

A star is born in BIM

As soon as they express their novel T-cell receptors (TCRs) in the thymus, immature T cells are screened for autoreactivity in a process known as negative selection. Although the precise signals that connect TCR stimulation to an apoptotic program are still being clarified (and might differ by developmental stage), there is general agreement that high-affinity interactions between the TCR and thymic MHC-peptide complexes ultimately trigger mitochondrial activation of a caspase complex and subsequent apoptosis. Bcl-2 family members are crucial upstream regulators of these mitochondria-mediated death signals. Antiapoptotic family members bcl-2 and bcl-xL can protect thymocytes from some TCR-mediated apoptotic signals and were the main focus of early studies into the regulation of negative selection. However, more and more attention has been paid to the proapoptotic family members, which include bcl-2-like members (e.g. bax and bak) and more distant cousins that share only one domain (BH3) with bcl-2 family members (e.g. the 'BH3-only members', bid, bik and bim). This attention appears well deserved.

There was a general expectation that redundancy within the large family of proapoptotic bcl-2 family members would confound efforts to assign a particular role to a specific bcl-2 family member. Surprisingly, however, Bouillet *et al.* [1] reveal that a single BH3-only member, Bim, is required for thymocyte negative selection. In each of six models of negative selection examined, Bim deficiency abrogated thymocyte apoptosis. *Bim*-knockout thymocytes are protected from *in vitro* TCR-mediated apoptosis. *Bim*-knockout mice are unable to delete thymocytes specific for superantigen and for peptide antigen *in vivo*. Negative selection of MHC class I- and MHC class II-restricted thymocytes does not occur in the absence of Bim. Although T-cell maturation is not always fully restored in *Bim*^{-/-} mice (raising the possibility that other mechanisms of negative selection can play a role at later stages of development), Bim is clearly a main actress in the thymocyte apoptosis drama.

How does Bim fit into the TCR-signaling cascade? Bouillet *et al.* provide evidence that TCR signals can upregulate *Bim* expression post-transcriptionally, and can

induce its translocation from cytoskeleton to mitochondria. However, the proximal TCR signals responsible for these crucial changes are unknown. Similarly, the downstream targets of Bim have not yet been identified. BH3-only family members generally appear to act as mitochondria-mediated apoptosis initiators by enhancing proapoptotic and/or inhibiting antiapoptotic activity of bcl-2-like family members. Although these investigators found no evidence for a physical association between Bim and the proapoptotic bcl-2 family members bax and bak, which gained recent prominence as crucial apoptosis mediators, it will be interesting to see if they have a functional relationship. Nonetheless, Bouillet *et al.* have made a star out of the previously under-appreciated Bim, and attention will now undoubtedly be riveted on its future.

1 Bouillet, P. *et al.* (2002) BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 415, 922–926

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Quantitative clonotypic analysis of antigen-specific T-cell responses

Antigen receptor diversity is an essential feature of adaptive immunity. This diversity is generated in immunoglobulin and T-cell receptor (TCR) variable regions by the process of V(D)J recombination from an array of germline gene segments. In T cells, subsequent editing by thymic selection governs the composition of the peripheral TCR repertoire. Each individual TCR within this repertoire exhibits specificity with a degree of cross-reactivity. Thus, a single TCR can recognize multiple peptide-major histocompatibility complex (pMHC) antigens, and a single pMHC complex can potentially be recognized by multiple TCRs. A T-cell response to a given individual antigen can therefore comprise more than one TCR clonotype. Although several methodological advances have allowed the detailed quantification and characterization of antigen-specific T-cell responses, technical limitations have hampered the

accurate determination of clonotypic structure within such T-cell populations.

A recent study reports on a new methodological approach to the issue of clonality within antigen-specific T-cell responses [1]. Following direct *ex vivo* peptide stimulation to induce an effector response, functional antigen-specific CD8⁺ T cells were isolated by flow cytometric sorting, using cell-surface interferon (IFN)- γ capture. Extracted mRNA was subjected to anchored reverse transcriptase (RT)-PCR with a TCRB constant region 3' primer. Clonotype-specific primers and probes designed on the basis of the TCRB sequences obtained were then used to quantify the frequency of each clonotype in peripheral blood mononuclear cells by real-time quantitative PCR. This produced a comprehensive, quantitative and unbiased picture of the clonal composition of antigen-specific CD8⁺ T-cell responses.

This methodology was used to study immunodominant HIV-specific CD8⁺ T-cell populations in chronically infected individuals. Substantial differences were observed between individuals with respect to the number of clonotypes targeting a single antigen. Further, the clonotypic composition of the dominant antigen-specific response varied within individuals over time, both in absolute terms, as a function of virus load, and in relative terms, as a function of mutation within the viral epitope.

HIV can escape from immune control that is mediated by CD8⁺ T cells through antigenic mutation; however, it is unclear why immune escape variants emerge to fixation in some individuals but not in others. The findings of Douek *et al.* provide a potential explanation. Theoretically, the more clonotypes that are present within an antigen-specific T-cell population, the more

probable it is that a given variant viral epitope will fall within the spectrum of agonist ligands for at least one of the constituent TCRs. This was confirmed experimentally in individuals with poly-clonotypic immunodominant responses, using a naturally occurring viral antigenic variant. These observations suggest that poly-clonotypic responses are best suited to control a virus that readily mutates its antigenic structure, and could also help to explain why most convincing demonstrations of viral escape from CD8⁺

T-cell-mediated control in HIV infection involve variant peptide epitopes that fail to bind HLA class I.

The remarkable achievement of Douek *et al.* lies primarily with the methodological advance that their work represents. With the approach they have developed, the clonotypic composition of T-cell responses to individual antigens can be examined both qualitatively and quantitatively. Furthermore, the method has the potential to be applied to situations in which the precise antigen is not known. Armed with

these capabilities, immunologists are now in a position to address many fundamental issues in the field of T-cell biology.

- 1 Douek, D.C. *et al.* (2002) A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T-cell responses reveals a potential mechanism for control of viral escape. *J. Immunol.* 168, 3099–3104

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Quo vadis synapse?

The current acute interest in T-cell signalling was emphasized at a recent Keystone meeting on T-cell biology, where approximately half of the talks addressed the contact zone between T cells and antigen presenting cells (APCs), known as the 'immunological synapse'. However, although the underlying cellular and molecular biophysics are being elucidated in more and more detail, a new report by a group led by Andrey Shaw might force us to re-evaluate the role of synapses [1].

An immunological synapse is characterized by several centrally located signalling molecules (TCR–MHC, CD28–B7) surrounded by a peripheral 'glue' of adhesion molecules (intercellular adhesion molecule 1, leukocyte function-associated antigen 1) on the T cell and APC. This molecular organization (the mature synapse) forms within 30–60 minutes after contact between the cells. To visualize early signalling during synapse formation between naive T cells and APCs, the Shaw group made use of an antibody that

recognizes the TCR signalling molecule LCK in its active form (tyrosine phosphorylated at position 394). The occurrence of this LCK species is one of the earliest signalling events after contact of a T cell with an APC. The striking finding was that within two minutes of first contact, active LCK was detectable, as was active ζ -chain-associated protein 70 (ZAP-70), another key signalling molecule of the TCR complex. However, at this time, no mature synapse had formed, as the entire pool of TCR was outside the central cluster (immature synapse). When the TCR had reached its final central position after 15–30 minutes, the entire pool of active LCK and ZAP-70 had already been lost. Therefore, the very early events of TCR signalling are completed well before a mature synapse has formed. Shaw and colleagues were also able to show that a T cell–APC contact of 150 minutes is sufficient to drive naive T cells into multiple rounds of proliferation, calling current models of prolonged contact (up to 20-hour T cell–APC contact required for T-cell activation) severely into question.

Thus, as the group writes in the paper: 'what then is the function of the immunological synapse?' Obviously, T cells do not need a very long contact period for an APC to be activated for division, as important signalling events are terminated well before synapses form. This paper suggests that TCRs are triggered immediately upon contact with the APC at the outer margin of the contact zone. The synapse might be the 'sink' for used TCRs that have been engaged by an MHC-peptide, rather than a structure that is required for efficient TCR triggering. Alternatively, one might have to start thinking about the synapse as a structure that is needed by the APC, but not by the T cell.

- 1 Lee, K.H. *et al.* (2002) T-cell receptor signalling precedes immunological synapse formation. *Science* 295, 1539–1542

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Epitope-dominance: survival of the joe-average T cell

Cytotoxic T cells (CTLs) are key weapons of the immune system that combat infections with intracellular pathogens. During most immune responses, CTLs proliferate extensively and expand up to 100 000-fold. This occurs within days to weeks of infection. Subsequently, usually after elimination of the pathogen, CTL memory is established with a population 10–100-fold smaller than observed during the peak response. Regulation of expansion and contraction of the specific

T-cell pool is complex and is the subject of intensive investigation. Questions of particular interest are which T cells enter the primary response and which T cells survive to make up the memory pool. Most data available pertain to acute infections and indicate that epitope load and TCR affinities determine which T cells are efficiently recruited in the early response. Moreover, the repertoire of specificities in the memory T-cell pool is similar to that observed in the primary response, perhaps

with a tendency to find higher-affinity T cells in the memory pool.

Davenport *et al.* [1] have examined in detail CTL responses during persistent infections. Their data show that EBV-specific CTL responses in humans follow a different pattern than for acute infections. Surprisingly, they observed that those specificities of either maximal or minimal abundance at the peak of the acute response were under-represented one year later (i.e. during the persistent phase of the