Significance and Biological Function of Class II MHC Molecules

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THE TRANSPLANTATION of allogeneic and even xenogeneic tissue grafts has been attempted unsuccessfully for centuries. In 1936, Peter Gorer and George Snell demonstrated the immunologic basis of allograft rejection and established that the rejection of tissue transplants between two incompatible individuals of a species is the result of a specific immunologic reaction directed against highly polymorphic cell surface antigens.[1]

The membrane components against which allograft immunity is directed were named histocompatibility antigens. Furthermore, Gorer and Snell made the important discovery that the stronger histocompatibility antigens, in the mouse, are coded for by a group of closely linked genetic loci, the major histocompatibility complex (MHC), H-2 in the mouse. The corresponding major histocompatibility complex of man, HLA, was identified subsequently by Dausset[2] and by Van Rood.[3] In addition to the rejection of tissue grafts, the genes of the MHC and the antigens they determine are responsible for all immune phenomena between histoincompatible individuals, the mixed leukocyte reaction (MLR) and the graft-versus-host (GVH) reaction.

I propose to review what has been learned concerning the genes of the MHC and the antigens they code for from the contributions of many laboratories. I shall then discuss their fundamental role in the regulation of immunity by thyumus-derived (T) lymphocytes, their contributions to the specificity of T lymphocytes, their requisite involvement in essential cell interactions in immune responses, and their biologic significance in the broadest evolutionary sense.

Structure of MHC Antigens

Two classes of MHC antigens have been identified: Type I molecules, expressed on the surface of all cells, and Type II molecules, expressed selectively on the cells of the immune system, such as antigen-presenting cells (macrophages and dendritic cells), B lymphocytes, and activated T lymphocytes. The molecular structures of these antigens are now well understood, based predominantly on the contributions of Strominger's laboratory.[4] Moreover, the genes coding for their polypeptide chains have been cloned and sequenced in the mouse and in man as the contribution of the efforts of many laboratories.

Type I antigens consist of a 45,000-dalton polymorphic chain complexed to β_2-microglobulin, whereas Type II antigens, also called Ia (immune-response–associated) contain two polymorphic chains with 33,000 and 28,000 daltons, respectively, and a recently discovered invariant chain. The structure of Class I and II antigens in the mouse and in man revealed considerable homology between the two species. More important, both the 45,000-dalton Class I chain and the 33,000- and 28,000-dalton Class II chains are constructed by domains similar to those of immunoglobulin chains. Moreover, significant homologies exist between Class I and Class II polypeptides and between these and immunoglobulins, suggesting a common ancestor for these immunologically important molecules.

The most important and characteristic property of histocompatibility antigens is their extensive polymorphism, which is not encountered in any other class of molecules in higher organisms. This polymorphism is so extensive that, in a randomly bred population, all individuals are expected to differ in at least some of the antigens of this complex and, as a consequence, to ex-

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press unique individualism and reject one another's tissues. The antigens of the MHC are also unique in their ability to elicit powerful immune responses from thymus-derived lymphocytes, which are responsible, indeed, for the prompt rejection of allogeneic tissues and cells. Alloreactivity toward allogeneic MHC antigens of the same species is such an important concern of T lymphocytes that a large fraction of these cells appears to be specific for MHC alloantigens, even previous to immunization.

Biologic Significance of MHC Antigens

The histocompatibility antigens of the MHC have presented a considerable challenge to immunologists, because it is evident that the rejection of allografts does not have survival value, and that this complex and costly system did not evolve for this trivial purpose. This complex system, which is found in many vertebrate species other than mammals, must have more important evolutionary significance and function. The answer to this question was provided by discoveries in fields other than transplantation immunology. The study of the genetic control of specific immune responses to thymus-dependent antigens revealed that such responses are determined by a class of MHC genes regularly expressed on accessory cells and B cells.[5] Moreoever, the analysis of the specificity of T lymphocytes and of their regulatory interactions with other cells of the immune system demonstrated the important role played by MHC genes in these phenomena.

The following points have been established:

1) MHC antigens are concerned with our ability to distinguish self from nonself.[6]

2) T cells react to conventional foreign antigens only when presented on cell surfaces in conjunction with autologous MHC antigens.[7] MHC molecules, therefore, govern the necessary cell interactions concerned with antigen-induced stimulation between the various cells of the immune system, T cells, B cells, and macrophages.

3) Alloreactivity and our inability to transplant tissues is the price we pay for the benefits to the species of this complex recognition system.[8,9]

4) The extensive polymorphism of MHC antigens has as its consequence that individuals differ in their ability to respond specifically to selected antigens[5,6] and to develop immunologic diseases.[10]

Insight into the biologic significance of MHC molecules arose from several experimental approaches. Initially, studies in our laboratory[11] and in McDevitt's[12] revealed that genes in the I region of the murine H-2 complex determine the ability of T lymphocytes to respond to thymus dependent antigens. We called these genes "immune response (Ir) genes." Different individual and different inbred strains varied, therefore, in their ability to respond to an antigen under Ir gene control and were classified as responders and as nonresponders. Numerous studies over the years, in our laboratory and others, established that Ir genes code for the polypeptide chains of Class II MHC (Ia) molecules expressed for this purpose on the membranes of antigen-presenting (accessory) cells and of B lymphocytes.

In an attempt to elucidate the function of Ir genes, several laboratories focused their attention on the role of Ia molecules in specific immune responses. Rosenthal and Shevach[7] demonstrated that T lymphocytes only react to foreign antigens if they are presented by antigen-presenting cells together with autologous Ia antigens. Moreover, the T-cell clones in F_1 animals were shown to be restricted specifically to one or the other parental Ia molecules and the foreign antigen.[13] Accordingly, anti-Ia antibody was able to block specifically the interaction of antigen-presenting cells and antigen-specific T lymphocytes.[14]

At the time when these findings were made, Kindred and Shreffler[15] and Katz et al[16] observed that the interactions between helper T cells and the B cells they regulate were equally restricted by Ia molecules. The helper T cell is restricted to interact with a B cell bearing the same autologous Ia molecule as the macrophage that originally presented it with antigen. In addition, this restriction is clearly concerned with Ir-gene function, because a (responder × nonresponder) T cell can provide help for a responder B cell and not for a nonresponder B cell.[17] We concluded that helper T lymphocytes are specific for both autologous Ia molecules and foreign antigens presented jointly, and that a T-cell clone so selected in the course of immunization is restricted to interact with B lymphocytes bearing antigen in the same manner. The more recent experiments with cloned B-cell and T-cell lines have substantiated these conclusions. Moreover, the observation that specific B cells are able to bind antigen and present it very efficiently to primed T cells accounts in part for the MHC restriction of T- and B-cell interactions.[18]

Following a different line of investigation, Zinkernagel and Doherty[19] arrived independently at substantially identical conclusions, with respect to Class I MHC molecules and cytolytic T lymphocytes. They reported that cytolytic T lymphocytes (CTLs) from mice immune to the lymphochoriomeningitis virus (LCMV) lysed LCMV-infected target cells bearing the same Class I MHC antigen as the immunized host, but not LCMV-infected allogeneic cells, bearing distinct Type I MHC antigens. They concluded that CTLs were restricted to

interact with foreign antigens only when presented in the context of autologous Class I MHC antigens.

Thus, different classes of T lymphocytes are restricted by different classes of MHC molecules in their ability to recognize and interact with foreign antigens.

Recent studies with human CTL cloned lines by Meuer, Schlossman, and Rhinehurst[20] have established that in man also different classes of T lymphocytes are specific for Class I and for Class II MHC antigens; CTLs specific for Class I alloantigens bear the T8 surface antigen, whereas CTLs specific for human Class II alloantigens (DR) bear T4 membrane glycoprotein. In addition, anti-T8 and anti-T4 antibody inhibit lysis of CTLs specific for Class I and Class II antigens, respectively, which suggests that in man T4 and T8 glycoproteins are directly concerned with the specificity of MHC restriction phenomena in this species.

The Origin of Alloreactivity

We can now consider the biologic mechanism for alloreactivity and therefore for our inability to accept allografts. Alloreactivity is the unavoidable consequence of the commitment of T lymphocytes to express receptors specific for autologous MHC antigens in relation to foreign antigens. Such receptors would be expected to cross-react with the variants of autologous MHC antigens expressed in other individuals of the same species. This hypothesis postulates that if one examines individual clones in a population of immune T cells specific for a foreign antigen "X" in conjunction with a self-MHC antigen, selected T-cell clones in this population should express reactivity for a particular allogeneic MHC antigen of the same class, in the absence of antigen X. Distinct clones, specific for the same foreign antigen X together with an autologous MHC antigen, should cross-react with different allogeneic MHC antigens. This type of cross-reactivity was detected initially in our laboratory[8] and has been verified since then in other laboratories as well[9] for both Class I and Class II MHC antigens in the mouse.

Our inability to accept allogeneic grafts results, therefore, from 1) the heterogeneity of clonally expressed T-cell receptors specific for autologous MHC antigens and foreign antigens and 2) the cross-reactivity that exists between autologous MHC antigens and foreign antigens and allogeneic MHC antigens of the same species.

The Interaction Between Class II MHC Molecules and Antigens as a Basis for Ir Gene Specificity

From the initial observations of Ir-gene control of specific immune responsiveness, it was evident that the specificity of Ir-gene function is considerable and is able to discriminate between single amino acid substitutions in protein antigens. However, there is lack of agreement concerning the mechanism by which MHC molecules, and more particularly Ia antigens, exert their specific control of selected immune responses. Two distinct, but in my opinion not mutually exclusive, mechanisms have been proposed to account for the manner in which MHC molecules restrict the responsiveness of T cells to foreign antigens to individuals bearing the appropriate MHC antigens. Rosenthal[21] and we,[22] impressed by the extent Ir genes are involved in the selection of the particular epitope on an antigen against which T-cell responses are directed, proposed that antigen or its processed fragment associates specifically with Ia molecules on the surface of the antigen-presenting cell before interaction with the T-cell receptor. Such an association was deemed to be necessary for the complex Ia-foreign antigen to be reactive with the T-cell receptor and to be responsible for the phenomenon of determinant selection, alluded to earlier. Alternatively, other investigators[23,24] postulated that the specificity of Ir-gene function is the indirect consequence of the selective effects of autologous Class II MHC molecules on the T-cell–specific repertoire during differentiation in the thymus. These authors proposed that one of the consequences of the induction of tolerance to autologous molecules presented in the context of self-MHC molecules in the thymus, is the loss of T-cell clones capable of reacting with selected foreign antigens presented with self-MHC molecules when these complexes resemble structurally the tolerizing complexes.

As I stated earlier, these two mechanisms are not necessarily exclusive.

The direct demonstration of the interaction of Ia molecules and antigen on the surface of the antigen-presenting cell has been very difficult to document by conventional techniques. The evidence for Ia-antigen interaction has therefore relied on a functional analysis of the effect of this postulated interaction on the reactivity of the resulting Ia-antigen complex, using selected T-cell clones and their receptors as specific probes. This type of experiment has depended upon the prior isolation of T-cell hybrid cell lines, with the appropriate combined specificity for the autologous MHC molecule and the foreign epitope. The reaction of these specific T-cell lines with the Ia-antigen complex on the antigen-presenting cell is generally detected by the secretion of IL-2, assayed on the proliferative capacity of an IL-2–dependent T-cell line. Another important feature of the experimental approach used to document Ia-antigen–specific interactions has been the prior antigen pulsing of the Ia-bearing, antigen-presenting cell,

which in certain selected cases can be a cloned B-cell line such as the A-20, H-2^d cell line, before addition to the reactive T-cell hybridomas, used as the specific probe. With this approach we can appropriately investigate the interaction of the Class II MHC molecules and the antigen on the antigen-presenting cell before the binding of the complex by the T-cell receptor.

In a first series of experiments, we explored whether the phenomenon of antigenic competition could be used to demonstrate Ia-antigen interaction at the level of the antigen-presenting cell (APC). This approach was suggested by the recent report by Werdelin[25] of antigenic competition for presentation by accessory cells to T cells, *in vitro*, in the guinea pig, between antigens under the control of the same Ir gene. We developed a series of T-cell lines specific for the copolymer of L-glutamic acid, L-alanine, L-tyrosine (GAT) from GAT-immunized H-2^d BALB/c or H-2^b Bl10 mice. These cells were hybridized with the myeloma BW5147.[26] The hybrid cloned lines were reactive with GAT presented by H-2^d or H-2^b APCs, respectively, and responded by the secretion of IL-2 to such stimuli. Moreover, a fine analysis of the restricting MHC subregion, with anti-Ia moloclonal antibodies, revealed that both H-2^d and H-2^b clones reacted with GAT in the context of I-A-subregion–coded molecules. None of these T-cell hybrid clones responded to the copolymers of L-glutamic and L-tyrosine (GT), L-alanine (GA), or L-lysine (GL).

We then investigated whether GT was able to inhibit stimulation of the GAT-specific clones by GAT. We were able to establish that GT at the nontoxic concentration of 0.1 mg/ml was able to significantly interfere with the stimulation of GAT-specific, H-2^d, BALB/c clones by GAT on syngeneic APCs[26] (Figure 1). However, the inhibition by GT of the GAT response was only observed with H-2^d but not with H-2^b T-cell hybrid lines. We then analyzed the site where the competition occurred by prepulsing with GT at the appropriate concentration either the antigen-presenting cells (which were either irradiated BALB/c spleen cells or the A20 B cell cloned line) or the T-cell GAT-specific clones before stimulation with GAT. These experiments revealed that the site of the competition was, without doubt, the antigen-presenting cell and not the specific T-cell clone. Furthermore, we noted that the inhibition by GT was totally reversible by an appropriate concentration of GAT added to the cultures.

The finding that the copolymer GT was able to compete with GAT on H-2^d but not on H-2^b APCs showed that the phenomenon of antigen competition is intimately related to that of MHC restriction. It also allowed us to design an experiment to eliminate the possibility that the antigenic competition observed could

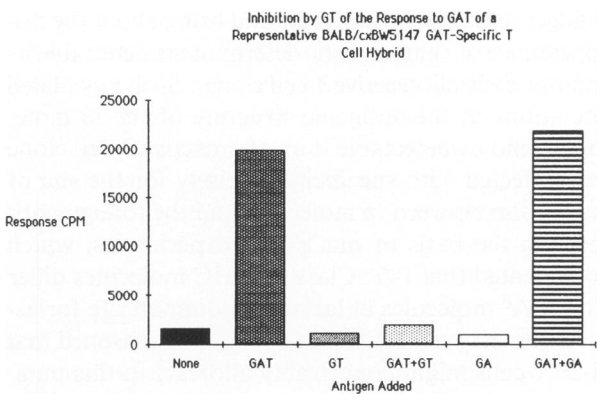

Figure 1—Inhibition by GT of the response to GAT of a representative BALB/c × BW5147 GAT-specific T-cell hybrid. Primary cultures were prepared with 5 × 10^4 cell hybrids in 0.2 ml with 10^6 x-irradiated BALB/c splenocytes as accessory cells and assayed for IL-2 content. Counts per minute (CPM) measure the incorporation of ^{3}H-thymidine by an IL-2–dependent cell line. Antigens were added at a final concentration of 0.5 mg/ml.

be explained on the basis of a common antigen-processing step in the accessory cell. We used the same irradiated (b×d)F$_1$ spleen cells as antigen-presenting cells with both H-2^d and H-2^b hybrid T-cell lines. The (b×d)F$_1$ cells were pulsed with GAT in the presence of a competing concentration of GT previous to being added to the H-2^d or H-2^b GAT-specific T-cell lines. GT interfered with the response of the H-2^d, but not of the H-2^b, T cells to the GAT-pulsed F$_1$ APCs, thereby demonstrating that the competition, at the level of the accessory cell, was an event which occurred after all common antigen-processing steps had taken place. We interpreted these data as strong evidence for a specific but reversible interaction between the I-A^d molecule and the antigens GAT and GT, respectively, on the surface of the antigen-presenting cells. Since completing these experiments, we have been able to demonstrate similar competition phenomena in another Ir-gene–controlled system.[27]

We have also sought to test some of the predictions of a model of specific interaction between Class II MHC molecules and antigens, which we advocate takes place previous to antigen presentation to T-cell receptors, more directly. One of the possible consequences of interaction between Ia molecules and antigen is a change in the structure of the Ia molecule itself. Minimally, this change could occur at the site of interaction between the Ia molecule and the antigen and could result in the effective covering of surface determinants or epitopes on the Ia molecule which otherwise would be exposed to interaction with allospecific T-cell lymphocytes. It is also conceivable that the postulated Ia molecule–antigen stable interaction could cause allosteric

changes in the MHC molecule and bring about the disappearance of characteristic determinants detectable by appropriately alloreactive T-cell clones. Such postulated alterations of the antigenic structure of the Ia molecule would be detectable if an alloreactive T-cell clone were selected with specificity precisely for the site of interaction between Ia molecules and the foreign antigen. On the basis of our earlier experiments, which documented that I-A^b Class II MHC molecules differ from I-A^d molecules in lacking a common site for association between I-A^d and GAT/GT, we reasoned that H-2^b T cells might conceivably alloreact to this putative GAT/GT interaction site on I-A^d accessory cells. We therefore undertook to generate a series of alloreactive, H-2^d-specific, H-2^b T-cell hybridomas to analyze selected determinants on the I-A^d molecule.[28,29] These alloreactive T-cell hybrids have been prepared by hybridizing alloreactive B10 T cells from mice immunized with H-2^d, BALB/c cells, with the myeloma BW5147. The resulting H-2^d alloreactive T-cell hybrid lines obtained were tested for reactivity with H-2^d stimulator cells by assaying for the production of IL-2, as described earlier for the GAT-specific syngeneic hybrid T-cell clones. A total of 77 H-2^d-specific alloreactive T-cell hybrids were obtained and studied. We then looked for those alloreactive hybrids which could be inhibited in their capacity to respond to H-2^d stimulators by the addition of the antigen GAT. Of the 73 hybrids so examined, three distinct stable clones that were isolated were markedly inhibited in their reactivity by the addition of GAT. These three clones were all I-A^d-specific, as would be expected because GAT is an I-A- and not an I-E-restricted antigen. These three GAT-inhibitable clones were thoroughly studied. The inhibition of alloreactivity was in all cases very significant and was maximally observed at 0.1–0.5 mg/ml of GAT. Furthermore, the inhibition depended on the concentration of GAT and occurred in precisely the same range of concentrations required to stimulate the response of GAT-specific I-A^d-restricted syngeneic hybrid T-cell clones (Figure 2). Indeed, the same GAT-pulsed antigen-presenting I-A^d cells acquired the ability to stimulate GAT-specific I-A^d-restricted clones, while it lost the ability to stimulate the three I-A^d-specific H-2^b-alloreactive clones which had been identified as GAT-inhibitable. Moreover, as we would have predicted from our earlier results on antigen competition, those three alloreactive clones inhibitable by GAT were equally inhibited by GT but not by other randomly selected antigens.

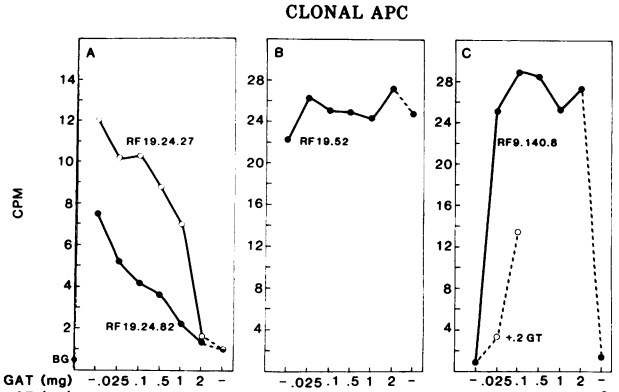

Figure 2—Effect of GAT on the activation of B10 anti-BALB/c representative I-A^d-specific alloreactive T-cell hybridomas by x-irradiated BALB/c splenic stimulator cells. RF19.24 (**A**) is a representative inhibitable cell line and RF19.52 (**B**) is a representative uninhibitable cell line. RF9.140 (**C**), a BALB/c × BW5147 anti-GAT hybridoma, is shown for comparison.

The data we have discussed, which illustrate that one can appropriately select a series of alloreactive clones capable of detecting the loss of an allodeterminant on an I-A-bearing stimulator cell, as a consequence of its interaction with an antigen such as GAT, is strong evidence of antigen–Ia interaction. These data are all the more convincing because the loss of the allodeterminant occurs concomitantly with the acquisition of the ability to stimulate GAT-specific clones. Moreover, the interaction between I-A molecules and GAT appears reasonably stable and seems to occur independently of the interaction with the T-cell receptor, although it is conceivable that the T-cell receptor might further stabilize the complex.

The fact that 3 of 77 alloreactive H-2^b anti-H-2^d hybrids were clearly inhibitable by GAT indicates that this phenomenon is not very rare. It is probable that similar inhibition of allodeterminants can be detected with other antigens by using the same approach we have followed.

I am not surprised that we have detected evidence of stable interaction between Class II MHC antigens and selected copolymers such as GAT or GT. It is, indeed, difficult to envisage how a receptor specific for both an Ia molecule and a foreign antigen could manage to interact with both components efficiently if these components had not been brought into some close relationship on the surface of the antigen-presenting cell previously. The precise mechanism of how this interaction is brought about remains to be elucidated and may indeed depend upon the ability of antigens or of their appropriately processed fragment to become integrated in the cell membrane through structures evolved for this purpose. Such membrane structures may also be related to those which anchor the I-A molecule to the membrane.

The Selective Expansion of Thymocytes Specific for Autologous Class II MHC Antigens

The thymus is a necessary organ for the maturation of functional T lymphocytes.[30] In this organ, striking T-cell proliferation and cell death are observed. It has been generally assumed that these phenomena are in some way responsible for the generation and selection of a mature T-cell population specifically able to bind foreign antigens in the context of self-MHC molecules.

We have proposed[31] that this process occurs in two stages:

1. T cells with receptors for self-MHC antigens are stimulated in the thymus to differentiate and replicate.
2. Only those T cells which bear low affinity receptors for self-MHC antigens can mature and leave the thymus as functional T cells.

We considered that the selective expansion of thymocytes reactive with autologous MHC antigens may proceed by the same mechanism which triggers mature peripheral T cells, that is, as a consequence of a specific interaction with Ia-bearing autologous accessory cells, resulting in the stimulation of secretion of IL-1 by these cells. However, it is well established that IL-1 is mitogenic for thymocytes, which has been exploited as a reliable assay for this factor. We postulated that the classic IL-1 assay, based on the proliferation of thymocytes to IL-1, is a reflection of this process. If our hypothesis is correct, the proliferative response of thymocytes to IL-1 should be dependent on the presence of Ia-bearing accessory cells.[32] As shown in Figure 3, the response of thymocytes to IL-1 is significantly inhibited if the responding population is treated with appropriately specific monoclonal anti-Ia antibody and complement or passed through nylon wool for elimination of Ia-bearing accessory cells. Moreover, the response of thymic T cells can be reconstituted with an adherent, Ia-bearing radioresistant accessory cell found equally in the low density fractions of the spleen and of the thymus.[32] The IL-1–dependent activation of thymocytes does not require the addition of foreign antigens but appears to involve the direct recognition of self-Ia molecules. Thymocytes were fractionated by peanut agglutinin (PNA) or steroid exposure into cortical (immature) and medullary (mature) subsets and tested for IL-1 responsiveness. Both mature and immature fractions proliferated in response to IL-1 and autologous accessory cells, with medullary thymocytes exhibiting the stronger response. These experiments establish that thymocytes with specificity for autologous Class II MHC molecules are selectively stimulated to expand upon interaction with self-Ia molecules.

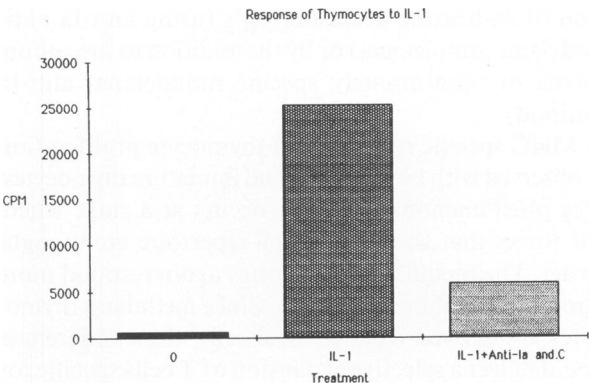

Figure 3—The response of thymocytes to IL-1 requires Ia-bearing accessory cells. To 1.5 × 10^6 normal or nylon-wool–passed BALB/c thymocytes treated with anti-Ia antibody and complement (*C*) was added a 1:8 dilution of IL-1 supernatant. Counts per minute (CPM) measure incorporation of ^{3}H-thymidine by the cultured thymocytes in response to IL-1.

These findings raised the following issues: 1) whether IL-1 is indeed produced in supernatants of cultures of thymocytes with either thymic or splenic accessory cells and 2) whether thymocytes are induced to proliferate in these cultures in the absence of added IL-1. We were able to demonstrate IL-1 activity in supernatants of cultures of thymocytes with accessory cells (Figure 4). Furthermore, thymocyte proliferation, to autologous accessory cells, was observed to coincide in time with the intrinsic production of IL-1 in the culture. Upon induction by autologous Ia-bearing accessory cells, IL-1 appears to be rapidly produced, being first detected at 96 hours at almost maximal levels. Proliferation of thymocytes follows at approximately 115 hours and becomes maximal at 136–164 hours.[32]

Both the production of IL-1 and the proliferative response of thymocytes could be inhibited by the dele-

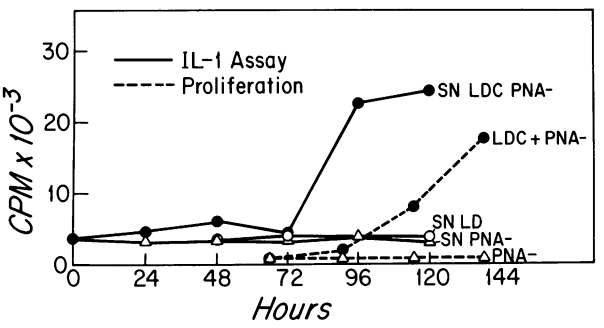

Figure 4—Kinetics of ILI-1 generation and thmocyte proliferation. PNA-treated C3H/Fej thymocytes, (5 × 10^5) and x-irradiated low-density splenocytes (4 × 10^5) were cultured by themselves (△, ○, respectively) or together (●) in 200 μl. At the indicated times, 50 μl of supernatant was removed and tested for IL-1 activity (———), and the proliferation in the same culture wells was measured (- - - -). The culture supernatant was assayed at a 1:4 final dilution. From Rock KL, Benacerraf B: Proc Natl Acad Sci USA 1984, 81:221.

tion of Ia-bearing accessory cells (using anti-Ia antibody and complement) or by the addition to the culture media of appropriately specific monoclonal anti-Ia antibody.

MHC specific spontaneous thymocyte proliferation is observed with both mature and immature thymocytes. The phenomenon, therefore, occurs at a stage where the forces that shape the T-cell repertoire are thought to act. The medullary thymocytes again respond more strongly than the cortical cells. Since medullary thymocytes are derived from cortical cells, these differences could reflect a selective expansion of T cells specific for self-Ia molecules.

We concluded from these experiments that some of the proliferation, which is normally observed in the thymus, reflects the stimulation and expansion of clones specific for autologous Class II MHC molecules. These clones react with Ia molecules on thymic accessory cells and stimulate them to secrete IL-1, which in turn acts as the second requisite signal for thymocyte proliferation.

As a consequence of these findings, it could be said that thymocytes, in contrast to peripheral T cells, comprise a relatively high fraction of cells very reactive with autologous Class II MHC antigens, in the absence of foreign antigens.[33]

To document the specificity of mature activated thymocytes for autologous Class II MHC molecules, we have analyzed the clonal nature of these responses by somatic cell hybridization techniques. Murine, medullary, PNA-treated, C3H/Hej thymocytes were activated *in vitro* with either exogenous IL-1 or autologous accessory cells. T-cell blasts from these cultures were subsequently fused to the T-cell lymphoma BW5147 to generate thymocyte hybridomas. The receptor specificity of these hybridomas was analyzed by assaying for IL-2 production upon challenge with a variety of accessory cells of various H-2 haplotypes. A high frequency of these hybridomas was triggered to produce IL-2 in the presence of syngeneic accessory cells. Using accessory cells from a series of recombinant mice, the specificity of this interaction could be mapped to either I-A^k or I-E^k or both. As expected from our hypothesis, on the origin of alloreactivity, many of these cells reactive with autologous MHC Class II antigens were also alloreactive (Table 1). Several clones were identified that appear to recognize public Ia determinants broadly shared by several alleles and genetic subregions. Such determinants appear to contrast markedly with those recognized by peripheral T lymphocytes whose specificity is dominated by the genetically polymorphic portion of the Ia molecule. Moreover, the frequency of self-reactive clones in these hybridomas

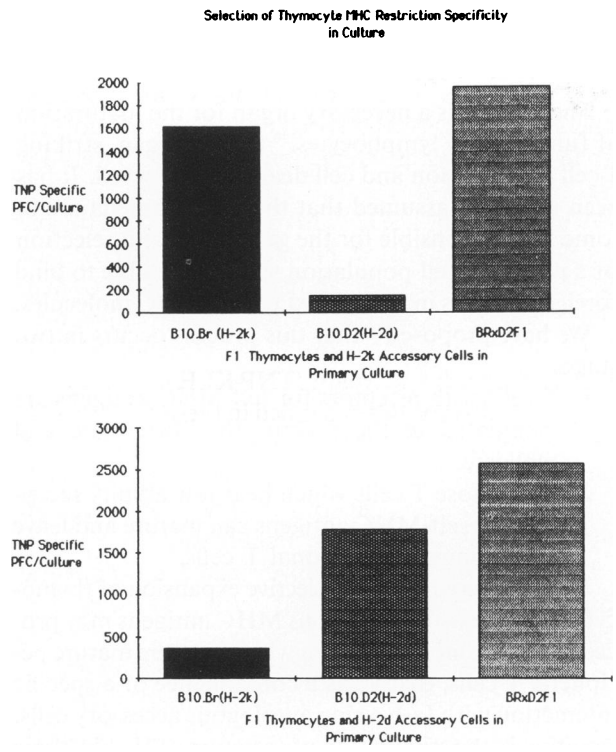

Figure 5—Analysis of *in vitro* selected thymocytes function and restriction specificity. Secondary cultures were prepared in 0.7 ml with 3 × 10^6 anti-Thy 1.2 + C'-treated TNP-LPS-primed B cells in the presence of 0.1 μg TNP-KLH, to which were added 10^4 BR × D_2 F_1 thymocytes obtained from 144-hour primary cultures with irradiated B10.BR or B10.D_2 accessory cells. The secondary cultures were incubated with rocking for 96 hours at 37 C. Aliquots were then assayed for TNP-specific plaque-forming cells (PFC).

Table 1—Specificity of 20 Autoreactive Hybridomas

	Number	%
MHC-specific*	20/20	100
I-A^k-specific†	9/20	45
I-E^k-specific‡	12/20	60
Restricted to self Ia§	8/20	40
Alloreactive‖	12/20	60
Reactive with		
2 MHCs	5/20	25
3 MHCs	3/20	15
4 MHCs	2/20	10
>4 MHCs	2/20	10

Twenty H-2^k hybridomas were tested with accessory cells of different genotypes.

* Reacts with both C3H/Hej (H-2^k) and B10.BR (H-2^k), but not with another B10 H-2 congenic.

† Reacts with B10.A(4R) (kkbb) and B10.AQR (qkkd) but not DBA-1 (qqqq).

‡ Reacts with B10.AQR (qkkd), but not B10.A(4R) (kkbb). Note: YH 2.38 is reactive to both I-A^k and I-E^k and is included in both categories.

§ Only reactive to H-2^k. The number of haplotype examined varied from 4 to 9.

‖ Cross-react with other haplotypes.

From Yeh et al.[33] By permission.

was very impressive, when compared with peripheral T cells.

In the next series of experiments we analyzed the functional capacity and specificity of the thymocytes expanded following interaction with autologous Ia-bearing accessory cells.

After culture of C3D2 F_1 MHC-heterozygous thymic T cells with irradiated parental B10.D2 (H-2^d) or B10.BR (H-2^k) cells, the ability of these cells to help trinitrophenyl–lipopolysaccharide (TNP-LPS)-primed B cells was assessed *in vitro*, in the presence of TNP keyhole limpet hemocyanin (TNP-KLH).[34] As shown in Figure 5, thymocytes expanded in these primary cultures helped a TNP B cell response in the presence of TNP-KLH. The F_1 thymocytes cocultured with B10.BR or B10.D2 accessory cells provided help selectively to B10.BR and B10.D2 B cells, respectively.

In other experiments we investigated and potential contribution of the selected thymocytes to the peripheral T-cell pool, as well as the stability of the imposed MHC restriction, upon adoptive transfer. F_1 thymic T cells were again selected in culture with parental accessory cells and subsequently adoptively transferred to lethally irradiated F_1hosts, where they were primed *in vivo* with a carrier antigen in adjuvant. Helper activity was recovered from the lymph nodes of such recipient animals and analyzed for MHC restriction. The MHC restriction observed matched the specificity imposed *in vitro* by the parental accessory cells.[34] Thus, the *in vitro* selection is maintained *in vivo* even upon antigen priming in the presence of both parental-MHC allelic gene products on recipient accessory cells.

We were therefore able to establish in these experiments the following: 1) Thymocytes stimulated in extended cultures with irratidated autologous accessory cells provide antigen nonspecific help for antibody responses. 2) F_1 MHC-heterozygous populations develop helper T cells that are capable of cooperating with B cells of either parental haplotype. 3) The MHC genotype of the accessory cell in the primary culture determines the resulting T helper MHC restriction specificity. 4) The *in vitro* expanded MHC-specific thymocytes with helper activity are capable of homing to peripheral lymphoid organs. 5) The *in vitro* MHC imposed restriction on the helper thymocyte population is stable *in vivo* even in an F_1 MHC heterozygous environment. 6) Lastly, *in vitro* selected, parentally restricted thymocytes, adoptively transferred, can be specifically primed to a nominal antigen, indicating that such a population contains clones which can be specific for autologous MHC plus X as well as for self-MHC alone.

The demonstration that thymocytes specific for autologous Class II MHC antigens can be selectively expanded when interacted with autologous Ia-bearing accessory cells and that these cells are functionally active in peripheral lymphoid organs raised the important issue of the mechanism whereby these autoreactive clones, potentially capable of initiating autologous GVH reactions are deleted from, or suppressed in, the peripheral lymphoid organs. Experiments are in progress, in our laboratory, to address this critical question. Contrasting with the susceptibility of thymocytes to selection by MHC Class II antigens, which accounts for the phenomenon of MHC restrictions, the ultimate mature T-cell population, which eventually populates the peripheral lymphoid organs, bears receptors with low affinity for self-Ia antigens and concomitantly. high affinity for antigens presented in the context of autologous Ia molecules on accessory cells.

The Critical Role of Accessory Cells Bearing Class II Molecules in the Normal Differentiation of T Cells and in the Development of Immunocompetence

The previous experiments have demonstrated the role of Ia-bearing cells in the generation of T cells committed to recognize foreign antigens in the context of autologous Class II MHC molecules and in the generation of alloreactivity.

Several years ago,[35] we reported that the treatment of adult mice with anti-Ia antisera interfered with the capacity of the mice to become immunized to foreign antigens, presumably by interfering with appropriate antigen presentation by accessory cells. The development of highly specific monoclonal anti-Ia antibodies and their availability in large amounts rendered possible a more effective approach to this problem by us and other investigators.[36,37]

Systematic studies were made of the immunosuppressive effects of treating mice with appropriate amounts of monoclonal antibodies directed to autologous Ia molecules, starting immediately after birth and continuing for several weeks until immunologic competence is normally developed. The results of these experiments were highly informative and very gratifying. After such treatments with anti-Ia antibody the generation of Ia-bearing cells was considerably suppressed both in peripheral lymphoid tissues and in the thymus. Moreover, such treated mice failed to develop immunologic competence, as demonstrated by their inability to mount cytolytic T-cell responses and mixed leukocyte responses to alloantigens.[36] In addition, an analysis of the T-cell subpopulation in the thymus and the spleen revealed a marked decrease in cells bearing the L3T4 surface an-

tigen normally associated with helper function and reactivity with Class II MHC antigens.[37]

These experiments dramatically illustrate the importance that Class II MHC antigens have in the development of mature T cells and normal immunocompetence. They also suggest an effective approach to the successful transplantation of tissue allografts. If, indeed, prolonged treatment with anti-Ia antibodies proves to be effective in suppressing the generation of alloreactive cells in adult individuals as well, possibly after sublethal radiation therapy, the transplantation of allogeneic bone marrow grafts depleted of T cells in these individuals will produce successful chimeras. Such chimeric individuals would then be prepared to accept allografts of any tissue from the donor of the bone-marrow graft.

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