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The Major Histocompatibility Complex: The Value of Extended Haplotypes in the Analysis of Associated Immune Diseases and Disorders

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Major histocompatibility complex antigens are critical to an animal's immune response. In most animals, the extreme polymorphism of MHC molecules complicates studies of the role of this complex in the immune response. In mice, however, MHC haplotype-homozygous inbred strains have been developed which are invaluable in the study of the immune system and the search for immune response genes. The human MHC bears many similarities to its murine equivalent with regard to antigen structure and polymorphism; furthermore, a number of combinations of specific MHC alleles between HLA-B and HLA-DR/DQ (extended haplotypes) are found in people more commonly than predicted by individual allele frequencies. Over 30 percent of Caucasian haplotypes are extended haplotypes, and over 55 percent of individuals have at least one extended haplotype. Examples of the same extended haplotype, even in unrelated individuals, should either all have or lack any gene within the MHC region. The value of considering extended haplotypes in searching for associations between the MHC and diseases, or immune response, is shown in three examples: congenital adrenal hyperplasia, hepatitis B immunization, and transfusion-associated graft-versus-host disease.

INTRODUCTION

An elaborate system of host defense has evolved in animals to allow an organism to defend itself from invasion by other species. Graft rejection has proven to be an excellent test for the presence of a functional immune system. Although the recognition and rejection of foreign grafted tissues may not be a normal physiologic function, graft rejection involves processes very similar to those used in identifying viral and other foreign organisms and antigens. Studies have shown that all vertebrate animals are capable of graft rejection, and that this phenomenon correlates with the presence of major histocompatibility complex (MHC) antigens. The MHC is a critical factor in this self:non-self immune recognition system: MHC cell-surface proteins, antigenic determinants, and the T-cell receptor must interact in order to initiate humoral and cellular immune responses. MHC proteins are highly polymorphic, and, in recent years, additional complexities have been uncovered with the use of techniques such as

Abbreviations: CAH: congenital adrenal hyperplasia GVH: graft-versus-host (disease) MHC: major histocompatibility complex

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isoelectric focusing and restriction fragment length polymorphisms [1–4]. Such intricacies have complicated study of the role of the MHC in antibody formation in response to specific antigens, susceptibility to disease, and transplantation problems, particularly in humans.

The mouse, on the other hand, has probably the most intensively studied and best understood MHC complex. Immunogenetic studies in this animal have been facilitated by the identification in the wild, and further development in the laboratory, of strains of animals which are homozygous for particular H-2 haplotypes (inbred mice). The use of these haplotype-homozygous animals, and their related variants, both congenic (one strain's H-2 haplotype inserted into a different inbred strain) and recombinant (parts of two different H-2 haplotypes inserted into an inbred strain), has been particularly valuable in the identification of immune response genes [5]. In the process of analyzing human MHC haplotypes for variations in complement alleles, Dr. Chester Alper and his research group at the Center for Blood Research (which includes Drs. Edmond Yunis, Zuheir Awdeh, and the author) have demonstrated that a relatively small number of gene combinations are surprisingly common in many unrelated individuals. Furthermore, approximately 1 percent of the human population is homozygous for one of these MHC gene combinations. Thus, as in mice, the human MHC might also profitably be considered from the standpoint of common haplotypes ("extended haplotypes"). In this review, I will compare the murine and human MHC, explain the concept of extended haplotypes, and demonstrate the value of this approach in understanding genetic linkage and aspects of the immune response important to transfusion medicine.

THE MHC IN MICE

The murine MHC is found on chromosome 17, and the complex of these genes is designated H-2. Haplotypes composed of specific antigen combinations are described by lowercase letters (for example, k, d, b, f). Inbred strains of mice are homozygous for one of these haplotypes. Three classes of antigen comprise the MHC complex. Class I antigens (K, D, and L) are heterodimers composed of a 45 kilodalton polypeptide (heavy chain) coupled with beta-2-microglobulin. Antigens of this class are present on the surface of nearly all cells in the mouse [6]. Extreme polymorphism exists among class I antigens; for example, over 100 variants have been identified at each of two loci (H-2K and H-2D) [6]. Class II antigens are heterodimers, consisting of an alpha (heavy) chain of 34,000 daltons and a beta (light) chain of 29,000 daltons. These I-A and I-E antigens, also highly polymorphic, are found on B lymphocytes, antigen-presenting cells, and activated T lymphocytes. The position of the antigen in the cell membrane results in each molecule having extracellular, transmembrane, and cytoplasmic regions. The polymorphism of these antigens is, however, found largely in the extracellular portion, supporting the critical role of this region in the specificity of the immune response in an animal of given haplotype [7]. The complement proteins Bf, C2, and two forms of C4 are encoded by genes located between class II and class I D regions. Because of this location, complement gene products have come to be called class III antigens, even though they are structurally distinct from the other MHC proteins, and are not cell-surface markers.

THE IMMUNE RESPONSE OF THE MOUSE IS DICTATED BY H-2 HAPLOTYPE

Class I and II proteins are critical ingredients in the process of foreign antigen recognition. Their primary role may be the binding and presentation of peptide antigens to T cells. T cells expressing the protein CD4 (helper T cells) recognize antigen only in the presence of class II gene products, leading to B-cell activation and antibody production. T cells which express CD8, on the other hand, recognize antigen in the presence of class I proteins as a prerequisite for cytotoxicity. Although class I and class II MHC proteins have the capacity to bind many different types of peptides, individual MHC proteins have binding preferences such that not all peptide antigens are bound equally well, and some are not bound at all (peptide specificity). This selectivity probably also affects the ability of the MHC-antigen combination to trigger T-cell recognition [8]. The development of MHC diversity is believed by many workers to be advantageous to an organism and species. Without such genetic variability, vulnerability to certain pathogens may emerge, with devastating consequences. For example, the cheetah is currently considered an endangered species. All cheetahs which have been studied have identical MHC markers. The species' lack of MHC polymorphism appears to have rendered cheetahs nearly uniformly susceptible to a fatal feline infectious peritonitis caused by a coronavirus [9].

In mice, antibody responses to a variety of different synthetic peptides have been shown to be determined by genes mapping to the region of the MHC. Thus, strains of mice bearing the haplotype $H\text{-}2^k$ (for example, CBA and C58/J strains) are poor responders to the synthetic antigen (T,G)-A-L, while animals with the haplotype $H\text{-}2^d$ (BALB/c and NZB strains) are intermediate, and those with the haplotype $H\text{-}2^b$ (C57) are high responders [10]. Subsequent experiments have suggested that numerous immune response genes exist, because the level of antibody response in a given inbred strain varies with the antigen used, and good response to one antigen does not guarantee good response with others. Current thinking suggests that the poor responsiveness occurs because an abnormal or absent immune response gene results in T cells from such animals being unable to recognize a particular antigen-plus-MHC-protein complex. This lack of recognition could be due to one of several reasons: the appropriate T-cell clone is not produced (a "hole" in the T-cell repertoire); such cells are produced but eliminated early in development ("negative selection"); the foreign antigen and MHC proteins are unable to bind in a manner sufficient for T-cell recognition; or the MHC-antigen pair leads to preferential stimulation of T suppressor rather than T helper cells.

THE MAJOR HISTOCOMPATIBILITY COMPLEX IN HUMANS

The human MHC is found on the short arm of the sixth chromosome and encompasses more than 3.5 million base pairs of DNA, or 2 percent of the total chromosome [11,12]. Similar to the MHC in mice, three classes of antigen are produced by human MHC genes. Class I antigens are heterodimers composed of a 45 kilodalton MHC-encoded heavy chain coupled with beta-2-microglobulin, a 12 kilodalton product of a gene on chromosome 15. These antigens (HLA-A, -B, and -C) are found on the surface of all nucleated cells and are critical to antigen recognition by cytotoxic T cells. The structure of the HLA-A2 molecule has recently been determined through crystallography; the alpha 1 and alpha 2 domains of the heavy chain form the sides of a cleft, which appears to bind antigenic peptides for recognition by T cells [13]. Class II antigens are

also very similar to their murine equivalents in size, structure, and location on B lymphocytes, macrophages and other antigen-presenting cells, and activated T lymphocytes. The antigens, including HLA-DR, -DQ, and -DP, are critical to soluble antigen recognition by helper T cells. There is much structural similarity between human and murine antigens—for example, some regions of hypervariability in the extracellular portion of class II antigens occur at the same residues [7]. Such conservation over evolution between species presumably occurred because the proteins served valuable, and similar, immunologic functions; such similarities strengthen the value of the mouse as an immunologic model. There are also differences—for example, the mouse does not appear to have the DP region, and man has more genes for the alpha chain of class II proteins (six) than the mouse (two) [7]. As in mice, genes for three complement proteins (Factor B, C2, and the two variants of C4) are found nestled between class I and class II genes. In addition to these genes, there are many others in the MHC region, including genes for the adrenal steroid 21-hydroxylase, tumor necrosis factor, a heat shock protein, proteins of unknown function, such as the so-called HLA-B-associated transcripts, and large stretches of genomic DNA without identified functional gene products.

As they are in mice, the genes of the human MHC are highly polymorphic, including at least 24 variants of HLA-A, 52 variants of HLA-B, 11 of HLA-C, 20 of HLA-DR, nine of HLA-DQ, and six of HLA-DP [14]. Such polymorphisms may have been important in an evolutionary sense in the development of immunity, although, ironically, at the expense of a greater likelihood of graft rejection [12].

THE SEARCH FOR IMMUNE RESPONSE GENES IN MAN.

While it is extremely likely that the human MHC is critical to immune response, both against foreign and self antigens, few experiments have yielded results as clear as those in mice. For most immunologic disorders where an association with the MHC has been sought, analysis of observations in populations have been hampered by MHC polymorphism, as well as by many confounding clinical factors such as ethnic variation in the distribution of MHC alleles, environmental factors, and the additive effects of non-MHC genes.

Our group and others have noted, however, that combinations of specific alleles are found together in some people much more commonly than individual allele frequencies would have predicted. For example, the frequency of HLA-B8-positive Caucasians in Boston is approximately 26 percent, and the frequency of HLA-DR3-positive individuals in the same population is 28 percent. Nearly all antigen-positive individuals are heterozygous, rather than homozygous, for these antigens; therefore the allele frequency of HLA-B8 can be estimated from the Hardy-Weinberg equation as being roughly half, or 13 percent, and the frequency of HLA-DR3 as 14 percent. If HLA-B8 and -DR3 were inherited independently, the combination of HLA-B8 and -DR3 should have a frequency of less than 2 percent (0.14×0.13), and in the Boston population fewer than 4 percent of individuals would have this allele combination. Instead, over 10 percent of individuals are B8,DR3-positive [14]. Furthermore, nearly all haplotypes also carry a specific combination of class III complement genes: *BF*S, C2*C, C4A*Q0,* and *C4B*1* (encoding for the BF protein S, the C2 protein C, an absence of C4A, and the C4B variant 1; for convenience, this combination is abbreviated as "SC01" in describing its presence in haplotypes). Population studies in our laboratory have revealed at least 12 such sets of alleles encompassing the region between HLA-B

TABLE 1
Common Extended MHC Haplotypes in Whites

Extended Haplotype	Haplotype Frequency in Whites (%)	Frequency of Heterozygous Individuals (A) (%)	Frequency of Homozygous Individuals (B) (%)	Chance of Transfusion Random Donor B to Recipient A (%)
[HLA-(A1),B8,SC01,DR3]	9.2	16.7	0.8	0.1
[HLA-(A3),B7,SC31,DR2]	6.8	12.7	0.5	0.1
[HLA-(A29),B44,FC31,DR7]	3.0	5.8	0.1	<0.1
[HLA-(A2),B44,SC30,DR4]	2.9	5.7	0.1	<0.1
[HLA-(A1),Bw57,SC61,DR7]	2.0	3.9	<0.1	<0.1
[HLA-(A33),B14,SC21,DR1]	1.4	2.7	<0.1	<0.1
[HLA-(A26),B38,SC21,DR4]	0.9	1.8	<0.1	<0.1
[HLA-(A2),B15,SC33,DR4]	0.9	1.8	<0.1	<0.1
[HLA-(A3),B35,FC3.20,DR1]	0.9	1.8	<0.1	<0.1
Total	28.1	53.0	1.6	0.2

In addition to HLA-B and -DR results, the haplotypes include the complement alleles BF, C2, C4A, and C4B, in that arbitrary order. Thus, the abbreviation SC01 stands for the S allele of BF, the C allele of C2, absence of C4A, and the C4B allele 1. The most frequently associated HLA-A allele for each haplotype is shown in parentheses (from [32]).

and HLA-DR,DQ (in most of these haplotypes, one HLA-A allele predominates, although there is some variability at this locus). We have called these combinations fixed or "extended" haplotypes; other groups have appellations such as "preferred allelic associations" or "ancestral haplotypes" [14]. Whether, and if so why, such combinations have been preferentially preserved over time is not clear, but some authors have hypothesized either crossover suppression, or restricted crossover capacity of extended haplotypes [15]. In total, these combinations are found in over 30 percent of Caucasian chromosomes; over 55 percent of individuals have one extended haplotype, and 1.6 percent of individuals are homozygous for such a haplotype [15]. Nine of the most common haplotypes are shown in Table 1.

Because the DNA appears fixed for each haplotype over at least the HLA-B through HLA-DR/DQ interval, we believe that these gene combinations are of great value in understanding the human immune response. For example, an individual MHC allele which appears to be a marker for a disease may be so not because the antigen is directly responsible, but only because it marks an extended haplotype, all or nearly all examples of which also bear another etiologic gene. In looking for the presence of an immune response or immune suppression gene in man, a search for correlation with the presence (or absence) of extended haplotypes can be a much more potent analysis than a relationship to an individual allele, since examples of the same fixed allelic combinations should all either contain or lack the putative gene. The three examples listed below offer testimony to the value of this concept.

Example 1: Congenital Adrenal Hyperplasia Is Associated with Two Extended Haplotypes

An illustrative example of the value of analyses which take into account extended haplotypes involves the syndrome of congenital adrenal hyperplasia (CAH). Even though not an immunologic disease, this rare, recessively inherited endocrine disorder was originally found to be linked to the MHC, and later to show linkage disequilibrium

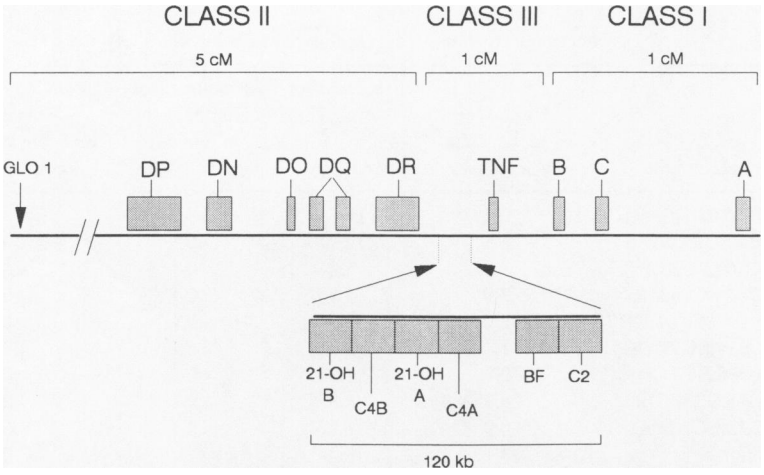

FIG. 1. A map of the human MHC on the short arm of chromosome 6, with the centromere to the left (from [11]).

with Bw47 [16], B14 (now Bw65), and DR1 [17–19]. Subsequently it was shown that the gene for 21-hydroxylase, whose deficiency was responsible for the various manifestations of the disease (salt- wasting versus simple virilization, for example), was located in the MHC complex adjacent to the C4B gene. The Center for Blood Researcc laboratory data on extended haplotypes then provided the solution for the MHC linkage. HLA-Bw47 was part of the extended haplotype [HLA-Bw47,FC91,0,DR7]; this rare haplotype, present in only 0.3 percent of control subjects, was present in 40 percent of patients with the salt-wasting form of CAH. The haplotype has a deletion of both C4B and 21-hydroxylase B (the active gene for the enzyme). Furthermore, Bw65 and DR1 were part of a more common extended haplotype [HLA-Bw65,SC2(1,2),DR1] with a duplicated 21-hydroxylase B gene, which produces a defective product, and the milder form of CAH [20,21]. Thus, in this case, MHC markers have no role in causing disease; the association simply reflects the presence of abnormal 21-hydroxylase genes in certain extended haplotypes (Fig. 1).

Example 2: At Least One Extended Haplotype Is Associated with Non-Response to the Hepatitis B Vaccine

As with other peptide antigens, different inbred strains of mice have variable responses to the hepatitis B surface antigen, depending on the H-2 haplotype. For example, strains with the haplotypes H-2^f and H-2^s respond poorly, whereas other strains develop normal or even elevated levels of antibody [22]. F1 heterozygotes of two different inbred mouse strains will respond to HBsAg provided that one parent is a responder. This effect suggests that responder haplotypes bear an immune response gene which is expressed dominantly.

In early studies of the efficacy of the hepatitis B vaccine in healthy human volunteers, the Center for Blood Research and collaborators found that approximately 4 percent of recipients made little or no antibody (<36 RIA units) following a three-injection course [23]. Similar proportions of non-responders have consistently been found by other groups [24,25]. To explore a possible relationship between poor response to the vaccine and the MHC, 20 non-responders were typed for HLA markers. Three of these individuals typed as apparent homozygotes for extended

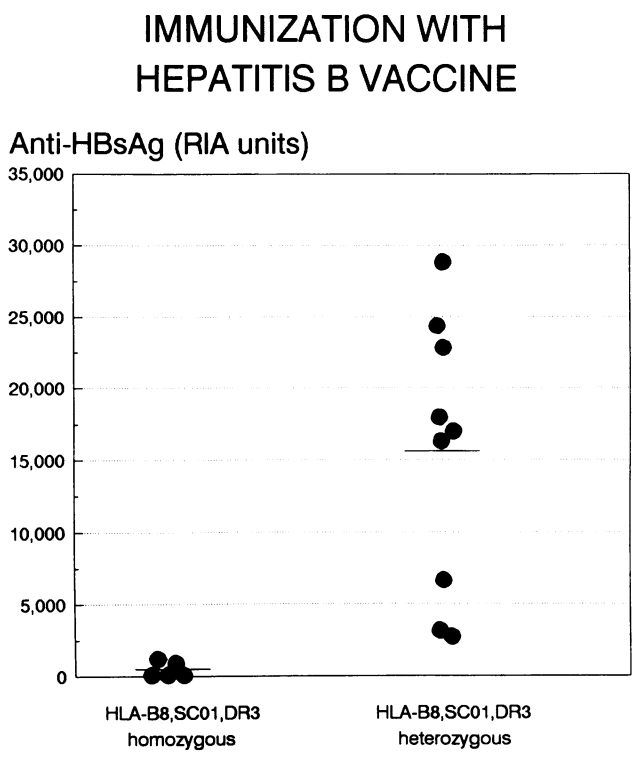

FIG. 2. Serum antibody to hepatitis B surface antigen measured two months after completion of a standard course of immunization with hepatitis B vaccine. Results in individuals homozygous for the extended haplotype [HLA-B8,SC01,DR3] on the left, and heterozygous for this haplotype, on the right [23].

haplotypes (15 percent), a tenfold excess over the expected incidence of individuals with homozygous haplotypes in the general population (1.6 percent, Table 1). Two non-responders were apparent homozygotes for the extended haplotype [HLA-B8,SC01,DR3], and one for [HLA-B44,FC31,DR7] [23]. Furthermore, when we prospectively vaccinated 14 individuals bearing either one or two [HLA-B8,SC01,DR3] chromosomes, the five homozygous individuals all had poor or absent antibody responses (mean, 467 RIA units; range, <8–1,266) two months following the third injection (Fig. 2). The heterozygous individuals had substantially different levels (mean, 15,608; range, 2,655–28,900) [26].

These results in humans suggest that the production of antibody to HBsAg is, as in mice, a dominant trait. Individuals homozygous for the extended haplotype [HLA-B8,SC01,DR3] appear to lack an immune response gene which recognizes the hepatitis B surface antigen. Further studies are in progress to document the mode of inheritance in families where at least one individual is a homozygote. This relationship between lack of response to a protein antigen and MHC homozygosity may be the first clear demonstration in man of an immune response gene. Cells and DNA from these haplotype-homozygous individuals offer a valuable tool for dissecting the location of this and other genes and their functions.

Example 3. Graft-Versus-Host Disease Following Transfusion Can Occur When the Blood Donor Is Homozygous for an Extended Haplotype, and the Recipient Is Heterozygous for It

In the mouse and other animal species, transfusion of immunocompetent T cells from a homozygous (inbred) parent to an offspring heterozygous for the parent's

haplotype regularly produces graft-versus-host (GVH) disease. This process occurs because the donor lymphocytes, being fully HLA-identical with markers encoded by one of the recipient's chromosomes, can escape detection and react against MHC markers encoded by the recipient's other chromosomes [27,28]. In humans, reactivity in mixed lymphocyte culture between unrelated individuals matched for common extended haplotypes has been shown to be as low as that between MHC-identical siblings [29]. Because any two examples of the same extended haplotype in unrelated individuals are immunologically similar, an individual homozygous for an extended haplotype can be considered analogous to inbred, MHC-homozygous (haplotype-identical) animals in terms of immunologic recognition.

HLA typing has only rarely been successful in transfusion-associated GVH in humans; samples are often unobtainable because the patient rapidly develops extreme lymphopenia or dies before the diagnosis is considered. In some of the cases where at least partial typing has been done, however, extended-haplotype-homozygous donors have been implicated. For example, a donor apparently homozygous for antigens contained in the haplotype [HLA-B8,SC01,DR3] caused fatal GVH in an unrelated recipient with Hodgkin's disease [30]. Another donor, apparently homozygous for HLA-B12 (parent group of B44, found in two common extended haplotypes), caused fatal GVH in a newborn [31]. Furthermore, in a recent report from Israel, a fresh red cell component from a donor homozygous for the antigens HLA-A26,B38,DR4 (and therefore very likely for the Caucasian extended haplotype [HLA-A26,B38,SC21,DR4]) caused fatal GVH in his haplotype-heterozygous, seemingly immunocompetent, father [32]. Blood from an unrelated haplotype-homozygous donor (the common Japanese haplotype [HLA-A24,CBL,Bw52,DR2,DRw52,DQw1] has been implicated in two cases of GVH in normal Japanese recipients [33,34].

The probability that blood from a donor homozygous for any extended haplotype might be transfused to a recipient heterozygous for the same haplotype can be calculated, and proves to be quite high in Caucasians (approximately 1 in 500; Table 1) [35]. The odds of such a donor:recipient combination occurring are increased when the donor is a first-degree relative of the recipient, explaining the recent rash of reports involving so-called directed donations [32,36]. Reports of transfusion-associated GVH are far less common than the calculations predict, however, and in fact still quite rare in immunocompetent individuals. Mitigating factors, such as the number and viability of transfused lymphocytes, may be important; alternatively, GVH may be underdiagnosed. Retrospective analyses of the incidence and recipient population characteristics of transfusion-associated graft-versus-host disease would help to answer this question. Prospective studies of the fate of transfused lymphocytes from haplotype-homozygous donors would be extremely valuable but may not be advisable, given the theoretical risk of GVH; other *in vitro* studies may need to be substituted to understand this syndrome.

CONCLUSION

Although the MHC is a complex system, the recognition of common gene combinations in humans, or extended haplotypes, has clarified, and sometimes identified for the first time, relationships between MHC genes and immunologic function and dysfunction. Many questions can be explored using this approach—the relationship between specific haplotypes and gluten-sensitive enteropathy [37], pemphigus vulgaris [38], and diabetes [39], for example. Others merit possible consideration—for example, could unrelated bone marrow donors be chosen based on extended haplotype matching

(which we would predict could be successful despite selective HLA-A allele mismatching)? The concept of extended haplotypes should provide a useful framework for interpreting data from a variety of studies of the immune system over the coming years.

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