Positive selection of T cells: fastidious or promiscuous?

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Several studies reported during the past year, most of which exploited novel *in vivo* positive selection systems, have addressed the basis of peptide involvement in positive selection of T cells. The very flexible, yet specific, requirements the studies demonstrate differ somewhat from the very specific requirements reported in earlier experiments relying on *in vitro* selection systems.

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Abbreviations

FTOC	fetal thymic organ culture
MCC	moth cytochrome c
TCR	T cell receptor
V	variable

Introduction

The fact that T cells are 'MHC restricted', that is preferentially respond to antigens offered by cells expressing particular alleles of molecules encoded in the MHC, is the landmark discovery [1,2] recognized by the 1996 Nobel Prize in Physiology or Medicine. This acknowledgement of the importance of MHC restriction coincided nicely with a final step in the elucidation of its molecular basis, the solution of the crystal structures of two TCR–peptide–MHC molecule ternary complexes [3••,4••]. In contrast, our understanding of the cellular basis of MHC restriction is still quite cloudy; we still do not know precisely how the peripheral T cell repertoire is shaped to enrich for CD8+ cells capable of recognizing antigens in the context of self-MHC class I molecules and for CD4+ cells which can see antigens in association with self-MHC class II molecules. The process is known to involve positive selection of immature T cells in the thymus and is determined by the specificity of the TCR expressed by individual thymocytes for the particular MHC molecules encountered on stromal cells. But some important questions remain either unanswered or their answers are controversial: precisely when during differentiation, and for how long, is a thymocyte subject to positive selection? What accounts for the multistep, or at least prolonged, nature of the selection process? What is the mechanism by which thymocytes commit to the CD8+ versus the CD4+ lineage? What is the exact nature of the ligand(s) involved? What underlies positive versus negative selection? Which of the two, positive or negative selection, has the greater impact on shaping the repertoire that emerges into the periphery?

Experiments touching on all of these questions have been reported during the 1995–1996 period covered by this review. We have chosen to focus on those addressing the nature of the ligand involved in positive selection because, in our opinion, results from these have been the most productively provocative.

The previous consensus

That the pivotal event in positive selection involves thymocyte recognition of MHC molecules on stromal cells was first suggested by a series of bone marrow chimera and thymus transplantation experiments performed in the 1970s and 1980s, but was more convincingly established using TCR transgenic and MHC knockout mouse strains produced in later years (reviewed in [5]). The precise nature of the ligand recognized during positive selection remained undefined, however, because this issue was so difficult to address using the existing experimental approaches. It was conceivable that the TCRs on differentiating thymocytes interacted with either MHC molecules alone or with MHC molecules carrying essentially any peptide, a specific subset of peptides or only a single peptide. It is important to distinguish between these possibilities because the nature of the ligand could have implications for the distinction between positive and negative selection in the thymus, the flexibility of the T cell repertoire in the periphery, and potential mechanisms of autoimmunity.

In 1993, a powerful approach to address this issue was reported by two groups [6,7]: the addition of peptides to fetal thymic organ cultures (FTOCs) derived from mouse strains carrying mutations which interfere with the peptide loading and/or surface display of MHC class I molecules, thereby heavily favoring the presentation of the added peptides. A major conclusion from both studies was that peptides play a specific role in positive selection. The approach was refined by using FTOCs derived from the same mouse mutants but crossed with TCR transgenic strains [8–10]. These experiments permitted the definition of peptides capable of selecting a particular TCR and solidified the conclusion that peptides are very specifically involved in positive selection. Unfortunately, the nature of the most effective peptides differed markedly with the two TCRs examined. In one case, low concentrations of the antigenic peptide or of agonist analogs (peptide variants also capable of stimulating the mature T cell) were the most efficient positive selectors; elevated concentrations of these peptides promoted negative selection [9,10]. In the other case, antagonist analogs (variants

incapable of stimulating the mature T cell, but able to prevent its stimulation by agonists) were the best positive selectors and, while higher concentrations of such peptides occasionally led to negative selection, this was not usual; the antigenic peptide itself could not promote positive selection of functional T cells at any concentration tested [8,11,12]. These observations had implications for the distinction between positive and negative selection: the former set was taken as strong evidence in support of a differential avidity model, whereby TCR–MHC–peptide interactions of moderate avidity result in positive selection while stronger interactions lead to negative selection, whereas the latter set argued for a more complex view, termed the efficacy model. The latter model included a consideration of the nature of the ligand, perhaps more accurately the type of signal it evoked, in addition to the effect of ligand affinity and concentration on selection.

The ingenuity of the FTOC systems prompted a rapid and general acceptance of the major conclusions from this series of studies [8–12]. The consensus was that positive selection is 'exquisitely peptide specific', being most effective with those peptides which exhibit minimal, or even no, structural divergence from the antigenic peptide.

But...

Deeper reflection suggested that this consensus might be a bit premature because the FTOC experiments actually had several limitations. First, they involved analyses of only a limited number of peptides and only two TCRs. Furthermore, both TCRs were specific for foreign peptide antigens in the context of MHC class I molecules. It was obviously important to assess a larger set of peptides and different types of TCRs, including ones that were alloreactive or MHC class II restricted. The latter, in particular, were important because their crystal structure [13] and peptide elution [14] data have hinted at a potentially different, more relaxed, mode of MHC–peptide recognition. Second, although the FTOC systems can give an indication of what peptides are capable of enhancing the positive selection of a given TCR, they do not address which ones are actually responsible for selection in the thymus *in vivo*. Third, as discussed by Ploegh and colleagues [15,16], such systems create an artificial situation in which thymocytes are exposed to very low numbers of MHC molecules heavily loaded with a single peptide. This is unlike the natural condition where higher numbers of MHC molecules carry a heterogeneous mix of peptides. Each of these limitations has been addressed by recently reported experiments.

FTOC systems supplemented with additional peptides or employing other TCRs

Sebzda *et al.* [17] have extended the panel of peptides examined in one of the FTOC systems mentioned above: stromal cells bearing the β_2-microglobulin null mutation; thymocytes expressing receptors specific for a peptide from the lymphocytic choriomeningitis virus glycoprotein presented by the D^b molecule. The new peptides used were again very similar to the antigenic peptide, single alanine substitutions of a strong agonist, but the results proved difficult to reconcile with either the avidity or efficacy models of selection. A clear correlation between the ability of a peptide to stimulate the mature T cell and to promote its positive selection was not observed, arguing against strict avidity considerations. On the other hand, contrary to what would be expected on pure efficacy considerations, one of the peptides which was a moderate agonist with no antagonist activity induced efficient positive selection over a broad range of concentrations, including those capable of stimulating the mature T cell. Such positive selection occurred in the absence of negative selection as assessed by clonal deletion. These perplexing results were taken as evidence that certain peptides have an 'undefined, intrinsic' capacity to promote positive selection, and predicted that peptides apparently unrelated in sequence to the antigenic peptide might be competent positive selectors.

Pawlowski *et al.* [18] have examined positive selection in FTOCs derived from a third TCR transgenic strain, one whose receptors have alloreactive specificity for the L^d molecule. Their results contrasted sharply with those previously reported for the other two TCR transgenic systems. They found that many peptides, some with no apparent structural similarity to the antigenic peptide, could promote positive selection. Furthermore, there was no correlation between the capacity of a peptide to stimulate mature T cells and its ability to positively select immature thymocytes. Thus, at least in this context, positive selection appears to be a highly promiscuous process. It remains to be determined whether the other FTOC systems are fundamentally different or whether it is just that the appropriate peptides have not yet been evaluated in these systems.

Systems assaying positive selection *in vivo* on a single peptide

The approach based on peptide addition to FTOC systems starts with T cells expressing a defined TCR and asks what peptides can positively select them. Although this is important information, it does not reveal what peptides actually positively select these cells in the thymus *in vivo*. In theory, such systems could be used to identify *in vivo* selecting peptides but this promises to be technically demanding. Thus, certain groups have reversed the approach, by starting with a defined peptide and asking what TCRs are expressed by the cells positively selected on them. To facilitate identification of the selected cells, initial studies have relied on novel mouse strains engineered to express only one type of MHC class II molecule loaded with essentially a single peptide.

In one such study [19•], mice that carry a transgene encoding an MHC class II molecule ($A_\beta{}^b$) covalently

linked to a particular peptide (Eα52–68) were produced. When null mutations of the $A_\beta{}^b$ and invariant chain (Ii) genes were introduced into the transgenic strain, which already bore a natural mutation of the Eα gene, the resultant mice expressed only $A_\alpha{}^bA_\beta{}^b$ complexes. There were about 5- to 10-fold fewer $A\alpha{}^bA_\beta{}^b$ complexes than in normal animals, but all were seemingly loaded with the Eα peptide. Surprisingly, a large and diverse repertoire of CD4+ T cells was selected in these transgenic mice, reaching about 20% of the CD4+ T cell numbers found in wild-type animals and including the full range of variable $(V)_\beta$ TCR segments with a diversity of sequences in the hypervariable region. Fully two thirds of hybridomas derived from the CD4+ T cell population of transgenic (but not wild-type) mice responded 'syngeneically' to A^b molecules from wild-type animals, suggesting that the TCRs on these cells were enriched for specificities with some affinity for self-MHC. Normally such cells would have been purged from the repertoire in the thymus by encounter with $A_\alpha{}^bA_\beta{}^b$ complexes occupied with a diverse complement of self-peptides. Two other strains of transgenic mice carrying the same type of $A_\beta{}^b$–Eα52–68 fusion were produced independently by another group [20•]. The two new strains differed from each other by 5- to 10-fold in expression levels of the fusion protein. Interestingly, the strain with the lower levels had a detectable population of CD4+ T cells displaying a diversity of TCR V_β segments whereas the higher expressor did not, thereby suggesting that the corresponding population of T cells had undergone negative selection. This observation was taken as *in vivo* evidence for the avidity model of selection. It is, however, rather surprising that a population of receptors with lower average affinities did not emerge instead. This might be explained by invoking a threshold affinity required for positive selection, which would be consistent with the quite narrow 'window' of affinities (a single log range) distinguishing positive selection from negative or no selection, according to direct measurements [21] for one of the TCRs and a panel of peptides previously assayed in an FTOC system [8,11,12].

A second set of *in vivo* studies made use of mice carrying a null mutation of the *H-2Ma* gene, resulting in normal numbers of $A_\alpha{}^bA_\beta{}^b$ molecules essentially all filled with a peptide derived from Ii termed class II associated invariant chain peptide (CLIP) [22•–24•]. Here again, large numbers of CD4+ T cells developed — as many as half the normal numbers — and these expressed the full range of V_β segments [22•] with a diversity of sequences (S Tourne, T Miyazaki, C Benoist, D Mathis, unpublished data). Once more, the peripheral CD4+ T cells had high 'syngeneic' reactivity to A^b molecules from wild-type mice loaded with the full complement of self-peptides. As might have been predicted, chimeric mice made up of H-2M-negative thymic epithelial cells and H-2M-positive bone marrow derived cells selected a much smaller compartment of CD4+ T cells, presumably reflecting the influence of negative selection on the latter (S Tourne, T Miyazaki, C Benoist, D Mathis, unpublished data). More recent studies have confirmed the broad repertoire of peripheral CD4+ T cells in H-2M null mice, as these cells were capable of responding to injection of several peptide antigens (e.g. ribonuclease peptide 95–110, hen egg lysozyme peptide 74–88). Yet, the repertoire clearly has 'holes' because CD4+ T cells displaying particular TCRs could not be positively selected, as assayed by crossing three TCR transgenic strains with the H-2M null strain (S Tourne, T Miyazaki, C Benoist, D Mathis, unpublished data).

In general, the studies using these quite different systems have produced amazingly concordant results: MHC class II molecules loaded with essentially a single peptide do not promote positive selection of an entirely normal repertoire of CD4+ T cells, but do select a large number of CD4+ cells with diverse TCR specificities. The fact that the repertoire is incomplete argues that peptides have a specific role in the positive selection of CD4+ cells *in vivo*; the fact that it is nonetheless large and diverse suggests that the specificity is muted and that positive selection is quite promiscuous. This result is reminiscent of one of the earliest, unexplained results from one of the FTOC systems: a surprisingly large number of CD8+ T cells of diverse V_β repertoire was selected upon addition of certain single peptides [7].

This approach has overcome the second limitation of the experiments employing FTOCs because it allows the identification of T cells selected *in vivo* on a defined peptide; however, it remains susceptible to the third in that these systems produce a situation where MHC molecules are loaded with essentially one peptide rather than the usual diverse complement.

A system which assays positive selection *in vivo* on one of a multitude of peptides

To surmount both these limitations, a novel experimental system was developed: Ii-negative mice were injected intrathymically with an adenovirus vector encoding different Ii–peptide fusions [25•]. Thereby, introduced 'neo-peptides' could be efficiently presented by the MHC molecules on thymic epithelial cells. Though somewhat reduced vis-à-vis normal mice, the level of MHC molecule expression was high, and these molecules could be occupied by many other peptides besides the neo-peptide. This system was used to study positive selection of the CD4+ T cell response to moth cytochrome c (MCC) in Ii-deficient B10.BR mice, a reactivity that is absent in animals lacking expression of Ii due to failure of positive selection. Selection of responses to MCC could be promoted by adenovirus-mediated delivery of the peptide itself into the thymus, reminiscent of some of the results from FTOC systems [9,10]. Closely related analogs also worked, even though they were devoid of measurable agonist and antagonist activity. Even ostensibly unrelated peptides could promote a response: an anti-MCC response

could be selected on a peptide derived from hemoglobin with no sequence similarity to MCC. Conversely, cells selected on the MCC peptide or similar peptide analogs could respond to an unrelated peptide from bovine ribonuclease. Thus, positive selection appeared promiscuous in this system although there were limits, as not all peptides promoted all responses (e.g. the MCC peptide could not select a response to a λ repressor peptide). Peptide specificity clearly shaped the repertoire because different patterns of fine antigen specificity and TCR usage characterized the MCC-reactive T cells selected on the following different peptides: the naturally occurring peptide(s) which normally select(s) this response, the antigenic MCC peptide, a single base substitution of the antigenic peptide and an unrelated hemoglobin peptide.

Where do we stand now?

Assembling this body of data into a coherent picture is not easy. It must constantly be kept in mind that the various experiments assessed positive selection of T cells expressing different TCRs, and rules for one receptor might not apply to all others. In addition, different assay systems were employed and vital features such as MHC expression levels, peptide diversity and T cell physiology were therefore dissimilar and could bias the outcome.

It seems clear, nonetheless, that peptides play a specific role in positive selection. A single peptide can promote selection of a large population of T cells, perhaps as many as 20–50% of the normal numbers [7,19•,22•–24•], but no peptide so far examined is capable of selecting a full repertoire. When multiple peptides can select a T cell population responsive to the same antigen, the different peptides select repertoires varying in patterns of fine antigen specificity and TCR usage. In some systems, the degree of specificity imposed by peptide seems rather limited [19•,20•,22•–25•] and perhaps reflects a general binding mode between the TCR and MHC molecules, as has been proposed on the basis of the crystal structure of TCR–peptide–MHC molecule ternary complexes [4••]. In other systems, peptide-imposed specificity appears to be more dominant [8,9,11], and this seems also to be true for the selection of T cells involved in restricted responses like the $V_\alpha 11V_\beta 3$-restricted response to MCC in B10.Br mice [25•]. Considering these differences, it might be instructive to draw an analogy with the range in degeneracy of the responses made by mature T cells. Reactivity to a single peptide may be restricted to a very limited subset of TCRs or involve a large diversity of receptors [26]; a T cell displaying a particular TCR may react to a single peptide or to multiple peptides with very different sequences [27–29]. Perhaps positive selection just exhibits a similar range in degeneracy. Indeed, such a range makes some sense for the organism. It might be useful to have the capacity to mount some very restricted responses against certain frequently encountered or particularly noxious pathogens, responses that can be quickly mobilized and are devoid of potentially autoreactive cells; it would also be advantageous to have the possibility of exploiting a broad range of specificities in order to meet newly encountered challenges. The answer to the question posed in the title would thus appear to be 'both'.

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