IMMUNOGENETICS AND TRANSPLANTATION

WHAT'S THE MHC GOT TO DO WITH TRANSPLANTATION? We have learned that the molecules of Class I and II MHC are involved in presenting antigenic peptides to the receptors of T cells. But that's actually fairly recent knowledge; they were first discovered decades earlier as the products of genetic loci that determined whether you could transplant tissues between members of the same species—mice and guinea pigs, originally. At first glance it might seem to you that those properties are unrelated (it did to most immunologists) but as we'll see in this Unit, they are really two sides of the same coin. To begin, we'll look at histocompatibility.

HISTOCOMPATIBILITY. The term is used to describe the outcome of grafts of living tissues between two individuals; they are histocompatible if the graft is accepted for a long time, and otherwise histo*in*compatible. George Snell[1] studied the genetics of tissue acceptance in mice using grafts of tumors and skin, and found that although it was influenced by many genetic loci, by far the most important was a region he called ***H-2*** (histocompatibility-2). Later it was shown that *H-2* itself is not a single locus, but a string of loci, close together on the mouse's 17th chromosome, encoding many factors responsible for tissue rejection. When it was demonstrated that rejection is an immunological process, it became clear *H-2* coded for histocompatibility **antigens**, some of which are expressed on the surfaces of all nucleated cells.

Studies in many species have shown that they all have their strongest histocompatibility antigens coded for by a family of genes on a single chromosome; ►the group was therefore named the **Major Histocompatibility Complex**, or **MHC**. In mice the MHC is H-2; in humans it is **HLA** (**H**uman **L**eukocyte **A**ntigen, because it is easiest to get leukocytes for typing). ►The strongest loci within HLA are **HLA-A, HLA-B,** and the HLA-D group, of which **HLA-DR** is the one of greatest concern to transplanters.

An extraordinary feature of the histocompatibility antigens is their ►genetic polymorphism (very large number of alleles at each locus within the species); the odds against any two unrelated humans having exactly the same combination of antigens might be about 1,000,000:1. No other genetic system is so diverse.

> **ASK YOURSELF:** You can assume that this polymorphism did not evolve just to make life difficult for transplant surgeons; so why, would you speculate, might it have evolved?

MAP OF THE MHC.

Human **HLA** (chromosome 6, short arm, p21.1-21.3)

Class II	Class III	**Class I**
● DP/ DQ/ **DR**/	C4/ Bf/C2/TNF	**B**/ C/ **A**/

There are more Class II and Class I loci than shown here; the rest (including HLA-C) are relatively minor. Several loci within MHC encode components of the complement system and certain cytokines; these are referred to as *MHC Class III*. ●= centromere.

[1] Snell discovered the H antigen system in the 1930s while working at Jackson Labs. He waited patiently nearly 50 years for his Nobel Prize (1980).

[Mouse: **H-2** (chromosome 17)

- **__K_/__I___/_S_/__D___/_Qa/_Tla_/___**

Mouse H-2I is equivalent to the human HLA-D family; they code for Class II antigens. H-2K and H2-D are equivalent to HLA-A and HLA-B; these are Class I MHC.]

Like most genes, each of these loci is expressed codominantly, so that at the HLA-A locus you have both paternal and maternal alleles expressed, etc. A typical **phenotype** then might be HLA-A1, A3/ HLA-B6, B7/ HLA-DR3, DR4, as it is for the father in this example. ►The MHC gene set that you inherited from one parent is called a **haplotype**:

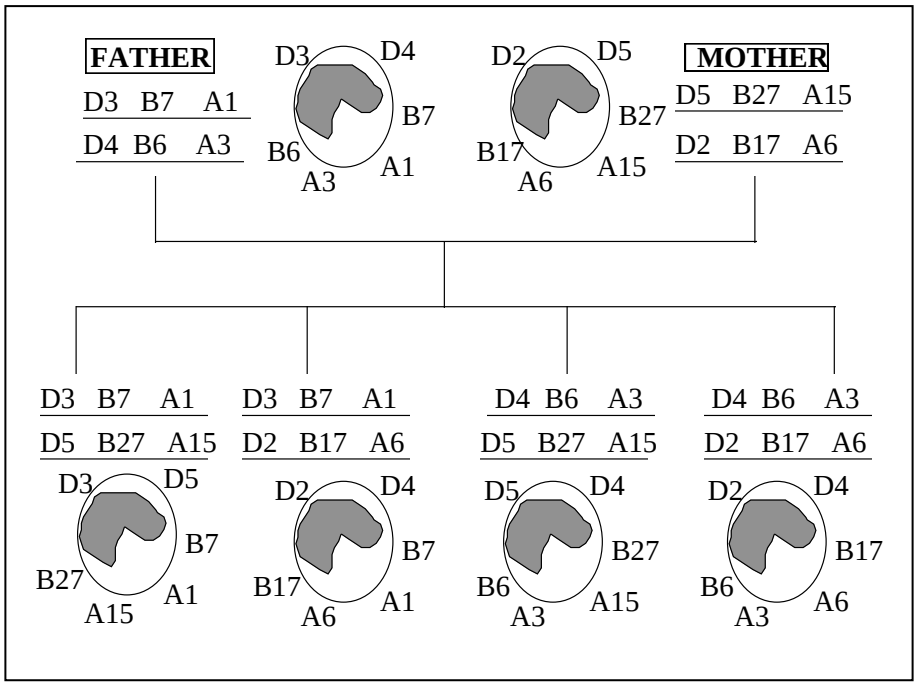


Fig. 1. D3 B7 A1 and so on are each individual's haplotypes. The cells show their phenotypes, the actual proteins expressed on the surface of their cells. Every cell expresses *both* alleles. Note that ►since MHC Class II antigens (called here 'HLA-D' for simplicity) are displayed, the cells shown must be antigen-presenting DC, macrophages, or B cells.

►**ASK YOURSELF:** A father's HLA **phenotype** is A1, A3/ B5, B7/ DR9, DR11. The mother's phenotype is A2, A4/ B6, B8/ DR10, DR12. Their baby's phenotype is A1, A4/ B6, B7/ DR11, DR12. What are the father's, mother's and child's **haplotypes**?

Now that you have worked out everyone's haplotypes, and assuming recombination within a haplotype does not happen, could they have a child who is

A1, A2/ B7, B8 / DR10, DR11?

Or one who is A1, A4/ B7, B8 / DR9, DR11?

Typing at the HLA-A and HLA-B loci used to be done by treating the patient's leukocytes with a panel of antisera to specific HLA alleles, and complement. If the cells expressed the allele that the antibody recognized, the complement lysed them, which was easy to observe. Nowadays, it is actually easier (and much more informative) to sequence the HLA *genes* themselves for typing.

> With sequence-based typing, what we thought was a single allele, say HLA-B27, turns out to be a lot of different closely-related alleles. This fine detail is not just for the amusement of sequencers; for example, HLA-B*2705 (the * means a DNA-sequenced allele; 2705 means the 5th subtype of HLA-B27) is highly associated with the arthritic disease ankylosing spondylitis, while HLA-B*2706 is not. With antisera, both typed simply as HLA-B27. There are ~100 known B27 alleles.

We can clarify MHC genetics by considering a simpler mouse model. In mice the situation is similar to that in humans, but because mice can be inbred it is possible to obtain a mouse, both of whose haplotypes are identical. Letters are used to designate the alleles in mice; so an inbred H-2s mouse is one who carries the original 's' strain's version of the gene (allele) at loci H-2K, H2-I, and H2-D, which correspond to HLA-A, HLA-DR, and HLA-B, respectively. The mouse's haplotypes are, in short, $H2^s + H2^s$, and in long, $\underline{K^s\ I^s\ D^s} + \underline{K^s\ I^s\ D^s}$; his genotype is, in very short, s/s. Note, then, that if an H-2s mouse, genotype s/s, is crossed with an H-2q mouse, genotype q/q, all the F1's will be s/q, that is, K^s/K^q; I^s/I^q; D^s/D^q (see Figure 2, below).
►F1's will all accept grafts from each other, **and from either parent**, because the parents, being homozygous s or q, present nothing foreign to an s/q hybrid. On the other hand, either parent will reject grafts from the F1's, since to the parent they are half self, and half foreign. This P→F1 and F1→P reciprocal system has greatly advanced our knowledge of graft rejection, and of the graft versus host phenomenon (discussed later).

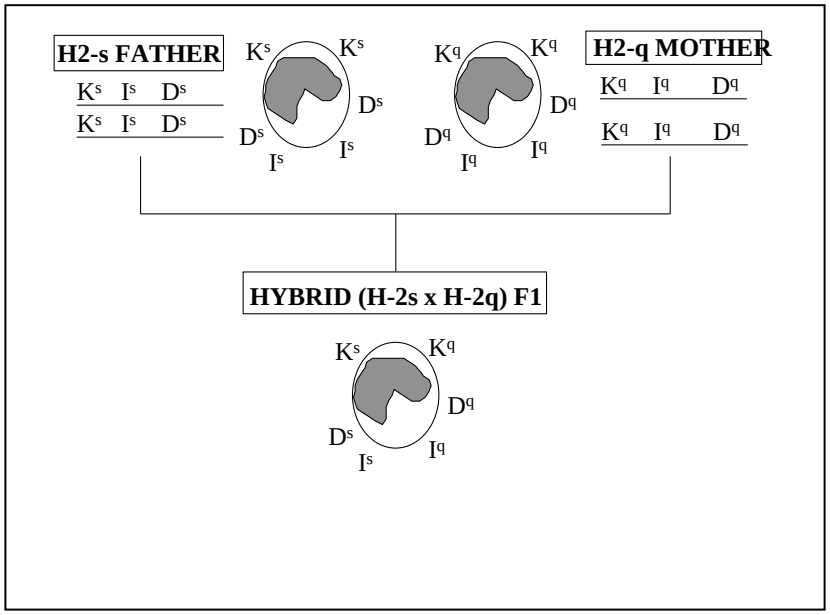


Figure 2

►**ASK YOURSELF:** Is this also true for people—will any child accept a graft from either parent, but parents can't accept a graft from a child? If so, why? If not, why? (As always, making a diagram will help.) Statistically speaking, who is likely to be your best organ donor?

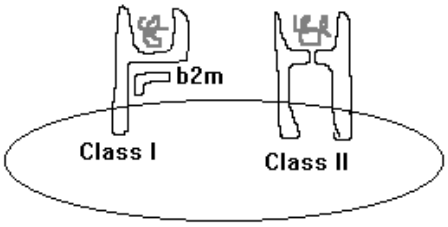
HLA-DR WAS DIFFERENT FROM HLA-A AND HLA-B. HLA-A and –B (and –C), the 'original' transplantation antigens, were fairly simple to study because they are on *all* nucleated cells and antisera to them were quite easily obtained. ►They are the human **Class I MHC** loci. But in 1965 Fritz Bach noted that grafts perfectly matched at HLA-A and -B were nevertheless quite often rapidly rejected; there must be another major locus involved. None of the antisera available when human transplantation began in the 1960s could detect the difference, but by accident it was observed that if leukocytes from the donor were mixed in cell culture with leukocytes from the recipient, there was, after a day or two, a burst of cell division in the culture…

…the **MIXED LEUKOCYTE REACTION**, or **MLR**, was positive. T cells were recognizing, and being stimulated to proliferate by, antigens on the other person's white cells that Bach did not have antisera against. Further studies identified these antigens as **Class II MHC**. The MLR in this form doesn't help us know how strongly the recipient's T cells are responding against donor Class II, because the recognition is bidirectional. But for transplanting what we want to know is: How strongly do the recipient's T cells recognize the Class II of this potential donor as compared to that one? ►So we create a **'one-way' MLR**, in which the cells from the donor are treated (DNA synthesis inhibitors or radiation) to prevent *their* division (after all, you really want to know, can the recipient recognize the donor's MHC?). What you then observe is recipient's Th cells dividing in response to the donor's Class II MHC (on monocytes/macrophages). A strong reaction may preclude doing the transplant.[2]

> **ASK YOURSELF:** Smith and Jones have both offered to donate a kidney to Brown. How would you set up the MLRs to see who'd be the better donor?

IMPORTANT: ►Remember that Class I antigens are found on all nucleated cells including platelets, which are honorary nucleated cells. Expression of Class II, on the other hand, is restricted to B cells, macrophages, dendritic cells, and a few related cells like Langerhans cells in the skin. The reason this is important will be clearer soon.

MHC ANTIGEN STRUCTURE. Both class I and class II MHC molecules are glycoproteins composed of two polypeptide chains. Class I consists of an allelically variable chain associated with an invariant chain called beta$_2$-microglobulin. Both of the class II chains (α and β) are variable[3]. There is enough sequence homology between classes I and II, and immunoglobulins and T cell receptors, to indicate that they *all* arose from a common ancestral gene, the famous immunoglobulin domain. It's fascinating to consider that the molecules that do the recognizing, and the molecules that present to them, originated in the same early structure. What might its function have been?



Class I loci A and B (and C) produce MHC molecules which are very similar in structure and function, and one arose from the other by gene duplication. Humans have duplicated Class II loci more than once, as well, so that we have DR, DP, and DQ loci. People sometimes just say 'HLA-D' as an equivalent of Class II; they usually are referring to HLA-DR, the most significant locus in solid organ transplantation.

[2] DNA sequencing is the usual way to type Class II as well as Class I. But the MLR is still an important predictive test, because we don't know how every pair of alleles in humans will respond.

[3] See very informative pictures in supplementary VISUAL MHC material on the web site.

MINOR HISTOCOMPATIBILITY ANTIGENS. There are about 30 minors, mismatch at any of which may cause slow (chronic) rejection. One is H-Y, coded for on the Y chromosome. It's not a transmembrane surface molecule, but an internal protein whose peptides are displayed on MHC Class I (only on male cells, of course). Because of H-Y, male mouse skin grafts will be slowly rejected by inbred syngeneic females; while males accept female grafts without a fuss.

ARCANE TERMINOLOGY. Grafts between genetically identical individuals (e.g., inbred mice, identical twins) are called **syngeneic** or **isografts**; between non-identical members of the same species (e.g., people) are **allogeneic** or **allografts**; and between members of different species (e.g., baboon hearts into babies) are **xenogeneic** or **xenografts**. Grafts from one individual to himself (e.g., hair transplants) are **autografts**.[4]

GRAFT REJECTION MECHANISMS. Grafts are rejected by T cell mechanisms with which you are already familiar, the most important being killer T cells and Th1 cells (via lymphokines and the monocyte/macrophage inflammatory response). Macrophage-derived pro-inflammatory cytokines make the reaction even more intense. As we have developed better immunosuppression regimens and drugs, graft survival has increased and morbidity decreased; but it's almost never been possible to taper and stop a patient's drugs without rejection.

Antibody and complement are not thought to be important, ►except in **hyperacute rejection**. In this case, a graft is given to a patient who has preexisting antibody, IgG or IgM, to it (either to its HLA, because of a prior graft or transfusions, or, in a mismatch, to ABO blood group antigens). Antibody immediately binds to the endothelial cells of the graft's blood vessels. Complement is activated and vasospasm results, via anaphylatoxins and histamine; the organ may never even become perfused with blood (a 'white graft'). This catastrophe can be avoided by making sure there are no cytotoxic (complement-activating) antibodies in the serum of the recipient when tested on the donor's leukocytes.

T CELL INTERACTION IN REJECTION. Th1 recognize foreign MHC antigens of the Class II, HLA-DR loci; killer T cells (CTL) recognize foreign MHC antigens of the Class I, HLA-A and HLA-B loci. *Why* they recognize them will be explained soon.

In rejection, what first happens is that Th1 cells recognize foreign HLA-DR on graft cells (Figure 3, below). Remember, not all cells express HLA-DR; the cells which do so are primarily macrophages and dendritic cells, of which most grafts have plenty. The Th1 proliferate (the phenomenon which is measured in the mixed leukocyte reaction). They will also secrete lymphokines (like IFNγ) that attract a macrophage inflammatory response. The macrophages will mostly be the graft recipient's, because there are more of them.

Meanwhile, CTL nearby are recognizing foreign HLA-A and HLA-B, which are on *all* the graft cells; this recognition is usually insufficient to activate them, though; they also require Th1-derived interleukins (IL-2, primarily) as a second signal. Once activated, the CTL become highly cytotoxic; they may proliferate although they don't have to, and they start killing cells in the graft.

[4] Some biological graft materials (pig heart valves, demineralized bone matrix, tissue-cultured skin) either have no living cells, or they are gradually replaced by the recipient's own cells while serving as a supporting matrix.

You have recognized, I hope, that ►this whole sequence of events is exactly parallel to the recognition of antigen in a normal immune response, for example to a virus. In a normal response, foreign peptide plus self-MHC is recognized; in rejection, it's foreign MHC, probably loaded with a self-peptide.

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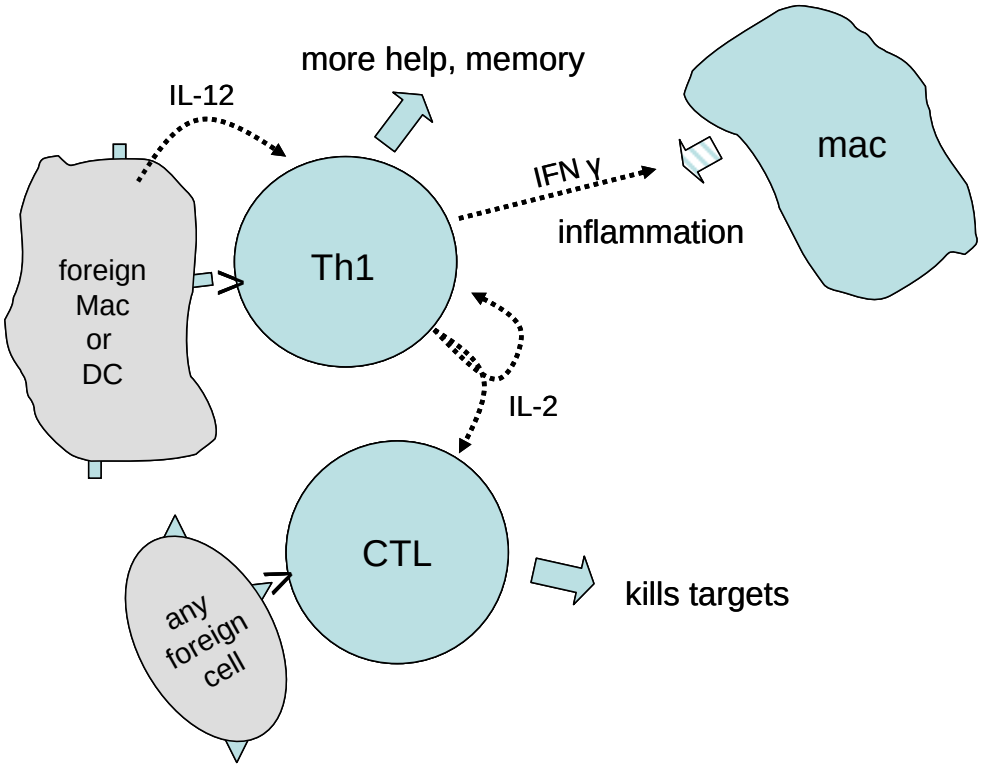

Fig. 3. The Th1, seeing foreign Class II, gets activated, attracting macrophages; and it also helps activate a CTL which is seeing foreign Class I.

Here are some **important** logical extensions of the above scheme: ►(1) If the donor and recipient are identical at Class I but different at Class II, Th1 will be activated; but no CTL will be activated, because there is no Class I difference for them to see. The graft will still be rejected, but since only Th1 and not CTL would be involved, rejection may be slower. ►(2) If the donor and recipient are different at Class I but identical at Class II, there will be no Th1 activated, no IL-2 will be generated, and so few CTL will be activated.

►***Therefore a good Class II match is the most important thing*.**

In general, transplant surgeons are more relaxed about the immunology than immunologists; they figure that a perfect match is impossible anyway, taking minor antigens into account, so they might as well rely on immunosuppressive drugs. It is true that, given the intensive and improved drug therapy that is currently available, the goodness of HLA matching has become less important. Nevertheless, kidney grafts identical at 6/6 alleles (HLA-A, B, DR) do better than those with 2/6 matches, which is the average quality of match in the USA. For bone marrow transplantation, where graft-versus-host (we'll cover that later) is a *huge* problem, we look for matches at A, B, C, DR, and DQ: A 10/10 match is desirable.

> **ASK YOURSELF:** If you could remove the macrophages and dendritic cells from a graft before transplantation, would the graft's chances for survival improve? Why?

LINKING ANTIGEN RECOGNITION AND ALLOREACTIVITY. Th cells normally see MHC Class II + peptide; they also are the ones that see foreign MHC Class II. CTL normally see peptide + Class I, and they also can see foreign Class I. **The recognition of foreign MHC is a chance cross-reaction; the receptors are actually selected to recognize self-MHC + antigen** (Figure 4, below). In fact, if you make a cloned T cell line specific for the donor's own HLA plus antigen X, the same cells can quite often be shown to also react with some foreign HLA (probably loaded with a different peptide, antigen Y).

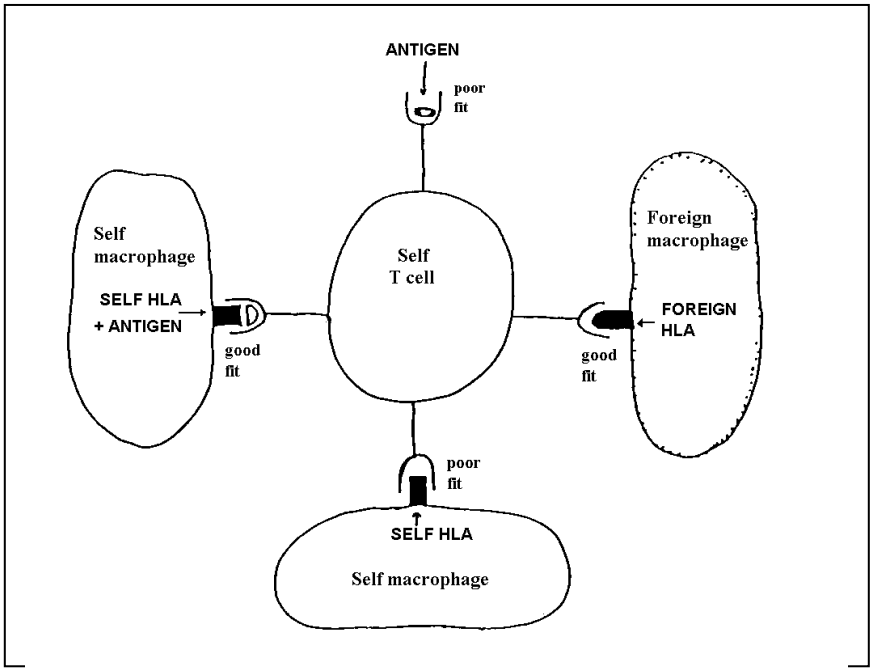

Fig. 4. Foreign HLA mimics self HLA + an antigenic peptide

In other words: My T cells were selected to see something that isn't quite me; it's me plus a foreign peptide. ►What else could look like not-quite-me? *You*. In fact, whoever you are, about 5 % of my T cells will respond in a one-way MLR against your leukocytes. T\For immune responses, that's a huge lot, so even if I've never seen you before, *I behave as though I'm immunized against you.*

Here's the paradox: Maybe 5% of my T cells will bind your MHC strongly enough to cause activation. But essentially none of my T cells that see me plus peptide X will also see you plus peptide X, since they were selected on *my* MHC, not yours. For these two reasons, we can't give one person's T cells to another.

HLA-ASSOCIATED DISEASES. Many diseases have been shown to be associated with a particular HLA allele. These can be Class I or Class II. ►Let us take **ankylosing spondylitis** as an example. This is an arthritic condition in which there is inflammation of the insertions of tendons into bones, followed eventually by calcification so that the affected joints may become inflexible (ankylosed). The spine and sacroiliac joints are most involved. About 92% of people with ankylosing spondylitis are HLA-B27 (8% of people in the USA without the disease are B27). Your risk of getting the disease is 90 times greater if you are B27 than if you are not. Rats made transgenic for the human HLA-B27 gene develop arthritis similar to ankylosing spondylitis.

Why the association? Is there an as-yet unknown pathogen with a surface antigen that so closely resembles the B27 molecule that B27+ people can't recognize it as foreign? Alternatively, the antigen might cross-react with B27, so that a response to the foreign antigen might somehow lead to autoimmunity. There is some intriguing evidence of cross-reaction between certain *Klebsiella* bacteria and B27, but a true functional relation is lacking. Finally, HLA-B27 is prone to misfolding; could this, like a prion, cause inflammation? We just don't know yet.

Another interesting association is between HLA-DR3 and -DR4 and Type 1 juvenile (insulin-dependent) diabetes. The relative risk factor is 5 if a child has one of these antigens. HLA-DR2 seems to *protect* against Type 1 diabetes. In these cases, the ►real culprit seems to be certain HLA-DQ alleles which are in strong linkage disequilibrium with those HLA-DR alleles.

ASK YOURSELF: Do I remember what linkage disequilibrium is? Or shall I look it up?

A growing body of evidence suggests that rare modifications of self-proteins (e.g., citrullination and deamidation in rheumatoid arthritis and celiac disease, respectively) may create novel epitopes that associate strongly with certain MHC alleles; the cells that respond to these 'neoantigens' cross-react with the normal protein.

In genome-wide surveys of single-nucleotide polymorphisms in various autoimmune diseases, by far the most 'hits' are in the MHC. Many genes affect disease risk, but your ability to distinguish foreign invaders from self is of overriding importance. Some examples:

Condition	HLA association	Relative risk
Ankylosing spondylitis	HLA-B27	90
Rheumatoid arthritis	HLA-DR4	4
Systemic lupus erythematosus	HLA-DR3	6
Type 1 diabetes	HLA-DR3	5
Type 1 diabetes	HLA-DR4	5
Type 1 diabetes	HLA-DQ6 or HLA-DR2	0.2
Goodpasture syndrome	HLA-DR2	16
Celiac disease	HLA-DQ2	30
Multiple sclerosis	(DRB1*15:01;DQB1*06:02) haplotype	2
Schizophrenia	Complement C4 (Class III)	not yet established

Learning Objectives for Immunogenetics & Transplantation

1. Define the Major Histocompatibility Complex. Distinguish between HLA-A and HLA-B antigens on the one hand, and HLA-DR on the other, in terms of: which associate with foreign antigens for recognition by helper T cells; which, in association with foreign antigens, are the targets for killer T cells.

2. Define:

alloantigen
haplotype

3. Distinguish Class I and Class II histocompatibility antigens.

4. Identify the chromosome on which the MHC is found in humans.

5. Discuss HLA-A and B typing in terms of how many antigens a person expresses at each locus; given two unrelated parents' haplotypes, predict their children's phenotypes.

6. Discuss the results of exchanging skin grafts P to F1 and F1 to P in inbred animals, and in humans.

7. Describe the one-way mixed leukocyte reaction (MLR) and discuss its use.

8. Distinguish between ‘HLA-D’ and HLA-DR, -DP, -DQ.

9. Explain the interaction of T cells recognizing HLA-D and T cells recognizing HLA-A or B in the generation of killer T cells. Include the roles of cytokines in your discussion.

10. Describe the cellular and molecular events which go on during graft rejection, both of the usual type and hyperacute rejection. Include:

cytotoxic T cells
Th1 cells
M1 macrophages
antibody + complement

11. Discuss how T cells selected to recognize ‘self + X’ also recognize foreign MHC (allorecognition).

12. Give an example of a disease whose incidence is tightly linked to a particular HLA allele. Speculate on mechanisms which might explain the linkage.