Fcγ Receptor-mediated Induction of Dendritic Cell Maturation and Major Histocompatibility Complex Class I-restricted Antigen Presentation after Immune Complex Internalization

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Summary

Dendritic cells (DCs) express several receptors for the Fc portion of immunoglobulin (Ig)G (FcγR), which mediate internalization of antigen–IgG complexes (immune complexes, ICs) and promote efficient major histocompatibility complex (MHC) class II–restricted antigen presentation. We now show that FcγRs have two additional specific attributes in murine DCs: the induction of DC maturation and the promotion of efficient MHC class I–restricted presentation of peptides from exogenous, IgG-complexed antigens. Both FcγR functions require the FcγR-associated γ chain. FcγR-mediated MHC class I–restricted antigen presentation is extremely sensitive and specific to immature DCs. It requires proteasomal degradation and is dependent on functional peptide transporter associated with antigen processing, TAP1-TAP2. By promoting DC maturation and presentation on both MHC class I and II molecules, ICs should efficiently sensitize DCs for priming of both CD4+ helper and CD8+ cytotoxic T lymphocytes in vivo.

Key words: Fc receptors • dendritic cells • antigen presentation • immune complexes • cross-priming

Major histocompatibility complex (MHC) class I molecules are generally complexed exclusively with peptides derived from cytosolic antigens (1). However, this picture is too restrictive to explain the priming of naïve CD8+ T cells by bone marrow (BM)-derived APCs (2): APCs also internalize exogenous antigens for processing and presentation on MHC class I molecules. The induction of CTL response due to exogenous antigen transfer was first examined in response to minor histocompatibility antigens, and was referred to as cross-priming (3). Recent results suggest that DCs may play a critical role in this process (4).

Indeed, dendritic cells (DCs) are the most potent APCs for inducing differentiation of naïve CD4+ and CD8+ T cells into helper and cytotoxic T cells, respectively, and for initiating primary and secondary immune responses (5, 6). To prime T cell responses, DCs require several separate signals. The first is provided by antigens themselves, which are processed into peptides and loaded intracellularly onto MHC molecules. Efficient T cell priming also requires a cell activation signal, delivered by either inflammatory cytokines (TNF-α or IL-1) or bacterial components (such as LPS). This signal induces expression of MHC and T cell costimulatory molecules at the DC surface and causes migration from peripheral tissues to secondary lymphoid organs, where T cell priming occurs. Cognate CD4+ T cell help is also required for efficient CD8+ T cell priming, with antigen recognition by both CD4+ and CD8+ T cells.

Abbreviations used in this paper: BM-DC, bone marrow–derived DC; CHX, cycloheximide; DC, dendritic cell; FcγR-ctg, chimeric receptor composed of the lumenal and transmembrane domains of FcγRII and the cytoplasmic tail of the γ chain; HEL, hen egg lysozyme; IC, immune complex; ITAM, immunoreceptor tyrosine-based activation motif; LLnL, aldehyde N-acyetyl-Leu-Leu-Leu-norleucinal; OVA-IC, OVA-containing IC; PTK, protein tyrosine kinase; TAP, transporter associated with antigen processing; wt, wild-type.
on the same DC (7–10). Therefore, this DC requires the simultaneous presentation of peptides from exogenous antigens on both MHC class I and II molecules.

Presentation of peptides derived from exogenous antigens on MHC class I molecules may occur through two different pathways (11). First, internalized antigens may exit endocytic compartments and, once in the cytosol, be processed by the proteasome into peptides which then reach the conventional transporter associated with antigen processing (TAP)1/2-dependent MHC class I antigen presentation pathway. Alternatively, internalized antigens may be processed in endocytic compartments, generating peptides which associate to preexisting MHC class I molecules, either in endosomes or at the cell surface after peptide regurgitation.

Regardless of the pathway, cross-priming in vitro after fluid phase antigen internalization is generally very inefficient, since it requires very high antigen concentrations—in the mg/ml range (11). Antigen coupling to or mixing with latex beads forces internalization by phagocytosis and strongly enhances the efficiency of MHC class I–restricted antigen presentation in macrophages or DCs (12, 13). Phagocytosis of bacteria (14, 15) or of apoptotic cells (4) also results in efficient MHC class I–restricted antigen presentation in macrophages and/or DCs. Thus, the pathway by which antigens are internalized appears to influence the efficiency of presentation on both MHC class I and II molecules.

In the case of MHC class II–restricted presentation, a major breakthrough came from the observation that antigens internalized through specific membrane receptors are more efficiently presented to CD4+ T cells than they are after fluid phase internalization (16). FcγR[s], which bind antigen–IgG complexes (immune complexes, ICs [17]), represent a privileged antigen internalization route for efficient MHC class II–restricted antigen presentation in DCs (18). Human DCs express several types of FcγRs, including type I (FcγRI, CD64 [19]) and type II (FcγRII, CD32 [18]). FcγR expression by murine DCs has not been fully examined. Importantly, in addition to IC internalization, FcγRI and FcγRII trigger cell activation (17) through the associated γ chain, which bears a motif called immunoreceptor tyrosine-based activation motif (ITAM), also required for IC internalization (20, 21).

Here, we examined the role of FcγRs in DC activation and in MHC class I–restricted presentation of peptides derived from internalized IgG-complexed antigens. We found that FcγR engagement in DCs triggers maturation and induces efficient MHC class I and II–restricted antigen presentation. These results suggest the existence of unknown connections between humoral and cytotoxic components of immune responses.

Materials and Methods

Mice. γ chain−/− mice were obtained on a B6 × 129 background (22) and TAP−/− mice from Centre National de la Recherche Scientifique (Orleans, France). TAP−/− mice were on a B6 × 129 background (23).

DCs and Culture Medium. Immature DCs were prepared as described (24). C57BL/6 and γ chain−/− BM cells were incubated 3 wk in IMDM (Sigma Chemical Co.) containing 10% heat-inactivated FBS (GIBCO BRL), 100 IU/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine (Sigma), and 50 mM 2-μM E with 30% conditioned medium from GM-CSF–producing N11/3T3 cells (R 1 medium). D1 long-term cultured cell line is an H-2b splenic DC cell line described by Wizniet et al. (24) and was also cultured in the same medium with 30% R 1 medium. The cells expressed MHC class I and II molecules, CD40, CD80, CD86, and 24G.2 FcγR, but not Gr1. The surface expression of all of these molecules, except FcγR s, was increased after LPS treatment for 24 h, indicating effective DC maturation.

Antibodies. Cell Surface Staining, and Immunofluorescence. The following antibodies were purchased from PharMingen: CD80/B7.1 (1G10), CD40 (HM 40–3), CD86/B7.2 (GL1), CD107a/Lamp-1. Before labeling experiments, FcγR blocking was performed by incubating cells with 2.4G2 supernatant. Staining was carried out according to standard techniques, and flow cytometry analysis was performed with a FACScan (using CellQuest software; Becton Dickinson). For intracellular immunofluorescence, cells were fixed for 20 min in 3% paraformaldehyde and then permeabilized for 30 min in PBS containing 1% saponin, 5% BSA, and then stained in PBS containing 1% saponin, 5% BSA.

A nitogen Presentation Assay. OVA batches from different companies were screened for the absence of presentation to B3Z cells with fixed cells. The selected batch (from Worthington) did not induce DC activation by immunofluorescence and flow cytometry. Presentation of OVA epitope 257–264 on K b was detected using the T cell hybridoma B3Z, which carries a β-galactosidase construct driven by NF-AT elements from the IL-2 promoter (25). For antigen presentation assays DCs were exposed to OVA, at the concentration and for the periods of time specified, in the presence of the T cell hybridoma B3Z. After exposure to OVA, cells were washed with PBS, and a colorimetric assay using ONPG (Sigma Chemical Co.) as a substrate was used to detect β-galactosidase activity in B3Z lysates. Where indicated, the following inhibitors were included in the antigen presentation assays.

MHC class II–restricted response to ovalbumin was detected using two T cell hybridomas, BO97.10, specific for OVA 323–339 on I-A d (26) on DCs, and 54.8, specific for the same peptide presented on I-A b, 20 h after antigen pulsing. DCs were fixed with glutaraldehyde 0.001%, and BO97.10 cells were added for 24 h. After 24 h, 50 ml of supernatant was harvested and the IL-2 production by BO97.10 was measured with [3H]thymidine incorporation by IL-2–dependent CTL L2 cell line.

Lactacystin and the peptide aldehyde N-acetyl-Leu-L-Leu-L-norleucinal (LLNl) were stored in DM SO as 1 and 2.5 mM stock solutions, respectively, and they were diluted in culture media for use. 5 × 104 D1 cells/well were incubated with the inhibitors for 1 h before antigen pulsing and during the antigen pulse. OVA-containing ICs (OVA-ICs) were prepared by incubation at 37°C, 15 min of soluble OVA at a final concentration of 0.4 and 20 μg/ml of anti-OVA IgGs purified from rabbit sera (Sigma Chemical Co.). As a control for inhibitor toxicity, we used the OVA 257–264 peptide at a final concentration of 10 ng/ml. After a chase of 12 h, D1 cells were fixed with glutaraldehyde 0.001%, overlaid with 5 × 106 B3Z cells/well, and incubated for 24 h. A divalent induction by ICs. 5 × 104 D1 cells/well were incubated with soluble OVA alone or in the presence of hen egg lysozyme (HEL)-ICs (at final concentrations: HEL 30 μg/ml, and mAbs anti-HEL, HyHEL5, and 5253C7, 15 μg/ml each [27]) and were overlaid with 5 × 106 B3Z cells/well and incubated for 24 h.
Results

ICs Induce DC Activation. To analyze the expression and function of FcγR in murine DCs, we first used a well-characterized, growth factor–dependent, spleen-derived DC line called D1 (24). D1 cells display all of the phenotypic characteristics of immature DCs: low levels of surface MHC and costimulatory molecules, and abundant endocytic MHC class II–containing compartments. Upon treatment with LPS or TNF-α, D1 cells show all of the phenotypical changes characteristic of DC maturation (24).

We first analyzed the nature of the FcγRs expressed by D1 cells. Western blot analysis after immunoprecipitation with the anti-FcγRII and FcγRIII 2.4G2 antibody showed that D1 cells expressed FcγRIIb1, FcγRIIb2, and FcγRIII (Fig. 1 A). The two intermediate bands between FcγRIIb1 and FcγRIIb2 most likely represent FcγRIIb1′ and an unidentified spliced variant (29). FcγRI are also expressed in murine DCs, since mRNA encoding this receptor was readily detected by reverse transcription PCR (not shown). These results indicate that murine DCs express FcγRI, II, and III.

Engagement of FcγRI or III in macrophages triggers cell activation, causing the production of various cytokines and chemokines, as well as changes in expression of cell surface proteins involved in antigen presentation (17, 30). To evaluate the ability of ICs to induce DC activation, D1 cells were incubated for 24 h in the presence of OVA complexed to specific polyclonal anti-OVA IgG antibodies (OVA–ICs) or LPS, which induces D1 maturation.

As shown in Fig. 1 B, OVA–ICs, like LPS, induced a marked increase in the surface expression of MHC class II, CD86, and CD40 molecules (Fig. 1 B), phenotypic changes characteristic of DC maturation (6, 24). Immunofluorescence and confocal microscopy analysis showed that both LPS (Fig. 1 C, middle) and OVA–ICs (bottom) also induced MHC class II redistribution to the plasma membrane (compared with Fig. 1 C, top), as lysosomes became devoid of MHC class II molecules. Incubation with OVA alone, or with the antibodies in the absence of OVA, induced no changes in the surface expression of any of the
markers analyzed or in DC morphology and MHC class II localization (not shown). Similar results were obtained with fresh BM-derived DCs (BM-DCs, see below). Like other maturation stimuli (31, 32), OVA-ICs induced an increase in MHC class II synthesis and a strong decrease in their rate of degradation (MHC class II half-life raised from 3–5 to >40 h; not shown). Therefore, like LPS or TNF-α, FcγR engagement by ICs induces murine DC maturation in vitro.

IC internalization results in efficient MHC class I-restricted antigen presentation. The other main consequence of FcγR engagement is IC internalization, which induces potent MHC class II-restricted presentation in various cell types, including DCs (18, 33). However, during cross-priming, DCs also need to present exogenous antigens on MHC class I molecules to initiate CTL responses. To determine if ICs may participate in the acquisition of antigens by DCs for MHC class I-restricted presentation, we next examined presentation of an OVA-derived peptide to a CD8+ T cell hybrid after FcγR-mediated internalization of OVA-ICs by murine D1 cells.

As shown previously (34–36), presentation of OVA 257–264/H-2Kb epitope to B3Z T cells (25) after OVA uptake by fluid phase was only observed at very high, non-physiological antigen concentrations of 1–10 mg/ml (Fig. 2). In contrast, presentation of the same epitope after internalization of OVA-ICs was observed at OVA concentrations ranging between 0.1 and 1 μg/ml (Fig. 2), i.e., three to four orders of magnitude lower antigen concentrations than uncomplexed OVA. As expected for ICs, the optimal antigen to antibody ratio was achieved at lower antigen concentrations as the antibody concentrations decreased (Fig. 2). The highly efficient OVA presentation observed after OVA-IC internalization was not due to FcγR engagement per se, since presentation of soluble OVA to B3Z T cells was not modified by the presence of irrelevant HEL-ICs (Fig. 2). HEL-ICs induced D1 maturation, as detected by surface immunostaining of MHC and costimulation molecules (not shown). Therefore, formation of ICs allows efficient acquisition of antigens for peptide presentation to CTLs on MHC class I molecules.

The capacity of DCs to ingest and process exogenous antigens for presentation by MHC class II molecules is down-modulated upon DC maturation (37). Therefore, it was important to determine whether MHC class I-restricted presentation after IC internalization was also modulated during DC maturation. Like D1 cells, immature (untreated) BM-DCs presented OVA epitope 257–264 to B3Z cells more efficiently after OVA-IC internalization than after uncomplexed OVA uptake (Fig. 3, top left and middle left panels). In cells treated with LPS for 18 h, presentation after both OVA-IC and soluble OVA internalization was transiently and reproducibly enhanced (Fig. 3, top center and right panels). In cells treated with LPS for 18 h, presentation after both OVA-IC and soluble OVA internalization was transiently and reproducibly enhanced (Fig. 3, top center and right panels).
Middle center panels). In contrast, MHC class I–restricted presentation after OVA-IC and soluble OVA internalization was almost completely abrogated in DCs treated with LPS for 48 h (Fig. 3, top right and middle right panels). Direct presentation of the 257–264 OVA peptide was not significantly modified by LPS treatment (Fig. 3, bottom panels). Therefore, as shown previously for MHC class II (31, 32), MHC class I–restricted presentation of exogenous antigens is first transiently upregulated and then shut down during DC maturation. Downregulation of MHC class I–restricted presentation by DCs upon maturation may prevent presentation of IgG-complexed antigens encountered after DC migration to secondary lymphoid organs.

MHC Class I Presentation after IC Internalization Is TAP Dependent and Lactacycin Sensitive. Two pathways for MHC class I–restricted presentation of exogenous antigens have been described (11). One reaches the so-called “conventional” pathway after exogenous antigen transfer from endocytic compartments to the cytosol. This pathway requires proteasomal degradation, TAP-dependent transport of the peptides into the lumen of the endoplasmic reticulum, and association to newly synthesized MHC class I molecules (11). The second pathway is TAP and proteasome independent; it is inhibited by NH4Cl (which neu-
molecules (11). The second pathway is TAP and protea-
some independent; it is inhibited by NH4Cl (which neu-
teralizes lysosomal pH) but not by cycloheximide (CHX; an inhibitor of protein synthesis), suggesting that the genera-
tion of peptides and their association to MHC class I may occur in endosomes (11). We next determined which of these two pathways was used for MHC class I–restricted presentation after IC internalization.

Antigen presentation by D1 cells after OVA-IC internalization was strongly inhibited by CHX (Fig. 4 A), lactacycin (a specific proteasome inhibitor [38]; Fig. 4 B), and the peptide aldehyde N-acetyl-Leu-L-Leu-Norleucinal (LLnL; Fig. 4 C), which inhibits both lysosomal proteases and the proteasome (38). Direct presentation of the 257–264 OVA peptide was not affected by any of these drugs (Fig. 4, A–C). To assess the specificity of the two protease inhibitors in DCs, lactacycin and LLnL were tested in parallel on the MHC class II–restricted presentation after OVA-IC internalization. Only LLnL, and not lactacycin, blocked MHC class II–restricted presentation of OVA peptide 323–339 on I-Ak to BO97.10–specific T cells (Fig. 4 D), indicating that lactacycin specifically inhibits proteasomal degradation at the concentrations used. MHC class I–restricted presenta-
tion after OVA-IC internalization was also sensitive to brefeldin A (which blocks protein transport from the endo-
plasmic reticulum, not shown).

MHC class I–restricted presentation after OVA-IC internalization also required the TAP1-TAP2 peptide transporter. BM-DCs derived from TAP1-deficient mice did not present the OVA epitope after internalization of OVA-ICs, whereas direct presentation of the 257–264 OVA peptide to B3Z T cells was as efficient as with DCs derived from wild-type (wt) C57BL/6 mice (Fig. 4 E). Therefore, MHC class I–restricted presentation after IC internalization reaches the conventional MHC class I presentation pathway.

MHC Class I–restricted Presentation after IC Internalization Is DC Specific. However, efficient IC internalization is not restricted to DCs. In B lymphoma cells, we showed that expression of endocytic FcγRs induces efficient MHC class II–restricted presentation after IC internalization (20, 39). To determine whether FcγR–mediated MHC class I–restricted presentation of exogenous antigens is DC specific, we next examined antigen presentation after OVA-IC internalization in B lymphoma cells.

![Figure 4](https://example.com/figure4.png)

Figure 4. Antigen presentation after IC internalization reaches the conventional MHC class I antigen presentation pathway. (A–C) D1 cells were preincubated alone or in the presence of CHX (3 μg/ml; A) for 3 h or lactacycin (10 μM, B) or LLnL (8 μM, C) for 1 h. Pretreated D1 cells were then incubated for an additional 7-h period with OVA-ICs (0.4 μg/ml of OVA and 20 μg/ml of rabbit anti-OVA IgGs; black bars) or with the 257–264 synthetic peptide (10 ng/ml; white bars) in the presence of the same concentrations of inhibitors. D1 cells were glutaraldehyde fixed before coculture with B3Z cells. (D) MHC class II–restricted presentation after OVA-IC internalization was tested in the presence of lactacycin (black bars) and LLnL (hatched bars) using the T cell hybridoma BO97.10 (specific for OVA 323–339 on I-Ak) on DCs fixed with glutaraldehyde after antigen pulsing. IL-2 production by BO97.10 was measured with [3H]thymidine incorporation by IL-2–dependent CTL.L2 cell line. (E) BM-DCs were generated as described (reference 33) from C57BL/6 or TAP-1–deficient mice and were incubated with 257–264 (SIINFEKL) peptide (10 ng/ml; white bars) or with OVA-ICs (0.4 μg/ml of OVA and 20 μg/ml of anti-OVA rabbit IgGs; black bars) in the presence of B3Z cells for 24 h. Cultures were then treated as described above (one representative experiment out of three is shown).
cells are an FcγR clone derived from A20 B lymphoma cells, one of the cell lines most widely used to analyze MHC class II–restricted antigen presentation (20, 39). We have shown previously that IIA1.6 cells expressing recombinant FcγRIIb2 or FcγRIII efficiently internalize IC and strongly promote MHC class II–restricted antigen presentation (20, 39). In addition, a chimeric receptor composed of the luminal and transmembrane domains of FcγRII and the cytoplasmic tail of the γ chain (FcγR-ctγ) presents all of the functional characteristics of FcγRIII in terms of internalization and antigen presentation (20, 33). I-Aδ-expressing B lymphoma IIA1.6 cells expressing recombinant FcγRIIb2 or FcγR-ctγ chimeras were supertransfected with H-2Kb, and compared with D1 cells for MHC class I and II presentation after OVA-IC internalization.

In FcγR-ctγ/H-2Kb-expressing B lymphoma cells, MHC class II–restricted presentation of the 323–339 OVA peptide on I-Aδ to 54.8 T cell hybridomas was strongly enhanced (three to four orders of magnitude) after OVA-IC internalization (Fig. 5 A). In D1 cells, presentation of the same peptide on I-Aδ to B097.10 T cell hybridomas was also enhanced by three to four orders of magnitude after OVA-IC internalization (Fig. 5 B). Similar results were obtained with cells expressing FcγRIIb2 (not shown). Therefore, internalization of ICs results in efficient MHC class II–restricted presentation in both B lymphoma cells and DCs.

In D1 cells, as shown previously, soluble MHC class I–restricted presentation was observed after soluble OVA at high concentrations and after OVA-IC internalization at low antigen concentrations (Fig. 5 D). In contrast, B lymphoma cells expressing FcγR-ctγ (Fig. 5 C) or FcγRIIb2 (not shown) were completely incompetent for MHC class I–restricted presentation of OVA-derived peptide 257–264, after internalization of OVA-ICs or uncomplexed OVA at high concentrations. In contrast, when incubated with the OVA 257–264 synthetic peptide, both D1 cells and B lymphoma cells activated B3Z cells (Fig. 5, C and D). Like B lymphoma cells, IFN-γ–treated peritoneal macrophages were capable of directly presenting OVA peptide 257–264 to B3Z cells, but not after OVA-IC or uncomplexed OVA internalization (not shown). Therefore, in contrast to MHC class II–restricted presentation, the FcγR-mediated pathway for presentation of exogenous antigens by MHC class I molecules is restricted to DCs.

Role of the FcγR-associated γ Chain in IC-induced DC Maturation and MHC Class I–restricted Antigen Presentation. The results presented thus far suggest that FcγRs are involved in both the induction of DC maturation and antigen uptake for efficient MHC class I–restricted presentation of exogenous antigens. Importantly, FcγRI and FcγRIII trigger cell activation in a variety of cell types through an ITAM found in the associated γ chain (17). To determine the nature of the FcγRs involved in the triggering of DC maturation by ICs, we prepared BM-DCs from wt mice and from mice deficient for the FcγRI- and FcγRIII-associated γ chain (22). Surface expression of FcγRII and III (as detected by the mAb 2.4G2) was decreased but not abolished in BM-DCs from the γ chain−/− mice (Fig. 6 A), confirming that

![Figure 5. MHC class I–restricted presentation after OVA-IC internalization is DC specific. D1 cells were tested concurrently with a B cell line expressing recombinant internalization–competent FcγR-ctγ chimeric receptors and supertransfected with cDNA encoding the Kb MHC class I molecule. B lymphoma cells (A and C) and D1 cells (B and D) were incubated with the indicated concentrations of OVA (squares) or OVA complexed to 20 μg/ml of anti-OVA rabbit polyclonal IgG (OVA–IC; circles). D1 cells were fixed with glutaraldehyde before coculture with T cells. MHC class II–restricted presentation of OVA was tested using the T cell hybridomas B097.10 (for I-Aδ on D1 cells B) or 54.8 (for I-Aδ on B lymphoma cells A), which both recognize the 323–339 epitope, in association with I-Aδ and I-Ad, respectively. MHC class I presentation by B lymphoma cells (C) or D1 cells (D) was tested using B3Z T cell hybridoma as described in the legend to Fig. 2. The 323–339 and 257–264 synthetic peptides (10 μg/ml) were used as positive controls (histograms).](image-url)
BM-DCs from the γ chain−/− mice still express FcγRII and suggesting that BM-DCs from wt C57BL/6 mice expressed both FcγRII and III.

To determine the requirement for γ chain in DC activation, the ability of LPS and OVA-ICs to induce maturation was analyzed. LPS induced a marked increase of the surface expression of MHC class II, CD86, and CD40 molecules on DCs from wt and γ chain−/− mice (Fig. 6 B), as well as all of the morphological modifications characteristic of DC maturation (not shown). In contrast, OVA-ICs did not induce any detectable maturation in BM-DCs from γ−/− mice, whereas they induced maturation of BM-DCs from wt mice (Fig. 6 B). Therefore, DCs from γ chain−/− mice presented a selective defect in IC-induced maturation.

The involvement of the γ chain in MHC class I-restricted presentation was tested next. As expected, BM-DCs from both wt and γ chain−/− mice presented soluble OVA and the 257–264 OVA peptide to the B3Z T cell hybridomas with similar efficiencies (Fig. 7). BM-DCs from wt mice also presented the OVA epitope after OVA-IC internalization at low antigen concentrations (Fig. 7). In contrast, DCs from γ−/− mice did not show any significant MHC class I-restricted presentation after incubation with OVA-ICs (Fig. 7). These DCs had also lost the ability to present OVA peptide 323–339 in association to I-Ab MHC class II molecules (not shown). In addition, FcγR-mediated IC internalization in BM-DCs from γ chain−/− mice was decreased, as measured both biochemically and by immunofluorescence and confocal microscopy (not shown), suggesting that the absence of antigen presentation by BM-DCs from γ chain−/− mice results from inefficient IC internalization.

Therefore, the FcγR-associated γ chain is required for both induction of DC maturation by ICs and promotion of MHC class I-restricted presentation, indicating that FcγRII and/or FcγRIII (the two γ chain-associated FcγRs) are required for the functions of ICs in DCs.

Discussion

Our results evidence a novel receptor-mediated pathway for antigen acquisition for MHC class I-restricted presentation. This FcγR-mediated cross-priming pathway is DC specific and is inactive in mature DCs. It requires the TAP1-TAP2 transporter and is sensitive to lactacystin, a proteasome inhibitor, indicating that after internalization, IgG-complexed antigens are transferred into the cytosol and reach the conventional MHC class I antigen presenta-
activation, which are both efficient in macrophages or DCs. The mechanism of this transfer is still unclear, it was shown previously that IgG-complexed antigens are transferred from endocytic compartments into the cytosol. Although the antigen is determinant for cross-priming with ICs, we observed antigen transfer to the cytosol is inefficient in these two cell types compared with DCs (Rodriguez, A., unpublished results). Therefore, the specificity of DCs for cross-priming might be related to a selective ability of DCs to deliver antigen from endosomes or lysosomes into the cytosol.

The other major effect of FcγR engagement in DCs is induction of maturation. Indeed, all of the phenotypical, morphological, and functional modifications caused by inflammatory cytokines or LPS were also induced by ICs. In addition, none of these modifications were observed with DCs from γ chain−/− mice, demonstrating the implication of the γ chain in the induction of DC maturation by ICs. We found here that mouse D1 cells (as well as BM-DCs) express the two γ chain–dependent FcγRs, FcγRI (CD64) and FcγRIII (CD16). It is not clear to date which of these two receptors is required for DC maturation, but it will be addressed using FcγRI and/or FcγRIII−/− mice.

Whether FcγRI or FcγRIII is used, the involvement of the FcγR–associated γ chain in DC maturation indicates that an ITAM–bearing receptor triggers DC activation. FcγR cross-linking causes activation of protein tyrosine kinases (PTKs) from the src family (17). These PTKs phosphorylate tyrosine residues in the ITAM, thus inducing association to src PTK, leading to Ca2+ release from intracellular stocks and to a wide variety of biological responses. Our observation represents the first evidence of induction of DC activation and maturation through an ITAM–containing receptor. Interestingly, the γ chain ITAM also bears an internalization signal that mediates endocytosis of FcγRIII (20), and more recently, we showed that the ITAM also determines γ chain–mediated transport from endosomes to lysosomes (41). Thus, FcγR may initiate DC maturation and simultaneously target antigen to the appropriate endocytic compartment, where peptides are loaded onto MHC class II and from where antigens are transferred into the cytosol.

Indeed, CD4+ T cells play an important role in antinfectious CD8+ T cell–mediated responses, even if they are dispensable in some of them (42–44). The antigen presentation on both MHC class I and class II molecules that we observed after IC internalization by DCs would ensure an optimal stimulation of both CD4+ and CD8+ T cells. At the end of primary responses and in the course of secondary immune responses, the production of specific antibodies induces formation of complexes between antigens derived from infected cells or tumor cells and specific IgGs. These complexes could be taken up by FcγRs on DCs. After internalization ICs, DCs would then present the antigen to specific CD4+ T cells, which activate them through interactions implicating costimulatory molecules like CD40-CD40L and convert them into DCs capable of priming CD8+ T cells (7–10). However, since FcγR engagement also induces efficient maturation, IL-activated DCs could potentially prime CD8+ T cells directly, bypassing cognate CD4+ T cell help. This mechanism could operate in certain antiviral and/or antitumoral immune responses. However, it could also induce inappropriate CTL responses,
since the absence of CD4+ T cells would not allow a control of specificity, i.e., a “double check.”

In what physiological situation might ICs trigger CTL responses? Specific antibodies, which may potentially form ICs, are produced during most immune responses, including those where the final effectors are CTLs. ICs may participate in DC-mediated CD8+ T cell priming in the case of secondary immune responses, when specific IgGs may be produced very rapidly. In the case of ongoing immune responses which in many cases correspond to situations of immunosuppression, such as chronic infections or tumors, cross-priming through ICs may contribute to the establishment of specific tolerance. ICs have also been reported to play critical roles in several autoimmune diseases. Amplification of anti-self CTL responses by DCs that have acquired autoantigens from ICs may contribute to the triggering or development of autoimmune pathologies.

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