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Commentary

Restrictions on the use of antigenic peptides by the immune system

Jonathan C. Howard

Department of Immunology, The Agricultural and Food Research Council Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, United Kingdom

The peptide binding grooves of major histocompatibility complex (MHC) molecules are an imperfect solution to the problem of presenting pathogenic epitopes to the immune system. Structural constraints limit the efficiency of each allelic product: for example, peptides assembled in HLA.B27 must have an arginine as an anchor at position 2 (1), while HLA.A3 needs a leucine at the same anchor position (2). Indeed, the situation is more restrictive still, since, while the specific anchor residues are positively necessary for efficient assembly of peptide, other positions probably have more complex positive and negative rules. Overall, the rules governing the assembly of peptides with a given class I allelic product probably normally prohibit the use as epitopes of more than one or two peptides per average protein. These limits are purely structural: immunological tolerance of self antigens may further prevent the immune system from using as an antigenic epitope a peptide that is perfectly well assembled. That these fundamental limitations on the use of pathogen polypeptides as a source of antigen must feed through into excess mortality from infection can be seen from the polymorphism of MHC molecules, a polymorphism that is focused on residues interacting directly with peptide (3). Each allele alone is far from perfect, but two alleles at each of three loci multiply the chance of a successful immune response by a factor presumably close to 6. In general, the efficacy of each MHC allele must be a limiting factor in disease resistance; if a way could be found of causing a MHC molecule to assemble successfully with twice as many peptides, this would be strongly favored by natural selection, while, by the same argument, if any process were to restrict further the successful assembly of peptides with MHC molecules, this should be strongly selected against.

The recent discovery of an accessory apparatus presumably required for efficient delivery of cytosolic peptides to the lumen of the endoplasmic reticulum (Fig. 1; refs. 4 and 5) has brought speculation that there may be further limitations on peptide availability for class I loading. How permissive is the accessory system? And if this too shows functional polymorphism, does this implicate the accessory system as a further limiting factor in disease resistance? Lobigs and Müllbacher (6) have attempted to show that there is no significant functional polymorphism in the endoplasmic reticulum peptide transporter TAP by inquiring whether the transporters of several different mammalian species are competent to deliver a specific peptide for loading into a transfected mouse class I molecule. This approach has enabled them to determine that indeed several mammalian transporters can deliver influenza and vaccinia virus peptides for mouse class I loading, a point already shown by implication for a number of peptides by others working between mice and humans with cross-species transfections without specific reference to the class I accessory apparatus. Lobigs and Müllbacher's view that polymorphism in transporter sequences is unlikely to play a large role in individual differences in peptide loading into class I molecules is already suspected to be generally correct for humans since fairly extensive studies have so far failed to demonstrate large-scale structural polymorphism in human TAP (7, 8). A total of five conservative coding substitutions in the two TAP chains provide a system of alleles segregating at various frequencies in the human populations studied. The substitutions are exclusively located in the C-terminal portions of the TAP polypeptides, regions of the protein viewed as less likely to determine the specificity of peptide transport than substitutions in the N-terminal membrane-spanning loops, which presumably form a transport channel. It must be emphasized, however, that there is no direct evidence whether or not the limited polymorphism of human TAP chains has any impact on peptide transport specificity; the matter is still *sub judice*. In the absence of explicit evidence to the contrary, the relative lack of polymorphism in TAP proteins might justifiably be interpreted to mean that TAP is permissive for all relevant peptides and therefore does not impose any further limitation on the already serious problem exposed by the polymorphism of class I molecules.

As Lobigs and Müllbacher well know, however, the situation is more complex than it seems at first sight because in the rat one of the two TAP chains, TAP2, has been shown to exist in two markedly different allelic forms (9). In this species, two abundant alleles of TAP2 differ by a total of 25 coding substitutions, the great majority of them in the N-terminal membrane-spanning loops of the polypeptide. It is perhaps worth adding that none of the human TAP2 polymorphic positions corresponds to any of the 25 rat TAP2 polymorphic positions. Furthermore, the polymorphism in TAP2 correlates with loading of a certain rat class I molecule with rather different sets of peptides, and there is clear evidence that one of the two TAP alleles is much more efficient than the other at loading this particular class I molecule (10). The inescapable implication of these observations is that neither of the two rat TAP alleles is fully permissive, that the class I molecule in question does not load efficiently with the peptides provided by one of the two TAP alleles (for some reason), and that this limitation is compensated for by the existence of the other allele.

These findings also have implications for understanding the situation in humans despite the contrary view of Lobigs and Müllbacher. For if neither of the two rat TAP alleles is fully permissive, we might suppose, as with class I molecules, that this was due to an inherent structural limitation in the peptide transport mechanism. And if this is so in the rat, we must also ask whether the apparently insignificantly polymorphic human TAP is indeed fully permissive. Both possible answers to this question raise troublesome issues. For if human TAP is in fact fully permissive, our supposition about an inherent structural limitation must be wrong, and we must ask why the rat has settled for the apparently less efficient solution of polymorphism for two partially permissive TAPs. On the other hand, if human TAP is not fully permissive, we must ask why our species has apparently not taken advantage of the extra coverage potentially available from the second TAP allele.

Inspection of the amino acid sequences of the two rat TAP2 alleles (9) suggests a preliminary answer to the question whether TAP2 sequences used by putatively functionally monomorphic species like humans are permissive. It is clear that one of the two rat alleles is identical to the typical human and mouse TAP2 at

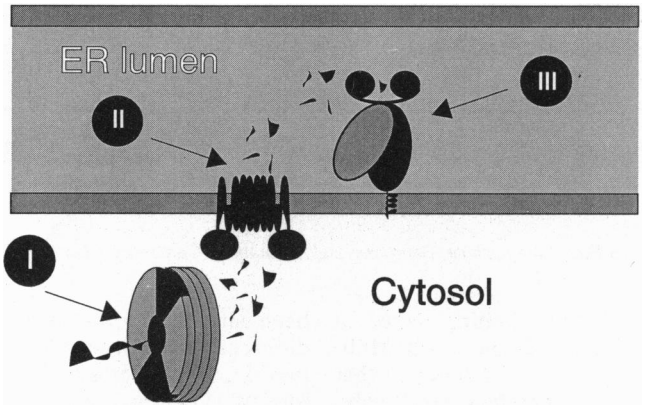

FIG. 1. Three potential restriction points operating during antigen processing and class I MHC molecule assembly, which may limit the opportunities that the T-cell immune system has to inspect the full sequence of polypeptides of pathogenic origin. I, the proteasome, a cytosolic protease, has two polymorphic subunits that map in the MHC. It is likely that the MHC-linked subunits affect the identity of peptides available for transport to the endoplasmic reticulum (ER) lumen. II, the MHC-linked peptide transporter TAP is also polymorphic in the rat and probably further restricts the free flow of peptides into the ER. III, of the peptides that finally reach the ER, the class I MHC molecule can only accept a subset that are conformable to the structure of the peptide-binding groove.

most of the 25 positions at which the two rat alleles differ. It therefore seems likely that if the rat allele is restrictive, so also will be the closely similar human and mouse sequences. Insofar as there is any experimental evidence on the matter, it supports this conclusion. Thus, when the rat class I molecule was transfected into a mouse cell, it expressed antigenic specificities suggesting that it had loaded with peptides typical of the rat transporter allele whose sequence the mouse TAP2 most closely resembled, while it failed to express antigenic specificities typical of the other rat TAP2 allele (10). The mouse TAP was thus apparently incapable of transporting the second set of peptides. Comparable experiments on the loading of rat class I could evidently be done to test human TAP for the restrictive properties which its sequence strongly suggests it will also possess.

It therefore looks as if the critical question is, why is it that the human species tolerates an apparently monomorphic TAP even though it is restrictive and even though a second allele that can transport a somewhat different set of peptides is structurally feasible? A testable explanation that seems to cover most of the bases is as follows. The form of TAP used by approximately all people, possibly all mice, and perhaps 50% of rats, is indeed fully permissive for peptides that can load into essentially all class I alleles of mice and humans. It is inefficient only in the rat (and, of course, in other undescribed species, which may have taken the rat route), because the rat has evolved a significant frequency of structurally peculiar class I MHC alleles. Many of these have specialized requirements for peptides, which are met much better by the unusual rat allele of TAP2 than by the conventional allele. Two additional points are essential to this argument: first, the unconventional allele of rat TAP2 should be less permissive for loading peptides into conventional class I alleles than the conventional allele of TAP2 (although the effect could be small); second, the human species should have essentially no class I alleles that are structurally peculiar in whatever sense the alleles of rat class I are peculiar.

If this argument is correct, it will be of the greatest interest to discover in what structural respect the critical rat class I alleles are peculiar and how this impacts on the classes of peptides that they can accommodate. Equally, the narrowly defined adaptive relationship between TAP2 sequence and class I structure implied by the rat phenomena seems to suggest that the TAP transporter protein must be involved in the transport of peptides to the endoplasmic reticulum lumen, which are close to, if not in, the final state for assembly. This conclusion would seem, incidentally, to argue against a view of peptide assembly into class I that involves extensive trimming by proteolytic enzymes of significantly longer precursors after transport into the endoplasmic reticulum.

Just as the peptide transport process is a key point at which further restriction on peptide presentation can be imposed over and above the structural limitations of individual class I alleles, so the proteolytic enzymes that generate peptides from polypeptide precursors may also, in principle, play a restrictive role. The fact that two β subunits of the proteasome map in the MHC closely adjacent to the two TAP genes (11–13) has justifiably fired enthusiasm for the view that the MHC-linked subunits of the proteasome may be involved in generation of cytosolic peptides for delivery to the endoplasmic reticulum. Experiments have so far failed to provide any support for this idea: human mutant cells lacking both the MHC-linked proteasome subunits and the TAP genes, but reconstituted with both the TAP genes by transfection, appear to be able to process and present viral polypeptides essentially as efficiently as wild-type cells (14). Indeed, the whole pattern of constitutive peptide loading of the HLA.A2 class I molecules in these cells was apparently restored to normal by the TAP genes alone (15). However, a key consideration that qualifies the results of these experiments is that they were done in the absence of γ-interferon. This lymphokine is known to modify substantially the pattern of proteasome subunit biosynthesis (16, 17), including increasing expression of both the MHC-linked subunits (11, 13). One might therefore urge that future experiments designed to display the activity of the MHC-linked proteasome subunits should look specifically at the effects of γ-interferon in addition to analysis of constitutive proteolytic activity.

In light of these results, it is important to consider the possible implications of polymorphism in the MHC-linked β subunits of the proteasome. Both these subunits were first discovered in the mouse because of protein polymorphism, which permitted allele-specific anti-proteasome antisera to be prepared inadvertently (18). Allele-specific subunit behavior on two-dimensional gels (16–19) showed that the allelic substitutions were unlikely to be conservative, and sequence analysis has now confirmed this expectation. As Zhou and colleagues show (20), allelic variation in the LMP-2 subunit sequence is not extensive, with a total of only three positions involved in coding substitutions, but all three substituents entail a loss or gain of charge. In particular, the substitution of a histidine for an arginine at position 60 seems of interest since this same position shows the same polymorphism in the human homologue (13). Discriminating assay systems are required by means of which subtle changes in specificity of proteasome cleavage may be detected. If, as Zhou *et al.* speculate, polymorphic sequence differences in LMP-2 (and also in the second MHC-linked proteasome β subunit, LMP-7) are associated with generation of distinct sets of peptides, then the overall peptide spectra loaded by different MHC class I alleles in the presence of different LMP-2 and -7 alleles may be a sensitive way of detecting the phenomenon, although not of understanding its structural basis.

Clearly, the issues raised by functional polymorphism in proteasome subunits are the same as for TAP chains. In all cases, they imply a lack of complete permissiveness in the accessory systems

by which peptides are made available for loading into class I MHC molecules and a concomitant further limitation on the range of peptides with which a given class I protein can assemble. The possibility cannot be excluded that this further limitation is itself minimized by coadaptation of accessory system alleles and peptide-binding grooves in the sense that in an ideal world the accessory system should generate and transport peptides with which the class I allele(s) in the same haplotype assemble efficiently. This notion has the attraction that it provides a coherent explanation for the mapping of TAP and proteasome subunit chains into the MHC, but for the time being it survives largely through lack of any relevant evidence either way.

1. Jardetzky, T. S., Lane, W. S., Robinson, R. A., Madden, D. R. & Wiley, D. C. (1991) *Nature (London)* **353,** 326–331.
2. DiBrino, M., Parker, K. C., Shiloach, J., Knierman, M., Lukszo, J., Turner, R. V., Biddison, W. E. & Coligan, J. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1508–1512.
3. Bjorkman, P. J., Saper, M. A., Samroui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 512–517.
4. Parham, P. (1990) *Nature (London)* **348,** 674–675.
5. Robertson, M. (1991) *Nature (London)* **353,** 300–301.
6. Lobigs, M. & Müllbacher, A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2676–2680.
7. Powis, S. H., Mockridge, I., Kelly, A., Kerr, L.-A., Glynne, R., Gileadi, U., Beck, S. & Trowsdale, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1463–1467.
8. Colonna, M., Bresnahan, M., Bahram, S., Strominger, J. L. & Spies, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3932–3936.
9. Powis, S. J., Deverson, E. V., Coadwell, W. J., Ciruela, A., Huskisson, N. S., Smith, H., Butcher, G. W. & Howard, J. C. (1992) *Nature (London)* **357,** 211–215.
10. Powis, S. J., Howard, J. C. & Butcher, G. W. (1991) *J. Exp. Med.* **173,** 913–921.
11. Glynne, R., Powis, S. H., Beck, S., Kelly, A., Kerr, L. & Trowsdale, J. (1991) *Nature (London)* **353,** 357–360.
12. Martinez, C. K. & Monaco, J. J. (1991) *Nature (London)* **353,** 664–667.
13. Kelly, A., Powis, S. H., Glynne, R., Radley, E., Beck, S. & Trowsdale, J. (1991) *Nature (London)* **353,** 667–668.
14. Momburg, F., Ortiz-Navarrete, V., Neefjes, J., Goulmy, E., Vandewal, Y., Spits, H., Powis, S. J., Butcher, G. W., Howard, J. C., Walden, P. & Hämmerling, G. J. (1992) *Nature (London)* **360,** 174–177.
15. Arnold, D., Driscoll, J., Androlewicz, M., Hughes, E., Cresswell, P. & Spies, T. A. (1992) *Nature (London)* **360,** 171–173.
16. Ortiz-Navarrete, V., Seelig, A., Gernold, M., Frentzel, S., Kloetzel, P. M. & Hämmerling, G. J. (1991) *Nature (London)* **353,** 662–664.
17. Young, Y., Waters, J. B., Früh, K. & Peterson, P. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4928–4932.
18. Monaco, J. J. & McDevitt, H. O. (1984) *Nature (London)* **309,** 797–799.
19. Monaco, J. J. & McDevitt, H. O. (1986) *Hum. Immunol.* **15,** 416–426.
20. Zhou, P., Cao, H., Smart, M. & David, C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2681–2684.