Past, Present, and Future Aspects of Histocompatibility

F. H. Bach, J. E. Gose, B. J. Alter, P. M. Sondel, and M. L. Bach

THE modern era of histocompatibility, ushered in during the early part of the century and so elegantly and critically focused by the pioneering studies of Little, Gorer, Snell, and their many students and followers, has expanded into the study of a dizzying panoply of diverse phenomena. From the early description of the existence of multiple different genetic loci that can influence graft rejection has emerged the concept that one of these loci is of quantitatively great importance and is usually referred to as the major histocompatibility complex (MHC). A thorough review related only to the MHC would have to include the importance of the various MHC loci in graft rejection, genetic control of virus susceptibility, disease associations, immune response to newly expressed or "altered-self" antigens on autologous or syngeneic cells, and the control of cell interactions, cell differentiation and, at an extreme, sexual mating preferences. One would have to delve into the genetic fine structure of what may well be the best example of a "super gene" that exists at the level of differentiation represented by mouse, guinea pig, rat, and man and analyze the possible selective forces that have maintained polymorphism and linkage disequilibrium.¹

Attractive as it is to conjure up models that may integrate all of these varied phenomena into a unifying role for the MHC, we have decided to focus on three areas that seem, to us, to be directly related to basic and clinical problems of transplantation.

HLA-ENCODED ANTIGEN

Many different loci very closely linked to the HLA super gene have been described. We shall focus in this discussion on HLA-A, B, C, and D since the products of these loci function as transplantation antigens. The HLA-A, B, C, and D antigens are usually divided into two groups based on tissue distribution and the T lymphocyte subsets responsive to them. Antigens of the HLA-A, B, and C loci, classically detected by serologic methods and thus frequently referred to as the serologically defined (SD) loci, are found on the surface of essentially all tissues. In contrast, products of HLA-D were first detected by a proliferative response in mixed lymphocyte culture (MLC); these antigens, found on relatively few tissues of the body, are frequently referred to as lymphocyte-defined (LD) determinants and serve as the primary activating factors for helper T lymphocytes. Antigens of the ABC region can be defined not only by serologic methods but also by cytotoxic T lymphocytes; these Tc cells recognize the cytotoxicity defined (CD) determinants. Likewise, antigens associated with HLA-D can be defined not only by studying a proliferative response in a primary MLC test, but also by the use of antisera against what are now called the HLA-DR (D-related or D region) antigens.

A major question that still confronts the HLA immunogeneticist is whether the serologically detected and cellularly detected determinants are one and the same. Evidence reviewed at the last International Transplantation Congress suggests that CD and SD determinants of the ABC region may be different.² We shall focus in this paper on the D region. (The D region is defined as that

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genetic material on both sides of the HLA-D locus that segregates with HLA-D but is to the left of HLA-B.

There are three methods by which the D region antigens can be detected. First, homozygous typing cells (HTC) can be used as a direct measure of the HLA-D locus product. Eleven different Dw antigens have been "defined," although it is generally agreed that this test is, for at least some "antigens," difficult at best.

Primed LD typing (PLT), a test introduced from our own laboratory in 1975, appears to measure primarily those determinants encoded in the D region. There has been some controversy in the literature as to whether the determinant(s) that is measured with the HTC is identical with, or even a part of, the determinant(s) that stimulates the secondary proliferative response in PLT. The serologic approach to the recognition of D region-encoded antigens has led to the enumeration of approximately seven different D region antigens.

One major area that needs intensive investigation regards the complexity of the D region. Evidence has been presented using HTC and PLT cells that the D region is, in all probability, complex in that more than a single determinant is encoded by the gene(s) of a single haplotype. Using PLT cells, Sondel and Bach in fact suggested that as many as five different determinants may be encoded by a single haplotype. (The demonstration that multiple determinants are encoded by a single haplotype does not, of necessity, provide evidence for more than a single locus.) Likewise, if one considers only the D region antigens as defined serologically, data presently available for both the mouse and guinea pig suggest that there are at least four and perhaps as many as seven Ia loci in the mouse (the presumed evolutionary homologue of the D region system in man) and three such loci in the guinea pig. It would seem most surprising if man were able to accomplish the multiple and varied functions associated with the I region in the mouse with only a single genetic locus representing the homologue of the H-2 I region in the mouse.

There is a very close correspondence between the various antigens defined with HTC, PLT cells, and anti-region sera. Thus, with regard to most haplotypes, a given Dw antigen is associated with a given PL antigen as well as a given DRw antigen. There are, however, exceptions to this rule. For instance, Dw2 is usually found associated on the same haplotype with DRw2 and PL2 (using the PL nomenclature from Madison). In some individuals, Dw2 can be associated with a different DRw antigen.

One way of demonstrating the flexible correspondence between Dw and PL antigens is to stimulate PLT cells prepared in one haplotype different family combinations with HTC. Results demonstrating the complexity of the D region are shown in Table 1. PLT cells defining antigen PL3 are not restimulated by HTC for Dw1 or Dw3 (nor with HTC for Dw2, Dw4, Dw5, and Dw7; data not shown); they are strongly restimulated, however, by HTC for Dw6, demonstrating a close association within the population tested between PL3 and Dw6 determinants. PLT cells defining antigen PL1 are restimulated by HTC for Dw3 as well as Dw6 but are not restimulated by HTC for Dw1, Dw2, Dw4, Dw5, and Dw7. These data would provide another line of evidence suggesting complexity of the D region since there is not a strict one-to-one correspondence among Dw, DR, and PL antigens. It is thus a major challenge for the future to analyze and dissect the D region.

<table>
<thead>
<tr>
<th>Restimulating Cell</th>
<th>PL1 78C</th>
<th>11BCx</th>
<th>11DBx</th>
<th>9ACx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dw1</td>
<td>774*</td>
<td>499</td>
<td>951</td>
<td>138</td>
</tr>
<tr>
<td>Dw3</td>
<td>3509</td>
<td>2181</td>
<td>841</td>
<td>1030</td>
</tr>
<tr>
<td>Dw3</td>
<td>6431</td>
<td>4833</td>
<td>213</td>
<td>1580</td>
</tr>
<tr>
<td>Dw6</td>
<td>3232</td>
<td>4331</td>
<td>3299</td>
<td>7948</td>
</tr>
</tbody>
</table>

The two PLT cells, 78C, and 11BCx both appear to measure an antigen PL1. Likewise, 11DBx, and 9ACx both measure PL3.

*cpm of H^3TdR incorporated by PLT cells restimulated with HTC.
region as well as to understand the interrelationshi
not only between the cellulary and
serologically defined determinants but be-
tween the various determinants and the func-
tions that they subserve.

IMMUNOLOGICALLY EVOKED RESPONSES
BY MHC-ENCODED ANTIGENS

In Vitro Studies

In 1972, we proposed that the dichotomy
of D and ABC antigen presentation could best
be explained by hypothesizing the response of
two functionally disparate T cell subpopula-
tions: proliferating helper T lymphocytes
(Th) responding to D-encoded antigens, and
precursor cytotoxic T lymphocytes (Tc)
responding to the ABC-encoded antigens.
This model has received experimental sub-
stantiation. In man, we showed that Tc can be
separated from the majority of proliferating
cells (presumably including Th) by mono-
layer absorption studies, in the mouse.
Cantor and Boyse demonstrated that Th are
Lyt 1\(^+\) Lymphocytes (Lyt 1\(^+\) Th) whereas Tc are
Lyt 1\(^-\) Lymphocytes (Lyt 2\(^-\) Tc). It is collaboration
between the Lyt 1\(^+\) Th and Lyt 2\(^-\) Tc that leads to
the generation of maximal cytotoxic responses.
We have recently obtained data, however,
that a second pathway of T lymphocyte activa-
tion obtains when responder and stimulator
cell differ not by an entire MHC difference
but only by a K or D region difference in the
mouse (the presumed analogues of the ABC
region antigens in man) without the strong I
region-encoded LD stimulus. Under these
conditions the proliferative and the cytotoxic
response are strongly dependent on the
presence in the precursor responding cell
population of an Lyt 1\(^-\) Lymphocyte (Lyt 1\(^-\) cell) which plays
little or no role in the generation of cytotoxic
responses when stimulating cells differ by an
entire MHC difference. The exact role of the
Lyt 1\(^-\) cell is not known. One could visual-
ize several possibilities, including the Lyt
1\(^-\) cell acting as a precursor or performing
some form of regulatory function.

It thus appears clear that there are at least
two alternative pathways of T lymphocyte
activation which can be differentially activ-
ated depending on whether a strong LD
stimulus encoded in the I region is present on
the stimulating cells. First, the collabora-
tive response between Lyt 1\(^+\) Th and Lyt 2\(^-\) Tc
and, second, the pathway that is dependent on
the presence of a Lyt 1\(^-\) Tc cell. Whether it is
the presence of the I region-encoded LD
antigens that determines which of these path-
ways predominates in a given response or
whether it is the overall strength of the
reaction that is implicated has yet to be investi-
gated.

The biologic significance of having two
alternative pathways of T lymphocyte differen-
tiation in response to alloantigens is not
clear. One possibility, which we advance
solely as a hypothesis, is that these two
pathways may be differentially responsive to
suppression. Suppressor T lymphocytes have
been thought to act at least in part via
interaction with the Lyt 1\(^+\) Th. To the extent
that this cell plays a major role in the interac-
tive pathway but may be relatively unimpor-
tant when the Lyt 1\(^-\) cells is involved, the
two pathways may be differentially respon-
sive to suppression.

In Vivo Studies

We have been interested in the function of
various antigens encoded by MHC genes
in vivo. A thyroid lobe that is taken from an
animal pretreated with cyclophosphamide
and x-ray and then left in culture for 10 days
prior to transplantation under the kidney
capsule is not rejected in an allogeneic H-2
incompatible recipient. That such a cultured
thyroid still does have target antigens (pre-
sumably on its parenchymal cells) is dem-
strated by the prompt rejection of that
cultured thyroid by a recipient animal specif-
ically presensitized by transplantation of a
fresh thyroid lobe or by administration of
lymphoid cells. To test whether the same
target determinants on the cultured thyroid
would be able to sensitize a recipient, we
transplanted a cultured thyroid to an allo-
geneic host, left the thyroid in place for varying periods of time (in some cases allowing several weeks of function), removed that cultured thyroid from the recipient animal, and retransplanted the host with a new cultured thyroid syngeneic with the “sensitizing” one. This second cultured thyroid was not rejected by the recipient, indicating that the target determinants that appear to be present on the parenchymal cells of a cultured thyroid are not there in an immunogenic form. A challenge for the future, it would seem, is to gain a further understanding of the relative “immunogenicity” and “antigenicity” of various MHC-encoded determinants and possible differences in this regard depending on whether the determinants are presented on parenchymal cells or on lymphoid cells.

**HLA PAIRING AND GRAFT SURVIVAL**

The enormous effort that has been expended on studies attempting to demonstrate the correlation between matching for the HLA-A and B SD antigens and graft survival has yielded relatively little in the way of encouragement. Although there is some evidence that in the nonsensitized recipient, diminishing the number of incompatibilities for the HLA-A and B SD antigens may have a statistically significant effect, this effect is very weak indeed.

In contrast, more than 10 different studies,\textsuperscript{15,16} using reactivity in a primary MLC, which is a reflection of the amount of HLA-D disparity, as well as more recent studies matching donor and recipient for D region antigens,\textsuperscript{17} suggest that very marked improvement in graft survival is associated with decreased amounts of D region disparity.

After having experienced the initial euphoria regarding results of matching for the HLA-A and B SD antigens with the subsequent disappointment as more data were analyzed, it would seem foolhardy to become too optimistic regarding the salutary effects of matching for HLA-D and D region. The almost uniform nature of the positive results in this regard, however, provide the basis for guarded optimism. Prospective and carefully controlled studies may at last fulfill the promise that has been anticipated based on the overriding importance of the HLA complex in determining graft survival. Whether, in the final analysis, it will be an interaction between the response to D region- and ABC-related target antigens will require much more study and analysis.

We would suggest at least one reason why matching for the D region has proved more useful than matching for ABC antigens. We recognize that the extrapolation we are about to make is several steps removed from actual data. In MLC-CML studies we have used three-cell protocols so that we can study the relative quantitative need for LD and CD stimulation for the development of CML. Our findings, shown in Table 2, are that any diminution in the amount of LD stimulus (holding the number of CD different cells constant) results in a rapid decrease in CML; if LD-stimulating cells are held constant, diminishing the number of CD different cells to the same extent causes very little decrease in CML. One could thus make the following argument. Matching for the D region (which would represent a decreased LD stimulus) might have an effect even if the LD stimulus is decreased by only one-half, for instance—thus the improved graft survival when only

<table>
<thead>
<tr>
<th>No. of 6R Responding Cells</th>
<th>Stimulating Cells</th>
<th>CML B10.A Target Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>B10.A&lt;sub&gt;uv&lt;/sub&gt;</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>AQR&lt;sub&gt;uv&lt;/sub&gt;</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>B10.A</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>AQR</td>
<td>26.12</td>
</tr>
<tr>
<td>50</td>
<td>B10.A&lt;sub&gt;uv&lt;/sub&gt;</td>
<td>27.22</td>
</tr>
<tr>
<td>50</td>
<td>AQR</td>
<td>27.22</td>
</tr>
<tr>
<td>50</td>
<td>B10.A</td>
<td>16.82</td>
</tr>
<tr>
<td>50</td>
<td>AQR</td>
<td>16.82</td>
</tr>
</tbody>
</table>

Table 2. Dilution of CD and LD Stimuli in a Three-Cell Experiment

Number of cells × 10<sup>6</sup> in flasks. The UV light-treated B10.A cells provide CD stimulus; the x-irradiated AQR cells, the LD stimulus. With equal numbers of B10.A and AQR stimulating cells, 50 × 10<sup>6</sup> responding cells and 25 × 10<sup>6</sup> each of B10.A and AQR cells yields maximal or near maximal cytotoxicity under these culture conditions.
one D region antigen is matched. Decreasing CD disparity might be much less effective in effecting a diminution in the development of a cytotoxic response.

SUMMARY

We have stressed problems attendant on studies of the MHC, ignoring non-HLA factors and their role in allograft immunity. Many other topics could have been chosen for discussion is any such overview; our selection reflects our own interests. We felt assured, however, that excellent coverage by our many colleagues of the diverse and varied aspects of histocompatibility not approached in this summary has allowed us this freedom.

REFERENCES

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