The Journal of Immunology

Putting a Face to MHC Restriction

Peter Parham

J Immunol 2005; 174:3-5; ;
doi: 10.4049/jimmunol.174.1.3
http://www.jimmunol.org/content/174/1/3

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Putting a Face to MHC Restriction

Peter Parham[1]

It is now an immunological fact that small peptides bound to polymorphic MHC molecules are the antigenic ligands recognized by $\alpha\beta$ TCRs. All of protective immunity is built from and fueled by these interactions, as are the chronic diseases of autoimmunity and hypersensitivity that afflict affluent society. Through positive and negative selection in the thymus, self peptides bound to autologous MHC molecules determine the repertoire of peripheral T cells, which then responds to infection by recognizing foreign peptides bound to those same MHC molecules. In looking back, the erratic voyage of serendipity that was made to discover this fact—-now said in a sentence—-involved a century of exploration and adventure. For those on board in 1987, two papers by Bjorkman et al. in the October 8–14 issue of *Nature* magazine gave a first sighting of the journey's end. The first paper was an immunologist's guide to the three-dimensional structure of the human MHC class I molecule, HLA-A2 (1); the second paper was a manifesto, laying out the implications of the structure for T cell biology (2).

History

That MHC differences cause the rejection of tissue transplants was unknowingly observed in laboratory mice toward the end of the nineteenth century. By the late 1930s, MHC class I alloantigens of mice were being defined serologically with alloantibodies (3); their human counterparts, the HLA class I Ags, were identified in analogous fashion in the 1950s. HLA-A2 was arguably the first HLA Ag to be defined. It was also common, being at frequencies of up to 50% in human populations. Such abundance and its ease of detection were to make HLA-A2 a magnet for research (4). The 1960s saw the first use in clinical transplantation of matching donors and recipients for HLA Ags (5). With the 1970s came discovery of MHC class II Ags and appreciation of the cellularly defined phenomenon of "MHC restriction" (6). The latter was defined by T cell reactivity, as illustrated here by influenza infection. Recovery from influenza requires influenza-specific CD8 T cells, which kill off the influenza-infected cells in the respiratory tract. In laboratory experiments, influenza-specific CD8 T cells from one person will kill influenza-infected cells from another person, but only if they share an HLA class I Ag. The cytotoxic function of the CD8 T cells is thus restricted to MHC class I type, as well as to the kind of virus. Likewise, the recognition of foreign Ags by CD4 T cells is restricted to MHC class II type. Conceptually, MHC restriction was a breakthrough; it liberated thought on MHC function from the manmade world of transplantation, and put it bang in the middle of the fight with infection.

In molecular terms, the simple way to explain MHC restriction was with two TCRs, one binding to MHC, the other to foreign Ag: a model called dual recognition. Much study of T cell specificity and thymic selection failed, however, to separate the two activities, such negative results favored the competing model in which MHC restriction and Ag specificity were the properties of a single TCR recognizing a composite ligand called "altered self." Although continued attempts were made to resolve the question using cellular systems, they never quite reached the mark. A molecular solution was needed, but where was one to start? At that time, the late 1970s, nobody had a grasp on a TCR or a T cell Ag. For MHC, the situation was better: glycoproteins were purified and sequences determined. So crystallographic work on HLA class I began in 1979. Crystals grew almost immediately, but diffracted poorly. Workable HLA-A2 and HLA-A28 crystals were eventually obtained and in 1985 Bjorkman, Strominger, and Wiley staked their claim in *The Journal of Molecular Biology* (7).

Between 1980 and 1985, both TCRs and T cell Ags were firmly taken in hand. After years of being elusive, the $\alpha\beta$ TCR of CD4 and CD8 T cells turned out to be comfortingly familiar, having structure, genetics, and diversity that paralleled those of the well-characterized Ig receptors of B cells (8). It so fitted the bill for a clonotypic T cell Ag receptor, that minds were instantly freed to worry about other things. For the T cell Ags, an elegant and simplifying principle was emerging, one that revealed common ground among the universe of foreign proteins that happen to be T cell Ags. Its essence was that the Ags restricted by MHC were short peptides produced by cellular degradation of antigenic proteins (9). This property of T cell Ags was first understood for CD4 T cells restricted by the MHC class II molecules on macrophages, cells specialized in the uptake, and breakdown of foreign material. Macrophages were now said to "process" and "present" Ags to T cells (10), and by 1985 it was seen that MHC class II molecules selectively bind such antigenic peptides (11). Similar properties were inferred for the Ags recognized by CD8 T cells, when it was shown that incubation of uninfected cells with synthetic peptides corresponding to influenza Ags would render the cells susceptible to attack by influenza-specific CD8 T cells (12). The years 1980–1985 were the time when molecular biologists and their methods were recruited to immunology. The changes they wrought were instrumental for description of the TCR and its diversity (8); they also drove a rapidly increasing knowledge of MHC polymorphism. Research on the three elements of MHC restriction, TCR, T cell Ag, and MHC, now came to confluence. In the resulting turbulence, each stream was challenged and tested by the others, culminating in the structure and synthesis of Bjorkman et al. (1, 2).

[1] Address correspondence and reprint requests to Dr. Peter Parham, Department of Structural Biology, Sherman Fairchild Building, 299 Campus Drive West, Stanford University School of Medicine, Stanford CA 94305-5126. E-mail address: peropa@stanford.edu

0022-1767/05/$02.00

Structure

The HLA-A2 protein that crystallized had been cleaved from the membrane of a human cell line with the protease papain; it consisted of a H chain fragment of approximately 300 amino acids and β_2-microglobulin (β_2m), the L chain of approximately 100 amino acids. The amino acid sequence of β_2m had been known for more than a decade to resemble that of an Ig constant domain (13) and the membrane-proximal α_3 domain of the HLA-A2 chain was more recently shown to be similarly Ig-like (14). In the three-dimensional structure, these two domains were indeed folded like Ig constant domains, together making a support for the membrane-distal α_1 and α_2 domains. Unlike β_2m and α_3, the primary structures of α_1 and α_2 had never yielded their secrets. There was a history of casting MHC in Ig's image (15), and in their 1985 paper, Bjorkman et al. (7) were still thinking along those lines. Ending the paper's summary was the sentence: "This domain organization [of HLA class I] is similar to the arrangement of domains in the Fab and Fc fragments for immunoglobulins" (7). Regarding α_1 and α_2, they were in for a welcome surprise; these two domains formed a platform of β-pleated sheet on top of which were two long and parallel α helices separated by a large groove. While sorting out the electron-density map of HLA-A2, Bjorkman et al. had encountered a region of electron density that could not be attributed to either of the two polypeptides or to the carbohydrate attached to the α_1 domain. In the final structure, this unanticipated extra density was seen to fill the groove between the two α helices, and to be in size and quality what might be expected from a bound peptide. Archimedes had a word for this.

That the extra density had comparable strength to that of the surrounding polypeptide was consistent with each HLA-A2 molecule binding a single peptide. That individual amino acids were not discerned in the extra density, pointed to the HLA-A2 molecules being bound to peptides of different sequence, giving a blurred composite image. The tops of the two α helices and the bound peptide formed a face that would project away from the cell membrane to which the MHC class I molecule is attached. Bjorkman et al. predicted that this face is what engages the TCR, allowing simultaneous contact to be made with peptide Ag and MHC. This was the face of MHC restriction, and it looked like altered self.

Synthesis

Although their crystals contained neither TCR nor foreign Ag, Bjorkman et al. (2) proceeded boldly in their second paper to elaborate the "The foreign Ag binding site and T cell recognition regions of class I histocompatibility Ags." The analysis built off of the assumption that the extra density in the groove represented peptides bound in the cells from which the protein was purified. They then proposed that in virus-infected cells, peptides derived from viral proteins would be similarly produced and bound by MHC class I to become targets for virus-specific T cells. Although speculative in the context of the structure itself, these assumptions were bolstered by what was then swirling through the crowd working on Ag processing and presentation (9–12).

Having established the groove and its environs as the foreign Ag-binding site, Bjorkman et al. then defined its immunologically functional positions: residues whose side chains could contact bound peptide (32 positions) or TCR (18 positions). Comparison of these positions to the 17 positions where polymorphic variation of HLA class I concentrates revealed that 15 of the 17 most variable positions were also functional positions: 12 contacting peptide and 3 contacting TCR. Variation in MHC class I molecules was seen to be mainly at sites that could change which peptides bind and, to lesser extent, which TCRs engage the resulting peptide-MHC complexes. Here was both mechanism for MHC restriction and function for MHC polymorphism.

Bjorkman et al. extended their synthesis by taking into account studies related to transplantation. In clinical transplantation, the extent of HLA difference between donor and recipient correlates with rejection of organ grafts or graft-vs-host disease following bone marrow grafts. These immune reactions involve alloreactive T cells and Abs that recognize specific HLA differences. By 1987, a substantial literature reported on substitutions within the α_1 and α_2 domains that distinguish MHC class I allotypes and their recognition by alloreactive T cells and Abs. Bjorkman et al. compiled these results to show that allotypic differences affecting T cell reactivity all involved peptide-binding residues of the Ag-binding site, some of which were deep in the groove and unlikely to contact TCRs directly. The inference was that T cell allorecognition plays by similar rules to the recognition of altered self, being dependent upon the type of peptide bound. By contrast, allotypic substitutions that determined the binding of alloantibodies did not necessarily involve peptide-binding residues. Those substitutions were located at exposed surfaces, mainly of the helices, where they could interact directly with Ab.

The concordance between the crystallographic structure and the accumulated immunology was uncanny, because it all made so much sense. The two papers were accepted for publication in *Nature* within 10 days of submission (1, 2). With these papers to guide, future experiment could then proceed with more purpose, but less adventure. From the choice and design of their figures, this is surely what Bjorkman et al. intended. Three of the figures in the structure paper (1) are incessantly reproduced—-in research papers, reviews, journal covers, textbooks, and company stuff. These icons of immunology are the ribbon diagram of the entire protein, with its resemblance to the head of a moose (a large North American or Siberian elk) (Fig. 2*a* of Ref. 1), the ribbon diagram of the α_1 and α_2 domains (Fig. 2*b* of Ref. 1), and the surface representation of the top of the molecule—-the face of MHC restriction—-with its gaudy, thought-provoking extra density (Fig. 6*b* of Ref. 1).

Outcome

Before the papers of Bjorkman et al. (1, 2), the impact and influence of protein crystallography on immunology was not so widely felt. Obtaining a structure was a long arduous process, limited by protein supply, crystal fragility, and the painstaking methods for collecting and analyzing the diffraction patterns. The HLA-A2 structure was a saga done mainly in the old heroic style and what Bjorkman et al. published was an unrefined structure at modest resolution, 3.6A. Having charted the waters with HLA-A2, structures for additional MHC class I molecules were obtained more quickly, each one giving additional insight: HLA-A28 in 1989, and HLA-B27 and the refined HLA-A2 structure in 1991 (16). The latter was masterfully encyclopedic, filling 43 pages of *The Journal of Molecular Biology* (17). In the following year, a complex of HLA-A28 reconstituted with a defined nonamer peptide from the influenza virus nucleoprotein gave a structure at atomic resolution. A similar progression for MHC class II structures began in 1991 (16).

The first HLA class I structures showed that the peptides bound by MHC class I were mostly nonamers that were bound tightly in register within the binding groove. Once bound they literally became restricted! This knowledge opened up a new cottage industry in which peptide pools were extracted from purified MHC class I and analyzed by high performance liquid chromatography, mass spectrometry, and protein sequencing (18, 19). Peptide-binding motifs for individual class I allotypes were defined and then used

to search the sequences of microbial proteins for candidate antigenic peptides for incorporation into vaccines. Similarly, peptide pools fractionated from tumor cells have been screened with immunological assays to identify tumor Ags (19). These too are being tested as potential cancer vaccines.

Because the HLA-A2 structure spoke so directly to T cell function, the paper by Bjorkman et al. marked the beginning of a period in which crystallographers and their methods were inexorably incorporated into quotidian immunology. Helping this happen were improved crystallographic methods and the abundance of proteins made possible by recombinant techniques. In Wiley's laboratory alone more than one MHC structure per year was averaged in the 14 years following Bjorkman et al. (1, 2). Landfall being reached in 1996, with the structure of HLA-A2 and a viral peptide bound to a TCR: journey's end—-a complete picture of MHC restriction (20). That same year, Rolf Zinkernagel and Peter Doherty were awarded the Nobel Prize in physiology or medicine for the discovery in Australia of MHC restriction (6).

References

1. Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature 329:506*.
2. Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibity antigens. *Nature 329:512*.
3. Klein, J. 1975. *Biology of the Mouse Histocompatibility-2 Complex: Principles of Immunogenetics Applied to a Single System*. Springer-Verlag, Berlin.
4. Terasaki, P. I., ed. 1990. *History of HLA: Ten Recollections*. UCLA Tissue Typing Laboratory, Los Angeles.
5. Terasaki, P. I., ed. 1991. *History of Transplantation: Thirty-Five Recollections*. UCLA Tissue Typing Laboratory, Los Angeles.
6. Zinkernagel, R. M., and P. C. Doherty. 1997. The discovery of MHC restriction. *Immunol. Today 18:14*.
7. Bjorkman, P. J., J. L. Strominger, and D. C. Wiley. 1985. Crystallization and X-ray diffraction studies on the histocompatibility antigens HLA-A2 and HLA-A28 from human cell membranes. *J. Mol. Biol. 186:205*.
8. Davis, M. M., and Y.-H. Chien. 1999. T-cell antigen receptors. In *Fundamental Immunology*. W. E. Paul, ed. Lippincott-Raven, Philadelphia, p. 341.
9. Germain, R. N. Antigen-processing and presentation. 1999. In *Fundamental Immunology*. W. E. Paul, ed. Lippincott-Raven, Philadelphia, p. 287.
10. Unanue, E. R. 1984. Antigen-presenting function of the macrophage. *Annu. Rev. Immunol. 2:395*.
11. Babbitt, B. P., P. M. Allen, G. Matsueda, E. Haber, and E. R. Unanue. Binding of immunogenic peptides to Ia histocompatibility molecules. 1985. *Nature 317:359*.
12. Townsend, A. R., J. Rothbard, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell 44:959*.
13. Peterson, P. A., B. A. Cunningham, I. Berggard, and G. M. Edelman. 1972. β_2-microgloblin: a free immunoglobulin domain. *Proc. Nat. Acad. Sci. USA 69:1697*.
14. Orr, H. T., D. Lancet, R. J. Robb, J. A. Lopez de Castro, and J. L. Strominger. 1979. The heavy chain of human histocompatibility antigen HLA-B7 contains an immunoglobulin-like region. *Nature 282:266*.
15. Peterson, P. A., L. Rask, K. Sege, L. Klareskog, H. Anundi, and L. Ostberg. 1975. Evolutionary relationship between immunoglobulins and transplantation antigens. *Proc. Nat. Acad. Sci. USA 72:1612*.
16. Stern, L. J., and D. C. Wiley. 1994. Antigenic peptide-binding by class I and class II histocompatibility proteins. *Structure 2:245*.
17. Saper, M. A., P. J. Bjorkman, and D. C. Wiley. 1991. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6A resolution. *J. Mol. Biol. 219:277*.
18. Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H. G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature 348:252*.
19. Cox, A. L., J. Skipper, Y. Chen, R. A. Henderson, T. L. Darrow, J. Shabanowitz, V. H. Engelhard, D. F. Hunt, and C. L. Slingluff. 1994. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science 264:716*.
20. Garboczi, D. N., P. Ghosh, U. Utz, Q. R. Fan, W. E. Biddison, and D. C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature 384:134*.