Serial engagement proposed

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THE sensitivity of an antigen-specific response by T cells is remarkable. Yet the number of cognate ligands formed by a peptide antigen complexed with a molecule of the major histocompatibility complex (MHC) on the antigen-presenting cell can be very small; and the interaction with the appropriate T-cell receptor (TCR) appears to be one of low affinity and short duration. How can this combination of high sensitivity and low affinity be reconciled?

On page 148 of this issue[1], Valitutti *et al.* present striking results which might offer a resolution to this puzzle. They show that TCR complexes disappear from the surface in numbers that are large multiples of the number of cognate peptide/MHC ligands available to them, and that irrelevant TCRs are largely unaffected. The model they propose is one of serial engagement, with the high 'off-rate' of the interaction allowing the same peptide/MHC combination to trigger up to 200 TCRs and thus amplify and sustain the activation response. The response, which is measured in this instance by production of the cytokine γ-interferon, correlates in magnitude and timing with the observed downregulation of the TCR. Thus the T cell seems to be 'counting' the number of receptors that have bound an appropriate ligand, formulating a proportional response, and then taking them out of service.

Off-rates

Serial engagement of a given peptide/MHC complex with what is typically a large excess of TCRs seems very likely, as the half-lives of the TCR/ligand interactions are of the order of 2–30 seconds (even when their affinities vary greatly)[2–4], and so over the 2–5 hour course of these cell-activation experiments one would expect that hundreds or even thousands of contacts would be made. These fast off-rates are not unique to TCR interactions, but instead seem to be a general property of all cell-surface receptors which 'receive' other cell-surface molecules[5]. Because of the highly polyvalent nature of molecular interactions between cells, the fast off-rates of these other molecules are probably important in allowing the cells to separate from each other. In the case of TCRs, however, whose contribution to the energy of the cell–cell conjugate seems negligible[6], this property seems to have been put to another purpose.

Although previous work has shown that both TCRs and surface immunoglobulins largely disappear from the cell surface upon antibody cross-linking or (in the case of immunoglobulins) ligand encounter, these events have been associated with wholesale 'capping' and internalization, and not with the specific, sequential process observed by Valitutti *et al.* Instead this seems more reminiscent of the receptor-mediated endocytosis described for epidermal growth factor and several other tyrosine-kinase receptors for soluble ligands[7]. The TCR is related to the tyrosine-kinase family of receptors by virtue of the tyrosine phosphorylation of its associated CD3 molecules which accompanies activation[8].

Although many of the tyrosine-kinase receptors depend on soluble-ligand-induced dimerization for activation[7], Valitutti *et al.* prefer a model of monovalent serial engagement for the TCR, and they do not see a role for receptor clustering. Nonetheless, it is clear that multimerization of TCR heterodimers and their non-covalently associated CD3 polypeptides can trigger T cells. The experiments showing this include cross-linking with antibodies against TCR or CD3 determinants and, more recently, cross-linking of chimaeric molecules consisting of a non-TCR extracellular domain linked to a CD3-ζ cytoplasmic dimer[8]. In addition, work with TCRs specific for fluorescence[9] or arsonate[10] has shown that these haptens can trigger specific T cells when highly multimerized but not in a monomeric or less multimeric state.

All of these data indicate that some multimerization involving CD3 domains is likely to be a component of activation, although reconciling a requirement for multimerization with the observed sensitivity of the T-cell response to its physiological ligand is not straightforward. I would argue that it is feasible, however, and that the data of Valitutti *et al.* do not preclude the involvement of dimerization in each serial activation and downregulation event.

To incorporate multimerization, one has to consider the question of how two TCRs can be aggregated by as few as 100 (refs 11–13) peptide/MHC ligands diluted by a sea of 50,000 or so complexes that are irrelevant. This is an especially acute problem if homogeneous peptide/MHC dimers are required to facilitate TCR dimerization on the T cell, as Brown *et al.*[14] have proposed (based, in part, on their finding that MHC class II molecules form dimers upon crystallization). Such dimers of heterodimers have also been observed in immunoprecipitates of class II MHC molecules[15]. The problem is that the random juxtaposition of peptide/MHC complexes into dimers would mean that only 1 in 250,000 pairs $(1/500)^2$ would have the same peptide, fewer than the number of MHC molecules on a given cell.

Enrichment

As Kupfer and Singer[16] have pointed out, however, the juxtaposition of two cells would cause all of the complementary molecules on their respective cell surfaces to accumulate at the interface, or 'co-cap'. In a T-cell context, this would enrich significantly for the 'correct' peptide/MHC but not for irrelevant ones[6]. If the area in contact represents about 10 per cent of the available surface there could be a 10-fold enrichment; a peptide/MHC ligand present as 1 in 500 would therefore become 1 in 50 and the chances of having a homogeneous dimer would rise to 1 in 2,500. So there would be an average of one or a few homogeneous MHC/peptide dimers at the cell–cell interface, and by serial engagement this might be able to trigger tyrosine phosphorylation and the internalization of TCRs that the authors see. (Although it would require off-rates generally to be at the lower end of the spectrum, a reasonable possibility under physiological conditions.)

All in all, one can argue over whether the TCRs are disposed of one by one, or as cross-linked groups of two or more. Nevertheless the data of Valitutti *et al.* clearly reveal a new aspect of T-cell activation in which the same peptide/MHC complexes seem to be used over and over again to engage and trigger a much larger number of TCR molecules. How these observations are eventually integrated into a complete molecular and cellular picture of T-cell activation will be a story worth following. □

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