Professionals and amateurs

Professional antigen-presenting cells, notably dendritic cells, play a key role in stimulating naive T cells — but nonprofessional antigen-presenting cells, such as fibroblasts, may also contribute to this process.

Stimulation of mature T cells generally requires the presence of specialized antigen-presenting cells, such as macrophages and dendritic cells [1–6]. These cells are strategically positioned in the T-cell-dependent areas of lymphoid tissues and express high levels of the costimulatory molecules required for optimal signaling by T cells. Despite the importance of specialized antigen-presenting cells, Kundig *et al.* [7] have recently reported that primary cytotoxic T lymphocyte (CTL) responses to viral antigens *in vivo* can be initiated by fibroblasts transfected with viral proteins. In view of this finding, the requirements for activating unprimed T cells need to be re-evaluated.

Functions of antigen-presenting cells

To present antigen to T cells, antigen-presenting cells first degrade native proteins into peptides and then load these peptides onto MHC molecules — class I MHC molecules for T cells expressing CD8 and class II molecules for CD4-bearing cells [5,8]. Peptide loading occurs predominantly in the endoplasmic reticulum for class I molecules and in endosomes for class II molecules. When displayed on the cell surface, peptide-bound MHC molecules on antigen-presenting cells are recognized by T cells via antigen-specific αβ T-cell receptors. In the case of activated T cells, this recognition signals the T cells to manifest their effector function. For example, a pre-activated, virus-specific $CD8^+$ CTL encountering class I–peptide complexes on virus-infected epithelial cells in the lung will be signalled to destroy the epithelial cells and thus promote clearance of the virus.

The situation with naive resting T cells is different. For these cells, signalling via the T-cell receptor (signal 1) is generally nonimmunogenic unless accompanied by 'second signals' resulting from contact with costimulatory molecules [4]. Specialized antigen-presenting cells express a spectrum of costimulatory molecules, such as B7, and these molecules interact with complementary molecules on T cells, such as CD28 or CTLA4 [3,4,6]. Although the signalling pathways initiated by T-cell-receptor–peptide–MHC and CD28–B7 interactions seem to be partly overlapping, the prevailing view is that the CD28–B7 interaction is crucial for the production of growth-promoting cytokines such as interleukin-2 (IL-2). Thus, blocking a T cell from contact with B7 using soluble CTLA4–immunoglobulin fusion protein (expressed from recombinant DNA constructs) generally causes a marked inhibition of the primary response, both *in vitro* and *in vivo*. Costimulation is not unique to the CD28–B7 interaction, however, and there is increasing evidence that a spectrum of molecules on antigen-presenting cells — such as intercellular adhesion molecule 1 (ICAM-1) and heat stable antigen (HSA) — can deliver costimulatory signals to T cells [9,10].

Although many different cell types express at least low levels of costimulatory molecules, high levels of expression of these molecules is largely restricted to professional antigen-presenting cells, especially dendritic cells [3]. These cells are scattered throughout the body, but are concentrated in the T-cell-dependent areas of the lymphoid tissues — the periarteriolar sheaths of the splenic white pulp and the paracortex of lymph nodes. Dendritic cells are thus ideally positioned to present antigen to naive T cells.

Cell types that have antigen-presenting function *in vitro*

Dendritic cells have potent antigen-presenting function *in vitro*, and some workers argue that stimulation of unprimed T cells is under the sole control of dendritic cells [3]. Much of the evidence on the antigen-presenting function of dendritic cells has come from studies on the response of naive T cells to cells expressing foreign MHC molecules (alloantigens). Here, there is general agreement that small numbers of dendritic cells are highly efficient at stimulating primary mixed lymphocyte reactions by unprimed T cells in the absence of added lymphokines [2,3]. Whether or not other cell types can stimulate such reactions has long been controversial. Some workers report that, unlike dendritic cells, macrophages and B cells have little or no capacity to stimulate naive T cells [3]. However, in the case of $CD8^+$ T cells, others have reported that primary mixed lymphocyte reactions to class I alloantigens can be elicited by a spectrum of cell types, including activated macrophages, T blast cells, transformed fibroblasts and mastocytoma (P815) cells [2]; there are also reports that reactions can be stimulated by vascular endothelial cells [11]. The antigen-presenting function of resting T and B cells is generally very low, but when lymphoid cells are activated and/or treated with neuraminidase, which reduces the net surface charge, antigen-presenting function is increased dramatically [2,12]. Indeed, after neuraminidase treatment, their function in presenting antigen to activated $CD8^+$ cells is almost as potent as that of dendritic cells [2].

Although some of the cell types discussed above lack the costimulatory molecule B7, virtually all cell types express at least low levels of molecules that have costimulatory function. Hence, the data described above do not challenge the view that T-cell activation requires costimulation. However, the data do suggest that the two-signal model for T-cell activation may be oversimplistic. This

writer favors a holistic model, in which T-cell activation leading to cytokine production and proliferation reflects the overall avidity of the interaction between T cells and antigen-presenting cells [2,13]. The avidity of T-cell–antigen-presenting cell interaction is presumably a reflection of many different factors, including the density of antigen (peptide–MHC complexes) on the antigen-presenting cell, the relative affinity of the T-cell receptor for antigen, the range of costimulatory molecules on the antigen-presenting cell and the net surface charge of these cells (see Fig. 1).

According to this model, high-avidity interaction promotes strong crosslinking of T-cell receptor–CD3 complexes on the T-cell surface, which in turn leads to strong signalling via various interconnected pathways within the T cell; costimulation feeds into this network and serves to boost the T-cell receptor–CD3 signal. High-intensity signalling within T cells stimulates production of cytokines (such as IL-2) and of the receptors for these cytokines (such as the IL-2 receptor). The T cells then proliferate and differentiate into effector cells. When the intensity of signalling is below a certain threshold — for example, when the density of antigen or the level of costimulation is low — the responding T cells express IL-2 receptors but not IL-2. Hence, the T cells fail to proliferate unless exposed to exogenous IL-2. This scenario is based on studies with $CD8^+$ T cells [2,14,15]; whether the model is also applicable to $CD4^+$ cells is less clear.

Antigen presentation *in vivo*

A corollary of the above model is that, under the conditions encountered *in vivo*, a very wide variety of different cell types have the potential to present antigen to unprimed $CD8^+$ cells. In the absence of exogenous help from cytokines, however, one would expect antigen-presenting function leading to overt T-cell stimulation to be limited to professional antigen-presenting cells. But this may apply only to the majority population of T cells that have 'average' affinity for antigen. In the case of high-affinity T cells, it is conceivable that contact with antigen on various nonprofessional antigen-presenting cells is sufficient to activate the T cells and stimulate endogenous IL-2 production, thereby bypassing the requirement for exogenous IL-2. In support of this idea, it has been found that exposing naive $CD8^+$ cells to class I alloantigens expressed selectively on non-bone-marrow-derived cells in bone marrow chimeras activates a small proportion of the $CD8^+$ cells to proliferate and differentiate into CTL in the absence of $CD4^+$ cells [16]. Likewise, injecting purified naive $CD8^+$ cells subcutaneously into heavily irradiated syngeneic mice together with allogeneic (MHC-incompatible) tumor cells leads to rapid destruction of the tumor cells: these cells initially act as direct antigen-presenting cells for the $CD8^+$ cells and then become targets for the CTL they induce [17]. Further evidence that professional antigen-presenting cells are not essential for T-cell responses *in vivo* is provided by the recent finding that mice that display a selective depletion of dendritic cells — through disruption of the *relB* gene

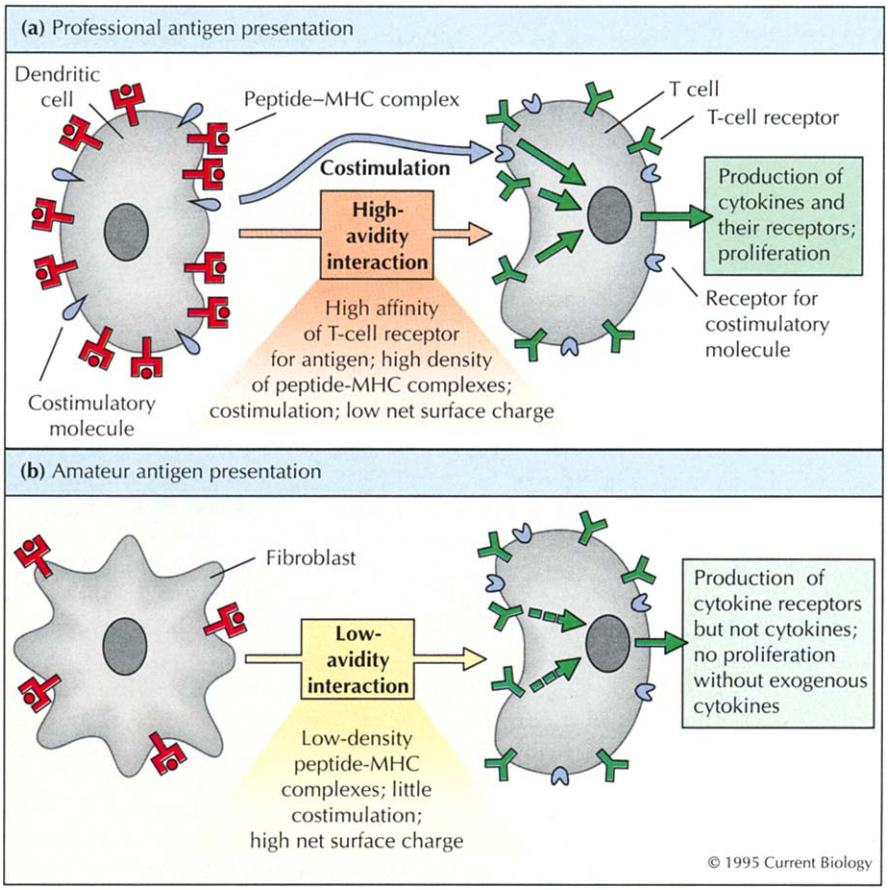

Fig. 1. The functional differences between professional and non-professional antigen-presenting cells. See text for further details.

— are capable of mounting low but significant antibody responses to T-cell-dependent antigens [18].

As argued above, stimulation of unprimed T cells *in vivo* by nonprofessional antigen-presenting cells is likely to be limited to a small subset of high-affinity T cells. In the experiments of Kundig *et al.* [7] mentioned earlier, mice were injected with a syngeneic, MHC class I-positive fibrosarcoma cell line transfected with the glycoprotein of lymphocytic choriomeningitis virus. When tested *in vitro*, the transfected cells failed to stimulate glycoprotein-specific T cells unless supplemented with exogenous IL-2. However, despite lacking several key costimulatory molecules (B7, ICAM-1, LFA-1), the transfected cells elicited quite strong induction of CTL *in vivo*, especially after direct intrasplenic injection. Various control experiments suggested that the injected cells acted as direct antigen-presenting cells and did not require 'processing' by host cells; 'help' from $CD4^+$ cells was not required because prior elimination of $CD4^+$ cells did not inhibit the response.

In light of these findings, the authors argue that nonprofessional antigen-presenting cells such as fibroblasts can act as a significant source of antigen presentation *in vivo* (but only if the nonprofessional cells reach the central lymphoid tissues). To explain the discrepancy with the data *in vitro*, the authors argue that antigen presentation by fibroblasts *in vivo* depends upon help from "the abundance of costimulatory cytokines in lymphoid organs...". The problem with this interpretation is that there is little if any direct evidence that the background level of IL-2 in normal lymphoid organs is sufficient to provide such bystander help. Three other possibilities may be considered. First, it is conceivable that the antigen-presenting function of fibroblasts *in vivo* reflects enhanced expression of MHC class I molecules [2] and/or up-regulation of costimulatory molecules. Second, adjacent host antigen-presenting cells could provide bystander costimulatory function for the responding T cells [4]. Third, harking back to the model considered earlier, the transfected fibroblasts may have been directly immunogenic for a small subset of high-affinity T cells which then underwent prolonged expansion followed by differentiation into CTL. This possibility could be assessed by testing the affinity of the CTL generated, for example by examining the susceptibility of the cells to inhibition with anti-CD8 antibody.

Whatever the explanation, the data of Kundig *et al.* [7] reinforce the view that stimulation of unprimed T cells *in vivo* is not necessarily limited to professional antigen-presenting cells. As emphasized by these workers, however, it is highly likely that primary immune responses are restricted to the primary lymphoid organs — the spleen and the draining lymph nodes, in the case of viral infections. In these organs, the range of cell types available for antigen-presenting function is quite small. Indeed, in the T-cell-dependent areas, dendritic cells are virtually the only cell type present (except for T cells themselves and a few resting B cells). Hence, naive resting T cells entering the lymphoid organs from the recirculating lymphocyte pool will encounter antigen largely, and perhaps exclusively, presented by dendritic cells, the quintessential professional antigen-presenting cells. Under normal physiological conditions, therefore, it is highly likely that initial antigen presentation is by dendritic cells. After their initial activation by dendritic cells, however, it is quite possible that activated T cells then move to adjacent areas of the lymphoid tissues, such as the red pulp of the spleen, and there receive subsidiary antigen presentation from other cells such as macrophages, activated B cells and stromal cells such as fibroblasts. This secondary contact with nonprofessional antigen-presenting cells could be crucial for expanding the responding T cell clones and inducing differentiation into effector cells.

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References

1. Sprent J, Webb SR; **Function and specificity of T cell subsets in the mouse.** *Adv Immunol* 1987, **41**:39–133.
2. Sprent J, Schaefer M; **Antigen-presenting cells for $CD8^+$ T cells.** *Immunol Rev* 1990, **117**:213–234.
3. Steinman RM; **The dendritic cell system and its role in immunogenicity.** *Annu Rev Immunol* 1991, **9**:271–296.
4. Schwartz RH; **Costimulation of T lymphocytes: the role of CD28, CTLA4, and B7/BB1 in interleukin2 production and immunotherapy.** *Cell* 1992, **71**:1065–1068.
5. Unanue ER; **Macrophages, antigen-presenting cells, and the phenomenon of antigen handling and presentation.** In: *Fundamental Immunology*, 3rd edn. Edited by Paul WE. New York: Raven Press; 1993:111–144.
6. Linsley PS; **Distinct roles for CD28 and cytotoxic T lymphocyte-associated molecule-4 receptors during T cell activation?** *J Immunol* 1995, **182**:289–292.
7. Kundig TM, Bachmann MF, DiPaolo C, Simmard JJL, Battegay M, Lother H, Gessner A, Kuhlcke K, Ohashi PS, Hengartner H, Zinkernagel RM; **Fibroblasts as efficient antigen-presenting cells *in vivo*.** *Science* 1995, **268**:1343–1347.
8. Germain RN; **Antigen processing and presentation.** In: *Fundamental Immunology*, 3rd edn. Edited by Paul WE. New York: Raven Press; 1993:629–676.
9. Dubey C, Croft M, Swain SL; **Costimulatory requirements of naive $CD4^+$ T cells: ICAM1 or B71 can stimulate naive CD4 T cell activation but both are required for optimal responses.** *J Immunol* 1995, **155**:45–57.
10. Liu Y, Jones B, Aruffo A, Sullivan KM, Linsley PS, Janeway CA Jr; **Heat-stable antigen is a costimulatory molecule for CD4 T cell growth.** *J Exp Med* 1992, **175**:437–445.
11. Geppert TD, Lipsky PE; **Antigen presentation by interferon-gamma-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression.** *J Immunol* 1985, **135**:3750–3762.
12. Cowing C, Chapdelaine JM; **T cells discriminate between Ia antigens expressed on allogeneic accessory cells and B cells: a potential function for carbohydrate side chains on Ia molecules.** *Proc Natl Acad Sci USA* 1983, **80**:6000–6004.
13. Sprent J, Gao EK, Webb SR; **T cell reactivity to MHC molecules: immunity versus tolerance.** *Science* 1990, **248**:1357–1363.
14. Heath WR, KjerNielsen L, Hoffmann MW; **Avidity for antigen can influence the helper dependence of $CD8^+$ T lymphocytes.** *J Immunol* 1993, **151**:5993–6001.
15. Cai Z, Sprent J; **Resting and activated T cells display different requirements for CD8 molecules.** *J Exp Med* 1994, **179**:2005–2015.
16. Kosaka H, Surh CD, Sprent J; **Stimulation of mature unprimed $CD8^+$ T cells by semiprofessional antigen-presenting cells *in vivo*.** *J Exp Med* 1992, **176**:1291–1302.
17. Sprent J, Schaefer M; **Capacity of purified unprimed $Lyt2^+$ cells to reject Ia H2-different tumor cells in the Winn assay.** *J Immunol* 1988, **140**:2899–2902.
18. Burkly L, Hession C, Ogata L, Reilly C, Marconi LA, Olson D, Tizard R, Cate R, Lo D; **Expression of *relB* is required for the development of thymic medulla and dendritic cells.** *Nature* 1995, **373**:531–536.

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