results by immunizing with cells bearing minor, i.e. non-H-2, alloantigens in mice, in which case cytotoxic T cells recognized only target cells bearing the same H-2 as the original stimulator cell. Thirdly, in the guinea pig, it has been known for some time that *in vitro* proliferation of sensitized lymphocytes in response to macrophage-associated antigen required H-locus identity between lymphocyte and macrophage [21]. More recently, ERB and FELDMANN [10] showed that *in vitro* macrophages exposed to antigens such as keyhole limpet hemocyanin were able to stimulate T helper cells only if the 2 cell types were identical at the I-A region of the MHC. Finally, as will be described in a subsequent section, genes of the MHC influence T cell dependent inflammatory responses, such as delayed type hypersensitivity [18].

MACROPHAGES AND T CELL ACTIVATION

Physical interactions between lymphocytes and macrophages have been observed when these cells are cultured together. The binding of lymphocytes to the surface of macrophages has been studied extensively in guinea pig models [21], and has been divided into 2 phases: antigen-independent and antigen-dependent. The features which characterize the antigen-independent phase are as follows. Maximal binding occurs within half to 1 h. The binding is reversible. It requires metabolically active macrophages but not metabolically active lymphocytes. It requires Ca^{++}. Trypsinization of macrophages, though not of lymphocytes, abolishes binding; this implies that binding occurs *via* a trypsin-sensitive receptor on the macrophages. It is not blocked by excess immunoglobulin and is thus not mediated by such a molecule. Both T and B lymphocytes bind in proportion to their frequency in the population. Further, both allogeneic and syngeneic lymphocytes bind, the binding being therefore not strain specific although it is species specific. As can be expected from this, the binding is not blocked by alloantisera. It is blocked by cytochalasin B. The function of the macrophage receptor for lymphocytes is thus likely to enable lymphocytes to be brought in close association with antigen processed by the macrophages. It may also be instrumental in bringing T and B cells close together thus facilitating antibody responses dependent on T and B cell cooperation.

The antigen-independent binding of lymphocytes by macrophages, which is reversible, can be followed by a phase of antigen-dependent binding, if both antigen and sensitized T lymphocytes are present [21]. This antigen-dependent phase has the following characteristic features. Maximal binding occurs at 20 to 24 hrs and is not reversible. It occurs only if both lymphocytes and macrophages are metabolically active. It is carrier-specific, not hapten-specific; i.e. it occurs only if the macrophage carries the

appropriate antigen for which the sensitized T lymphocyte bears the corresponding immunospecific receptor. Both sensitized T cells and macrophages must share identity at the MHC (I-region identity) and, as can be expected, binding is blocked by alloantiserum (presumably by antibody directed against I region determinants). The binding is not reversed by cytochalasin B, although it does not occur if the antigen-independent phase was first blocked by this drug. When conditions are appropriate for the antigen-dependent phase of the binding, the sensitized lymphocytes proliferate *in vitro* and this can be measured by incorporation of precursors of DNA such as tritiated thymidine. This proliferative reaction has been observed with sensitized lymphocytes from a variety of animal species including man, guinea pig, rats and mice and is generally accepted to represent an *in vitro* correlate of cell-mediated immunity, i.e. it is a response of sensitized T cells. It is dependent on antigen processing by macrophages as functionally significant interaction of sensitized T cells and native soluble antigen fails to occur.

In the early work on T and B cell cooperation in antibody responses in mice, the technique of radioactive antigen induced suicide was used to determine if both T and B cells were specifically involved in the cooperative response [2]. Suspensions of purified T and B cells were incubated with an antigen such as fowl gammaglobulin (FGG) which can be heavily radioiodinated. The ability of the washed cells to cooperate in irradiated recipients to produce antibody to both FGG and an unrelated antigen was then assessed. It was observed that both T and B cell responsiveness to FGG was specifically impaired by the preincubation with radioactive FGG. The conditions for effective suicide did however differ considerably for the two types of cells [1] (tab. 1). Firstly, T cell suicide, unlike B cell suicide, could be achieved only if antigen was presented, not in soluble form, but in association with other cell types, probably macrophages. Secondly, suicide occurred with T cells only if incubation was made at 37 °C., not at 4 °C. even though the latter temperature was effective for B cell suicide. Thirdly, T cells could be protected from suicide by preincubation with alloantiserum directed against relevant MHC components whereas anti-immunoglobulin had no protective effect. By contrast, B cell suicide could be prevented by anti-immunoglobulin, not by anti-H-2 pretreatment. Finally, certain inhibitors of metabolism such as azide, had no effect on B cell suicide but prevented T cell suicide. These findings may explain why so-called 'T-independent' antigens [23] or antigens composed of D-amino acids [3] do not induce T helper cells or allow delayed type hypersensitivity (DTH), although they can elicit IgM responses from B cells. Such antigens are either poorly metabolized or nondegradable and hence may be unable to activate T cells since this appears to require antigen processing by metabolically active macrophages.

	T cell suicide	B cell suicide
antigen presentation	cell associated (probably on macrophage membrane)	soluble
temperature	37 °C.	4 °C.
anti-immunoglobulin	no effect	protects
anti-MHC antibody	protects	no effect
metabolic inhibitors	prevent	no effect

Table 1 - Conditions required for inactivation ('suicide') of primed T and B cells by radioactively labeled antigen (for detailed data see reference no. 1).

The different requirements for T and B cell suicide in mice and the protective influence of alloantisera on T but not on B cell suicide [1], prompted experiments on the role of the MHC in T cell activation, e.g. in DTH. It is generally considered that DTH is a model of T cell mediated inflammatory responses [26]. Mice have, however, been notoriously bad animals to assess DTH because the structure of their skin is such that inflammatory responses are difficult to visualize externally. Recently, a semiquantitative radioisotopic assay technique has been used to measure the extent of DTH in mice by prelabeling the monocyte pool with a radioisotopic precursor of DNA (^{125}I-UdR). Monocytes are derived from a rapidly replicating precursor cell pool [29] and are the predominant inflammatory cells recruited in an area where a DTH inflammatory response is elicited by antigen challenge [15]. According to this technique [17, 27], 10 µl of the appropriate concentration of antigen is injected intradermally in the left *pinna* and a control solution in the right; 10 hrs later, 0.1 ml of 1 mM solution of 5-fluorodeoxyuridine (5-FUdR) is injected intraperitoneally (to block subsequent I-UdR incorporation into *de novo* nucleotide synthesis and thus increase labeling efficiency for cells in DNA synthesis) and 20 min later 2 µCi of ^{125}I-UdR in 0.1 ml solution (specific activity 90-110 µCi/µg) is given intravenously. After a further 16 hrs, i.e. 26 hrs after antigen challenge, the mice are killed, the *pinnae* cut off at the hairline, counted in a γ spectrometer and checked histologically for mononuclear cell infiltration. The results are expressed as the ratio of the radioactivity in the left ear to the radioactivity in the right ear (L/R ^{125}I-UdR uptake). It is unusual to obtain a ratio greater than 1.2 in mice that are either not sensitized or that have not received sensitized cells.

This radioisotopic assay indeed affords a measure of DTH because of the following reasons [17, 27]: 1) the ear reaction is apparent at 24 and 48 hrs, not at 6 hrs; it is therefore delayed in contrast to immediate hypersensitivity reactions which appear at 6 hrs,

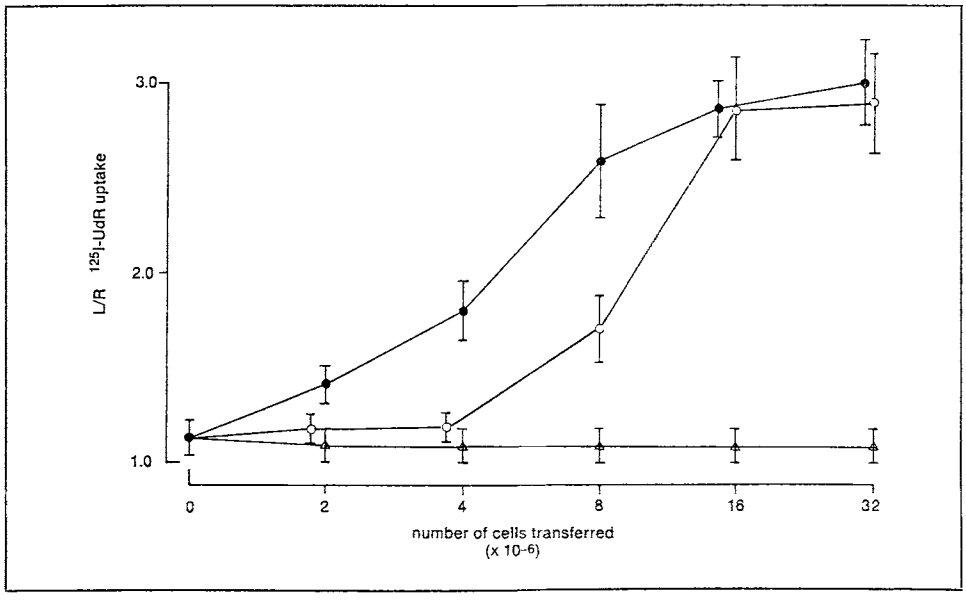

Fig. 2 - DTH response in naive CBA (●), (CBA × BALB/c)F_1 (○) and BALB/c (△) recipients of various doses of FGG sensitized CBA lymph node cells.

group	source of sensitized cells	recipients	MHC of recipients *						region of MHC identity	L/R ^{125}I-UdR uptake **	
			K	I-A	I-B	I-C	S	D		DNFB	FGG
1	A.TL	A.TL	s	k	k	k	k	d	whole	3.70 ± 0.79	1.96 ± 0.12
	A.TL	A.TH	s	*s*	*s*	*s*	*s*	d	K and D	2.58 ± 0.30	1.20 ± 0.04
2	A.SW °	A.TL	s	*k*	*k*	*k*	*k*	*d*	K	2.03 ± 0.06	1.15 ± 0.07
	A.SW	A.TH	s	s	s	s	s	*d*	K, I and S	2.78 ± 0.60	1.76 ± 0.16
3	C57BL	C57BL	b	b	b	b	b	b	whole	5.50 ± 0.53	2.67 ± 0.26
	C57BL	B10.A(2R)	*k*	*k*	*k*	*d*	*d*	b	D	3.21 ± 0.34	1.12 ± 0.03
4	CBA	CBA	k	k	k	k	k	k	whole	2.06 ± 0.10	2.48 ± 0.16
	CBA	A.TL	*s*	k	k	k	k	*d*	I and S	2.72 ± 0.21	2.36 ± 0.28

* letters in italics point to region of difference between donor and recipient mice;
** values are arithmetic mean ± 1 SEM. 5-6 mice/group;
° the MHC of A.SW mice is s s s s s s.

Table 2 - MHC imposed constraints on DTH transfer (for detailed data see references nos 18 and 19).

are not T cell mediated and are associated with a polymorphonuclear cell infiltration rather than a monocytic infiltration; 2) the ear reaction is associated with a typically mononuclear cell infiltration, the predominant cell types being monocytes which are labeled in autoradiographs; 3) the ear response is T cell dependent since it fails to occur in athymic mice or in normal mice given antigens known not to activate T cells; 4) the ear reaction can be transferred from sensitized to naive mice by purified T cells, not by non-T cells nor by serum antibody.

Since DTH could be transferred from sensitized to naive mice by T lymphocytes, the question of the influence of the MHC on this transfer was examined. This could be tested easily in histoincompatible mouse recipients since the DTH reaction is at its peak within 48 hrs whereas any reaction leading to the rejection of the sensitized cells in a recipient that is not preimmunized would not be expected to occur before 48 hrs. Lymph node cells from mice sensitized to fowl gammaglobulin (FGG) were injected into recipient mice which were then challenged in the ear and tested for DTH. Maximal responses occurred in syngeneic and minimal or no response in allogeneic animals [18]. The response in semi-allogeneic recipients of 8 million cells was of an intermediate degree (fig. 2). To determine whether transfer of DTH is linked to the MHC, congenic strains of mice and mice with recombinant chromosomes were used. The data summarized in tab. 2 show that I region identity is essential for the transfer of DTH to protein antigens such as FGG. The subregion involved was mapped to I-A [18]. On the other hand, in the case of chemical contactants such as dinitrofluorobenzene (DNFB), either I, K or D region identity alone could permit DTH transfer [19] (tab. 2). This difference in genetic constraints imposed on the transfer of sensitivity to protein antigens and to reactive contact chemicals may be related to different subsets of T cells, some reacting to antigens processed and presented by macrophages (e.g. in the case of FGG), and others reacting to compounds able to modify a variety of MHC gene products on cell surfaces (e.g. in the case of DNFB). The details of these interactions have not yet been worked out, but the evidence that DTH transfer is restricted by components of the MHC is clear.

The inability to transfer DTH between strains of mice incompatible at some regions of the MHC may be attributed to one of the following: 1) rejection of the injected cells; 2) their recruitment into areas such as the spleen; 3) their engagement in a mixed lymphocyte reaction with host cells pre-empting other activities; 4) their inhibition by host suppressor cells; or, 5) restriction of interaction between allogeneic host macrophages and donor sensitized T cells. There are reasons to believe that the first 4 possibilities are not supported by available data. For example, the experiments with F_1 cells or with F_1 hosts tend to exclude rejection, recruitment or engagement in an MLR. Additional evidence against rejection of allogeneic sensitized cells comes from experiments in which BALB/c mice were grafted with skin from the MHC incompatible strains CBA and C57BL. These grafts are rejected within 10 days. When the grafts were vascularized, i.e. 2 days post grafting, the mice received sensitized lymph node cells from CBA, C57BL or BALB/c donors and an intradermal challenge of antigen within the graft itself. As shown by ^{125}I-UdR incorporation, the reaction was always greater in grafts syngeneic with the sensitized cells, even when both graft and cells were allogeneic to the host (tab. 3).

In order to determine whether reactions between donor and host lymphocytes might influence the ability of sensitized cells to transfer a DTH response, the B10.A(2R) and B10.A(4R) mouse strains were used. These were derived after recombinational events had occurred between two MHC chromosomes such that the MHC regions in both strains have the same ends but differ in the midregion. The 2 independent crossovers did not occur in the same position and it has been shown that cells of the 2R strain do not respond to cells of the 4R strain in a MLR, whereas 4R strain cells do respond significantly to 2R stimulating cells [14]. When transfer of DTH was investigated between these 2 strains, it was successful in both directions [18] (tab. 4).

The possibility that allogeneic cells suppress the ability of the injected syngeneic sensitized cells to induce DTH was investigated by inoculating mixtures of allogeneic cells from sensitized donors into recipients syngeneic with one of the donors. As can be seen from tab. 5, no evidence of allogeneic suppression was obtained.

The requirement for compatibility at some genes of the MHC for DTH transfer may be taken to indicate that effective interaction between sensitized lymphocytes and cells presenting antigen (probably macrophages) are governed by cell surface structures coded by MHC genes. The genetic constraints imposed on this interaction may be explained in one of two ways: 1) first, it is possible that the antigen binding receptor on the T cell has a combining site directed towards a structure on the macrophage representing an antigen specific modification of the I region determinant or, alternatively, an I region modification of the processed antigen. This is shown schematically in model I of fig. 3 and is based on the idea favoured by DOHERTY et al. [8] in regard to the genetic constraints imposed by the K and D regions of the MHC for cytotoxic T

donor of DNFB sensitized lymph node cells	recipient	anterior skin graft	posterior skin graft	cpm in anterior graft — cpm in posterior graft
CBA	BALB/c	CBA	C57BL	172 ± 71 (12) [1] *
C57BL	BALB/c	CBA	C57BL	—102 ± 95 (6) [1, 2]
BALB/c	BALB/c	BALB/c	CBA	239 ± 112 (5) [2]

* values represent arithmetic mean ± 1 SEM. Number of mice/group is given in brackets. Absolute counts of the order of 1,000 (± 10-20 % in various groups). P values: groups 1 and 2 < 0.05.

Table 3 - Transfer of DTH by sensitized cells in allogeneic mice.

donor strain	recipient strain	L/R ^{125}I-UdR uptake
B10.A(4R)	B10.A(4R)	1.48 ± 0.20 (4) *
B10.A(4R)	B10.A(2R)	1.59 ± 0.05 (5)
B10.A(2R)	B10.A(2R)	2.25 ± 0.12 (5)
B10.A(2R)	B10.A(4R)	1.87 ± 0.15 (5)
none	B10.A(4R)	1.14 ± 0.04 (6)
none	B10.A(2R)	1.05 ± 0.03 (5)

* values are arithmetic mean ± 1 SEM. Number of mice/group is given in brackets.

Table 4 - Influence of mixed lymphocyte reaction on the transfer of DTH (data from reference no. 18).

lymphocytes; 2) a second alternative postulates a compound T cell receptor with one part being the combining site for antigen and the other part, not a receptor for antigen, but a cell interaction molecule coded by the I region. The T cell would become active only if 2 reactions occurred at its surface: the binding of the antigen to the combining site and some conformational change induced in the cell interaction molecule by its combination with an identical molecule on the macrophage surface. This model (shown as model II in fig. 3) is based on the postulate by KATZ and BENACERRAF [12] of the existence of cell interaction molecules to explain the failure of cooperative interactions between allogeneic T and B cells in secondary antibody responses. To allow some decision to be made between these 2 alternative models, 3 different experimental systems were investigated [19], as will now be briefly described.

The first system was devised to answer the following question. If a T cell population of one particular genotype (say from strain X) is primed to a given antigen in an environment where the antigen is presented on macrophages of 2 different genotypes

group *	recipients	L/R ^{125}I-UdR uptake in recipients given		
		15×10^6 FGG-CFA sensitized syngeneic cells	15×10^6 FGG-CFA sensitized syngeneic + 15×10^6 FGG-CFA sensitized allogeneic cells **	15×10^6 FGG-CFA sensitized syngeneic + 15×10^6 CFA sensitized allogeneic cells
1	CBA	2.17 ± 0.13 (5) °	1.99 ± 0.22 (5)	2.16 ± 0.32 (5)
2	C57BL	2.65 ± 0.20 (5)	2.42 ± 0.07 (5)	2.40 ± 0.15 (5)
3	CBA °°	2.52 ± 0.23 (8)	1.58 ± 0.22 (8)	1.67 ± 0.10 (8)

* in groups 1 and 2 all cells were given simultaneously, in group 3 allogeneic cells were given 24 hrs before the syngeneic ones;

** in each group it was confirmed that the allogeneic cells used were sensitized to fowl gammaglobulin (FGG), CFA = complete Freund's adjuvant;

° arithmetic mean ± 1 SEM. Number of mice/group shown in brackets;

°° these recipients were challenged when the allogeneic cells were given.

Table 5 - Allogeneic mixtures of sensitized cells do not suppress DTH transfer by syngeneic cells.

(X and Y), would the sensitized T cell then be able to transfer DTH to only syngeneic (i.e. X) or to both syngeneic and allogeneic recipient mice (i.e. X and Y)? If transfer were restricted to syngeneic recipients, we would be in a position to consider the idea that matching of the I-A region gene product between sensitized T cell and macrophage bearing antigen is a prerequisite for T cell activation. If, on the other hand, the transfer was not restricted, matching of identical I-A region gene products is unlikely to be a *sine qua non* for T cell activation. Rather we would think that sensitization of 2 separate T cells must have occurred, the antigen binding receptors of one type being directed to the I region associated antigen on macrophages of one genotype, and the other type being directed to the I region associated antigen on macrophages of the second genotype.

Tetraparental bone marrow chimaeras (TBMC) were used to provide an environment where a given antigen may be presented on macrophages of 2 different genotypes. To produce such mice, lethally X-irradiated F_1 hybrids were repopulated with bone marrow cells from which contaminating T cells were removed by pretreatment with anti-Thy.1 serum and complement. (CBA $\times$ C57BL)F_1 mice were given equal numbers of marrow cells from the 2 homozygous, histoincompatible parents CBA and C57BL. Cells from such TBMC are unresponsive in both the mixed lymphocyte reaction and cell-mediated lysis to either parent's histocompatibility antigens but have unimpaired reactivity to third party determinants [30]. The cells of the TBMC do, however, express K and D serological specificities of the MHC since they can be lysed by specific alloantisera, and also I determinants since they can be used as stimulator cells in the mixed lymphocyte reaction [30]. TBMC made from CBA and C57BL mice and containing roughly 50 % CBA cells and 50 % C57BL cells in their hemopoietic system, were sensitized to FGG. Lymph node cells, taken 5 days later, were separated into CBA and C57BL cells by using relevant anti-H-2 sera able to kill either CBA or C57BL cells. The viable cells were then washed and tested for their capacity to transfer DTH to naive recipients. For simplicity, we show here the results obtained with the

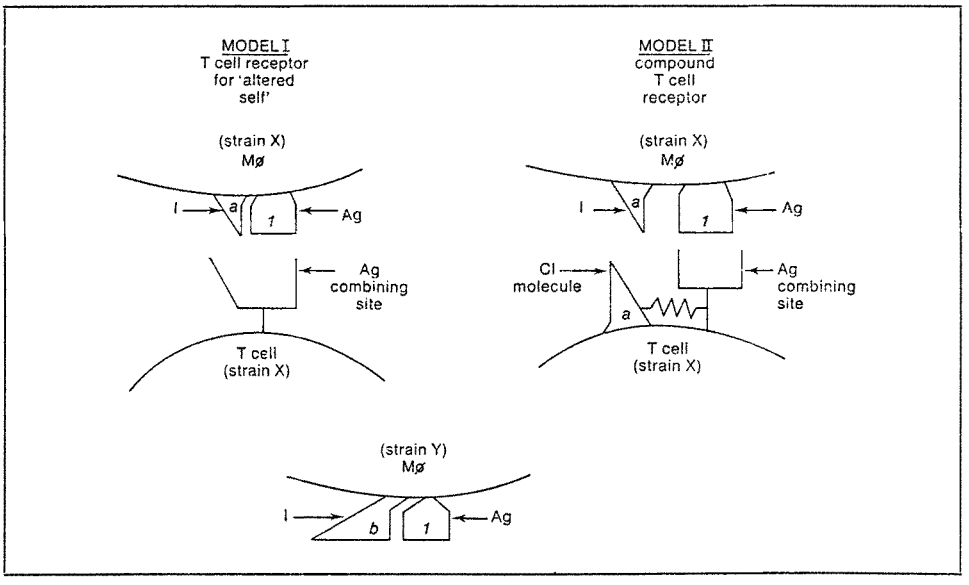

Fig. 3 - Hypothetical models used to illustrate genetic constraints imposed on T lymphocyte-macrophage interactions (see text). X and Y indicate hypothetical MHC incompatible strains of mice. I = I region determinant *a* or *b*; Mø = macrophage; Ag = antigen *1*.

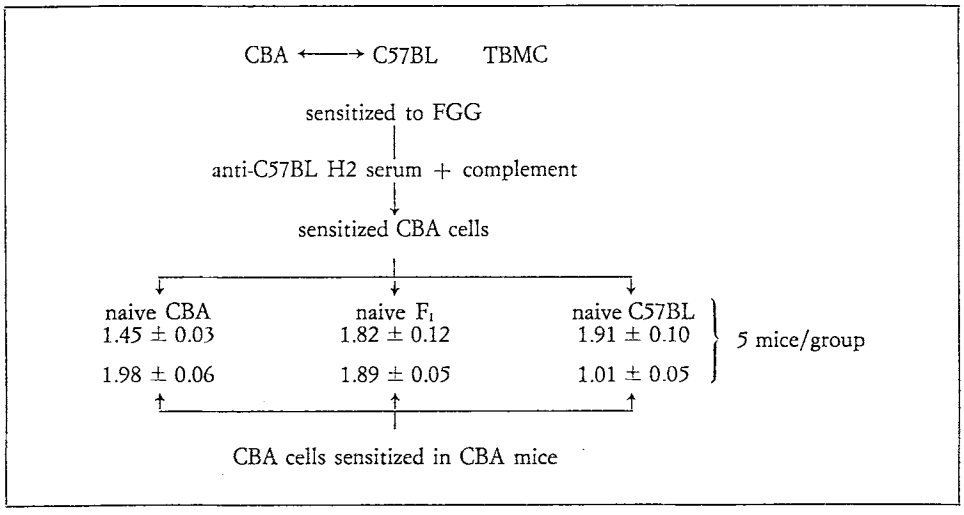

Table 6 - T cells sensitized in chimaeric environment (TBMC) transfer DTH to MHC-incompatible parental recipients (data from reference no. 19).

transfer of CBA cells sensitized to FGG in the chimaeric environment (tab. 6). These transferred DTH as effectively to allogeneic C57BL as to syngeneic or F_1 mice. By contrast, CBA cells sensitized in normal, non-chimaeric CBA mice failed to transfer DTH to allogeneic C57BL mice. These findings argue in favour of the notion that T cells primed in a chimaeric environment made up of 2 different strains are primed to antigen *1* and I region gene product *a* (fig. 4) as well as to antigen *1* and I region gene product *b*. Thus, in the TBMC environment, CBA T cells would be composed of 2 different subsets of primed T cells, one with specificity towards the antigen and the I region of CBA and another with specificity towards the antigen and the I region determinant of C57BL. The results make it increasingly more difficult to rescue the

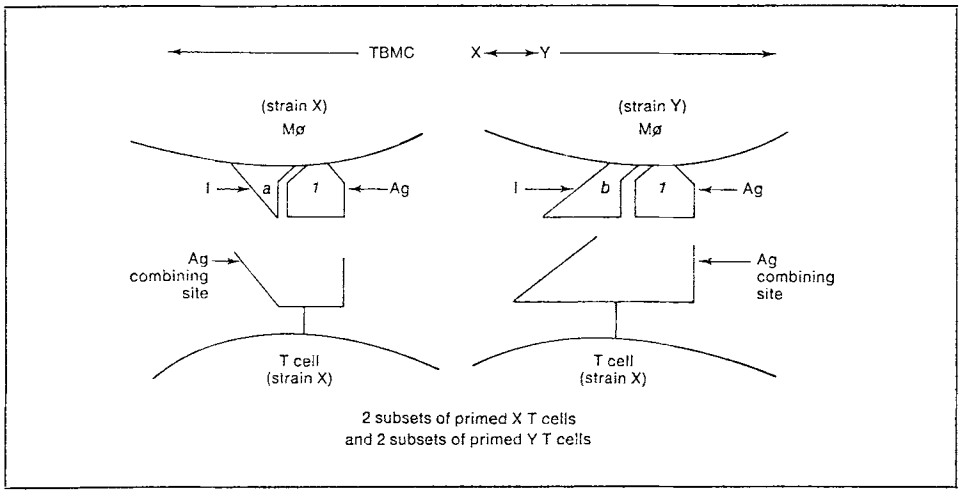

Fig. 4 - Scheme to show possible different types of reactive T cells in sensitized tetraparental bone marrow chimaeras (TBMC) (see text). Abbreviations as in fig. 3.

hypothesis that successful interaction depends on the matching of I region coded cell interaction molecules on T cells and macrophages. The TBMC experimental design does not however allow us to define more precisely the exact specificity of the sensitized T cell. Does it reside in a single molecule with a combining site directed toward a common region formed by the interaction of the antigenic determinant and the I region gene product? Or is it due to two separate molecules or receptors, one with specificity for the antigenic determinant and the other for the I region gene product?

Sensitized T cells from F_1 mice will successfully transfer DTH to both parental strains as well as to the syngeneic F_1 (see above and reference no. 18). The question was therefore asked: can F_1 T cells sensitized, not in the F_1 animal, but in one of the parental strain (say X) transfer DTH to both parental strains (X and say Y) or only to that in which it was originally sensitized, *viz.* X? In order to sensitize F_1 cells not in the F_1 but in one of the parental strains, athymic nude mice, which are available on several congenic backgrounds, were used. DTH could not be elicited in these mice unless they were first reconstituted with a source of T cells (tab. 7). To sensitize F_1 T cells in the parental environment, T cells from naive, *nonsensitized*, (CBA × BALB/c)F_1 mice were used to reconstitute BALB/c.nu and CBA.nu. These mice were then sensitized to FGG and their lymph node cells transferred to naive BALB/c and CBA mice in order to test their ability to transfer DTH. It is evident that F_1 cells sensitized in BALB/c.nu could transfer DTH successfully only to BALB/c, not to CBA. On the other hand, F_1 T cells sensitized in CBA.nu could transfer DTH successfully only to CBA, not to BALB/c (tab. 8). These results strongly support the notion that sensitization of T cells in an F_1 leads to the activation of 2 subsets of T cells with respect to the specificity of the antigen combining site: one with its site directed towards the antigen in association with the I-A region gene product of one parental strain, the other with a site directed towards the antigen in association with the I-A region gene product of the other parental strain (fig. 5). Sensitization of F_1 T cells in the parental environment would thus lead to the activation of only one of these 2 subsets of T cells. The alternative hypothesis which demands matching of identical I-A region gene products can be substantiated only if these products were subject to allelic exclusion. There is, however, no evidence for this phenomenon with regard to MHC components. Again, the experiment with F_1 T cells sensitized in parental strains cannot tell us whether the specificities involved are encompassed in 1 or 2 molecules/T cell.

In a third experimental system, macrophages from CBA or BALB/c mice were pulsed with FGG *in vitro*, washed and injected into (CBA × BALB/c)F_1 mice. This allowed levels of sensitivity to be achieved with doses of antigen that were calculated (using radiolabeled FGG) to be 100-1,000 less than those required for aggregated antigen. Lymphoid cells from mice sensitized with such macrophage associated antigen were tested for their ability to transfer DTH into parental naive recipients. The results

strain of nude	T cells given	L/R ^{125}I-UdR uptake
BALB/c.nu	none	1.05 ± 0.11
BALB/c.nu	BALB/c	4.10 ± 0.06
BALB/c.nu	(CBA × BALB/c)F_1	3.82 ± 0.10
CBA.nu	none	0.94 ± 0.08
CBA.nu	CBA	3.23 ± 0.50
CBA.nu	(CBA × BALB/c)F_1	3.70 ± 0.30

Table 7 - DTH to FGG in athymic nude mice (5 to 6 mice/group. Data from reference no. 19).

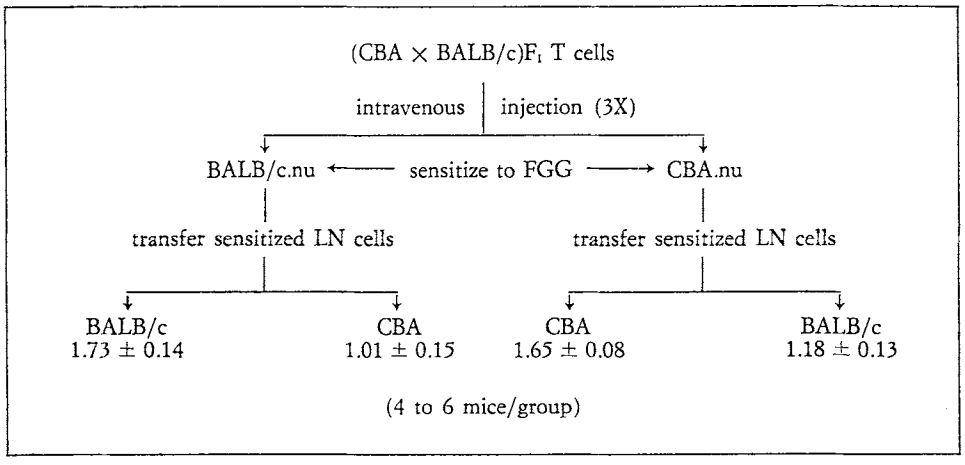

Table 8 - Evidence for 2 types of sensitized T cells in F_1 (data from reference no. 19).

shown in tab. 9 indicate that cell transfer of DTH was possible provided the macrophages used for sensitization were of the same genotype as the recipients [19]. This again strongly supports the notion that sensitization is directed not to the antigen as such but to a cell surface structure on the macrophage determined partly by a MHC gene product (I-A) and partly by the processed antigen.

MHC imposed restriction of T cell activities in DTH is thus likely to operate at the level of antigen processing by macrophages. It is possible that a unique biochemical pathway, dependent on MHC coded enzymes, is responsible for the processing of both antigen and MHC membrane specificities. As a result the antigenic determinant finally displayed to the T cell could become associated with some of the MHC determined specificities. Alternatively, T cells may utilize two recognition systems, one for antigen, the other for the MHC gene product.

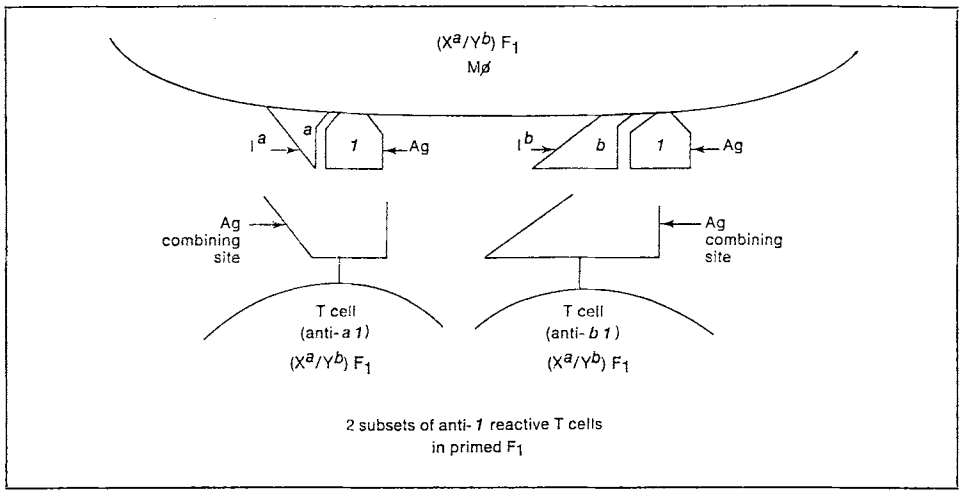

Fig. 5 - Scheme to show possible different types of reactive T cells in sensitized F_1 (see text). Abbreviations as in fig. 3.

Ir-GENE CONTROL OF T CELL DEPENDENT RESPONSES

The genes which influence, not whether an antigen can be seen as such, but whether it will be recognized as an immunogen, are localized in the I region of the MHC [4]. They have thus been termed histocompatibility or H-linked Ir genes. They determine whether T cell dependent responses (cellular immunity or antibody responses requiring T and B cell cooperation) can occur to various antigenic determinants [4]. The mode of action of these Ir genes is unknown but it has been postulated that they may represent the structural genes for the T cell receptor for antigen. The data, described above in relation to transfer of DTH from mice sensitized by macrophage associated antigens, suggest one possible hypothesis to explain Ir gene control of T cell dependent responses. Low responders may lack the appropriate I region gene which is essential to produce in the macrophage the product with which a particular antigenic determinant can become associated after the antigen is processed by that cell. The defect in the low responder may thus be an inadequate repertoire of I-A region gene products expressed on macrophages. Alternatively, the defect could be in corresponding antigen specific receptor dictionary of the T cells. Neither hypothesis, however, appears to be able to account for recent observations on the DTH responsiveness of mice to the enzyme lactic dehydrogenase B (LDH_B) which is under Ir gene control. As shown in fig. 6 a good DTH response was obtained in responder C57BL mice but none, at any time, with non-responder CBA mice. If, however, the mice were pretreated 2 days prior to antigen sensitization with 200 mg/kg cyclophosphamide, DTH could be elicited from CBA mice, but the period of sensitivity was short lived [19]. It seems, therefore, that low responder mice do not lack T cells with appropriate specificities, nor the mechanism by which macrophages effectively present antigen to T cells, presumably in association with the I-A region gene product. Under normal conditions, however, this stimulation potential is suppressed by a cyclophosphamide sensitive system which has yet to be identified. Candidates for such a suppressive mechanism are, of course, suppressor T cells but their role in this particular system has not been established. An alternative possibility is that cyclophosphamide may temporarily allow macrophages to process antigen in a more immunogenic form, irrespective of the responder status.

THE T CELL RECEPTOR FOR ANTIGEN

The nature of the antigen specific receptor on T lymphocytes has generated much debate and controversy. A number of investigations have shown that T and B cells may differ in the spectra of their pattern of reactivities to antigens (e.g. reference no. 22), and this has raised the possibility that different recognition systems may be involved. On the other hand, such a difference in antigen reactivity would be expected from the observations described here which indicate that T cells will react only if antigen is presented in association with cell membrane MHC gene specified products. The recognition molecule may thus be the same on T and B cells. Only the requirements for antigen processing and presentation may differ.

An obvious candidate for the T cell receptor for antigen is a molecule identical to B cell immunoglobulin, referred to as IgT [16]. In view of the difficulty that some investigators have had in isolating IgT, it has been suggested that the antigen specific receptor on T lymphocytes may not be classical immunoglobulin but a different molecule. This may in part be coded by C region genes located in the MHC and thus different from the C genes of classical immunoglobulin [20]. The molecule must, however, use the same V region genes as immunoglobulin since receptors for certain antigens on T and B cells have identical V region idiotypes as do circulating antibodies directed against the same antigens [6, 9]. Although a DNA translocation model for sharing of

source of FGG pulsed macrophages	mice sensitized with FGG pulsed macrophages *	naive recipient of 6×10^7 sensitized (CBA × BALB/c)F_1 spleen cells	L/R ^{125}I-UdR uptake
CBA	(CBA × BALB/c)F_1	CBA	1.62 ± 0.09
CBA	(CBA × BALB/c)F_1	BALB/c	1.11 ± 0.12
BALB/c	(CBA × BALB/c)F_1	CBA	1.12 ± 0.05
BALB/c	(CBA × BALB/c)F_1	BALB/c	1.63 ± 0.17

* the L/R ^{125}I-UdR uptake in (CBA × BALB/c)F_1 when sensitized with CBA macrophage associated FGG was 3.71 ± 0.29 while with BALB/c associated FGG it was 5.91 ± 0.61 on the day of cell transfer.

Table 9 - Transfer of DTH with cells from mice sensitized with macrophage associated antigen (6 mice/group Data from reference no. 19).

V region genes by various C regions linked on the same chromosome is quite likely [11], it is more difficult to envisage translocation between unlinked V region genes and MHC genes. It seems more reasonable to assume that the MHC is involved, not in coding for part of the T cell receptor, but in the activation of T lymphocytes as discussed above.

The T cell system in modern vertebrates has presumably evolved from primitive hemocytes. These must, at some stage, have diverged into 2 separate functional cell lines: one would be represented by the phagocytic, antigen processing or antigen presenting macrophage; the other by the nonphagocytic, antigen sensitive T lymphocyte. Other evidence indicates that the ability to discriminate between various self and non-self molecules was possessed by gametes and somatic cells in invertebrates long before the divergence of the T cell and macrophage occurred. The basis for such discrimination in the marine colonial tunicate, *Botryllus*, is a single genetic locus with multiple alleles [7]. These must dictate in some way the structure of cell surface components invol-

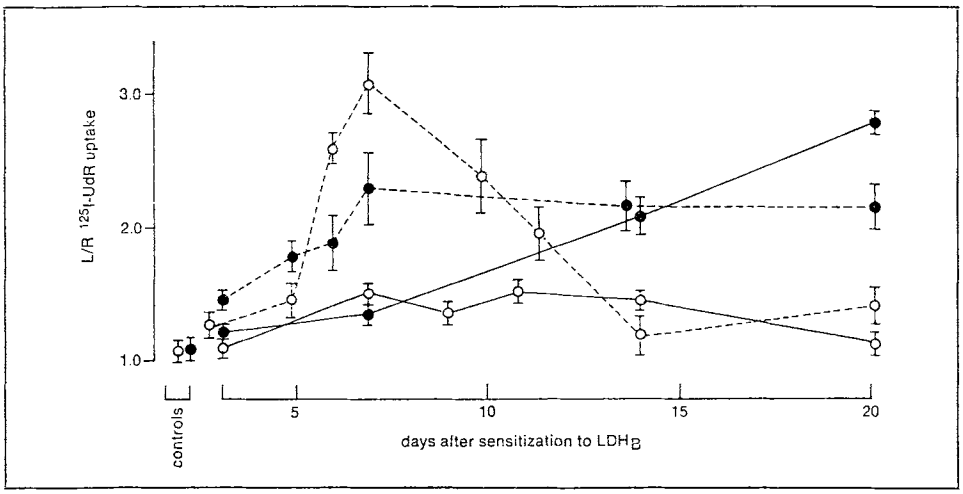

Fig. 6 - DTH response in C57BL (●) and CBA (○) mice sensitized with LDH_B. Mice not pretreated with cyclophosphamide (———); mice pretreated with cyclophosphamide (- - - -). Controls were not sensitized. Data from reference no. 19.

ved in intercellular recognition and in prevention of self-fertilization in this hermaphrodite species. Such a gene locus is the logical precursor of the 4 loci on chromosome 17 of the mouse, T/t, H-2K, H-2D and TL (fig. 1), each of which specifies surface membrane glycoproteins of similar structure and molecular weight [28]. From this point of view, it may be argued that the T cell dictionary is derived by the evolution of variations of the gene products used firstly, for enabling recognition of nonself in the process of fertilization; secondly, in the recognition of self in the process of cell association for the growth of particular tissues; and thirdly, in the production of self histocompatibility antigens. If MHC gene products did evolve to allow cell recognition for allofertilization and for tissue growth and development, there must have been an associated parallel evolution of complementary recognition elements. It is tempting to suggest that from these complementary structures T cell receptors for antigen evolved. The possibility that at least part of this receptor is coded by V genes which are also responsible for classical immunoglobulin diversity [6, 9] suggests an alternative to the 'altered self' hypothesis shown in model I of fig. 3 (DOHERTY, personal communication). Activation of T cells may require recognition by two separate receptors, one having specificity for antigen, the other having complementarity for a particular MHC gene product.

SUMMARY

There is considerable evidence that T cell activation to soluble antigens occurs only if this is processed by macrophages and displayed appropriately on the plasma membrane in association with products of the genes of the major histocompatibility complex. The genes responsible differ according to the antigens involved. For cytotoxicity, targets and killer T cells must share K or D region gene products. For delayed type hypersensitivity to fowl gammaglobulin in mice, I-A identity is necessary; for dinitrofluorobenzene, identity at either the I, K or D region is sufficient. Experiments using three different approaches do not support the notion that these genetic constraints are due to the necessity for the T cell and stimulator cell to match an identical gene product or 'cell interaction molecule'. Rather they favour the hypothesis that activated T cells recognize antigen and products of genes of the major histocompatibility complex. The implications of the results are discussed in terms of the mode of action of immune responsiveness (Ir) genes and of the possible parallel evolution of T cell receptors for antigen and gene products of the major histocompatibility complex.

REFERENCES

1) BASTEN A., MILLER J. F. A. P., ABRAHAM R.: Relationship between Fc Receptors, Antigen-Binding Sites on T and B Cells and H-2 Complex Associated Determinants - J. exp. Med. *141*, 547, 1975.
2) BASTEN A., MILLER J. F. A. P., WARNER N. L., PYE J.: Specific Inactivation of Thymus-Derived (T) and Non-Thymus-Derived (B) Lymphocytes by ^{125}I-Labelled Antigen - Nature (New Biol.) *231*, 104, 1971.
3) BENACERRAF B., KATZ D. H.: Failure to Induce Tolerance to 2,4-Dinitrochlorobenzene Contact Sensitivity with a 2,4-Dinitrophenyl (DNP) Conjugate of a Copolymer of D-Glutamic Acid and D-Lysine, a Specific Tolerogen for DNP B Cells - J. Immunol. *112*, 1158, 1974.
4) BENACERRAF B., KATZ D. H.: The Histocompatibility-Linked Immune Response Genes - Advanc. Cancer Res. *21*, 121, 1975.
5) BEVAN M. J.: The Major Histocompatibility Complex Determines Susceptibility to Cytotoxic T Cells Directed against Minor Histocompatibility Antigens - J. exp. Med. *142*, 1349, 1975.
6) BINZ H., WIGZELL H.: Shared Idiotypic Determinants on B and T Lymphocytes Reactive against the Same Antigenic Determinants. II. Determination of Frequency and Characteristics of Idiotypic T and B Lymphocytes in Normal Rats Using Direct Visualization - J. exp. Med. *142*, 1218, 1975.
7) BURNET F. M.: 'Self-Recognition' in Colonial Marine Forms and Flowering Plants in Relation to the Evolution of Immunity - Nature (Lond.) *232*, 230, 1971.
8) DOHERTY P. C., BLANDEN R. V., ZINKERNAGEL R. M.: Specificity of Virus-Immune Effector T Cells for H-2K or H-2D Compatible Interactions: Implications for H-Antigen Diversity - Transplant. Rev. *29*, 89, 1975.

9) EICHMANN K., RAJEWSKY K.: Induction of T and B Cell Immunity by Anti-Idiotypic Antibody - Europ. J. Immunol. *5*, 661, 1975.
10) ERB P., FELDMANN M.: The Role of Macrophages in the Generation of T-Helper Cells. II. The Genetic Control of the Macrophage-T Cell Interaction for Helper Cell Induction with Soluble Antigen - J. exp. Med. *142*, 460, 1975.
11) GALLY J. A., EDELMAN G. M.: Genetic Control of Immunoglobulin Synthesis - Ann. Rev. Genet. *6*, 1, 1972.
12) KATZ D. H., BENACERRAF B.: The Function and Interrelationship of T Cell Receptors, Ir Genes and Other Histocompatibility Gene Products - Transplant. Rev. *22*, 175, 1975.
13) KINDRED B., SHREFFLER D. C.: H-2 Dependence of Cooperation between T and B Cells *in Vivo* - J. Immunol *109*, 940, 1972.
14) LIVNAT S., KLEIN J., BACH F. H.: Graft-Versus-Host Reaction in Strains of Mice Identical for H-2K and H-2D Antigens - Nature (New Biol.) *243*, 42, 1973.
15) LUBAROFF D. M., WAKSMAN B. H.: Bone Marrow as Source of Cells in Reactions of Cellular Hypersensitivity. II. Identification of Allogeneic or Hybrid Cells by Immunofluorescence in Passively Transferred Tuberculin Reaction - J. exp. Med. *128*, 1437, 1968.
16) MARCHALONIS J. J.: Lymphocyte Receptors for Antigen - J. Med. *5*, 329, 1974.
17) MILLER J. F. A. P., VADAS M. A., WHITELAW A., GAMBLE J.: A Radioisotopic Method to Measure Delayed Type Hypersensitivity. II. Cell Transfer Studies - Int. Arch. Allergy *49*, 693, 1975.
18) MILLER J. F. A. P., VADAS M. A., WHITELAW A., GAMBLE J.: H-2 Gene Complex Restricts Transfer of Delayed-Type Hypersensitivity in Mice - Proc. nat. Acad. Sci. (Wash.) *72*, 5095, 1975.
19) MILLER J. F. A. P., VADAS M. A., WHITELAW A., GAMBLE J.: Role of Major Histocompatibility Complex Gene Products in Delayed-Type Hypersensitivity - Proc. nat. Acad. Sci. (Wash.) *73*, 2486, 1976.
20) MUNRO A. J., TAUSSIG M. J.: Two Genes in the Major Histocompatibility Complex Control Immune Responses - Nature (Lond.) *256*, 103, 1975.
21) ROSENTHAL A. S., LIPSKY P. E., SHEVACH E. M.: Macrophage-Lymphocyte Interaction and Antigen Recognition - Fed. Proc. *34*, 1743, 1975.
22) SCHIRRMACHER V., WIGZELL H.: Immune Responses against Native and Chemically Modified Albumins in Mice. I. Analysis of Non-Thymus Processed (B) and Thymus-Processed (T) Cell Responses against Methylated Bovine Serum Albumin - J. exp. Med. *136*, 1616, 1972.
23) SELA M., MOZES E., SHEARER G. M.: Thymus-Independence of Slowly Metabolized Immunogens - Proc. nat. Acad. Sci. (Wash.) *69*, 2696, 1972.
24) SHEARER G. M., REHN T. G., GARBARINO C. A.: Cell-Mediated Lympholysis of Trinitrophenyl-Modified Autologous Lymphocytes. Effector Cell Specificity to Modified Cell Surface Components Controlled by the H-2K and H-2D Serological Regions of the Murine Major Histocompatibility Complex - J. exp. Med. *141*, 1348, 1975.
25) SHREFFLER D. C., DAVID C. S.: The H-2 Major Histocompatibility Complex and the I Immune Response Region: Genetic Variation, Function and Organization - Advanc. Immunol. *20*, 125, 1975.
26) TOSOLINI F. M., MIMS C. A.: Effect of Murine Strain and Viral Strain on the Pathogenesis of Lymphocytic Choriomeningitis Infection and a Study of Footpad Responses - J. infect. Dis. *123*, 134, 1971.
27) VADAS M. A., MILLER J. F. A. P., GAMBLE J., WHITELAW A.: A Radioisotopic Method to Measure Delayed Type Hypersensitivity in the Mouse. I. Studies in Sensitized and Normal Mice - Int. Arch. Allergy *49*, 670, 1975.
28) VITETTA E. S., ARTZ K., BENNETT D., BOYSE E. A., JACOB F.: Structural Similarities between a Product of the T/t-Locus Isolated from Sperm and Teratoma Cells, and H-2 Antigens Isolated from Splenocytes - Proc. nat. Acad. Sci. (Wash.) *72*, 3215, 1975.
29) VOLKMANN A., GOWANS J. L.: The Production of Macrophages in the Rat - Brit. J. exp. Path. *46*, 50, 1965.
30) VON BOEHMER H., SPRENT J.: T Cell Function in Bone Marrow Chimeras: Absence of Host-Reactive T Cells and Cooperation of Helper T Cells across Allogeneic Barriers - Transplant. Rev. *29*, 3, 1975.

Requests for reprints should be addressed to:

JACQUES F. A. P. MILLER
The Walter and Eliza Hall Institute of Medical Research
P.O., Royal Melbourne Hospital
Victoria, 3050 - Australia