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Edited by

GEORGE KLEIN

SIDNEY WEINHOUSE

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INFLUENCE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON T-CELL ACTIVATION

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I. Introduction

The major stimulus for the activation of most T lymphocytes does not seem to be antigen alone, but antigen in association with one or the other of the gene products of the major histocompatibility complex.

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These products are present on the surface of the body's own cells and some of these, for example, cells of the monocyte-macrophage series, are particularly well equipped to present antigen in the appropriate form to T lymphocytes and to deliver the activating signal. Once activated, the T cells have their specificities directed, not to antigen alone, but to both the antigen and some histocompatibility gene product ("H-2 restriction," Zinkernagel and Doherty, 1974a). This discovery has stimulated a vast number of investigations aimed at defining the precise mechanism by which T cells are activated, the specificities of the receptors that enable T cells to recognize antigenic determinants, and the means by which the repertoire of T-cell reactivities may be generated in the genome. This work has led to a better understanding of the function of histocompatibility gene products and of the advantages of polymorphism of the loci which code for these products. It is the purpose of this review to consider some of these investigations and to offer certain models which may elucidate the role of the histocompatibility gene products in T-cell activation. The effect these genes may have in determining resistance to certain tumors will be briefly considered.

II. The Major Histocompatibility Complex: A Brief Description

The features of the major histocompatibility complex (MHC)[1] relevant to this discussion are shown in Fig. 1. The MHC, H-2 in mice or HLA in man, codes for antigens (glycoproteins) many of which are expressed on the surface membrane of most cells. If we disregard S and G, we can divide H-2 antigens into two major classes: those controlled by the peripheral K or D regions (or classical H-2 antigens) and those controlled by the central I region [or I-associated (Ia) antigens]. The genetic control of the classical H-2 antigens is determined by the loci H-2K in the K region and H-2D in the D region. Due to polymorphism, there is a large number of alleles of H-2. Each allele is designated by a small letter as a superscript: e.g., H-2K^b, H-2K^d, H-2K^k, etc., for the H-2K locus, and H-2D^b, H-2D^d, H-2D^k, etc., for the

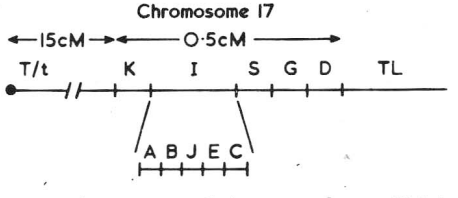

FIG. 1. The major histocompatibility complex or H-2 in the mouse.

H-2D locus. Each haplotype (a particular combination of H-2 antigens controlled by a single chromosome) is designated also by a small letter as a superscript: e.g., H-2^b, H-2^d, etc.

I-associated antigens or Ia antigens are genetically controlled by the I region and are distributed among five loci (so far detected): I-A, I-B, I-J, I-E, and I-C. Antigens coded by different I regions behave as on separate molecules. In contrast to H-2K and H-2D products which are represented on most cells, I-region products are found predominantly on cells of the lymphoreticular system (subsets of T cells, B cells, macrophages, etc.) and also on the epidermal cells of Langerhans. There are differences in the tissue distribution of individual Ia antigens coded by different I subregions (e.g., Ia antigens coded by the I-J locus are found predominantly on suppressor T cells). Many of the MHC-linked *Ir* genes have been mapped in the I-region.

On the right of D is the TL locus which codes for antigens expressed on thymocytes and on cells of some thymus-derived lymphomas. On the left of K is the T/t complex. The genes of this complex mediate a series of essential steps in embryogenesis probably by coding for cell surface components at relevant stages in development. There is a reciprocal relationship between one of these antigens, F9, and the H-2 antigens. F9 but not H-2 is present on sperms and on cells of the very early embryo. Later H-2 is present but not F9. TL has also some reciprocal relationship with H-2D: less H-2D is present when TL antigens are expressed on thymocytes. TL is not expressed on mature T cells. F9, H-2K, H-2D, and TL antigens are glycoproteins of similar molecular weight and three of these have the small molecule β_2 m associated with them. It is therefore of great interest that four loci, within a region of some 15 cM on chromosome 17, specify analogous cell membrane molecules playing an important role in cell to cell interactions. This has led to the hypothesis that this family of four loci are the logical descendants of an ancestral locus which acquired the property of cell surface perception as a condition for metazoan evolution.

Further details on the genetic organization of the MHC will be found in Jan Klein's excellent monograph (1975) and in a number of reviews (e.g., Shreffler, 1977; McKenzie, 1977; Peterson *et al.* 1977; Artz and Bennett, 1975).

III. T-Lymphocyte Subsets

T cells act in different ways in immune responses and may be divided into various subsets (Simpson and Beverley, 1977). Some T cells are directly cytotoxic (T_C) if they can make intimate contact with ap-

propriate targets. Some are involved in inducing the inflammatory lesions of delayed-type hypersensitivity (DTH, T_D cells). Some help B cells produce IgG antibody (T_H) and some regulate the immune response by suppressing the activities of other T and B cells (T_S). The various subsets can be distinguished by cell surface markers, such as the differentiation antigens of the Ly series and the differential expression of antigens coded by the MHC (Vadas *et al.*, 1976; Cantor and Boyse, 1977). Thus, in general, T_H and T_D cells are Ly-1$^+$ and Ia$^-$; T_C cells are Ly-2, 3$^+$ and usually Ia$^-$, whereas T_S cells are Ly-2, 3$^+$ and express Ia antigens coded by the I-J locus of the MHC. The exact way in which the various T-cell subsets exert their effect is not known but evidence points to the production, after antigenic stimulation, of factors which may be antigen specific or nonantigen specific. For example, T_H cells are thought to influence IgG-producing B cells either by a nonantigen-specific factor acting indirectly via macrophages (Miller, 1975), or by releasing antigen-specific factors of various types (Feldmann and Nossal, 1972; Munro and Taussig, 1975). Activated T_D cells release lymphokines such as the migration inhibitory factor (MIF), which are nonantigen specific and influence the recruitment of cells of the mononuclear-macrophage series (Bloom and Bennett, 1970). T_C cells presumably kill by some nonantigen-specific influence against target cells for which they have antigen-specific receptors (Cerottini and Brunner, 1974). Finally, antigen-specific helper or suppressor factors have been obtained from T_H and T_S cells and carry determinants coded by the I-region of the MHC. Unfortunately, none of the factors thought to be elaborated as a result of stimulation of the various T-cell subsets has yet been precisely characterized chemically.

IV. MHC Gene Products Influence T-Cell Activities

The MHC exerts a profound influence on T-cell activities. This is clear from the following.

(1) The frequency of alloreactive T cells (i.e., those directed against H-2 antigens) is generally thought to be 100 to 1000 times as high as the frequency of T cells reactive to non-H-2 antigens (Simonsen, 1967; Lindhal and Wilson, 1977).

(2) Different subsets of T cells exhibit the phenomenon of H-2 restriction, i.e., identity at certain MHC gene products is required between cells responsible for immune induction of T cells and targets of immune T cells (Zinkernagel and Doherty, 1974a; Bevan, 1975; Miller *et al.*, 1975; Katz and Benacerraf, 1975; Section V).

(3) The *Ir* genes, linked functionally to the MHC, influence the responsiveness of T cells or T-cell dependent functions (Benacerraf and Katz, 1975; Benacerraf and Germain, 1978; Section VII).

(4) There are I-region coded determinants on antigen-specific "helper" and "suppressor" factors which can be released or extracted from T_H and T_S cells, respectively (Feldmann *et al.*, 1977; Tada *et al.*, 1977).

The first three phenomena can all be accommodated in one general model of T-cell recognition and activation. This will be attempted in Section IX. On the other hand, the antigen-specific helper and suppressor factors will not be considered in detail in this review. It is felt that much more work is required to characterize these factors physiologically and biochemically to appreciate their special role in the network of immune interactions.

To account for the high frequency of alloreactive T cells, Jerne (1971) proposed that the repertoire of T-cell reactivities is coded by a set of germline *v* genes (genes coding for the *variable* region of T-cell antigen-binding units) which code for structures essentially complementary to the H-2 alleles of the species. Ater entering the thymus, potentially alloreactive T cells, which form a relatively large proportion of the T-cell pool, need not be influenced. By contrast, T cells with anti-self-H-2 reactivities would proliferate in response to H-2 structures present in the thymus. This must not be allowed to continue, for it was argued that such cells could kill self-H-2-bearing cells. Random somatic mutations in the genes coding for structures complementary to self-H-2 would thus be allowed to accumulate. This would decrease the strength of anti-self-H-2 binding and finally, only those T cells without anti-self-H-2 reactivities would mature. Such cells would have their reactivities directed to non-H-2 antigens and each specific set would thus constitute a much smaller proportion of the total T-cell pool than alloreactive T cells. As will be discussed later (Section IX), the discovery of the phenomenon of H-2 restriction has rekindled interest in this negative selection theory of Jerne. It has in fact been reformulated to account for a variety of the new findings (Langman, 1978; Blanden and Ada, 1978; von Boehmer *et al.*, 1978).

V. H-2 Restriction Phenomena

Most immune phenomena associated with T cells are subject to H-2 restriction. This has been well documented for T cells involved in cytotoxicity, delayed-type hypersensitivity (DTH), and helper func-

tions. By contrast, there is no evidence for H-2 restriction of B cell or natural killer (NK) cell activity.

A. Cytotoxicity

T_C cells may have their reactivities directed to H-2 antigens (other than self) or directed to non-H-2 antigens. For example, CBA ($H\text{-}2^k$) mice immunized to DBA/2 ($H\text{-}2^d$) produce T_C cells which kill any targets bearing either $H\text{-}2K^d$ or $H\text{-}2D^d$, no matter what other cell surface antigens these targets may have. Alloreactive T cells are thus clearly not self-*H*-2 restricted but rather "allo-H-2" restricted in view of their specificities to alloantigens. On the other hand, CBA mice recovering from infection by the virus of lymphocytic choriomeningitis (LCM) have T_C cells which attack LCM-infected $H\text{-}2^k$ targets, not uninfected $H\text{-}2^k$ targets or LCM-infected targets of any other H-2 type. There thus appears to be a requirement for H-2 matching between T_C cells and targets. This constitutes the phenomenon of "self-H-2 restriction" (hereinafter referred to as H-2 restriction) as originally described by Zinkernagel and Doherty (1974a). By using inbred strains of mice, congenic lines and recombinant lines, the genes imposing H-2 restriction of T_C cells could be mapped. The H-2K and the H-2D genes were found to impose H-2 restriction of T_C cells generated against virus-specified antigens (Doherty *et al.*, 1976a), hapten-modified antigens (such as trinitrophenyl, TNP) (Shearer *et al.*, 1976), and minor histocompatibility (H) antigens (non-H-2 H antigens designated H_1, H_3, H_4, etc., perhaps up to H_{100}, and the H-Y antigen which is a sex-linked minor H antigen) (Bevan, 1975; Gordon *et al.*, 1975).

T_C cells have clonally distributed receptors (the word receptor is used loosely to describe a cell surface structure with a site having some degree of complementarity to a determinant on an antigen molecule). In other words, one particular T_C cell has a receptor endowed with a unique specificity directed against either H-2K or H-2D gene products, but not both. There is therefore genic and allelic exclusion of receptors on the T cell (in contrast to the presence of both H-2K and H-2D gene-coded antigens on the same cell) (Zinkernagel and Doherty, 1975).

B. Delayed-Type Hypersensitivity

To achieve successful transfer of DTH, there is a requirement for H-2 matching between donors of sensitized T cells and naive recipients (Miller *et al.*, 1975). In the case of protein and polypeptide anti-

gens, the MHC region involved in restriction was I-A, but in the case of contact chemicals such as dinitrofluorobenzene, the genes were either H-2K, H-2D, or in the I region (Vadas *et al.*, 1977). These restrictions were interpreted as a requirement for H-2 matching between T_D cells and cells, such as macrophages or epidermal cells, responsible for presenting antigen to the T cells. More formal evidence for this was obtained in experiments which showed the necessity for Ia matching between antigen-pulsed macrophages used for sensitization and for elicitation of sensitivity in an *in vivo* system (Miller *et al.*, 1978). This is in line with observations of other investigators showing a requirement for Ia matching between antigen-pulsed macrophages and sensitized T cells *in vitro* to allow these to proliferate (Yano *et al.*, 1977; Thomas *et al.*, 1977b). The *in vitro* system enabled the investigators to use specific antibodies to determine the role played by antigen and MHC components in stimulating the proliferation of sensitized T cells. Incubation of T cells, themselves, with anti-Ia antibody had no effect. Antibody directed to Ia antigens, of the appropriate specificities, present on macrophages blocked the ability of the antigen-pulsed macrophages to stimulate proliferation (Shevach *et al.*, 1972; Thomas *et al.*, 1977a). The blocking was haplotype-specific in the F_1, indicating that it did not result from some nonspecific effect of the binding of antibody to a cell surface component (Schwartz *et al.*, 1976a). By contrast, antibody to the native antigen failed to block proliferation (Ellner *et al.*, 1977; Thomas *et al.*, 1978). The antigenic determinants which stimulate T cells therefore appear to do so in association with Ia determinants but are not readily accessible to antibodies directed against *native* antigen. Similar observations have been made in cytotoxic systems: the specific inhibition of lysis of target cells by T_C cells was achieved by antibody directed to the H-2K and H-2D gene products of the targets (Germain *et al.*, 1975; Schmitt-Verhulst *et al.*, 1976). Antiviral antibodies, on the other hand, consistently failed to protect the target (Blanden *et al.*, 1976b; Doherty *et al.*, 1976a; Braciale, 1977), except in one case (Koszinowski and Ertl, 1976). The implications of these observations are that native antigen is not recognized as such by T lymphocytes.

C. Helper Functions

It was initially observed that T_H cells and B cells must share I-A gene products for successful cooperation in antibody responses (Katz and Benacerraf, 1975). The restriction of T_H cells occurred both at the level of induction (presumed to reflect activation of T_H cells by

macrophage-associated antigen) and during T- and B-cell cooperation (Sprent, 1978a). T-cell recognition of MHC-associated antigen on macrophages and on specific B cells may thus be either identical or very similar. If, as discussed above, the antigen recognized by T cells on macrophage surfaces is not native antigen, the same must be true of antigen presented by B cells. T_H cells may recognize the B cells which they help, only if these can associate antigen with their surface Ia determinants in the same way as the antigen-pulsed macrophages. T- and B-cell cooperation thus might not depend on carrier-hapten "focusing" between T- and B-cell receptors specific for determinants of the carrier and the hapten, respectively. The T cells specific for Ia and "processed" antigen would seek out those B cells displaying identical structures. Of course, only B cells with specific receptors for either hapten or carrier determinants should "capture" sufficient hapten-carrier conjugates via their surface immunoglobulin receptors. Only these B cells should be able to "process" carrier determinants and associate them with Ia determinants on their cell surface, thus displaying the correct structure for specific carrier-reactive T_H cells. Binding of the specific T-cell receptors to such structures on the surface of the B cells may be associated with the delivery of an activating signal (nonantigen specific) from the T_H cell to the B cell, and the induction of synthesis of the corresponding specific antibody by the B cell. Hence, antibodies to both carrier and hapten determinants would be produced by carrier-specific and hapten-specific B cells.

There may be a requirement for H-2 matching (also in the I-A region) between T_H cells and T_C cells, as suggested by recent work in chimeric mice (Zinkernagel *et al.*, 1978a) (see Section VII).

D. Suppression

The antibody response to protein antigens may be suppressed by an antigen-specific factor, bearing an I-J-coded determinant and obtained from T_S cells. Identity at the I-J subregion between donor and recipient resulted in more effective suppression in some (Tada *et al.*, 1977) but not all cases (Kapp, 1978). When T_S cells were induced to hapten-modified cell membranes as, for example, when mice were tolerized with dinitrophenyl (DNP)-modified lymph node cells, the T_S cells suppressed recipient mice only if these shared H-2D with the strain providing the lymphoid cells (S. D. Miller *et al.*, 1978). The reactivity of the T_S cells in this system thus appeared to be directed to DNP-modified H-2D products. Somewhat similar results were obtained with soluble suppressor factors released from T_S cells

(Moorhead, 1977). A requirement for H-2D compatibility was also reported for T_S cells active in virus-infected systems (Pang and Blanden, 1976; Kumar and Bennett, 1977).

E. Possible Exceptions to H-2 Restriction

A number of phenomena have not exhibited the classical H-2 restriction described above. They are listed under the following headings and some will be referred to again in Section X.

1. T_C *Cells for I-Region Determinants*

I-region determinants can serve both as stimulator and as targets of T_C cells (Wagner *et al.*, 1975; J. Klein *et al.*, 1977; Billings *et al.*, 1977a). H-2K or H-2D compatibility is not required for the activity of these T_C cells. In addition, I-region determinants are susceptible to the same type of antigen modification (e.g., by TNP) as H-2K or H-2D gene products. In this case, the modified Ia determinants serve as targets for specific T_C cells raised against I-region determinants (Billings *et al.*, 1977b).

2. T_C *Cells for F9 Antigen*

OTT 6050 is a teratocarcinoma line, derived from strain 129 mice. It does not express H-2K or H-2D antigen specificities but produces an antigen, F9, genetically linked to the T/t locus of the MHC (Jacob, 1977). Although virus-infected $F9^+$ cells cannot serve as targets for T_C cells generated *in vivo* by infection with the virus (Zinkernagel and Oldstone, 1976; Doherty *et al.*, 1977), *in vivo* priming of mice with H-2 negative $F9^+$ cells followed by *in vitro* restimulation with $F9^+$ cells triggers the generation of anti-F9 immune T_C cells able to lyse specifically H-2 negative $F9^+$ cells (Wagner *et al.*, 1978). Since there may be some evolutionary relationship between the H-2 and T/t complexes, both located on chromosome 17 (Artz and Bennett, 1975), $F9^+$ cells may code for a functional analog of the H-2K and H-2D gene products. Whether it is possible to induce F9-restricted virus-specific or hapten-specific T cells has yet to be demonstrated.

3. *Cross-Reactive Lysis by* T_C *Cells*

T_C cells activated to allogeneic cells *in vivo* or *in vitro* in mixed lymphocyte cultures are very heterogeneous as evidenced by their cross-reactivity patterns. The effector cells kill not only targets syngeneic to stimulator cells but also third-party allogeneic targets with different H-2 antigens (Lindahl *et al.*, 1975). Cross-reactive lysis has

also been demonstrated both against minor H antigens (Bevan, 1977) and when normal spleen cells were cultured with irradiated TNP-conjugated syngeneic spleen cells. This generated T_C cells which lysed syngeneic TNP-targets efficiently and, to a lesser extent, allogeneic TNP targets (Burakoff *et al.*, 1976). Cold target inhibition experiments indicated the existence of clones of cross-reactive T_C cells and treatment of targets with anti-H-2 antibody blocked lysis thus demonstrating the requirement for recognition of H-2 on targets. If the repertoire of T-cell reactivities evolved to recognize modified self-H-2 antigens (Jerne, 1971), T_C cells with reactivities to alloantigens or xenoantigens might consist of clones of cells with specificities directed to modified autologous MHC products which cross-react with allogeneic and xenogeneic antigens. In an experimental investigation of this possibility, alloreactivity generated by xenogeneic stimulation was shown to result from the activities of separate T_C cell clones, each specific for an allogeneic target (Burakoff *et al.*, 1977). Moreover, alloreactive T_C cells exhibited cross-reactivities with chemically modified target cells syngeneic to the responders (Lemonnier *et al.*, 1977). Such cross-reactive T_C cells may account for the apparent breakdown of H-2 restriction observed in some tumor immunity systems (see Section X).

4. *Anomalous Cytotoxicity*

"Anomalous" cytotoxicity has been detected in a variety of experimental systems. Thus, for example, heterospecific cytotoxic cells were generated in the first 3 to 5 days of acute LCM infection in mice. These cells killed many types of infected or uninfected cultured cells, including syngeneic cells (Pfizenmaier *et al.*, 1975; Blanden and Gardner, 1976; Welsh and Zinkernagel, 1977). Likewise, Epstein–Barr virus (EBV)-genome carrying lymphoblastoid cell lines (LCL) stimulated autologous lymphocytes to generate cytotoxic cells reactive not only against the autologous cell line but also against other unrelated cell lines, whether or not these carried the EBV genome (Viallat *et al.*, 1978a,b). A somewhat analogous situation was described in human mixed lymphocyte cultures (Seeley and Golub, 1978). Here, two distinct types of cytotoxic activities were generated during sensitization to normal allogeneic peripheral blood lymphocytes: (a) allospecific cytotoxicity directed against alloblasts only; (b) "anomalous" cytotoxicity directed against serologically unrelated LCL and against the autochthonous LCL. The peak anomalous cytotoxicity occurred 1–2 days earlier than the peak allocytotoxicity and declined earlier. The anomalous cytotoxicity was not limited to targets sharing HLA