Role of MHC Gene Products in Immune Regulation

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The immune system has evolved the capacity to react specifically with a very large number of foreign molecules with which it had no previous contact, while avoiding reactivity for autologous molecules, naturally antigenic in other species or in other individuals of the same species.

Immunological research has been directed to the elucidation of this phenomenon ever since Ehrlich (*1*) proposed that immunocompetent cells bear receptors for antigen identical with the antibodies to be produced. Gowans *et al.* (*2*) identified lymphocytes as the cells responsible for immune phenomena. Burnet (*3*) proposed the clonal selection theory of immunity which postulated that (i) lymphocytes differentiate as clones bearing antibody receptors of unique specificity and (ii) antibody responses reflect the selective expansion of specific lymphocytes, following the binding of antigen, and their differentiation as secretors of antibody, identical in specificity with the antigen binding receptors on the original clones. The Burnet hypothesis was verified experimentally (*4–6*) and was accepted as a major advance, concerned primarily with the response of antibody-producing cells, later identified as B (or bursa equivalent) lymphocytes (*7*) and plasma cells. Accordingly, studies on the specificity of antibodies and on the structure of immunoglobulins revealed that these molecules (*8, 9*) and their structural genes (*10, 11*) evolved in a way that ensures the enormous diversity of antibody combining sites observed.

The discovery by Miller (*12*) and by Good *et al.* (*13*) that lymphocytes differentiate into two separate classes of cells (T, thymus-derived, and B) with distinct functions, the identification of cellular immune phenomena mediated by T cells (*14*), and the demonstration that immune responses are regulated by helper (*15–17*) and suppressor (*18, 19*) T cells and by macrophages (*20*) emphasized the complexity of the immune system and the critical role played by T lymphocytes in the regulation of immunity.

It became increasingly apparent that the clonal selection theory, although correct, did not take into account the complex cellular and molecular interactions essential to immune phenomena or the restrictions these interactions dictate in the specificity of T cells. An additional system, beside specific immunoglobulins, involving the products of the major histocompatibility complex (MHC) was shown to be critically involved in the manner by which T cells perceive antigens on the surface of cells and therefore in the nature of immunogenicity.

I give here a historical account of how our present understanding of T cell immunity and of T cell immune regulation has evolved with particular emphasis on the genes of the MHC and the molecules for which they code that regulate essential immune mechanisms.

Carrier Function and the Specificity of T Lymphocytes

The pioneering experiments of Landsteiner (*21*) established that antibodies can be produced against any type of molecule provided it is presented to the immune system coupled to an immunogenic carrier molecule. The determinants against which antibodies can be made were termed "haptens," and "carriers" were the essential immunogenic molecules required to initiate immune responses. Landsteiner's experiments implied the existence of a complex process involving the recognition of a carrier function by an entity distinct from antibody to initiate immune responses.

Spurred by Landsteiner's observations of the carrier effect, Gell and I (*22*) investigated the specificity of cellular immune responses to hapten-protein conjugates. We noted a fundamental difference between the specificity of cellular immune reactions and of antibodies. Immune cells displayed classical carrier specificity in contrast to antibodies which can be largely hapten-specific. This was later shown to be a general property of T cell mediated immune responses (*23*). Moreover, we also demonstrated another critical difference between the type of determinants reactive with antibodies and with T cells. Extensive denaturation of protein antigens capable of decreasing drastically reactivity with specific antibody had little effect on the ability of such proteins to initiate or elicit delayed-type hypersensitivity (DTH) to the intact molecules (*24*). This indicated again that T and B lymphocytes may not be specific for the same determinants and that T cells react preferentially with sequential determinants on proteins. These observations were confirmed by Schirrmacher and Wigzell (*25*) and by Ishizaka *et al.* (*26*).

Discovery of Immune Response Genes

The identification of the genes that determine biological phenomena and the study of the control they exert on these phenomena has proved to be the most successful approach to a detailed understanding of the mechanism of biological processes. Some of the most significant advances in molecular biology have relied on the methodology of genetics. The same statement may be made concerning our understanding of immunological phenomena.

Immunologists had not infrequently observed that certain individuals are weak responders to selected antigens. The complexity of most antigens and the marked heterogeneity of the antibody response did not encourage a genetic analysis of specific immune responsiveness. However, when synthetic polypeptides with relatively restricted structural heterogeneity were synthesized (*27*), the appropriate antigens were available to immunologists to study the genetic requirements for immunogenicity. The response of outbred guinea pigs to hapten conjugates of the poly-L-lysine homopolymer [dinitro phenyl (DNP)-PLL] was

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the first specific immune response documented to be under the control of a single dominant autosomal gene (*28*). We introduced the terms "responders" and "nonresponders" to distinguish animals possessing or not possessing the gene, and the gene responsible was referred to as an immune response or Ir gene. Fortunately, two inbred strains of guinea pigs developed originally by Sewell Wright were available at the National Institute of Allergy and Infectious Diseases, strain 2 and strain 13. Strain 2 animals responded to DNP-PLL and strain 13 guinea pigs did not, whereas the first filial generation produced by crossing the strains, that is, (2 × 13)F_1, were responders. The phenomenon was extended to other polypeptide antigens (Table 1), the random copolymers of L-glutamic acid and L-lysine (GL), L-glutamic acid and L-alanine (GA), and L-glutamic acid and L-tyrosine (GT) (*29*).

The response to conventional antigens, weak isologous antigens (*30*), or foreign protein antigens, administered at limiting immunizing doses (*31, 32*) to ensure response to only the most immunogenic determinants, is under similar control of individual Ir genes.

The phenomenon was extended to other experimental species. McDevitt and Sela demonstrated the Ir gene control of the response of inbred mice to an interesting set of branched copolymers synthesized by Sela: (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L (where H and Phe are histidine and phenylalanine, respectively). These differed from each other in only one of the amino acids on the side chain (*33*). The responses to these copolymers were under the control of distinct Ir genes. In collaboration with Maurer we also demonstrated Ir gene control of the response of inbred mice to linear random copolymers of L-amino acids (*34*). Genetic control of immune responsiveness was also reported in rats (*35, 36*) and rhesus monkeys (*37*), illustrating the generality of this phenomenon for different antigens and in different species.

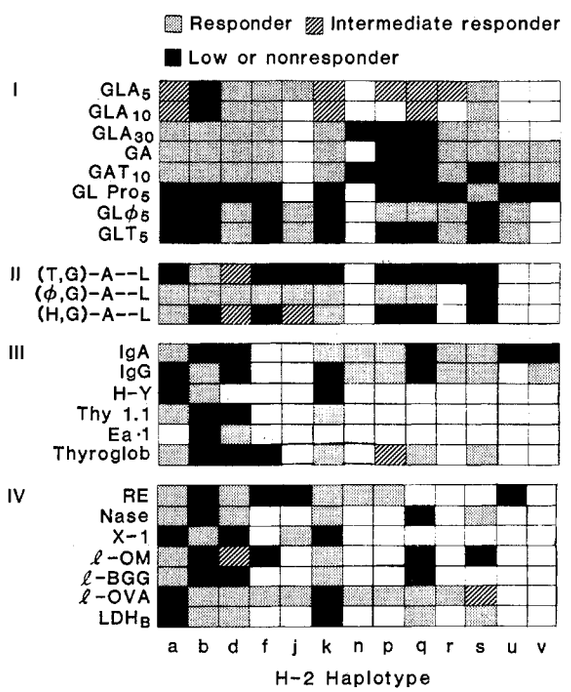

Fig. 1. Immune responsiveness to linear random and branched copolymers of L amino acids, to isologous antigens, and to foreign antigen administered at limiting immunizing doses is determined by the H-2 haplotype.

Table 1. Inheritance of specific Ir genes and of the major histocompatibility locus of strain 2 and strain 13 guinea pigs by (2 × 13)F_1 and backcross animals. Symbols: +, responsiveness and presence of major histocompatibility specificities; −, nonresponsiveness and absence of major histocompatibility specificities of the inbred strains; BSA, bovine serum albumin.

Antigens	Strain			(2 × 13)F_1 × 13		(2 × 13)F_1 × 2	
	2	13	(2 × 13)F_1	50 percent*	50 percent	50 percent	50 percent
DNP-PLL, GL	+	−	+	+	−		
GA	+	−	+	+	−		
GT	−	+	+			+	−
BSA (0.1 μg)	+	−	+	+			
HSA (1 μg)	+	−	+				
DNP-BSA (1 μg)	+	−	+	+	−		
DNP-GPA (1 μg)	−	+	+			+	−
Major H locus							
Strain 2	+	−	+	+	−		
Strain 13	−	+	+			+	−

*Column identifies the same group of backcross animals. From Benacerraf (*122*).

Linkage of Ir Genes to the Major Histocompatibility Complex

The availability of inbred strains of mice and guinea pigs permitted the rapid mapping of Ir genes. McDevitt and Chinitz (*38*) made the exciting finding that responsiveness of inbred mice to (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L could be predicted on the basis of their H-2 genotype. The linkage of murine Ir genes with the H-2 complex was confirmed for numerous antigens by many laboratories and is appropriately considered one of the distinctive features of specific Ir genes (*39*). A summary of the data is shown in Fig. 1 (*40*). Identical linkage between guinea pig Ir genes and MHC specificities in that species was documented in our laboratory (*41*). The strategy employed in these experiments is illustrated in Table 1. The genes for the responses to PLL, GA, and bovine serum albumin were observed to be linked to the locus controlling the major histocompatibility complex of strain 2 guinea pigs. Similarly, the GT gene and the genes controlling responsiveness to limiting doses of DNP–guinea pig serum albumin (GPA) were found to be linked to the major H locus of strain 13 guinea pigs. Linkage of Ir genes to the MHC of the rat (*35, 36*) and rhesus monkey (*37*) was also established, illustrating the general significance of the finding. In contrast, Ir genes were shown not to be linked to the structural genes for the H chain of immunoglobulins (*39*).

The availability of congenic resistant (CR) mouse strains developed by Snell (*42*) and of strains with documented recombinant events within the H-2 complex permitted McDevitt, Deak, Sheffler, Klein, Stimpfling, and Snell (*43*) to map the murine Ir-1 locus controlling responsiveness to (T,G)-A--L to a new region of the mouse H-2 complex termed the I region (Fig. 2).

Mapping of individual murine Ir genes by several laboratories [reviewed in (*40*)]

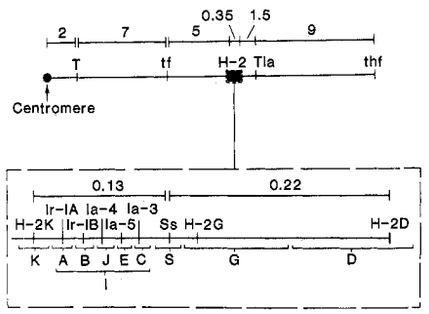

Fig. 2. Genetic map of the H-2 complex showing the various loci and the subregions of I. Note that Ir genes have been mapped in I-A, I-B, and I-E.

revealed that most Ir genes map in I-A, a smaller number map in I-B, whereas responsiveness to some antigens map in both I-A and I-E. These last cases provide the genetic basis for the molecular identification of Ir gene products. Whereas most immune responses investigated are under the control of single loci, complementation of Ir genes for the response to certain antigens is observed in rare cases. Thus Dorf and I (*44*) showed that the response to the terpolymer of L-glutamic acid, L-lysine, and L-phenylalanine (GLϕ) is determined by two Ir genes which complement in both the cis and trans configuration to permit a response to GLϕ to develop. These genes which we termed α and β map in the I-E and I-A subregion (Fig. 2), respectively (Table 2). Possession of either α or β genes alone does not confer responsiveness to GLϕ which requires the presence of both genes. Response to several other antigens follows the pattern of the GLϕ response (*45*).

I discuss later the evidence that Ir gene complementation for GLϕ responses reflects the molecular complementation of the α and β subunits of an immune response–associated (Ia) glycoprotein. This molecule must be expressed on the surface of macrophages and B lymphocytes for the response to GLϕ. In the case of this Ia molecule, the α and β chains will be shown to be coded, respectively, in I-E and I-A. When Ir genes map in a single region such as I-A, distinct α and β subunits, the Aα and Aβ chains, are coded in the same A subregion.

Ia Molecules and Histocompatibility Antigens

Advantage was taken of the existence of mouse and guinea pig strains that differ solely at the I region of their MHC, such as the ATL and ATH strains of mice and the guinea pig strains 2 and 13, in that attempts were made to produce antibodies specific for the Ir gene products by cross-immunization with lymphoid tissue. Alloantiserums prepared in this manner by Shreffler and David (*46*), Klein and Hauptfeld (*47*), and McDevitt and associates (*48*) in mice and Schwartz *et al.* (*49*) in guinea pigs reacted with Ia alloantigens expressed on B lymphocytes and a significant fraction of macrophages (*50*). A study by Shreffler and David (*46*) of the specificities detected by antiserums to Ia revealed the considerable polymorphism of these molecules.

Cullen *et al.* (*51, 52*) studied the structure of murine Ia antigens expressed on B lymphocytes, and analyzed the membrane antigens specifically reactive with antibodies to Ia. Such antibodies bound

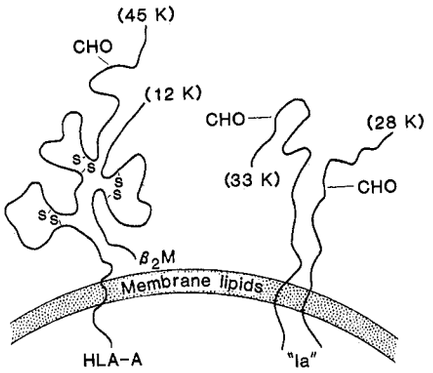

Fig. 3. Graphic representation of the chain structure of an Ia molecule (right) compared with the structure of a histocompatibility antigen (left). Essentially comparable results were obtained for the mouse H-2 and the human HLA and the guinea pig GPLA complexes. Abbreviations: *CHO*, carbohydrate moiety; *S–S*, intrachain sulfur bonds; β_2M, microglobulin β_2.

glycoproteins from B cells composed of an α and a β chain with molecular weights of 33,000 and 28,000, respectively.

Similarly, 13 alloantiserums to strain 2 and two alloantiserums to strain 13 detected homologous Ia molecules with corresponding α and β chain subunits on guinea pig macrophages and B lymphocytes (*49, 53*).

A graphic representation of an Ia molecule is shown in Fig. 3 and compared with a classical transplantation antigen of the MHC expressed on all cells and comprised of a 45,000 dalton polymorphic chain associated with β_2 microglobulin (*54*).

An analysis of the immunological properties of the highly polymorphic Ia molecules on macrophages and B lym-

Table 2. Complementation of α and β Ir genes for responses to GLϕ. Vertical bars indicate position of crossing-over. GLϕ genes are indicated by underline. Adapted from Dorf and Benacerraf (*44*).

Strain	H-2 haplotype	H-2 region formulas								GLϕ response (percentage binding ± standard error)
		K	I-A	I-B	I-J	I-E	I-C	S	D	
B10	b	b	b	b	b	b	b	b	b	1 ± 3
B10.BR	k	k	k	k	k	k	k	k	k	5 ± 3
(B10 × B10.BR)F_1	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	68 ± 16
B10.S	s	s	s	s	s	s	s	s	s	−1 ± 1
B10.D2	d	d	d	d	d	d	d	d	d	61 ± 5
B10.A	a	k	k	k	k	k	d	d	d	4 ± 2
3R	i3	b	b	b	b	k	d	d	d	59 ± 7
5R	i5	b	b	b	k	k	d	d	d	73 ± 5
18R	i18	b	b	b	b	b	b	b	d	5 ± 2
7R	t2	s	s	s	s	s	s	s	d	4 ± 2
9R	t4	s	s	?	k	k	d	d	d	71 ± 10
A.TL	t1	s	k	k	k	k	k	k	d	4 ± 3
B10.HTT	t3	s	s	s	s	k	k	k	d	77 ± 7
			β			α				

Table 3. Treatment with monoclonal antibody to Ia and complement (C) eliminates macrophages required for GAT induced T cell proliferation.

Responding cells*	Added antigen-presenting cells†	Response (count/min)
C treated	None	53,656
Antibody to Ia plus C treated	None	722
Antibody to Ia plus C treated	γ-Irradiated spleen	36,146
Antibody to Ia plus C treated	α-Thy 1 plus C treated γ-irradiated spleen	56,505

*Lymph node T cells (4×10^5, strained through nylon) from GAT-CFA primed BALB/c mice, treated with C alone, or M5/114 + C, then cultured with or without 100 μg of GAT per milliliter 3 days, exposed to [^{3}H]thymidine for 18 hours, harvested, and counted. † Syngeneic spleen cells (3×10^5) gamma-irradiated with 1500 R.

phocytes revealed that these products stimulate the alloreactive proliferation of unprimed clones of T lymphocytes in a test in vitro termed the mixed leukocyte reaction (MLR) (*55*). The ability of Ia bearing cells to stimulate MLR responses is effectively blocked by antibodies to Ia (*56*). Differences in the I region and the Ia molecules on cells stimulate strong graft versus host reactions (*57*) and vigorous homograft rejections (*58*).

Function of Ir Genes

The study of Ir gene function contributed to our understanding of the intricate regulatory mechanisms evolved by T cells and macrophages to regulate specific immune responses. Experiments were initially designed to identify the cells of the immune system in which Ir genes are expressed and the nature of the process they control. H-linked Ir genes were shown to determine both humoral and cellular immune responses (*28*). A further analysis revealed that the genes control the recognition of the "carrier" molecule as an immunogen (*59*), a property of T lymphocytes. Thus, responder guinea pigs that make antibody to DNP upon immunization with DNP-PLL are equally able to make antibody to benzylpenicilloyl (BPO)–PLL, whereas nonresponder guinea pigs to DNP-PLL do not (*59*). Similarly, nonresponder animals who failed to make antibody to DNP in response to DNP-PLL, make antibody to DNP when immunized with DNP conjugates of a conventional antigen. Moreover, Ira Green in my laboratory made the significant observation that the DNP-PLL genetic defect could be bypassed and nonresponder animals induced to form antibodies to DNP-PLL if DNP-PLL is treated as a macromolecular hapten and administered coupled to an immunogenic carrier such as ovalbumin (*60*). Consistent with the critical role of the carrier in cellular immunity, the genetic defect for cellular immunity was not bypassed and the nonresponder guinea pigs immunized with DNP-PLL–ovalbumin did not develop delayed-type sensitivity to DNP-PLL in spite of making large amounts of antibodies to DNP-PLL antibodies. Dunham, Unanue, and I (*61*) then verified the presence of B cells with antibody receptors for nonimmunogenic polypeptides in the spleens of nonresponder mice. We concluded from these experiments that the process governed by specific H-linked Ir genes controls T cell immune responses and affects antibody production only as a result of the need of helper T cells for B cell responses. In agreement with this conclusion, H-linked Ir genes were shown to control only the response to T dependent antigens (*39*). T independent responses, which result from the direct activation of B lymphocytes by antigen, are not under H-linked Ir gene control.

The involvement of Ir genes in T cell responses could result from either the expression of Ir genes in T cells and their coding for the T cell receptor, or the expression of Ir genes in macrophage

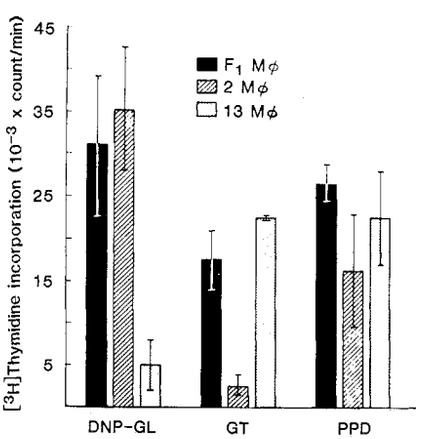

Fig. 4. Ir genes are expressed in Ia bearing macrophages. Guinea pig $(2 \times 13)F_1$ T cells primed to both DNP-GL (to which strain 2 responds) and GT (to which strain 13 responds) were cultured in vitro with strain 2, strain 13, or F_1 macrophages exposed to GT, DNP-GL, or PPD (tuberculin purified protein derivative). The proliferative responses are recorded as the incorporations of [^{3}H]thymidine into DNA. Adapted from Shevach and Rosenthal (*62*).

and B cells and their role in determinant selection, antigen presentation, and T cell–B cell interaction. The latter alternative was shown to be correct in every respect. Shevach and Rosenthal (*62*), working with the guinea pig systems we developed, made use of the finding that primed T cell clones proliferate in vitro and incorporate [^{3}H]thymidine when presented with antigen on macrophages. When $(2 \times 13)F_1$ guinea pigs were immunized with two antigens, DNP-GL, controlled by a strain 2 Ir gene, and GT controlled by a strain 13 Ir gene, and their T cells were exposed to DNP-GL or GT on macrophages of strain 2, strain 13, or F_1 origin, the results were unequivocal. Primed $(2 \times 13)F_1$ T cells responded to DNP-GL on strain 2 or F_1 macrophages but not on strain 13 macrophages. In contrast, the same primed cell populations responded to GT on strain 13 or F_1 but not strain 2 macrophages (Fig. 4). These experiments were extended in mice by Sredni *et al.* (*63*) using a GLϕ-specific T cell line cloned from a responder B10.A(5R) mouse. Such GLϕ-specific clone lines only proliferated when presented GLϕ by antigen-presenting cells (macrophages) from high responder mice [B10.A(5R) or $(B10.A \times B10)F_1$] expressing both Ir-GLϕ α and β genes in the same cell. The need for Ia-bearing macrophages for T cell stimulation was further documented in our laboratory by Germain and Springer (*64*). Treatment of antigen-presenting cells with monoclonal antibody to Ia and complement abolished the ability of the cells to present antigen for proliferative responses to primed T cells (Table 3).

Another approach to the role of Ir genes in the presentation of antigen to T cells by macrophages involved the use of antiserums to Ia without complement to block antigen presentation. The original experiments were carried out in guinea pigs by Shevach, Paul, and Green (*65*) and later in mice by Schwartz and associates in collaboration with our laboratory (*66*). T cells from $(2 \times 13)F_1$ guinea pigs primed to DNP-GL and GT were exposed to DNP-GL or GT in vitro together with $(2 \times 13)F_1$ macrophages and alloantiserums directed to strain 2 or strain 13 Ia specificities. Antiserums to strain 2 Ia blocked the response to DNP-GL but not that to GT, whereas antiserums to strain 13 Ia blocked the response to GT but not to DNP-GL (Table 4). These experiments led to the conclusion that (i) Ir genes are expressed on antigen-presenting cells with the morphology of macrophages and (ii) T cells detect antigen on the surface of antigen-pre-

senting cells and are specific for foreign antigens perceived in the context of autologous Ia molecules.

Determinant selection for T cell responses clearly results from such a process. Thus, even in the case where two inbred strains are responders to the same T dependent antigen, the studies of Barcinski and Rosenthal (*67*) on the immune response of guinea pig to insulin revealed that strains 2 and 13 respond to distinct determinants; strain 2 T cells respond to a determinant on the A chain of insulin (A_8-A_9-A_{10}), whereas in strain 13 guinea pigs the response is directed to sequential determinants on the B chain of insulin involving the histidine at position 10 (*68*). Similar data concerning determinant selection in other antigens was reported by Berzofsky *et al.* (*69*) and Kipps *et al.* in our laboratory (*70*).

The genetic restrictions dictated by I region–controlled antigen presentation to T cells can also be observed when attempts are made to transfer DTH. I had made the puzzling observation with Paul and Green that delayed-type hypersensitivity to DNP-PLL in random bred guinea pigs could only be adoptively transferred to recipients that were also responders to this antigen (*71*). Using CR inbred strains of mice, Miller *et al.* (*72*) later showed that the successful transfer of delayed-type reactivity requires I region identity between the sensitized T cell and the recipient mice which provide the antigen-presenting macrophage when the test antigen is injected. Moreover, as expected, sensitized cells from (responder × nonresponder)F_1 mice did not transfer DTH to nonresponder recipients lacking the antigen-presenting cells (*73*).

At the same time as the group at the National Institutes of Health documented the importance of Ir genes and Ia molecules in antigen-presenting cells and their critical role in the presentation of antigen to T cells, experiments were carried out by Katz, Hamaoka, Dorf, and me (*74*) and by Kindred and Shreffler (*75*) demonstrating the role of I region genes in the control of T cell–B cell interactions in antibody responses.

Table 4. Alloantiserums to Ia block antigen presentation to primed T cells. The responding cells were (2 × 13)F_1 guinea pig T cells primed to DNP-GL and GT. Their response to DNP-GL was blocked by strain 13 antiserum to strain 2 and their response to GT was blocked by strain 2 antiserum to strain 13. These alloantiserums are specific for Ia antigens on strains 2 and 13, respectively. Adapted from Shevach *et al.* (*65*).

Antigen	Antiserum added	Increased DNA synthesis
DNP-GL	None	++++
DNP-GL	To strain 2	+
DNP-GL	To strain 13	++++
GT	None	++++
GT	To strain 2	++++
GT	To strain 13	+

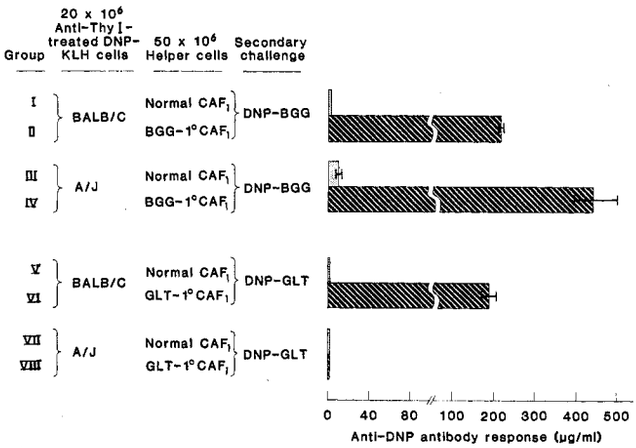

Fig. 5. Failure of GLT primed (*GLT-1°*) CAF_1 T cells to cooperate with nonresponder hapten primed parental A/J B cells, in contrast with the ability of BGG primed T cells to cooperate equally well with both parental A/J and parental BALB/c hapten primed B cells for secondary responses of antibody to DNP. From Katz *et al.* (*76*).

We devised a double adoptive transfer protocol whereby hapten-specific B cells and carrier-specific T cells from either the same parental strain or distinct parental strains were transferred to irradiated F_1 recipient mice prior to their receiving a secondary challenge (*74*). The results were unequivocal. Carrier-specific helper T cells and hapten primed B cells need to share I region genes for antibody response to develop to the hapten-carrier conjugate. Successful T cell–B cell interactions were observed between F_1 T cells and parental B cells, or parental T cells and F_1 B cells which only need to share one haplotype for successful responses provided both strains are responders to the carrier antigen used (Table 5).

When an antigen under Ir gene control was used, such as the copolymer GLT, (responder × nonresponder)F_1 T cells specific for GLT helped the responder but not the nonresponder hapten-specific B cells when they were challenged with DNP-GLT (*76*), indicating the critical role of Ir gene expression in B cells in T cell–B cell interactions (Fig. 5).

The need for I region identity for T cell–B cell interactions was confirmed by Sprent (*77*) and Kappler and Marrack (*78*) with different systems. More recently, Chiller, working with clonally derived antigen-specific helper T cell lines, observed the same I region requirement for successful T-B cell interaction.

The data of Singer *et al.* [see (*79*)] indicate that in certain experimental conditions where the antibody response involves solely unprimed B cells of the Ly b5 phenotype, antibody responses may be helped by T cells, across I differences. It is clear, however, that most of the responses of primed B cells require the type of I region–controlled T cell–B cell interaction discussed earlier. The specificity of the interaction for Ia is determined by the specificity of the T cell clones stimulated when antigen is originally presented by the Ia-bearing macrophages.

What is the mechanism of I region–controlled T cell–B cell interaction and Ir gene function at this level? The data are not as definitive as in the case of macrophage–T cell interaction discussed earlier. I feel, nevertheless, that substantial evidence exists for the view that murine Ly 1^+ T cells are specific for antigen perceived in the context of Ia molecules on antigen-presenting cells. The cells are stimulated to proliferate and differentiate into DTH or helper T cells. The helper T cells will in turn interact with Ia-bearing B cells which bound antigen through

Table 5. The I region genes restrict T-B cell cooperative interactions. Carrier primed T cells were adoptively transferred to (a × b)F_1 recipients that were irradiated; then hapten primed spleen cells (B cells) treated with antibody to Thy 1 and with C were adoptively transferred to the same (a × b)F_1 recipients. The animals were challenged with the hapten-protein conjugates and the secondary response to antibody to hapten was measured as an indication of T-B cell cooperative interactions. Adapted from Katz *et al.* (*74*).

H-2 haplotype of		Secondary responses in F_1 irradiated recipients
Hapten primed B cells	Carrier primed T cells	
a	a	++++
b	b	++++
a	b	–
b	a	–
b	(a × b)F_1	++++
a	(a × b)F_1	++++
(a × b)F_1	a	++++
(a × b)F_1	b	++++

their immunoglobulin receptors. The helper T cells deliver their differentiating signal by interacting with antigen and Ia molecules on B cells in a similar manner as on antigen-presenting cells.

The Ia Molecules Are the Ir Gene Products

There is now convincing evidence that Ia molecules are the Ir gene products and determine specific immune responsiveness to thymus dependent (T) antigens.

1) Ia molecules, the surface glycoproteins composed of α and β chains, expressed primarily on a population of macrophages and B lymphocytes, are coded for in precisely the same subregion of I in which Ir genes map: I-A and I-E.

2) Antiserums to Ia and particularly monoclonal antibodies to I-A specifically block responses in vitro by interacting with Ia molecules on antigen-presenting cells.

3) The mapping of the structural genes coding for the α and β chains of Ia molecules in the I region indicates an intimate correlation between chain structure and the control of the response to GLϕ by complementing α and β genes in I-A and I-E.

The structural analysis of Ia molecules and of their component chains in the mouse was carried out by several laboratories using the techniques of two-dimensional gel electrophoresis and peptide mapping to analyze the basis of polymorphisms.

Jones *et al.* (*80*) observed that, in strains bearing the appropriate H-2 haplotype, a gene in the I-E subregion controls the cell surface expression of an Ia molecule whose polymorphic determinants are largely controlled by the I-A subregion. Cook, Vitetta, Uhr, and Capra (*81*) and later Silver *et al.* (*82*) confirmed these findings and demonstrated that the I-E subregion controls the synthesis of the Ia Eα chain which on the cell surface is noncovalently associated with the β chain determined in the I-A subregion (Figs. 2 and 6). Strains with the H-2^b haplotype in the I-E subregion fail to synthesize this α chain and as a consequence the corresponding Ia molecule is not expressed on the cell membrane, although the β chain coded in I-A is synthesized and found in the cytoplasm.

The genetic control of the polypeptide chains of this class of Ia molecules by I-E and I-A correlates completely with the Ir gene complementation observed in the response to GLϕ discussed earlier (*44*).

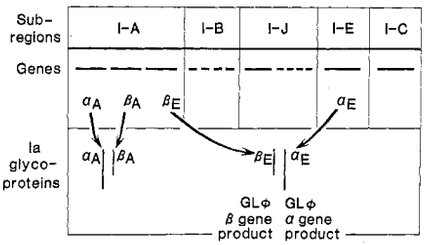

Fig. 6. Model for the genetic and structural basis of Ir gene complementation in the response to GLϕ. The products of the α and β Ir genes required for GLϕ presentation are postulated to consist, respectively, of an Ia.7 bearing α chain (designated α_E) encoded in the I-E subregion and of a β chain (designated β_E) encoded in the I-A subregion which interact selectively to form a functional Ia molecule on the cell membrane of the antigen-presenting cells.

Table 6. Three different types of MHC restriction of GAT-specific T cell colonies from (B10.A × B10)F_1 mice primed to GAT. (B10.A × B10)F_1 mice were immunized with GAT; their T lymphocytes were stimulated in vitro with GAT and cloned in soft agar. From each colony, 2×10^4 T cells were stimulated with 100 μg of GAT in the presence of antigen-presenting cells from B10, B10.A, or (B10.A × B10)F_1 mice. Adapted from Sredni *et al.* (*63*).

Colony number	Proliferative response to GAT on spleen cells from			H-2 restriction
	B10.A	B10	(B10.A × B10)F_1	
1	++++	−	++++	B10.A
4	++++	−	++++	B10.A
8	++++	−	++++	B10.A
12	++++	−	++++	B10.A
2	−	++++	++++	B10
3	−	++++	++++	B10
5	−	++++	++++	B10
9	−	++++	++++	B10
10	−	++++	++++	B10
13	−	++++	++++	B10
6	−	−	++++	(B10.A × B10)F_1
11	−	−	++++	(B10.A × B10)F_1
14	−	−	++++	(B10.A × B10)F_1

Moreover, those strains that exhibit a responder α gene at I-E synthesize an α chain controlled by this locus. It is indeed fortuitous that we called the I-E gene α and the I-A gene β at a time when we did not know that they determined, respectively, the α and β chains of the corresponding Ia molecule.

The availability of cloned lines of antigen-specific T cells and of monoclonal antibody to Ia provided still more conclusive evidence for the identity between Ia molecules and Ir gene products. Sredni, Schwartz, and associates (*63*) cloned a GLϕ-specific T cell line derived from a B10.A(5R) responder mouse which was stimulated only by GLϕ presented on B10.A(5R) or (B10 × B10.A)F_1 cells. The response of this clone to GLϕ in vitro is blocked by a monoclonal antibody to Ia specific for the conformational determinants (*83*) and the Ia molecules resulting from the interaction of the I-E coded α chain with the I-A coded β chain.

The other major murine Ia molecule has both α and β chains coded for in I-A. The possibility still exists for genetic complementations at the molecular level corresponding with Ir gene complementation in animals heterozygous at I-A, which is precisely what occurs. Such complementation is more difficult to detect and depends on clonal analysis of T cells specific for an antigen, the response to which is controlled at I-A, such as the terpolymer GAT. Cloned T cell lines specific for GAT were selected by Fathman and co-worker (*84*) and by Sredni *et al.* (*63*) from a (B10.A × B10)F_1 mouse immunized with GAT. Some of these clones responded to GAT when the polymer was presented by B10.A macrophages, other clones responded to GAT on B10 macrophages, and a third type of clone responded to the antigen only when presented on (B10.A × B10)F_1 macrophages (Table 6). We can conclude that the response to GAT in (B10.A × B10)F_1 mice is determined by three types of genetically distinct I-A coded Ia molecules which, together with antigen, specifically select the three types of clones stimulated. The extent to which these three Ia molecules interact with the same determinant on the GAT antigen or with different ones has not been ascertained.

Significance of Ir Gene Specificity

We have made considerable progress in our understanding: (i) of Ir gene function in antigen-presenting cells and in B cells, (ii) of the identity of Ia molecules

and Ir gene products, and (iii) of the commitment of murine T cells with the Ly 1^+ phenotype (*85*) to react with autologous Ia molecules and antigens. But an important issue remains unresolved which concerns the process by which the specificity of Ir gene function is imparted, that is, why certain Ia molecules on antigen-presenting cells determine T cell response to some antigens and not to others. The issue can also be presented in other terms, that is, what mechanism determines the development of T cell clones with combined specificity for autologous Ia molecules and selected antigens.

T cells bear receptors coded at least in part by the immunoglobulin H chain linkage group as shown by Binz and Wigzell (*86*), Eichmann (*87*) and our laboratory (*88–90*). Ir genes do not need to be expressed in T cells for responses to occur. T cells become committed to host MHC specificities as they differentiate. Thus nonresponder parental T cells can be turned into responder T cells by being developed in (responder × nonresponder)F_1 irradiated recipients (*73, 91*). Such T cells respond to the putative antigen if it is presented on responder macrophages bearing the appropriate Ia molecules with which the T cells interacted during differentiation.

Two types of hypotheses have been formulated to account for Ir gene controlled restrictions. Von Boehmer, Haas, and Jerne (*92*) proposed that T cells generate their repertoire for foreign antigens from their receptors for autologous MHC antigens and that Ir gene defects reflect the absence of clones bearing receptors for certain antigens, based on the restriction placed on the repertoire by the commitment of T cells to a particular set of autologous MHC antigens. As T cells differentiate and are selected to react with different MHC antigens in different individuals, different H-linked Ir gene defects result.

An alternative hypothesis was proposed independently by Rosenthal (*93*) and myself (*94*). It postulates (i) that Ia molecules are capable of reacting selectively with certain amino acid sequences on protein antigens and (ii) that such a selective interaction in antigen-presenting cells results in the formation of an Ia molecule–antigen complex reactive with T cell clones differentiated to bear receptors for autologous Ia and antigen (Fig. 7).

A limited number of such binding sites on a relatively small number of Ia molecules can generate from available antigens an almost unlimited number of determinants specifically recognized by T

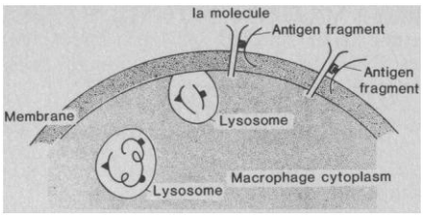

Fig. 7. Graphic representation of the specific interaction proposed between Ia molecules and antigen fragments on the surface of antigen-presenting cells (macrophages) required for specific interaction with T cells.

cells. The size of the binding site on the Ia molecule for the antigen or its fragment should encompass a limited number of amino acids in order not to impose undue restrictions on the system and to permit a given site to bind to a great variety of foreign proteins. The likelihood that such a sequence is present in a given protein varies inversely with the size of the sequence and is considerable for a postulated size involving three or at most four amino acids. The location of the binding sites will vary in different proteins. This dictates the antigenic determinants with which T cells react in conjunction with Ia molecules. A given Ia molecule could thus react with a large number of antigens and yet impose unigenic restriction to immune responsiveness.

The identification of the amino acid sequences critical for immunogenicity may provide some indication of the size of the postulated site of interaction with the Ia molecules. The studies of Schlossman and associates (*95, 96*) on the immunogenicity of DNP-oligo-L-lysines for strain 2 guinea pigs is very informative. The smallest oligolysine polymer which is immunogenic has seven lysines. However, a peptide containing only four lysines and a sequence of L-alanines, terminated with a DNP-lysine, is equally immunogenic in strain 2 guinea pigs, although the specificity of the response is different. It would appear, therefore, that the critical interaction site in this antigen for strain 2 guinea pigs may consist of at most four lysines. The data on the immunogenicity of insulin A chain for strain 2 guinea pigs and of B chain for strain 13 guinea pigs (*68*) and on the precise amino acids responsible suggest also an interaction site involving three or at most four amino acids.

Table 7. H-2 (I region) control of suppressor T cell responses to the copolymer GT. The GT (100 μg) was administered intraperitoneally; 3 days later the experimental and matched control groups were immunized with GT coupled to the immunogenic carrier, methylated bovine serum albumin, and the antibody responses compared to determine the suppression elicited by prior immunization with GT. Dominant GT-specific suppression can be generated in mice with H-2^d, H-2^f, and H-2^s but not the H-2^a or H-2^b haplotypes. The responsible genes mapped in the I region. In transfer experiments the GT suppression was shown to be mediated by suppressor T cells. Adapted from Debré *et al.* (*123*).

Strain	H-2	Percentage suppression	*P* values
A/J	a	0	< .4
A.By	b	0	< .3
C57BL/J	b	0	< .1
129/J	b	0	< .1
BALB/c	d	80	< .000001
DBA/2	d	81	< .001
D1.C	d	76	< .002
A.CA	f	100	< .00009
SJL	s	72	< .000001
A.SW	s	68	< .001
CAF_1	a/d	74	< .0001

Although the second hypothesis appears more compatible with the available findings, definite evidence of Ia molecule–antigen interaction is lacking. Some recent experiments of Nepom and Germain in our laboratory may also be interpreted to indicate a necessary interaction between Ia molecule and antigen for binding of the complex to T cells. We have indeed observed that when F_1 T cells specific for antigens under Ir gene control such as GAT or GLϕ are stimulated to proliferate by antigen, they selectively bind autologous Ia molecules of precisely the type which determined responsiveness to the antigen. Considering that some of the T cells in the population studied should have been specific for the allelic Ia molecule, the selective binding observed may indeed imply a requisite interaction between Ia molecules on macrophages and antigen.

I Region Control of T Cell Suppressor Responses

Selected antigens such as the terpolymer GAT induce preferentially suppressor T cells in certain nonresponder mouse strains, which contribute to the unresponsiveness observed (*97*). These T cells, adoptively transferred, suppress the GAT antibody response to GAT that is coupled to an immunogenic carrier. Preferential suppressor T cell responses were also observed for other antigens, such as for the copolymer GT (*98*) reported by us and hen egg lysozyme reported by Sercarz and associates (*99*).

The ability to develop specific suppressor T cells also proved to be con-

trolled in the I region of the murine H-2 complex (Table 7). The analysis of the genes responsible, of their products, and of the processes involved in the generation of specific suppressor T cells has not yet permitted a definitive understanding of these complex phenomena. As in the case of I region control of T cell responses, immunogenicity of a complex antigen is determined by the determinants it bears. Apparently certain determinants in mice of the appropriate H-2 haplotype induce selectively suppressor T cell responses (*99, 100*). Moreover, the presence of determinants that stimulate suppressor T cells preferentially may result in suppression of responses to other determinants on the antigen which otherwise would stimulate helper T cells and thereby antibody responses.

In addition to this type of I region control of suppressor T cell responses which is specific and determinant oriented, the I region affects suppressor T cell responses by coding for specificities expressed on all suppressor T cells. Murphy *et al.* (*101*) and Tada *et al.* (*102, 103*) discovered that a new subregion of I, the I-J, controls alloantigens expressed only on suppressor T cells. All the T cells in the suppressor T cell circuits bear I-J coded specificities. Moreover, antigen-specific suppressor factors were extracted from antigen-specific suppressor T cells (*104, 105*). Such factors were shown to stimulate the generation of suppressor T cells (*106*) and to bear on the same molecule determinants coded by the I-J subregion as well as idiotypic determinants coded for by the immunoglobulin heavy chain linkage group (*88, 89*).

Specificity of T Cells for MHC Antigens

I have already discussed the commitment of helper and DTH T cells to react with antigen and autologous Ia molecules. An analogous commitment of cytolytic T cells (CTL) to histocompatibility antigens of the MHC has been demonstrated by Zinkernagel and Doherty (*107*), by Shearer *et al.* (*108*), and by Bevan (*109*) illustrating the general nature of the commitment of T lymphocytes to react with antigen only on cell surfaces and in relation with gene products of the MHC.

Zinkernagel and Doherty (*107*) demonstrated that CTL from mice immune to lymphocytic choriomeningitis virus (LCM) only lyse LCM-infected target cells which share H-2 antigens with the killer cell. The MHC loci concerned map at either K or D of the H-2 complex.

Table 8. Mouse cytolytic T cells (CTL) specific for Sendai-infected syngeneic target cells also lyse noninfected allogeneic target cells. Adapted from Finberg *et al.* (*119*).

Target cells	Specific ^{51}Cr release (%)
B10.D2-Sendai (H-2^d)	78
B10.G (H-2^b)	37
B10.G (H-2^q)	12
B10.B2 (H-2^k)	38
B10.RIII (H-2^r)	46
B10.S (H-2^s)	28
B10.D2 (H-2^d)	1

Thus CTL recognize antigen in the context of the K or D histocompatibility antigens, like helper cells react with antigen and Ia molecules.

The evolutionary significance of these restrictions and of the role played by MHC antigens becomes readily apparent when we consider that T cell immune responses are primarily responsible for monitoring self and nonself on cell surfaces. T cells need to determine when an autologous cell becomes malignant or virally infected and must be destroyed. This surveillance function is optimally performed if a large number of T lymphocyte clones are specialized to detect small variants on MHC molecules. Taking advantage of this process, T cells have also evolved the capacity to regulate immune responses as a consequence of their ability to recognize antigen on cell membranes. Because unregulated immune responses can be very harmful, we have developed highly specific T cell mediated mechanisms of immune regulation which require the recognition by T cells of clones of other immune cells bearing antigen.

Because of the two types of MHC specificities exhibited by helper and cytolytic T lymphocytes, two types of Ir gene defects can be observed in CTL responses. A major type of Ir gene defect maps in the I region (*92, 110*) and concerns the generation of helper cells, as in the case of antibody. The other type maps in K (*111*) or D and reflects the ability of CTL clones to react with antigens on cell surfaces as they are presented in relation with K or D gene products.

The major topic here has been the specificity of T cells for autologous MHC antigens and the manner in which foreign antigens are perceived by T cells in the context of MHC gene products. We postulate that MHC antigens have evolved for precisely this function. Yet they were originally discovered by Gorer, Lyman, and Snell (*112*) and identified in a different context as the major antigens, within a species, responsible for alloreactivity and the rejection of allografts, a phenomenon which is of limited evolutionary value. The issue of the origin of alloreactivity can now be appropriately addressed as it appears to be closely related to the process whereby T cells become committed as a class to reactivity with autologous MHC antigens during differentiation.

Jerne (*113*) proposed a theory which was further elaborated by ourselves (*114, 115*) to explain the generation in the thymus of T cells specific for autologous MHC antigens. According to the theory, in the first stage T cells initially specific for self MHC gene products are selected in the thymus to differentiate and proliferate. Then, in a second stage, only those T cells which bear low-affinity receptors for self MHC antigens are allowed to mature and leave the thymus as functional T cells. Such T cells, having low reactivity for self MHC antigens, have concomitantly high affinity for variants of self MHC antigens. These variants appear to be the same as or similar to the allogeneic MHC antigens expressed in the same species. Weaker affinity for xenogeneic MHC antigens would thus be expected. Simultaneously and independently these T cells develop recognition for determinants on conventional thymus dependent antigens.

The high degree of reactivity to MHC antigens that constitute the polymorphic population encountered in the same species (that is, alloantigens) and the lower reactivity to xenogeneic MHC antigens may be attributed to the fact that low-affinity receptors for self MHC antigens are expected to react optimally with allogeneic MHC antigens, but much less so with xenogeneic antigens. This would account for the paradox that the strongest T cell responses are not elicited by antigens further removed phylogenetically from the responder. Two predictions from this theory are: (i) that clones of T cells induced by xenogeneic MHC antigens should be highly cross-reactive with allogeneic MHC antigens, even to the extent that they may demonstrate a heteroclitic specificity. This has indeed been demonstrated by Burakoff *et al.* when mouse antiserum to rat CTL was shown to be comprised of clones cross-reactive with allogeneic target cells (*116*). (ii) Alloreactive T cells should be expected to be highly cross-reactive with modified syngeneic cells. This was also shown to be the case when we observed considerable cross-reactivity by alloreactive cells for trinitrophenyl (TNP) conjugated target cells syngeneic to the responder (*114*).

Since the T cell repertoire for MHC

specificities is normally determined by the self MHC antigens of the thymus, we should expect the T cell repertoire to vary according to the MHC of the thymus in which T cells differentiate. Recent experiments with radiation-induced chimeras by Zinkernagel and associates (*117*) and Bevan (*118*) have demonstrated this to be the case.

The postulate that alloreactivity results from T cells differentiating in the thymus that are strongly reactive for variants of self MHC antigens leads to the expectation that immunization with virally infected syngeneic cells should result in the stimulation of T cell clones reactive with the virally infected syngeneic cells used for immunization and also reactive with uninfected allogeneic target cells.

Finberg *et al.* in our laboratory have recently shown that immunization of BALB/c (H-2^d) mice with syngeneic cells coated with Sendai virus stimulates CTL which lyse Sendai-coated BALB/c target cells but also lyse uncoated H-2^b, H-2^q, H-2^k, H-2^s, and H-2^r allogeneic target cells to an appreciable degree (*119*) (Table 8). We further demonstrated by the cold target inhibition technique that the same clones that lysed Sendai virus–coated BALB/c targets also cross-reactively lysed the allogeneic targets (Fig. 8). Furthermore, separate CTL clones lysed each of the different allogeneic targets. In addition, there was significantly less lysis of target cells bearing the H-2^q haplotype than of target cells bearing the H-2^k or H-2^s haplotypes. This latter finding suggests that the association of Sendai virus antigens with the H-2^d gene products of BALB/c mice creates determinants that are more cross-reactive with H-2^k and H-2^r than with H-2^q gene products. Using cloned T cell lines, von Boehmer *et al.* (*120*) confirmed the alloreactivity of CTL specific for virally infected syngeneic cells and Sredni and Schwartz (*121*) extended the observation to T cells reactive with autologous I region products and foreign antigens. B10.A T cells specific for DNP-ovalbumin (DNP-ova) were cloned on macrophages exposed briefly to DNP-ova. Such cloned lines proliferated specifically when exposed to DNP-ova on syngeneic macrophages. Some of these clones could also be stimulated to proliferate by H-2^s allogeneic macrophages in the absence of DNP-ova (Table 9). Therefore, the same clone selected on DNP-ova exposed B10.A macrophages reacted identically to DNP-ova exposed B10.A macrophages or B10.S macrophages without antigen. In both cases the ability to stimulate mapped in the I-A subregion, demonstrating that alloreactivity to I region antigens also arises as a consequence of the commitment of T cells to autologous MHC specificities.

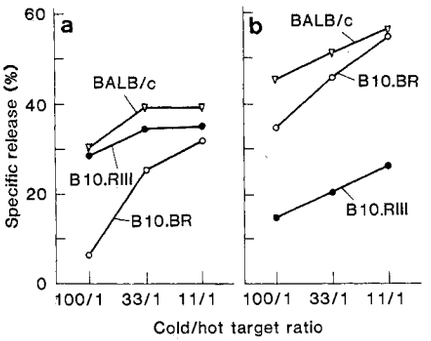

Fig. 8. Mature cytolytic T cells specific for Sendai-infected syngeneic target cells cross-reactively lyse noninfected allogeneic target cells. By using cold target inhibition of lysis by CTL, this experiment illustrates that distinct populations of Sendai-specific CTL lyse different allogeneic targets. In (a), targets are $^{51}C_2$-labeled B10.BR; in (b) targets are $^{51}C_2$-labeled B10.RIII. Adapted from Finberg *et al.* (*119*).

The remaining issue concerns the precise nature of the T cell receptor and how the specificity for foreign antigens and MHC coded molecules is concomitantly maintained. On the basis of idiotypic and genetic evidence, the variable region of immunoglobulin heavy chains appears to be responsible for both the specificity directed to MHC and the specificity for foreign determinants, when these are analyzed independently. The problem still remains whether T cells have one receptor or two coupled receptors and whether one or two variable, heavy chain regions are involved. Moreover, the significance of I region coded determinants on antigen-specific, idiotype bearing regulatory products on T cells must be clarified. I am not tempted to guess at the answer considering the present availability of cloned lines of specific T cells, and of T cell hybrids. A definitive answer should be forthcoming from the laboratory, and the genes coding for the T cell receptors will soon be identified.

Table 9. B10.A clones specific for antigen and self Ia may also be selectively alloreactive. A colony of DNP-ova specific proliferating T lymphocytes was derived from lymph node cells of a B10.A mouse immunized with DNP-ova. A cloned line was derived which was subcloned. Such cells at a concentration of 10^4 or 2×10^4 show reactivity both to DPN-ova or B10.A cells and to B10.S without antigen. Adapted from Sredni and Schwartz (*121*).

Source of spleen cells	H-2	Proliferative response (count/min)	
		Clone 5	Subclone 5.6
B10.A	a	150	180
B10.A + DNP-ova	a	14,700	39,200
B10	b	130	233
B10.D2	d	236	410
B10.S	s	15,900	36,300

Conclusions

The evolutionary significance of the commitment of T cells to MHC antigens should be assessed from several vantage points. From the point of view of the individual concerned, the existence of such a broadly polymorphic system to determine specific responsiveness and suppression will unescapably result in individuals with different immunological potential to a given challenge. Some will clearly be at greater risk, whereas others will be better prepared to resist certain infectious agents, and it is not surprising that immunological diseases are linked to the MHC. As far as the species is concerned, this polymorphic defense system results in a very significant survival advantage to unforeseen challenges and a better possibility for the immune system to adapt to evolutionary pressures.

As biologists we contemplate with admiration and awe the wondrous array of sophisticated cell interactions and recognitions evolved in the T cell immune system which must be a model for other similarly complex biological systems of highly differentiated organisms.

I would like to express my appreciation and affection for my numerous students and associates who have shared with me in the toil and should now equally share in the honor. I am also grateful to the National Institute of Allergy and Infectious Diseases which supported my work faithfully and generously since 1957, and housed me from 1968 to 1970, and to the National Cancer Institute which supported my program for the last 10 years. I owe also a debt of gratitude to New York University Medical School and to Harvard Medical School which provided the stimulating academic environment without which our work could not have progressed.

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