Cross-priming

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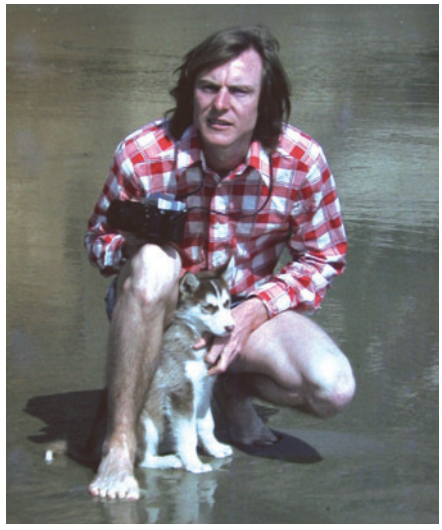
The stimulation of antigen-specific CD8$^+$ T cells by the presentation of antigen acquired from outside the cell was the startling conclusion of Michael Bevan's work done more than 30 years ago.

Cross-priming, a mechanism by which CD8$^+$ T cells are primed, is well established and much studied now, but there were two periods when the idea that noninfectious material could be 'taken up' by antigen-presenting cells and then be processed and presented on major histocompatibility complex (MHC) class I molecules to cytotoxic CD8$^+$ T cells was, to say the least, a hard concept to swallow. In the 1970s, when the phenomenon of cross-priming was first noted, the mechanism seemed to challenge the simplest form of the 'altered-self' hypothesis of T cell recognition, that a self-restricting element plus antigen was the functional unit of T cell recognition. Then, 10 years later, the 1980s brought the idea of cross-priming to the fore again because it seemed to violate the neat and tidy distinction between the MHC class I (endogenous) and MHC class II (exogenous) pathways of antigen presentation to cytotoxic T cells and helper T cells, respectively.

I was a postdoctoral fellow in Mel Cohn's lab at the Salk Institute in La Jolla, California, in 1974 when Peter Doherty and Rolf Zinkernagel proposed the altered-self hypothesis to explain the MHC restriction of cytotoxic T cell recognition[1]. According to that hypothesis, cytotoxic T cells induced by viral infection have a single receptor that recognizes self MHC products that have been altered by the virus. This was a beautiful insight and much too good not to be true! The explanation could easily extend to the observed MHC-restricted killing, by cytotoxic T lymphocytes, of target cells chemically modified by haptens[2]. I had been studying alloreactive cytotoxic T cell responses induced in mixed-lymphocyte cultures. Only when responder and stimulator cells differed at the MHC was killer activity observed, a finding that was true even though the monumental work of George Snell and others had shown there were dozens of other loci in the mouse genome that were associated with tissue graft rejection[3]. Those non-MHC loci were referred to as 'minor histocompatibility' loci (or antigens). At some stage before the altered-self bombshell exploded, I began to wonder whether killer cells could be induced across a difference in minor antigens if the responder animal had been primed *in vivo* before setting up the mixed lymphocyte culture. Thinking back, I had some BALB/c mice primed with cells from the MHC-identical B10.D2 strain ready for use. Liz Simpson's group in London was probably also at a similar point in their study of cytotoxic responses to the male-specific transplantation antigen H-Y. Working separately, we both showed that cytotoxic T cell responses to differences only at minor histocompatibility antigens can be detected after *in vivo* priming and *in vitro* boosting; notably, they are MHC restricted[4,5].

In those days so little was known of the molecular basis of T cell recognition that it was easy to suppose that viral gene products, haptens and even unknown products of minor histocompatibility loci (which had been suggested to be endogenous viral genomes) might irreversibly modify the MHC-restricting protein. In the system that I was using, the observed MHC restriction of minor histocompatibility antigen recognition was very strict (specific) at the effector level *in vitro*. I was very surprised, then, when further experiments showed that the strict MHC restriction was not apparent after *in vivo* priming. The experiment was as follows: a responder mouse expressing H-2^b and H-2^d alleles at the MHC (H-2^b/H-2^d mouse), primed *in vivo* with cells homozygous for H-2^b and differing in minor histocompatibility antigens ('minor different') demonstrated a strong *in vitro* secondary boost killing response against not only 'minor-different' H-2^b targets but also against 'minor-different' H-2^d targets[6]. Unexpectedly, I obtained that result even though the H-2^b-restricted cytotoxic T lymphocyte killer cells could not recognize the H-2^d targets and vice versa.

My colleagues Mel Cohn and Rod Langman welcomed those findings as evidence that the altered-self hypothesis was wrong and that MHC restriction was better explained by dual recognition of two independent entities, self MHC and foreign antigen, expressed



Mike Bevan on the beach in La Jolla, California, in 1975.

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on the target cells, and two separate receptors on the CD8 T cells, one for self MHC and one for the foreign antigen. However, I was not so ready to abandon the principle of one T cell receptor recognizing altered self (that is, self MHC plus foreign antigen) and proposed instead in my first paper on cross-priming that the observation might be explained as a 'carrier' effect. According to that hypothesis, naive cytotoxic T cells are in fact not cross-primed, but helper CD4 T cells are, which could explain the secondary cytotoxic response in much the same way as primed helper T cells are known to help B lymphocytes respond faster. Actually, considerable previous work on the immunogenetics of tissue transplantation had demonstrated cross-priming at the level of accelerated skin graft rejection, and the 'helper' explanation could be applied there also[3]. Fortunately, I was prepared to test that hypothesis and, more directly, to test whether cross-priming did or did not occur at the level of the cytotoxic CD8 T cell precursors.

With help from Cohn and Langman, I had devised a way to activate T cells 'polyclonally' (in an antigen-independent way) and to assay for specific killer activity against alloantigens[7]. CD8 T cells that had been activated with concanavalin A were washed free of the mitogen and then were 'titrated' for lysis of labeled targets. When the CD8 T cells from a naive mouse were prepared in this way, the only specific killing found was against targets that differed at the MHC (a true 'allo-response'), whereas no killing of MHC-identical targets that differed only at minor histocompatibility antigens could be detected. If, however, concanavalin A–simulated CD8 T cells came from a mouse that had been primed months earlier with 'minor-different' cells, killing of cells expressing the minor antigens could be found.

Using that powerful polyclonal activation technique, I was able to demonstrate that spleen cells from the H-2^b/H-2^d mouse that had been primed with 'minor-different', homozygous H-2^b cells contained increased numbers of both H-2^b- and H-2^d-restricted cytotoxic CD8 T cells. That result showed that cross-priming of cytotoxic CD8 T cells had occurred[8]. Not satisfied with that positive result alone, in another experiment I sought to determine whether helper CD4 T cell priming (via the carrier effect) could explain the original cross-priming result, but I could find no such evidence[9]. After obtaining those exciting results, I devoted more of my attention to the positive selection of the T cell repertoire in the thymus. In addition, cross-priming was mostly ignored by those working on cytotoxic T cell responses induced by viral infection.

After leaving Mel Cohn's lab, I spent 5 years at the Massachusetts Institute of Technology before returning to La Jolla, California, and the Scripps Research Institute. Great progress had been made in the meantime in defining altered self-recognition at the molecular level. It had become apparent that T cell receptors recognize foreign peptide bound in the groove of MHC class I or class II molecules. Studies of the cell biology of antigen processing and presentation had also made great strides and had shown that MHC class I and class II molecules are loaded with peptides in different cellular locations. MHC class I molecules, according to the paradigm developing at that time, are loaded with peptides derived from cytosolic degradation before they exit the endoplasmic reticulum. In contrast, the groove of nascent MHC class II molecules is 'protected' until the molecules have trafficked through the Golgi to endosomal or lysosomal vesicles. Thus, the array of peptides bound by MHC class I are derived from endogenous protein substrates degraded into peptides in the cytosol and shunted into the endoplasmic reticulum, whereas foreign peptides loaded onto MHC class II molecules are derived mainly from exogenous proteins taken up via endocytosis and degraded in lysosomes. That dichotomy made perfect sense because MHC class I–restricted killer T cells were thought to target virus-infected or damaged cells that synthesize (in a host cell) 'foreign' antigens, whereas the job of MHC class II–restricted T cells was thought to be to provide help, or activation signals, to B lymphocytes and other MHC class II–expressing cells that take up foreign antigen and process it in lysosomes to peptides that are presented on the cell surface. At the time, it seemed that the division of the antigenic world as 'seen' by T cells was complete: cytotoxic T cells recognize endogenous foreign antigens, whereas helper T cells recognize exogenous foreign antigens.

I think I was the only person at the time who felt more than a little uncomfortable with the absolute categorization of antigens as being strictly CD8 endogenous or CD4 exogenous. One reason for this was that I knew from the old work on minor histocompatibility antigen priming that exogenous cellular material does in fact access the MHC class I pathway of antigen presentation, thereby causing very efficient induction of a cytotoxic T cell responses. Yet the biochemistry of processing was correct and the logic was undeniable. How could both the new and old views be preserved?

The key events for me were the experiments by Frank Carbone and my discussions with him. He had established ovalbumin, which had long been a favorite exogenous antigen for priming helper CD4 T cells, as an MHC class I–restricted antigen for cytotoxic T cells. Using that system, he had shown that although *in vivo* injection of large amounts of soluble antigen fail to prime MHC class I–restricted T cells, injection of nanogram amounts of antigen associated with dead or dying cells does prime MHC class I–restricted cytotoxic T cells[10]. Suddenly it was clear: exogenous antigen could prime both CD4 helper cells and CD8 killer cells. The logic of focusing solely on cellular antigens for CD8 killer cells, moreover, could be preserved if phagocytosis of apoptic and necrotic cellular material (not pinocytosis of soluble material) allowed antigen to access the endogenous pathway of MHC class I–restricted antigen presentation[11]. The endogenous-exogenous division at the level of 'professional' antigen presentation could thus be refined as phagocytosis of exogenous cellular or particulate antigen leading to the MHC class I pathway and pinocytosis of soluble antigen leading to MHC class II loading (writing this now, I know that there are examples of soluble antigen priming of cytotoxic T cells, but the logic still seems strong to me).

Subsequent work showed that a subset of dendritic cells is in fact specially equipped to phagocytose dying cells and to shunt the antigen into the MHC class I processing and presentation pathway to initiate cross-priming[12,13]. Also, in another exciting application, this pathway of antigen presentation to MHC class I–restricted T cells has been shown to be a process occurring continuously in the body, even in the absence of foreign antigenic challenge. Dendritic cells in lymphoid tissue cross-present self antigens from other cells constitutively and by this means enforce self-tolerance of MHC class I–restricted cells[14]. Even now, however, the molecular basis of cross-priming–cross-presentation (how antigen passes from the phagosome to the cytosol) remains obscure.

Real-time movies are widely available of immunofluorescence-labeled CD8 and CD4 T cells scanning and interacting with dendritic cells in lymph nodes. It is hard to imagine that a tumor cell or an infected stromal cell could compete with a dendritic cell for engaging naive T cells and recruiting them to a response. Consequently, it is equally hard to imagine that 'professional' antigen presentation by means of cross-presentation is not a chief part of priming T cell responses.

1. Zinkernagel, R.M. & Doherty, P.C. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. *Nature* **251**, 547–548 (1974).
2. Shearer, G.M., Rehn, T.G. & Garbarino, C.A. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modified cell surface components controlled by H-2K and H-2D serological regions of the murine major histocompatibility complex. *J. Exp. Med.* **141**, 1348–1364 (1975).
3. Snell, G.D., Wheeler, N. & Aaron, M. A new method of typing inbred strains of mice for histocompatibility antigens. *Transplant. Bull.* **4**, 18–21 (1957).
4. Bevan, M.J. Interaction antigens detected by cytotoxic T cells with the major histocompatibility complex as modifier. *Nature* **256**, 419–421 (1975).
5. Gordon, R., Simpson, E. & Samelson, L. In vitro cell-mediated immune responses to the male specific (H-Y) antigen in mice. *J. Exp. Med.* **142**, 1108–1120 (1975).
6. Bevan, M.J. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J. Exp. Med.* **143**, 1283–1288 (1976).
7. Bevan, M.J., Langman, R.E. & Cohn, M. H-2 antigen-specific cytotoxic T cells induced by concanavalin A: estimation of their relative frequency. *Eur. J. Immunol.* **6**, 150–156 (1976).
8. Bevan, M.J. Minor H antigens introduced on H-2 different stimulating cells cross-react at the cytotoxic T cell level during in vivo priming. *J. Immunol.* **117**, 2233–2238 (1976).
9. Bevan, M.J. Priming for a cytotoxic response to minor histocompatibility antigens: antigen specificity and failure to demonstrate a carrier effect. *J. Immunol.* **118**, 1370–1374 (1977).
10. Carbone, F.R. & Bevan, M.J. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. *J. Exp. Med.* **171**, 377–387 (1990).
11. Bevan, M.J. Antigen recognition. Class discrimination in the world of immunology. *Nature* **325**, 192–194 (1987).
12. den Haan, J.M., Lehar, S.M. & Bevan, M.J. $CD8^+$ but not $CD8^-$ dendritic cells cross-prime cytotoxic T cells in vivo. *J. Exp. Med.* **192**, 1685–1696 (2000).
13. Iyoda, T. *et al.* The $CD8^+$ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. *J. Exp. Med.* **195**, 1289–1302 (2002).
14. Heath, W.R. & Carbone, F.R. Cross-presentation in viral immunity and self-tolerance. *Nat. Rev. Immunol.* **1**, 126–134 (2001).