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Editors

THE HLA SYSTEM

Second of Two Parts

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DEFICIENCIES OF HLA MOLECULES

A clinician's attention is normally drawn to a system only when it malfunctions. The HLA system is no exception in this regard, but in contrast to other systems, it also arouses interest when it functions well — too well, in fact. The most dramatic malfunction of the HLA system occurs when its genes falter in their expression, resulting in HLA class I or class II deficiencies (the bare lymphocyte syndrome, in which “bare” refers to the low level of HLA molecules on the cell surface). Severe cases of HLA deficiencies are, in fact, not caused by defective class I or class II genes, but by defects in other genes that influence the expression of HLA molecules on the surface of cells.

The HLA class I deficiency is caused by a defect in the *TAP* (transporter associated with antigen processing) genes, either *TAP1* or *TAP2*.²⁶⁻²⁸ In the absence of either the *TAP1* or the *TAP2* subunit, the delivery of peptides to the emerging class I molecules largely stops, the molecules that lack peptides become unstable and are inefficiently transported through the Golgi apparatus, and their numbers on the cell surface drop to 1 to 3 percent of the normal level. The consequences of this reduction appear in late childhood in the form of chronic bacterial infections of the respiratory tract, progressive degradation of the lung tissues, and bronchiectasis, leading to respiratory insufficiency.

The HLA class II deficiencies are the result of defects in genes that regulate the transcription of the class II genes.²⁹

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ABNORMALITIES OF GENES LINKED TO THE HLA COMPLEX

A large group of diseases involve genes in the HLA region that are linked to (or associated with) specific class I and class II alleles or combinations of alleles (haplotypes). In some of these diseases, the responsible genes are unrelated to the class I and class II genes, but in other diseases, the class I and class II genes are involved.

Narcolepsy

Narcolepsy is a debilitating neurologic condition characterized by excessive daytime sleepiness and sudden, uncontrollable periods of sleep. The disease is caused by a defect in the gene coding for the hypocretin type 2 receptor (*HCRTR2*).^{30,31} Hypocretins (orexins) are neuropeptides expressed by a small cluster of neurons in the lateral hypothalamus. To act, hypocretins must bind to receptors, one of which is rendered nonfunctional by a mutation carried by patients with narcolepsy. The mutation apparently arose in a common ancestor of these patients, in the *HCRTR2* gene located in the vicinity of the HLA-DQB1*0602 and DQA1*0102 alleles. Since the mutation occurred relatively recently, there has not been enough time for it to be separated from the two HLA-DQ alleles by crossing over in the patients with the most severe cases of narcolepsy. The three genes therefore appear together at frequencies higher than would be expected from random combinations of their frequencies in the population, a situation referred to as linkage disequilibrium.

Hemochromatosis

A defective class I gene, *HFE*, is responsible for hereditary hemochromatosis, one of the most frequent hereditary anomalies among whites.^{32,33} The gene codes for a class I α chain that has lost its ability to bind peptides and therefore no longer participates in immunity.^{34,35} Instead, it has acquired a new function — an ability to form complexes with the receptor for iron-binding transferrin and thus regulate the uptake of dietary iron by cells of the intestine.³⁶

An estimated 60 to 70 generations ago, a mutation occurred in the *HFE* gene of a Celtic person who is the ancestor of the more than 5 percent of whites now carrying the allele.³⁷ The mutation replaced cysteine with tyrosine at position 282 in the $\alpha 3$ domain of the *HFE* gene, thereby altering the chain's conformation and destroying its ability to associate with beta₂-microglobulin. As a consequence, the defective α chain fails to associate with the trans-

ferrin receptor after its synthesis in the lumen of the endoplasmic reticulum and the complex formed by the α chain and the transferrin receptor cannot be transported to the surface of the duodenal crypt cells. In the absence of this complex on these cells, two to three times the normal amount of iron is absorbed from food by the intestine of patients with hemochromatosis. The mutation is in linkage disequilibrium with the HLA-A*03 allele, with which hereditary hemochromatosis was originally found to be associated.

HLA GENES AND INFECTIOUS DISEASES

For an efficient immune response to a pathogen to occur, HLA molecules must bind peptides derived from microbial proteins and the T-cell repertoire must include clones that can be activated by such HLA-bound peptides.³⁸ Nonfulfillment of either of these requirements may render a person carrying a particular combination of HLA alleles more susceptible to given infectious diseases than one who has a different combination of alleles.

The best example of this resistance is the association of specific class I and class II alleles with protection against severe malaria in sub-Saharan Africa.^{39,40} In the Gambia, infection with *Plasmodium falciparum*, which causes malaria, is extremely common, although the mortality rate among children with malarial anemia or cerebral malaria is low. Both complications are believed to be the consequence of a failure to clear the parasites from the blood, leading to increased hemolysis and blockage of cerebral blood vessels by parasitized erythrocytes. HLA typing of the relevant population revealed the presence of the HLA-B*53 allele at a frequency of approximately 25 percent among healthy persons or children with mild malaria (the allele is rare in non-African populations). By contrast, the frequency of HLA-B*53 among patients with severe malaria was approximately 15 percent. The comparison suggests that possession of the HLA-B*53 allele reduces the risk of death from severe malaria by approximately 40 percent. Presumably, the HLA-B53 molecules bind very efficiently certain peptides produced by processing the malarial circumsporozoite protein and present them to CD8+ T cells, whose progeny attack the liver-stage parasites. Such cytotoxic T cells have indeed been found in patients with malaria, and circumsporozoite peptides have been eluted from the HLA-B*53 molecules of these patients.⁴⁰ Protection against severe malarial anemia is also afforded by possession of the class II HLA-DRB1*1302/DQB1*0501 haplotype. In other sub-Saharan populations, different class I and class II alleles are involved in the resistance to severe malaria.

Other examples of associations between infectious diseases and specific HLA alleles have been reported,³⁸ and resistance-conferring alleles are prevalent in areas in which the disease is endemic. Stronger re-

sistance of persons who are heterozygous for specific HLA alleles associated with an infectious disease has also been noted and has been explained in terms of the fact that a heterozygote can present a wider range of peptides to T cells than a homozygote can.⁴¹ In general, however, the associations are difficult to demonstrate, because they are often obscured by numerous complicating factors, which may overcome the resistance an HLA allele would otherwise confer. A striking example of this effect is the production by the parasite or infectious agent of variant peptides, or altered-peptide ligands, that differ from T-cell-stimulating peptides by one or more amino acids.⁴²⁻⁴⁴ The variant peptides bind to HLA molecules, but the assemblage does not stimulate a T-cell response: they act as antagonists of the response-stimulating peptides.

AUTOIMMUNE DISEASES

Genetic studies have shown that persons who have certain HLA alleles have a higher risk of specific autoimmune diseases than persons without these alleles (Table 1).⁴⁵⁻⁴⁷ The associations vary in strength, and in all the diseases studied, several other genes in addition to those of the HLA region are likely to be involved. One of the strongest associations is between HLA-B27 and ankylosing spondylitis.^{48,49} Approximately 90 percent of whites with the disease carry this allele, whereas its frequency in a control sample is only approximately 9 percent. The disease therefore occurs 10 times as often among persons carrying the HLA-B27 allele as among persons without this allele.

Direct evidence implicating HLA molecules in the development of autoimmune diseases has been provided by experiments involving transgenic animals. In one such experiment, in rats genetically manipulated to integrate two human genes, HLA-B27 and B2M, into their genomes, spontaneous inflammatory disease developed that resembled human spondyloarthropathies.⁵⁰ (B2M codes for beta₂-microglobulin, the light chain of the class I molecule.) The development of the disease correlated with the number of copies of the genes and the quantity of HLA-B27 expressed in lymphoid cells.

In another experiment, spontaneous diabetes developed in inbred mice whose own class II genes had been replaced by the HLA-DQA1*0301 and HLA-DQB1*0302 genes, provided that their pancreatic cells were made to express the B7 molecule.⁵¹ (The B7 molecule is required to send a costimulatory signal from an antigen-presenting cell to a T cell during the interaction between the HLA-peptide complex and a T-cell receptor.) The expression of the HLA-DQ8 molecule consisting of the α and β polypeptide chains encoded in the HLA-DQA1*0301 and HLA-DQB1*0302 genes predisposes persons to type I diabetes.

The nature of the association with HLA markers has not been fully elucidated in any of the autoim-

TABLE 1. ASSOCIATION BETWEEN THE PRESENCE OF VARIOUS HLA MARKERS AND SELECTED AUTOIMMUNE DISEASES.

DISEASE	ASSOCIATED HLA MARKER*	RELATIVE RISK OF DISEASE†
Ankylosing spondylitis	B27	87.4
Reactive arthropathy, including Reiter's syndrome	B27	37.0
Rheumatoid arthritis	DR4	4.2
Behçet's syndrome	B51	3.8
Systemic lupus erythematosus	DR3	5.8
Insulin-dependent (type 1) diabetes mellitus	DR3	3.3
	DQB1*0201	2.4
	DR4	6.4
	DQB1*0302	9.5
	DR2	0.19
	DRB*1501‡	
	DRB*0101‡	
	DQB1*0602	0.15
Idiopathic Addison's disease	DR3	6.3
Graves' disease	DR3	3.7
Hashimoto's disease	DR11	3.2
Postpartum thyroiditis	DR4	5.3
Celiac disease	DR3	10.8
	DQB1*0201‡	
	DQA1*0501‡	
	DR7, 11	6.0–10.0
	DR7, DQB1*0201‡	
	DR11, DQA1*0501‡	
Dermatitis herpetiformis	DR3	15.9
Sicca syndrome	DR3	9.7
Myasthenia gravis	DR3	2.5
	B8	3.4
Idiopathic membranous glomerulonephritis	DR3	12.0
Goodpasture's syndrome	DR2	15.9
Multiple sclerosis	DR2	4.1
	DRB1*1501‡	
	DRB5*0101‡	
	DQB1*0602‡	
Pemphigus vulgaris (among Ashkenazi Jews)	DR4	14.4
Psoriasis vulgaris	Cw6	13.3
Birdshot retinochoroidopathy	A29	109.0

*Symbols with asterisks indicate alleles, and symbols without asterisks indicate serologically defined antigens. For each disease, the marker or markers with the strongest associations are given. In many cases in which it is difficult to decide whether HLA-DR or DQ markers are responsible for the association, both markers are given.

†The relative risk indicates the frequency of a disease in persons with the HLA marker as compared with persons without the marker. A positive association (i.e., when the HLA marker is more frequent in persons with the disease than in those without it) is indicated by a relative risk of more than 1.0, a negative association by a relative risk of less than 1.0, and no association by a relative risk of 1.0.

‡The risk has not been assessed separately for this allele.

immune diseases. Autoimmunity apparently occurs because only the most strongly self-reactive T cells are deleted in the thymus; the autoreactive T cells that escape negative selection are normally kept under control by largely unidentified regulatory mechanisms. But when these mechanisms fail, self-reactive T cells can be activated by complexes of certain HLA molecules

with particular self peptides. Possible sources of the self peptides have been identified in the case of some diseases — for example, type II collagen in rheumatoid arthritis, glutamic acid decarboxylase in type 1 diabetes, myelin basic protein and proteoline protein in multiple sclerosis, acetylcholine receptors in myasthenia gravis, and thyrotropin receptors in Graves' disease.⁴⁵ The fact that self peptides, like all other peptides, are bound and presented by some HLA molecules but not by others presumably provides the basis for the association of each disease with a specific allele or alleles. Strong support for this interpretation is provided by the findings in patients with pemphigus vulgaris.

In some ethnic groups, the autoimmune disease pemphigus vulgaris is strongly associated with the HLA-DRB1*0402 allele, but in others it is associated with different alleles.⁵² More than 90 percent of Ashkenazi Jews who have pemphigus vulgaris carry this allele, but the allele is rare in other groups.⁵³ Some of the DRB1*0402 molecules in patients with pemphigus form a complex with one of two peptides produced by the processing of desmoglein 3, a cell-adhesion protein that glues epidermal keratinocytes together. The specificity of the HLA-peptide interaction is determined primarily by the negatively charged amino acid residues at positions 71 (aspartic acid) and 74 (glutamic acid), which make up the P4 pocket in the peptide-binding groove of the class II β chain.⁵⁴ The P4 amino acid residues of the two desmoglein peptides (lysine and arginine) carry a positive charge; thus, they not only fit neatly into the pocket, but also interact electrostatically with its lining residues. The interaction determines the association of pemphigus vulgaris with DRB1*0402: the bound peptides stimulate specific T cells, which secrete cytokines and trigger the production of desmoglein 3-specific antibodies; the autoantibodies attack the adhesion molecules of the keratinocytes; and the loss of cell adhesion leads to life-threatening blistering of the skin and mucosal membranes, which is characteristic of the disease. Since other HLA-DRB1 molecules have different residues in their P4 pockets, they do not bind the desmoglein peptides, and the disease does not arise. This interpretation, however, does not explain why the activation of T cells specific for peptides derived from the HLA-DRB1*0402-bound desmoglein 3 occurs only in some persons with the HLA-DRB1*0402 allele. The nature of the trigger remains unresolved in the case of virtually all the autoimmune diseases.

CANCER

The involvement of the HLA system in the development of cancer is still poorly understood. Cancer cells express a number of genes that their normal counterparts do not, and peptides from some of the protein products of these genes bind to HLA mole-

cules.⁵⁵ For a variety of reasons, however, the T-cell responses stimulated by these HLA-peptide complexes are not effective enough to eliminate the tumor cells, and the challenge of cancer research is to develop ways of specifically boosting the antitumor response.

One reason for the ineffectiveness of the immune response against tumor-associated antigens is that cancer cells tend to down-regulate the expression of some HLA molecules or stop expressing them altogether, thus rendering them poor targets for cytotoxic T cells.⁵⁶ This phenomenon may be amenable to corrective interventions.⁵⁷ On the other hand, the loss of HLA molecules from the surface of tumor cells can lead to the activation of natural killer cells, which are in a position to provide a backup system when the cytotoxic T cells fail.⁵⁸

TRANSPLANTATION

In addition to situations in which a malfunction of the HLA system occurs, problems can arise when it functions too well. This is the case when one attempts to transplant tissues or organs between genetically disparate individuals of the same or different species (allografts and xenografts, respectively). T cells are selected in the thymus to recognize self HLA molecules, but when artificially confronted with allogeneic HLA molecules, they not only are activated by them but also respond in large numbers, mounting a formidable attack that culminates in the destruction of the graft.⁵⁹ The T-cell receptors of the recipient's lymphocytes recognize either the donor's (allogeneic) HLA molecules on antigen-presenting cells of the graft (a process known as direct presentation) or the donor's HLA molecules on antigen-presenting cells of the recipient (a process known as indirect presentation).⁶⁰ In the former case, most of the T cells also recognize the peptide bound to the allogeneic HLA molecule, but some are rather liberal in this regard, and others show a total disregard for the content of the peptide-binding groove, even recognizing HLA molecules without peptides.⁶¹ During indirect presentation, the peptides are generated by the degradation of the allogeneic HLA molecules released from the graft.⁶² The T cells that respond to alloantigens (the alloreactive cells) are cells originally selected because they recognized self HLA molecules that had self peptides bound to them.⁶³ The recognition of alloantigens is therefore a form of immunologic cross-reactivity arising because of the relatively low affinity of the interaction between T-cell receptors and ligands and the flexibility of the loops of the receptor's complementarity-determining regions, in particular the loop of region 3.⁶⁴ The strength of the response can be attributed to the existence of a large number of such cross-reactive clones in the recipient.

Among HLA-identical pairs of donors and recipients, donor-derived peptides may differ from the peptides of the recipient because of mutations in the

genes. These small differences can be recognized by the recipient's T cells as minor histocompatibility antigens, and the activated cells can then effect the rejection of the graft.⁶⁵ Individual persons may differ with respect to several hundred minor histocompatibility antigens, but in donor-recipient pairs that are HLA-matched but mismatched for minor antigens, the response to a few of these antigens predominates.⁶⁶ Differences in minor histocompatibility antigens present a formidable obstacle to transplantation, particularly certain forms, such as bone marrow grafting.

APPLICATIONS AND CONCLUSIONS

Knowledge of the HLA system paves the way for numerous applications, both in immunodiagnostics and in immunotherapy. Although the affinity of the interaction between T-cell receptors and ligands is too weak to exploit it for a direct visualization of T cells with unmodified ligands, aggregated ligands increase the avidity sufficiently to achieve a stable, specific binding of HLA-peptide complexes to T-cell receptors.^{67,68} To prepare such complexes, HLA class I molecules are made from human genes introduced into *Escherichia coli* and allowed to fold, in the presence of labeled peptides, into complexes that each consist of four class I molecules and four peptides and that are referred to as class I tetramers.

It has proved technically difficult to prepare HLA class II tetramers by this method, but two other approaches have been more successful.^{69,70} In these new methods, HLA class II genes are introduced into insect cells either with the help of recombinant baculovirus or by direct transfection. Insect cells, like all other nonvertebrate cells, lack MHC molecules, but when transfected, they express HLA class II molecules at high levels in the absence of antigen processing. The application of such techniques has revealed the existence of surprisingly large numbers of CD8+ (class I tetramer) and CD4+ (class II tetramer) virus-specific T cells in the blood of patients with chronic or acute infections.⁷¹ Several variants of the method are already in use in diagnostic tests designed, for example, to detect T cells specific for the human immunodeficiency virus in the blood of asymptomatic persons who are infected with the virus.

In immunotherapy, numerous trials are under way to apply reagents based on HLA-peptide complexes to each of the clinical situations discussed above. Specific aims include the development of vaccines to prevent and treat major infectious diseases, such as malaria and the acquired immunodeficiency syndrome; rendering the antigen-specific autoreactive T cells that are involved in autoimmune diseases unresponsive; the augmentation of the responses of T cells and natural killer cells to tumors in patients with cancer; and the induction of alloantigen-specific tolerance among recipients of organ transplants.⁷² An added incentive to these attempts is the observation that HLA-bound

peptides in which a particular amino acid residue has been replaced by another (referred to as altered-peptide ligands) often act as partial agonists or antagonists of T cells.⁷³ The partial agonists activate the T cells suboptimally so that they carry out only some of the functions they normally perform when fully activated by the wild-type peptide (a full agonist). The antagonists not only prevent activation, but also shift the T cells into a state in which they are unable to respond to a challenge by the wild-type peptide. Because of these properties, altered-peptide ligands can be used to influence the quantitative and even the qualitative nature of an immune response.

The HLA system is highly complex genetically, biochemically, and functionally. To understand it well is a great challenge. It is, however, a challenge worth meeting, for although our knowledge of the HLA system has already been translated into many clinical applications, all signs indicate that the real windfall is still to come.

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