Commentary

Fcγ Receptors and Cross-Presentation in Dendritic Cells

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M. Bevan showed in the mid-seventies that cytotoxic T lymphocyte (CTL) responses may be initiated by antigen-presenting cells that do not express the antigens themselves (1). He called this process cross-priming. The antigen-presenting cells involved in cross-priming must therefore internalize and present antigens to $CD8^+$ T cells in the context of MHC class I molecules. This process is often referred to as "cross-presentation." Cross-presentation by antigen-presenting cells in vivo results in either cross-priming (initiation of $CD8^+$ T cell responses) or in cross-tolerance (induction of $CD8^+$ T cell unresponsiveness; reference 2). These results raised the question of the nature of the "cross-presenting cells." In vitro, dendritic cells cross-present antigens more efficiently than any other antigen-presenting cell (3). They are also the only antigen-presenting cells that activate naive T lymphocytes (4). Dendritic cells, indeed, are sufficient for cross-presentation in vivo (5).

Fc Receptors (FcRs) and Cross-Presentation in Dendritic Cells. If cross-presentation is how dendritic cells initiate CTL responses, antigen targeting to and internalization by dendritic cells must represent a critical step in cross-priming. In vitro, targeting antigens to receptors for the Fc region (FcγR) of IgG, dramatically increases the efficiency of cross-presentation (3).

FcγRs are a family of membrane glycoproteins expressed on hematopoietic cells (6). Most FcγRs do not bind IgG, unless IgGs are themselves bound to multivalent-specific antigens (i.e., immune complexes). Thus, FcγRII (CD32) and FcγRIII (CD16) bind monomeric IgG quite inefficiently, but bind immune complexes with very high affinity. FcγRI (CD64), in contrast, binds monomeric IgG with high affinity, but, like high affinity receptors for IgE, it does not signal unless IgGs are cross-linked by their specific polymeric ligands. Thus, FcγRs may be functionally considered as antigen receptors.

Targeting antigens to FcγR promotes cross-presentation by several orders of magnitude in mouse bone marrow–derived dendritic cells (7, 8). The intracellular mechanisms leading to cross-presentation after FcγR-mediated uptake have been analyzed. In dendritic cells, but not in other cell types, FcγR-mediated internalization very efficiently targets antigen for a unique dendritic cell–specific antigen transport pathway resulting in delivery to the cytosol. Once in the cytosol, internalized antigens are degraded by the proteasome. The resulting peptides are translocated into the lumen of the ER and loaded on MHC class I molecules (9). These results suggested that antigen-specific humoral immune responses may promote the generation of specific CTLs.

Protective Roles of Antitumor Abs In Vivo. In the course of most CTL-mediated immune responses, including antitumor immune responses, specific Abs are also produced. The biological role of these Abs, however, is poorly understood. In the past few years, the relative efficacy of antitumor Abs for the treatment of certain breast cancers or B cell lymphomas, renewed the interest of immunologist in antitumor humoral responses (10). The Abs used in the clinic are directed against Her2/neu (a cellular proto oncogene, trastuzumab) and CD20 (a B cell marker, rituximab). They interfere with tumor growth in vitro, but their mechanism of action in vivo is not fully understood.

In mice, antimelanoma Abs inhibit tumor growth in a FcγR-dependent manner (11). Human tumor treatment in a mouse model with trastuzumab or rituximab is independent on T cells, but requires activation FcγRs and is limited by inhibitory FcγRs (12). The main mechanism of Ab treatments was, most likely, Ab-dependent cellular cytotoxicity (ADCC). It is also most likely that ADCC also represents the main effector mechanism of Ab-based therapies in cancer patients.

These results, however, do not exclude the possibility that antitumor Abs induce specific CTL responses by promoting dendritic cell–mediated cross-presentation of tumor antigens. $CD8^+$ CTL responses were indeed found in mAb-based therapy of solid tumors in mice. Dyall et al. showed that $CD8^+$ T cell depletion in vivo prevents treatment of established solid tumors with antitumor mAbs (13). Therefore, antitumor Abs may also induce effective CTL responses in vivo. Tumor-specific CTLs, however, have not yet been reported in Ab-treated cancer patients.

In vitro experiments described in this issue by K.M. Dhodapkar and colleagues (14), suggest a role for antitumor Abs in the induction of antitumor CTLs. Coating of myeloma cells with anti–syndecan-1 Abs did not increase phagocytosis by dendritic cells. By contrast, cross-presentation of two tumor antigens (NY-ESO1 and MAGE3) and

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specific CTL cross-priming were strongly enhanced. Cross-presentation after opsonized tumor cell phagocytosis required FcγRs and was more efficient for cross-priming than phagocytosis of apoptotic cells, tumor cell lysates, or treatment with synthetic peptides.

These results may both modify our understanding of antitumor humoral responses, and, most likely, encourage new approaches for dendritic cells loading with tumor antigens for active cancer immunotherapy. Different methods allow to sensitize dendritic cells with total tumor antigens: tumor cell lysates, apoptotic tumor cells, tumor cell–derived exosomes, and total tumor cell RNA. Few studies, however, directly compared the efficiency of CTL priming using these different dendritic cell sensitization methods. The present results should encourage the use of Ab-coated tumor cells for dendritic cell sensitization in cancer immunotherapy.

FcγRs, Cross-Presentation, and Autoimmunity. Like in cancer, in most autoimmune diseases, including those with pathogenic CTLs, abundant pathogenic autoAbs are also produced. These Abs bind to either soluble autoantigens or self-tissue fragments, thus forming immune complexes, which may engage FcRs. In the past 10 years, the generation of mice lacking one or several FcγRs, demonstrated their role in different autoimmune diseases (6).

Two types of FcγR exist in both human and mouse: activation and inhibitory FcγR. Activation FcγRs signal through an amino acid motif, called immunoreceptor tyrosine–based activation motifs, found in the cytosolic domain of the receptor itself (for FcγRIIA), or on the FcR-associated γ chain (for FcγRI and FcγRIII). Activation FcγRs include mouse and human FcγRI, human FcγRIIA, and FcγRIII. Mouse and human FcγRII isoforms other than FcγRIIA, inhibit cell activation through immunoreceptor tyrosine–based inhibitory motifs when cocross-linked to activation receptors (FcγRs or many other receptors, including B and T cell receptors). All FcγRs, but one isoform of FcγRII (the B1 isoform), very efficiently internalize their ligands.

Deletion of activation FcγRs protects against immune complex–induced inflammation (6). Inhibitory FcγRs (FcγRIIB) knockout mice, by contrast, are more susceptible to immune complex–induced inflammation, which favors autoimmunity (including glomerulonephritis, collagen-induced arthritis, and hemolitic anemia, for example). Inhibitory FcγRIIB is thought to maintain peripheral B cell tolerance by blocking B cell activation when FDC present immune complexes to specific B cells in germinal centers.

Also in this issue, H. Kita et al. (15), propose a novel role for FcR-mediated cross-presentation in primary biliary cirrhosis (PBC). The authors identified a $CD8^+$ T cells epitope in the E2 component of pyruvate dehydrogenase (PDC-E2), and showed that the frequency of CTL precursors for this epitope is increased in PBC patients. FcR-mediated internalization of PDC-E2 complexed to Abs by dendritic cells results in effective cross-presentation to specific $CD8^+$ T cell clones. Importantly, anti–PDC-E2 Abs purified from patient's sera also promoted efficient cross-presentation, suggesting the involvement of autoAbs in the pathogenesis of this autoimmune disease: in promoting cross-presentation by dendritic cells, auto Abs could either participate to breaking $CD8^+$ T cell tolerance, or to the amplification and development of ongoing autoimmune CTL responses. These findings should focus our attention on FcγR expression and function on dendritic cells from patients bearing CTL-dependent autoimmune diseases.

Cross-Priming, Cross-Tolerance, and FcγR-induced Dendritic Cell Maturation. One critical aspect of FcγR function in dendritic cells, is the induction of maturation. In mouse dendritic cells, engagement of either FcγRI or FcγRIII induces maturation in an FcR-associated γ chain–dependent manner (7). In the studies published here, however, the authors did not see FcγR-mediated induction of dendritic cell maturation. This discrepancy could, of course, be due to species differences in FcγR function in mouse and human dendritic cells. Nevertheless, Geissman at al. showed that engagement of FcR specific for IgA does induce maturation of human monocyte–derived dendritic cells (16).

FcγR-mediated cell signaling results from a delicate balance between activation and inhibition signals triggered by different FcγRs (6). The same immune complexes or opsonized particles may simultaneously engage activation and inhibitory receptors. Coaggregation of these two types of receptors results in inhibition of cell signaling. Therefore, the outcome of FcγR engagement depends on the relative expression of activation and inhibitory receptors. In mouse, IL4 (a cytokine used for the differentiation of monocytes into dendritic cells) promotes the expression of FcγRIIB, an inhibitory FcγR isoform (17). IFN-γ, by contrast, promotes the expression of activation FcγR isoforms, such as FcγRI. In addition, the extent and specificity of FcγR engagement depend on the size of the immune complexes, and on the isotype and species origin, of the Abs used to form the immune complexes.

The pattern of FcγR expression in vivo, in dendritic cell subsets or during maturation, is unclear. Immature monocyte– and CD34-derived dendritic cells express CD32 (18), and occasionally, low levels of CD64. The relative expression of activation and inhibitory isoforms of CD32 (FcγRIIA and B/C, respectively) have not been analyzed. Dendritic cells purified from the blood, in contrast, express abundant CD64 (19). Therefore, it is not very surprising that depending on the type and maturation status of the dendritic cells used, the effect of immune complexes on maturation may differ. Neither FcγR expression, nor the effect of immune complexes on dendritic cell maturation have been yet analyzed in vivo (in mice or humans).

This point is particularly important, because different subpopulations of dendritic cells and dendritic cells at different stages of maturation have different functions. For example, mature dendritic cells induce T cell priming, whereas immature dendritic cells are believed to induce tolerance. In the context of autoimmunity, large immune complexes uptake by dendritic cells could simultaneously result in sensitization with autoantigens and induction of maturation. These mature dendritic cells, bearing specific

peptides from autoantigens, could then contribute to break tolerance and initiate the autoimmune responses. In the case of solid tumors, when natural CTL responses are often ineffective, the nature of the immune complexes and/or the expression of inhibitory FcγRs in dendritic cells, could prevent induction of maturation. The uptake of immune complexes should then result in sensitization of immature dendritic cells with tumor antigens, which could result the induction of immunological tolerance.

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