New facts on HLA genetics

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New Facts on HLA Genetics: Are They Relevant in Bone Marrow Transplantation?

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THE application of bone marrow transplantation has been severely limited because for almost two decades it was considered by most teams to be successful only if the donor and recipient were HLA identical siblings. This axiom, based upon animal experiments and the early very poor results of bone marrow transplantation in severe combined immune deficiency between HLA non-identical donor recipient pairs,[17] is being slowly replaced by the realization that HLA-incompatible grafts (from haploidentical family members or unrelated (partially) HLA-identical donors) can be successful in a considerable number of cases. Unfortunately, it is impossible to predict which incompatible graft will be safe for the patient and which will not be. Such insight could come from two sources (1) from improved knowledge of the structure and function of the histocompatibility systems in general and the HLA system in particular, and (2) from clinical experience. The first source will be summarized in this chapter, the latter, the use of HLA mismatched grafts and their clinical outcome, is of too recent date, too heterogeneous, and too limited to allow immunogenetic advice to be formulated. The available clinical experience is reviewed in this issue[76,84] and elsewhere.[21,37,39,46,64]

We will first review the products and genetics of the HLA system emphasizing recent developments, its function, and its role in the recognition of non-MHC determinants.

THE HLA SYSTEM: GENE PRODUCTS

The HLA system, the human major histocompatibility complex (MHC), designates a set of linked genes on chromosome 6 that are highly conserved in evolution. The molecules encoded by the HLA system can at present be divided into three groups or classes on the basis of their structure and function (Fig. 1).[7]

Class I

Class I molecules are present as integral membrane glycoproteins of nearly all nucleated cells, and consist of a glycosylated heavy chain of 44,000 daltons[101] binding noncovalently to β_2-microglobulin (β2MG), a nonglycosylated light chain of 12,000 daltons encoded on chromosome 15.[38] The heavy chain can be divided into three extracellular regions, called alpha-1, alpha-2, and alpha-3, and two intracellular regions (Fig. 2).[61] All three extracellular regions are folded into domains, as is β2MG. β2MG does not penetrate the membrane, and the manner in which β2MG is associated with the domains of the heavy chain of the molecule is not known. The heavy chain genes are highly polymorphic and are encoded by multiple alleles at the HLA-A, HLA-B, and HLA-C loci (Table 1). The β2MG subunit is probably nonpolymorphic.

By amino acid sequencing it has been shown that the sequence homology between the heavy

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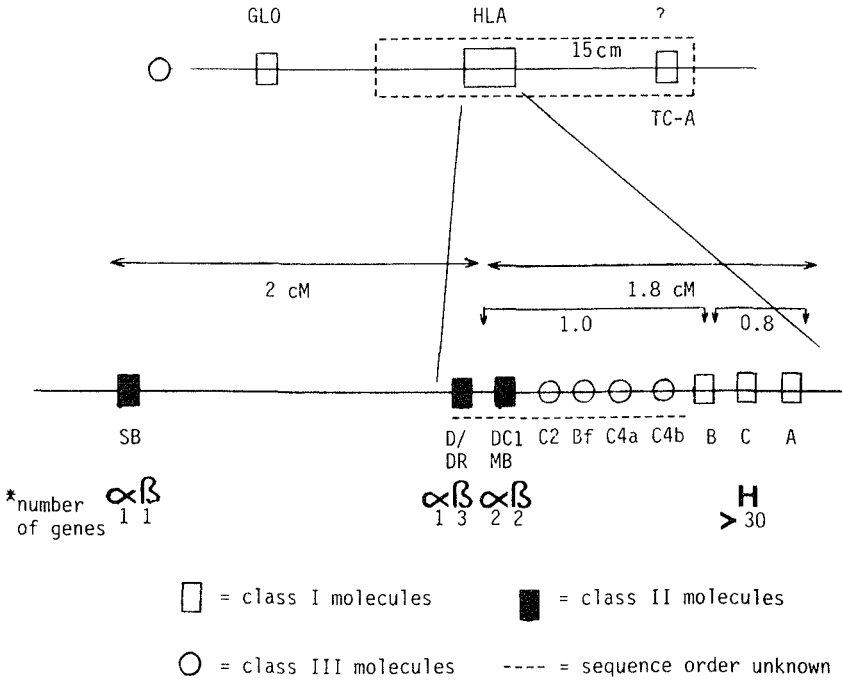

Fig. 1. The expanding HLA supergene.

chain of HLA-A2 and HLA-B7 is in the range of 80% to 85%.[78] Comparing the HLA-A2 and HLA-B7 sequences, amino acid differences occur throughout the molecules. However, three regions occur in which there is a clustering of differences: one in alpha-1, (residue 65 to 80), and two in alpha-2, (residues 105 to 115 and 174 to 178).[78] Of interest is that the animo acid sequence of the alpha-3 region is highly conserved in these molecules. Moreover, the amino acid sequence of alpha-3 and β2MG is homologous to that of immunoglobulin (Ig) C region domains, especially that of domain CH3 of IgG.[24] Among the functions of constant Ig domains are activation of complement and binding to the Fc receptor. The alpha-3 region might, by analogy, possess functional characteristics related to those of Ig constant region domains. It is in this context of special interest that the three-dimensional structure of the class I molecules might resemble that of the immunoglobulins.[63] Furthermore, in the three-dimensional structure of the immunoglobulins the antigen binding site is located at a site similar to that of the epitopes* or immunogenic determinants that define the class I allelic specificities.[60]

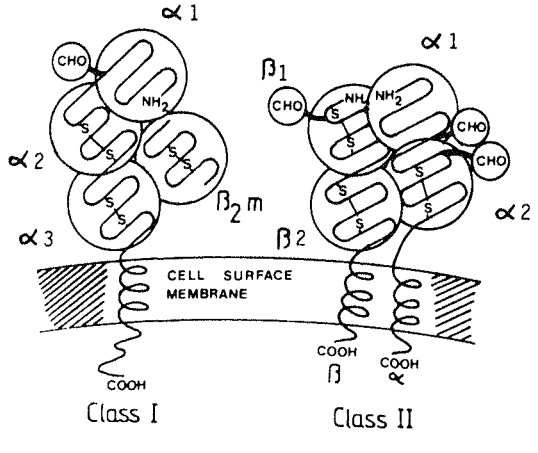

Fig. 2. Arrangement of the class I and II antigen domains, which may adopt a similar overall structure.[61]

The relevance of these data will be discussed in the following section. In addition to the "classical" class I molecules, recent evidence suggests that genes linked to HLA code for class I-like molecules on the surface of human T lymphocytes.[23,33,109] Because of their limited tissue distribution, these class I-like molecules are defined as

*An epitope is a defined area on a molecule, which can induce the formation of and interact with an alloimmune antibody.

Table 1. Complete Listing of Recognized HLA Specificities*

HLA-A	HLA-B	HLA-C	HLA-D	HLA-DR
HLA-A1	HLA-B5	HLA-Cw1	HLA-Dw1	HLA-DR1
HLA-A2	HLA-B7	HLA-Cw2	HLA-Dw2	HLA-DR2
HLA-A3	HLA-B8	HLA-Cw3	HLA-Dw3	HLA-DR3
HLA-A9	HLA-B12	HLA-Cw4	HLA-Dw4	HLA-DR4
HLA-A10	HLA-B13	HLA-Cw5	HLA-Dw5	HLA-DR5
HLA-A11	HLA-B14	HLA-Cw6	HLA-Dw6	HLA-DRw6
HLA-Aw19	HLA-B15	HLA-Cw7	HLA-Dw7	HLA-DR7
HLA-Aw23(9)	HLA-Bw16	HLA-Cw8	HLA-Dw8	HLA-DRw8
HLA-Aw24(9)	HLA-B17		HLA-Dw9	HLA-DRw9
HLA-A25(10)	HLA-B18		HLA-Dw10	HLA-DRw10
HLA-A26(10)	HLA-Bw21		HLA-Dw11	
HLA-A28	HLA-Bw22		HLA-Dw12	
HLA-A29	HLA-B27			
HLA-Aw30	HLA-Bw35			
HLA-Aw31	HLA-B37			
HLA-Aw32	HLA-Bw38(w16)			
HLA-Aw33	HLA-Bw39(w 16)			
HLA-Aw34	HLA-B40			
HLA-Aw36	HLA-Bw41			
HLA-Aw43	HLA-Bw42			
	HLA-Bw44(12)			
	HLA-Bw45(12)			
	HLA-Bw46			
	HLA-Bw47			
	HLA-Bw48			
	HLA-Bw49(w21)			
	HLA-Bw50(w21)			
	HLA-Bw51(5)			
	HLA-Bw52(5)			
	HLA-Bw53			
	HLA-Bw54(w22)			
	HLA-Bw55(w22)			
	HLA-Bw56(w22)			
	HLA-Bw57(17)			
	HLA-Bw58(17)			
	HLA-Bw59			
	HLA-Bw60(40)			
	HLA-Bw61(40)			
	HLA-Bw62(15)			
	HLA-Bw63(15)			
	HLA-Bw4			
	HLA-Bw6			

*The listing of broad specificities in parentheses after a narrow specificity, eg, HLA-Aw23(9) is optional.

"differentiation" molecules (with unknown function). They might be analogous to the murine Qa and Tla molecules, which are referred to as class IV molecules.[32,96]

Class II

Class II molecules are encoded by several loci in the HLA-D/DR region. Using serological and cellular techniques, class I molecules can be detected (as membrane glycoproteins) on cells with an immunological function such as B lymphocytes, macrophages and activated T lymphocytes[110,114,122] (Table 1). A class II molecule consists of two polypeptide chains, both spanning the cell membrane.[54] The heavy, or alpha-chain of 32,000 to 36,000 daltons is tightly, but noncovalently linked to the light or beta chain of 27,000 to 29,000 daltons. Peptide mapping studies indicate that the light chain of a class II molecule is more polymorphic than the heavy chain.[55] The amino acid sequences of those parts of the alpha and beta chains that are adjacent to the cell mem-

Table 2. Complotype Frequencies and Linkage Disequilibria Among 623 Random Normal Chromosomes from Caucasians

Complotype	Frequency
SC31	0.430
SC01	0.127
FC31	0.096
SC30	0.053
SC42	0.040
SC61	0.034
FC30	0.031
FC01	0.029
SC02	0.029
SC21	0.022
SB42	0.019
SC33	0 014
SC22	0.013
SC32	0.011

Complotypes are given as abbreviated letters and numbers in arbitrary order· BF, C2, C4A, and C4B.[125]

brane show, as do the class I molecules, remarkable and significant homology to the immunoglobulin C region.[58,123]

Class III

Class III molecules are recognized as polymorphic plasma proteins that belong to the complement system and can be recognized by electrophoresis.[6] The structural genes for BF, C2, and C4 (C4A and C4B) are localized in the HLA system.[2,48,6] Crossovers between the class III genes have not been observed so that their sequence order is unknown. Table 2 lists the most common class III gene combinations, which are also called complotypes.[125]

GENETIC ORGANIZATION

The HLA system contains a set of tightly linked genes that are usually inherited as one group. Such a group of genes is called a haplotype. Each individual has a maternal and paternal copy of chromosome six and, thus, a maternal and paternal HLA haplotype and the overall chance that two siblings inherit the same haplotype is 25%. The HLA system from the HLA-A to the HLA-DR locus spans on chromosome 6, a region of around 1.8 centimorgan† or recombination units,[121] which is probably equivalent to about half a promille of the total human genome.[86] The genetic distance of 1.8 centimorgan between HLA-A and HLA-DR, may be an underestimation. Recent studies suggest that class I DNA sequences occur telomeric from the HLA-A locus,[33,77] and class II genes have been detected centromeric of HLA-DR.[92,52] A schematic representation of the genetic map of the HLA system is shown in Fig. 1. From right (telomeric) to left (centromeric), there is first a group of loci that code for class I-like molecules or T cell differentiation antigens.[23,33,109] The second group of loci codes for the "classical" HLA-A, HLA-B, and HLA-C class I molecules.[7] Further to the left, four class III genes have been identified, which code for the complement components BF, C2, C4A, and C4B, respectively. The order of the class III loci is not known, but they are located between HLA-B and HLA-DR.[121] Centromeric of the class III genes is a set of genes coding for class II molecules. By employing serological and cellular techniques it has been possible to define at least three segregant series: DR, DC (LB-E, MB), and SB.[90–92,104,106,111] However, the definition of a segregant series is complicated by the fact that the heavy and light chain of a class II molecule is encoded by an alpha gene and by a beta gene.[53] There may be more genes coding for the alpha and beta chains of SB, DR, and DC, respectively. The beta genes carry the allelic specificity and the alpha genes do not, with the exception of the alpha gene coding for DC. The estimated number of alpha and beta genes present varies from a conservative one alpha for SB, one alpha for DR, and two alpha for DC, to almost double these figures. The number of beta genes is at least one for SB, three for DR, and two for DC. The interpretation of the data should take into account the DNA probes used and whether hybridization conditions were stringent or not. If the probes used can hybridize because of cross-homology with the $\alpha 3$ exons of class I, the $\beta 2$MG gene and the $\alpha 2$ and $\beta 2$ exons of the other class II coding sequences, the estimate will be far too high. This disregards the possibility of hybridization with Ig heavy genes.

These separate gene products can form dimers, as has been shown in the mouse MHC H-2-I region,[72] both for genes on the same haplotype (ciscomplementation)[53] and for genes on

†The distance between two loci is measured by recombination frequency. One centimorgan equals one recombinant in 100 meioses.

different haplotypes (transcomplementation).[27] The principle of ciscomplementation and transcomplementation is important for both our genetic and functional understanding of the class II gene products. It could imply, for instance, that a class II molecule is expressed in the offspring, which is encoded for by an alpha gene of the father and a beta gene in the mother, and thus is not expressed as such in either of the parents.[27,53,72]

Returning to the genetic map, the characterization of the DR beta molecules indicates a sequence homology with the mouse I-E products.[3] The DC alpha chain shows homology with the mouse I-A alpha chain.[12] Table 3 summarizes the number of class I and II loci that have been recognized by DNA technology or protein chemistry, cellular culture techniques, or serology.[4,7,11,14,26,28,58,59,62,66,77,104,117,118] The table serves to exemplify two points. First, protein chemistry indicates that the number of different class I and II molecules on the cell surface is twice as high as detected by current routinely used serological and cellular techniques. Second, the number of molecules coded for at the DNA level might again be at least twice as high as the number of molecules at the cell membrane. We know next to nothing about the transcription at the DNA level under normal conditions, let alone in disease or situations of intensive proliferation and differentiation such as occur during bone marrow repopulation.

POLYMORPHISM

We now come to one of the most striking features of the MHC, which sets the MHC apart from other genetic systems, and which we have mentioned earlier: its extreme balanced polymorphism.

Balanced polymorphism occurs when two or more alleles are maintained in a population by selection. The degree of polymorphism can be expressed as the average heterozygosity for a given locus.[73] In humans, the average heterozygosity for HLA-A and HLA-B is above 90%.[10] There is suggestive evidence that selection is a driving force in maintaining the polymorphism of the MHC.[10] Microbial agents are a likely candidate in this respect and De Vries et al[25] have shown that epidemics of typhoid and yellow fever resulted in a shift from HLA gene frequencies among survivors as compared to a nonaffected control group.

Another genetic characteristic of the MHC is the existence of linkage disequilibrium. When two alleles of different loci are considered, then the expected haplotype frequency can be calculated from the respective gene frequencies.[10] If the gene combinations occur at the predicted frequencies, then these are said to be in equilibrium; any deviation from the equilibrium frequencies is referred to as disequilibrium (parameter is "delta") and defined by the equation: delta = observed haplotype frequency minus expected haplotype frequency. Linkage disequilibrium can extend to more than two loci and a classical example in the MHC is the greater-than-expected occurrence of the haplotype HLA-A1—B8-DR3. The MHC seems thus to fulfill the criteria for a genetic system, according to Mi and Morton:[70] a unit of closely linked genetic information, whose phenotypic factors are nonrandomly associated in panmictic populations of higher organisms.

The enormous polymorphism of the HLA system has obviously important implications in bone marrow transplantation. Because of its great polymorphism, many parents will be heterozygous at the class I and II loci, and segregation of haplotypes in their offspring can be determined unequivocally. This has led to the unfortunate

Table 3. HLA Class I and II Defined Loci and Molecules

	Class I	Class II
Detected by		
DNA technology	More than 30 heavy chain genes[77]	4 (−6) α chain genes[4,62,118] 8 (−15) β chain genes[11,66]
Protein chemistry	8 heavy chains[117]	6–8 α–β dimers[58]
Cellular techniques	3 allelic series[14,59]	3 allelic series[28,104]
Serology	3 allelic series[7] A, B, C	3 allelic series[26] D/DR, DC, SB

situation in which some centers rely solely on the phenotypic HLA identity of donor and recipient to select a donor without checking whether genotypic identity is present as well. If one of the parents is homozygous for the class I and II antigens that are routinely typed for, such an assumption can be erroneous (Fig. 3). One is faced with this dilemma in about 10% of transplants. Phenotypic identity should always be confirmed by family segregation studies even if the MLC test between donor and recipient is negative.

In the unrelated donor–recipient situation two other points should be taken into account as well. The first is that in humans, as well as in the mouse, variants or mutants exist that cannot be detected by serology but only by cellular techniques or protein chemistry analysis.[9,16,41,42,49,67,117] For instance, about 10% of individuals who are A2 seropositive carry one out of four different A2 variants that can only be recognized by cellular techniques. Such variants have also been described for B27[16] and B35,[41] but a systematic analysis of all the class I antigens is lacking. The equivalence of these variants in the mouse can lead to strong homograft sensitivity and graft versus host reactions.[56,69] The second is the existence of splits or subgroups of the different HLA alleles such as HLA-Aw23 and HLA-Aw24 of HLA-A9. These are probably misnomers because they reflect the existence of two separate epitopes on the HLA-A molecule: one reactive with anti-A9 and one with anti-A23 antibodies. Table 4 summarizes the splits of the HLA-B alleles and their relation to the supertypic HLA-Bw4 and HLA-Bw6 antigens. A HLA-Bw22 positive cell might, therefore, carry not only that determinant but also the Bw6 and the e.g. Bw55 determinants. It was thought that the inventory of these splits of the class I antigens was rather complete but recent experience with monoclonal antibodies indicates that there might exist far more than described to date.

For the class II antigens the situation is different. An indication of the existence of variants recognized by DNA technology has been described only recently and the mapping of the splits has just been started.[80,102] Class II restricted T cell lines might also be an ideal test system with which to identify such variants.

The above serves to illustrate that the poly-

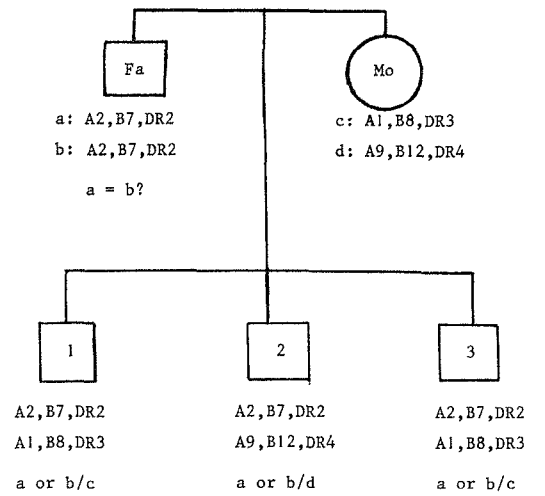

Fig. 3. Example of a family where one parent is homozygous for the class I and II (HLA-A,B and DR) antigens. Child 1 and 3 seem identical for HLA. Genetic identity cannot be proven on the basis of this HLA serotyping. Using cytotoxic lymphocytes, which recognized subgroups of A2, it could be shown that the a haplotype carried the most common A2 group A2.1 and the haplotype b the A2.3 variant (van der Poel et al.). Child 1 and 3 are thus not class I identical.

Table 4. HLA-B Locus Antigens and Their Inclusions in Bw4 and Bw6[89]

Bw4	Bw6
B5 Bw51 Bw52	Bw35
Bw53	B18
Bw44 Bw49	B12 Bw45 Bw21 Bw50
Bw63 (15A) B17 Bw57 (17.1) Bw58 (17.2)	B15 Bw62 (15b) Bw46
B37	
Bw38	Bw16 Bw39
B13	B40 Bw60 (40.1) Bw61 (40.2)
Bw47	Bw48
B27	Bw41 B7 B42
	Bw22 Bw55 (22.1) Bw56 (22.2) Bw54
Bw59 (B82)	B8 B14

morphism of the HLA system is much greater than suggested by the presently officially identified alleles.[1] This will further complicate the identification of unrelated class I and II identical donors for a given patient. However, the importance of the different variants and splits for activation of the homograft or graft versus host reaction has not been systematically inventoried. Some of them might be neutral and others might actually activate suppressor cells[47,100,124] and thus diminish the reactivity.[116]

Summarizing this first section, we can conclude that the complexity of the HLA system is even greater than the nomenclature of the officially recognized loci and alleles indicates, and that each HLA molecule carries an unknown number of different epitopes. Their function and importance in the induction of GVHD remains to be established.

One final point should be made. It has been shown that the enormous polymorphism of the MHC has a function in protecting a species from extinction by a given virus or microbial agent.[25] Although some individuals in a species will be susceptible to the infectious agent and die, others will survive because the MHC alleles they carry allowed them to develop an adequate immune response. It has also been shown that genetic factors coded for by HLA predispose for certain diseases mainly but not exclusively of immunopathological origin. The polymorphism of HLA can thus be used to identify individuals who are at risk for certain diseases or complications. It is really amazing that this powerful tool has not been more frequently applied for a study of the occurrence of GVH, interstitial pneumonia, or leukemic relapse. In the previous issue a first analysis was given of the occurrence of GVHD in bone marrow grafted aplastic anemia patients; HLA-B18 appears to increase the risk, and B8 and B35 seem to protect from it.[98,99] Because the data were only significant before correction for the number of antigens tested, confirmation of these findings is eagerly awaited. The authors justly point out that the association might not be primarily with the HLA-B locus but with the class II loci. If on further analysis the results reinforce the importance of the HLA-B locus antigens, then this might suggest that a viral agent plays an important role in (some) cases of GVHD. If a class II determinant is the determining factor an Ir gene effect seems more likely.

THE ANTIGEN BINDING PROPERTIES OF HLA MOLECULES

The finding that parts of the class I and II molecules are similar to that of the immunoglobulins, the at least partial similarity of the three dimensional structure and location of the antigen binding sites on immunoglobulins and epitopes, which determine the specificity on HLA molecules, indicate that the HLA molecules might have evolved from the same primordial gene as the immunoglobulins. By implication, they might have similar functions. Earlier studies failed to substantiate this[18,68] but recently, positive evidence has been found showing that HLA molecules themselves, or as part of a receptor, have binding properties, and that they might have a transmembrane transport function. So far, the data almost exclusively relate to class I molecules.

A study performed by Peterson's group is especially convincing. They showed that adenovirus-infected rat fibroblasts express on their sur-

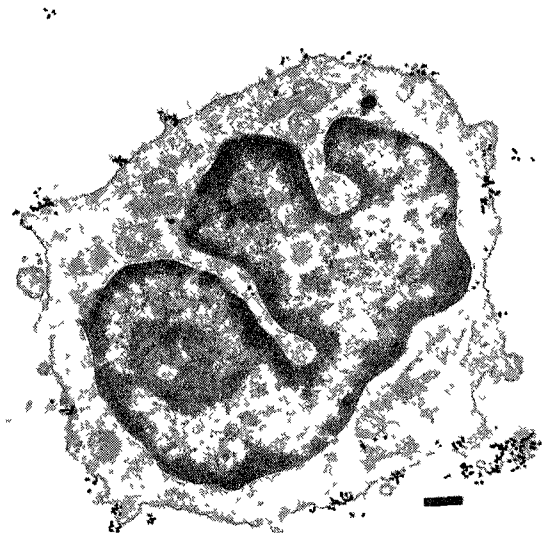

Fig. 4. Lymphocytes, sensitized with ^{125}I-anti HLA-A2 at room temperature were cultured at 37°C for various periods.

Figs. 4 through 7 show subsequent stages of the localization of the immune complexes during culture as visualized with electron microscopical autoradiography. In this photograph a lymphocyte is shown prior to culturing. The label is found associated with the plasma membrane. Bar represents 0 5 μ

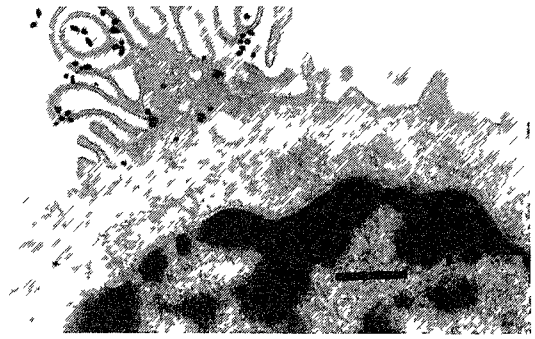

Fig. 5. This photograph was taken after 30 minutes of culture. The label is found in loops at the cell surface. Bar represents 0.5 μ. (See also legend to Fig 4.)

face a complex of a viral protein[94] and the heavy chain of class I molecules. This complex can be endocytosed, passes the multivesicular bodies, and ends up in the lysosomes, where it is degraded. In other words, the heavy chain of the class I molecule functions as a transport molecule. An identical sequence of events was observed by Giphart when he studied the fate of radiolabeled, highly purified anti-A2 antibodies after their interaction with HLA-A2 on mononuclear cells (Figs 4 through 7) [34] To be relevant, the assumption must be made that the polymorphic epitopes that interact with the antibody can in fact also interact with antigen.[113] We will refer to this point later.

Although in many instances such precise biochemical and electron microscopic studies have not been performed, there is agreement that HLA antigens are involved in the presentation of foreign, eg, viral, antigens to the immune system.[8] The combination of MHC and antigen

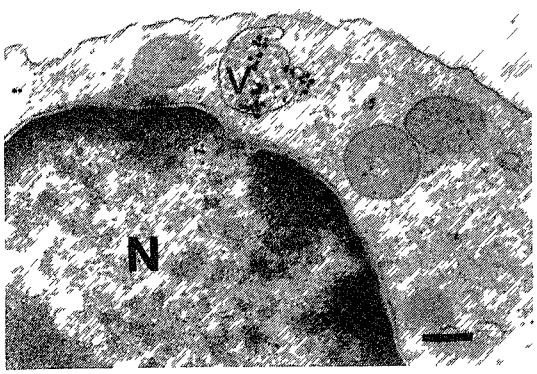

Fig. 6. This photograph was taken after three hours of culture The label is found associated with so-called multivesicular bodies (v) N = nucleus Bar represents 0.5 μ. (See also legend to Fig. 4.)

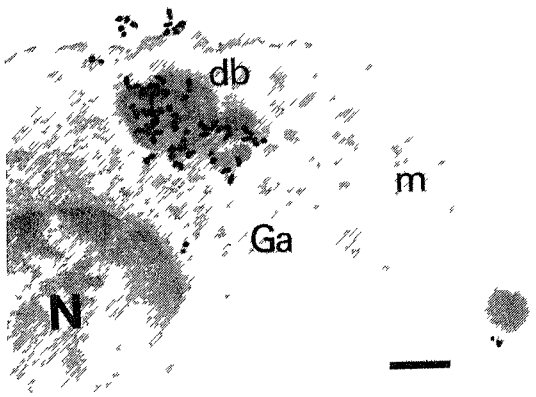

Fig. 7 The process of internalization ends in the lysosomes, which can be recognized as a cluster of dense bodies, which are associated with the label.[34] Ga = Golgi apparatus, N = nucleus, db = dense body, m = mitochondrium. Bar represents 0.5 μ. (See also legend to Fig. 4.)

activates T helper cells, which in turn initiate the cellular and humoral immune responses, which destroy the infected cells. The point that may be of importance in bone marrow transplantation is that the same activated T killer cells do not only recognize virus-infected autologous cells, but also allogeneic uninfected cells. In other words, the complex of self-MHC plus a foreign antigen, eg, virus, resembles an allo-MHC antigen. This is often referred to as altered self. It could be an important mechanism in the pathogenesis of GVHD and also of GVHD-like disease after transplantation.[22,71] If the patient's cells are "altered" through a viral infection, drugs, or irradiation, such cells might resemble alloantigens and activate the donor lymphocytes. Because during repopulation of the bone marrow the subtle balance of T helper and suppressor cells is easily disturbed, the ensuing activation may not be self-limited

That this is not only conjecture is borne out by the findings by Claas[19] (see also Brand et al[15]) that polymorphic determinants on platelets and neutrophils can interact with a large number of commonly used drugs (for instance cotrimoxazole, salazopyrine, dyta-urese, aldacton, and carbenicillin). These interaction products are recognized as foreign and the cells carrying the complex of polymorphic determinants and drugs are destroyed by antibodies directed against this complex. So far this has only been studied for polymorphic non-HLA antigens but it is likely that it can also happen for HLA.

A model study exemplifies this (Table 5).

Table 5. Affinity of Penicillin for Allotypic Determinants on Class I Antigens as Compared to That of HLA Antibodies[19]

Sequence of Incubation	Percentage of Lymphocytes Dead HLA Antibodies α-A2	α-B5
A. (1) Penicillin + cells (15 min) (2) HLA antibodies	100	10
B. (1) Penicillin, HLA antibodies + cells simult.	100	10
C. (1) HLA antibodies + cells (15 min) (2) Penicillin	100	80
Control. HLA antibodies + cells (30 min) without penicillin	100	100

Penicillin was able to block the interaction of anti-B5 antibody with the HLA-B5 antigen but not of anti-A2 antibody with HLA-A2. In other words, penicillin can interact and, through steric hindrance, block the polymorphic epitope on the B5 molecules but not on the A2 molecule.[20] The specificity of the blocking correlates with the binding affinity of the HLA molecules with a foreign molecule, in this case penicillin.

That such interaction products might alter the cellular immune response is suggested by studies on the effect of penicillin on the outcome of the cell-mediated lympholysis (CML) test (Fig. 8). The presence or absence of penicillin in the induction or effector phase of the CML test, or during the preparation of the target cells, influences to a large extent the outcome of the test.[51] Also, in this system an influence of the presence or absence of class I variants can be demonstrated.[50]

The common denominator of the above is the finding that certain viral molecules or drugs can alter the expression of self-MHC molecules and thereby initiate an immune response that can lead to GVHD.[106] This might explain some of the cases of GVHD not only in HLA-identical sibling combinations but also in monozygotic and autologous bone marrow transplants. Furthermore, as shown in animal models, irradiation alone can induce GVHD-like syndromes as well.[22,71] The finding that gut decontamination can lead to diminished GVHD is in accord with such an assumption. The mechanism by which bacteria could lead to GVHD is unclear.[108] Perhaps a process similar to that which has been described in the pathogenesis of ankylosing spondylitis is responsible. Geczy and his associates have published studies that suggest that plasmids in certain *Klebsiella, Shigella,* and *Coli* strains can, after gut infection, modify the expression of HLA-B27 leading again to a state of altered self.[75] Their findings must still be confirmed.

Evidence that HLA molecules can act as ligands, ie, molecules that can bind other struc-

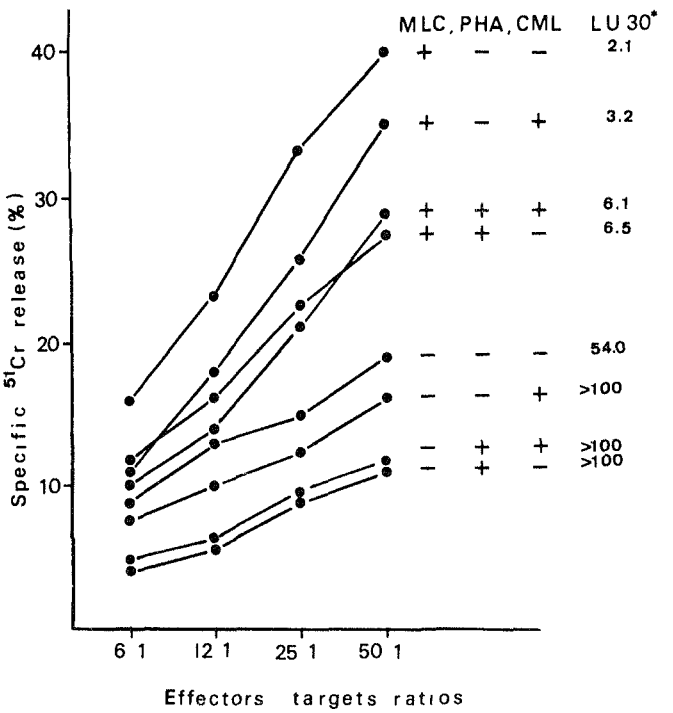

Fig. 8. CTLs were generated in the presence or absence of penicillin (100 IU/mL). After six days of coculture, CTLs were collected and washed three times with antibiotic-free RPMI 1640. Target cells were also cultured both in the presence and in the absence of penicillin and washed three times with antibiotic-free RPMI 1640. The CML assay was performed both with and without penicillin. The presence or absence of penicillin in the different phases of the assay is indicated as + and −, respectively. * LU30 is the number of effector cells × 10^5 necessary to obtain 30% specific lysis of 10^4 target cells. These values were estimated by linear regression analysis.[49]

tures, is thus increasing.[29,102] This is probably not only the case in immunology but also in other physiological processes such as endocrinology. Evidence from four different groups indicates that class I molecules form an integral part of the insulin[74] (Chvatchko et al, unpublished data), epidermal growth factor[88] and gamma-endorphin receptor (Claas et al, unpublished data). In the first three studies monomorphic monoclonal antibodies were used, in the latter, alloimmune sera. This made it possible to determine whether the different alleles of the class I molecules influenced the effectiveness of the gamma-endorphin receptor. Surprisingly, there existed a significant correlation between the ability of alpha-endorphin to block the interaction of class I antibodies and their corresponding antigens and the response in vivo of gamma endorphin treatment on schizophrenia. To what extent such findings are of relevance in bone marrow transplantation (receptor for interleukins) or hematology (receptor for poietines) remains to be ascertained.

In concluding, we would like to propose the following working hypothesis. HLA molecules can interact with a variety of foreign or self-molecules, either per se or as part of a hormone receptor. The interaction product of HLA and foreign molecules can be endocytosed and degraded. When the number of complexes on the membrane exceeds the capacity of this process, the complexes will remain on the surface and activate T helper cells.

Not only microbial agents but also polymorphic non-HLA histocompatibility determinants can be recognized in this way. Their recognition and role in GVHD will be discussed in the last section.

MHC-RESTRICTED NON-HLA ANTIGENS

It is of course logical to assume that differences of non-HLA-determinants between donor and recipient on their own alone or in conjunction with the factors described in the previous paragraph can lead to GVHD. Systematic studies in the mouse have documented this.[45,57] However, studies in humans have, with a few exceptions, been negative.

Using the CML test, Goulmy has systematically investigated to what extent hyperimmunized patients had MHC-restricted killer cells. In patients suffering from aplastic anemia, she[44] found cytotoxic lymphocytes (CTLs), which were MHC restricted and directed against the male minor histocompatibility antigen H-Y. The first case concerned a woman who suffered from aplastic anemia and had received a large number of blood and platelet transfusions. It was found that her lymphocytes were able to kill the lymphocytes of her HLA identical brother. The cells of this woman killed all A2 positive male cells and (virtually) none of the female A2 positive cells.

This was a typical example of an anti-H-Y/A2 restricted cytotoxic lymphocyte and since then several other examples have been found.[30,40,82,95] These CTLs react with class I antigens and H-Y, and for this reason reactivity will not always segregate with HLA. Of course, if families in which all the children are males are studied, the reactivity of such CTLs will segregate with the HLA haplotypes.

Although these findings have been confirmed, it is uncertain to what extent they are really of clinical importance. Originally, it was reported that female bone marrow donors lead more often to complications than male donors[13,36,97] but more recent analyses do not confirm this.[99] Furthermore, in such patients a correlation with the presence of MHC-restricted H-Y CTLs has not been found although this might be due to inadequate testing circumstances. The clinical importance of these HLA-A2 restricted anti-HY CTLs thus remains open. This might be different for a newly detected non-MHC determinant. A male patient (designated HA) was transplanted because of an acute myeloid leukemia. He received a bone marrow transplant from an HLA-identical sister. Their HLA type was A2, B27, Bw62, Cwl, Cw3, DR1, DR4; MLC and CML pretransplantation were negative; and full chimaerism was induced. The clinical course was complicated by grade III acute GVHD followed by severe chronic GVHD. This patient was studied by using his posttransplantation cells as responder cells.[43,44]

Table 6 shows that posttransplant effector cells of the patient were able to lyse the pretransplantation cells. Posttransplantation patient cells and cells of the bone marrow donor were not lysed. It was concluded that the bone marrow donor cells (after being primed in vivo) identified

Table 6. Percentage Lysis Obtained With Posttransplant Effector Cells of Patient HA[43]

Target Cells	% Lysis
Patient HA (pretransplant)	+59
Patient HA (posttransplant)	−1
Bone marrow donor	−3
Unrelated individual A	+5
Unrelated individual B	−7
Unrelated individual C	+26
Unrelated individual D	+35

something on the patient's cells, which was absent from donor cells.

Next, the family of the patient was studied (Fig. 9). The cells of both parents were killed by the CTLs of the patient taken posttransplantation. The patient was positive before transplantation, the donor was negative, and of the three siblings haploidentical to the patient, the cells of two were killed and of one were not. Thus, in this one family, two examples in which HLA-identical siblings reacted differently with the CTL cells recognizing the HA determinant were encountered. To test the specificity of the CTL, a panel of over 100 people was typed (Table 7). There was a clear correlation with A2, 90% of the A2-positive cells were killed. However, variants of A2 as defined by CTL typing[49,112] were not killed. The A2-positive cells, which were not HLA-A2 variants and were not killed, were obtained from the bone marrow donor, the haploidentical sister, and three unrelated individuals. Since then other examples have been found as well. Additionally, the lymphocytes of the patient also contained CTLs, which reacted with a part of the B27- and Bw62-positive cells.

The situation appears to be very similar to that of HY with a number of important differences. First, this minor HA antigen might have a very high frequency, at least in A2-positive individuals. Whether this minor antigen is localized on the sixth chromosome is not known. Second, it is also unknown whether the clones, which are restricted to B27 and Bw62, are directed against the same HA antigen as the A2-restricted clone.

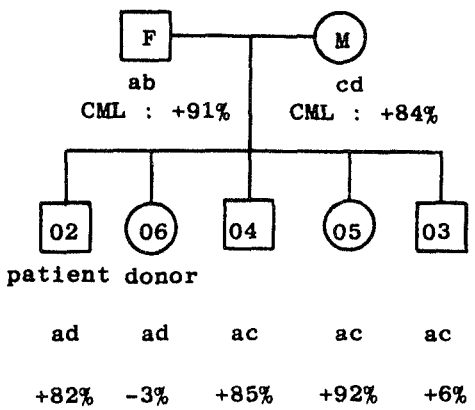

Fig. 9. The percentage of lysis in family HA.

Following this observation, a systematic study was performed in part in collaboration with the bone marrow transplant team in the hospital Saint Louis (Paris, France).[115] The data collected to date indicate that especially chronic GVHD correlates with the presence of incompatibility between the donor and recipient for the minor antigen HA. Using a prolonged sensitization phase, MHC-restricted CTLs can be detected posttransplantation in about half of the patients with GVHD. They are directed against HA and other non-HA determinants. So far they seem not to be identical to or to be associated with the known blood group systems, complement markers or intracellular enzymes.

The study of these MHC-restricted non-HLA markers is likely to have important consequences for our understanding and prevention of GVHD. How they can contribute to our understanding is self-evident. As far as prevention is concerned, the following applies. If the logistics of the use of unrelated donors have been resolved, such donors might in some cases be preferred above an HLA-identical sibling donor, if the sibling donor is mismatched for a minor histocompatibility antigen such as HA and the unrelated donor is not.[116] As long as partial HLA class I and II identity between donor and host exists it does not seem likely that mϕ-T-B cell interaction would become

Table 7. Analysis of HLA Restricted Anti-Minor HA Antigen Lysis[43]

		HLA Serotyping of Target Cells			
		HLA-A2 + Bw27 − B62 −	B27 + A2 − Bw62 −	Bw62 + A2 − B27 −	Others
	+	38	2	7	0
CML	−	5	5	3	44

so hampered as to severely inhibit immunological reconstitution. Perhaps a more serious problem might be the increased chance of leukemic relapse in patients without GVHD.[120]

CONCLUSIONS

If we summarize what has been discussed in the three previous sections and assess its importance in bone marrow transplantation, the following picture emerges.

The class I (and, in all probability, the class II molecules) carry not one immunogenic determinant or epitope, but perhaps as many as several dozen. Furthermore, the number of class I and II loci that code for molecules that are expressed and, thus, present on the cell membrane, is at least twice as high as the number now routinely typed for, ie, the HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DC or HLA-MB loci. Because some of these loci are located outside the region between HLA-A and HLA-DR, unidentified crossovers can occur. As a result, some presumed HLA-identical siblings will in fact not be identical for these loci. This has been documented for the SB locus, which is not routinely typed for.[93,105] Because HLA-identical siblings are identified by serotyping, class I variants that can thus far be detected only by cell-mediated lympholysis cannot be detected either. This will be especially relevant if a class I antigen occurs as a homozygote (by serotyping) in one of the parents (Fig. 3). This can be another reason that apparently HLA-identical siblings are in fact not identical.

Another complication can arise through the fact that a class II antigen, eg, HLA-DR is coded for by one of at least four alpha and five beta genes, which can also combine in transposition. This means that alpha genes of the father can combine with beta genes of the mother and, thus, give rise to an antigen that is absent in either parent. Because it is known that during proliferation and differentiation the expression of the only two class II molecules on which such information is available can differ,[31] one could speculate that during the repopulation of the bone marrow, donor and recipient, although they are genetically HLA identical, differ in their phenotypic expression of the class II molecules. Thus, crossovers of unidentified class I or II loci, the presence of a class I variant in one of the parents and differential expression of class II determinants could be the reason that apparently HLA-identical siblings are in fact different. The techniques to identify these differences have only recently become available. How important they are as a cause for GVHD remains to be assessed.

HLA molecules show amino acid sequence homology with immunoglobulins and this has lead to a quest to identify possible immunoglobulin-like functions of the HLA molecule. These have indeed been found. HLA molecules can interact with a variety of drugs, hormones, and viral proteins. They might be important as transmembrane transport molecules. In bone marrow transplantation it is of special interest that the interaction product of a given class I molecule and a viral protein can resemble another allelic class I molecule.[83] This might cause GVHD both in HLA-identical sibling donor-recipient pairs as well as in autologous or monozygotic bone marrow transplants, which are known to be triggered by viral infections.[35,85]

Non-HLA determinants, which are HLA restricted and thus far only detectable by the cell-mediated lympholysis test can cause GVHD as well. They seem especially relevant in chronic GVHD.

Thus, a better understanding of the genetics of HLA (crossover of unidentified loci, variants, and differential expression of loci), its function, (the complex of a class I antigen and a viral protein resembles an alloantigen), and its importance in the recognition of non-HLA determinants are all relevant in bone marrow transplantation.

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