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Antigen-Antibody Immune Complexes Empower Dendritic Cells to Efficiently Prime Specific CD8\(^+\) CTL Responses In Vivo\(^1\)

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Dendritic cells (DCs)\(^4\) comprise a family of professional APCs that are present in low numbers in most body tissues (1). DCs are crucial in the initiation of primary CD4\(^+\) and CD8\(^+\) T cell responses. Immature DCs lack the costimulatory signals for efficient T cell activation, but they are well equipped to capture Ags. DC maturation can be induced by different microbial and inflammatory products as well as CD4\(^+\) Th-dependent CD40 ligation. Following DC maturation, Ag uptake capacity is decreased, whereas the expression of costimulatory molecules and MHC molecules is increased, and concomitantly T cell stimulatory capacity is enhanced. Furthermore, mature DCs produce IL-12, a key cytokine in the induction of Th1 and CTL responses (2, 3). We have recently shown that maturation of DCs is required for the induction of efficient CTL responses in vivo (4).

DCs internalize exogenous Ags by fluid-phase pinocytosis or by receptor-mediated endocytosis. Peptides derived from exogenous Ags are presented efficiently in MHC class II molecules (5). Although MHC class I molecules are generally complexed with peptides derived from cytosolic Ags, exogenous Ags can be processed and presented in MHC class I molecules after internalization (6, 7).


Materials and Methods

Mice

Female C57BL/6 (B6; H-2\(^b\)) mice were obtained from IFFA Credo (Paris, France). Mice were maintained under specific pathogen-free conditions and used at 6–10 wk of age.
Cell lines and reagents

Human adenosivirus type 5 (Ad5) E1-transformed B6 mouse embryo cells (MECs) and B6 MECs transfected with murine CD80 (B7.1), H-2K^b, and a construct expressing an endoplasmic reticulum targeting signal sequence, followed by the OVA_{257-264} CTL epitope SIINFEKL (SigSIINFEKL), were generated as described (17, 18). B3Z is a T cell hybridoma specific for the SIINFEKL epitope, which carries an IgG1 kappa chain and is generated by NF-AT elements from the IL-2 promoter (19). Cell lines were cultured in IMDM (BioWhittaker, Verviers, Belgium) supplemented with 8% heat-inactivated FCS (Greiner, Alphen, The Netherlands), 100 IU/ml penicillin, 2 mM l-glutamine, and 20 mM 2-ME. D1 cell line, a long-term growth factor-dependent immature splenic DC line derived from B6 mice, was kindly provided by P. Ricciardi-Castagnoli (University of Milano-Bicocca, Milan, Italy) and cultured as described (20). Bone marrow-derived (BM) primary DC cultures (BM DCs) were generated as described (21). Both floating and adherent DCs (detached using 2 mM EDTA) were collected and used. LPS of Escherichia coli (serotype 026:B6) was purchased from Sigma-Aldrich (St. Louis, MO). Synthetic peptides used were: OVA_{257-264}, SIINFEKL; vesicular stomatitis virus nucleoprotein (VSV NP_{52-59}), GRYVVQGL; Ad5 E1A_{234-243}, SGPSNTTPPE; and human papillomavirus type 16 E7_{49-57}, KAHTYIVTF.

Abs and cell surface immunofluorescence

The following Abs were purchased from BD PharMingen (San Diego, CA): FITC-coupled anti-CD86/B7.2 Ab (GL1), FITC-coupled anti-CD8/Ab (Ly-3.2), allophycocyanin-coupled anti-CD86 Ab (Ly-2). PE-conjugated Ad5 E1A_{234-243}-loaded H-2D^d and PE-conjugated SigSIINFEKL-loaded H-2K^b tetramers were prepared as described (22, 23). Ad5 E1A_{234-243} and H-2D^d tetramers were kindly provided by T. Schreiber (NKI, Amsterdam, The Netherlands). Staining for H-2D^d/E1A_{234-243} tetrameric complexes and anti-CD8α allophycocyanin was conducted at 37°C for 15 min. For H-2K^b-SIINFEKL tetrameric complexes, cells were incubated for 30 min at room temperature with tetrameric complexes, washed, and incubated for another 30 min at room temperature with FITC-coupled anti-CD8/Ab. Stained cells were analyzed using a FACScan flow cytometer equipped with CellQuest software (BD Biosciences, Mountain View, CA).

Incubation with ICs, MHC class I-restricted Ag presentation assay, and IL-12 ELISA

ICs were prepared by incubating soluble OVA (grade V; Sigma-Aldrich) with 25 μg/ml polyclonal OVA-specific rabbit IgG (rlgGroVA; Sigma-Aldrich) for 30 min at 37°C in 96-well flat-bottom plates. As a control, soluble OVA was preincubated with 25 μg/ml control rabbit IgG. A total of 10^5 D1 cells/well was added and incubated for 24 h at 37°C. Supernatants were harvested, and 5 × 10^5 B3Z cells/well were added to the D1 cells and incubated for another 24 h at 37°C. Presentation of SIINFEKL in H-2K^b was detected by activation of B3Z cells, measured by a colorimetric assay using chlorophenol red-β-galactopyranoside as substrate to detect lacZ activity in B3Z lysates. Harvested supernatants were tested for IL-12 p40/p70 content using a standard sandwich ELISA. Coating Ab: rat anti-mouse IL-12 p40/p70 mAb (clone 15/6; BD PharMingen). Detection Ab: biotinylated rat anti-mouse IL-12 p40/p70 mAb (clone C17/8; BD PharMingen). Streptavidin-HRP and ABTS (Sigma-Aldrich) were used as enzyme and substrate, respectively.

Uptake of OVA-FITC and confocal microscopy

OVA-ICs were preformed as described in the previous paragraph, using FITC-labeled OVA (Molecular Probes, Leiden, The Netherlands), in 96-well plates. D1 cells were added to wells containing OVA-ICs or soluble OVA with control rabbit IgG and incubated for different time periods at 37°C. Cells were harvested and resuspended in the absence or presence of 0.4% (v/v) trypan blue (Sigma-Aldrich), which quenches extracellular, but not intracellular, fluorescence (25). Flow cytometry was performed with FACScan. For analysis by confocal microscopy, D1 cells were grown on 3-cm petri dishes for 2 days and then incubated with OVA-ICs or OVA with control rabbit IgG for 1 h at 37°C. After washing, cells were fixed by incubation with 1% paraformaldehyde for 15 min, followed by blocking with 0.2 M glycine. Cells were analyzed by confocal microscopy (Carl Zeiss microscope; Zeiss, Oberkochen, Germany). Analysis of the cells by Z-scan analysis, showing images of optical slices from the top to the bottom of the cells, was performed using LSM 510 Image browser software (Carl Zeiss Microimaging, Inc., San Diego, CA).

Induction of CTL responses in vivo

To induce CTL responses in vivo against exogenously loaded Ad5 E1A_{234-243} (SGPSNTTPPEI) or against the endogenously processed MHC class I-binding peptide OVA_{257-264} (SIINFEKL) derived from the OVA protein Ag present in the ICs, D1 cells or BM DCs were incubated for 48 h with OVA-ICs, with soluble OVA alone or in the presence of control rabbit IgG, or 10 μg/ml LPS, followed by loading with 1 μg/ml Ad5 E1A_{234-243} for 2 h at 37°C. After five times washing, 10^5 D1 cells were injected i.v. in B6 mice in PBS with 0.5% BSA. Mice were depleted of CD4^+ cells by i.p. injection of 100 μg purified anti-CD4 Ab GK1.5 in PBS at days 5, 3, and 1 before, and at days 1 and 7 after injection of D1 cells. CD4^+ cell populations were below detection level by flow cytometry. Depletion was performed to prevent endogenous CD4^+ T cells from activating the D1 cells in vivo (our unpublished results). After 10 days, spleen cells were used for detection of responses by direct ex vivo staining with tetrameric complexes. In addition, spleen cells were cocultured (5 × 10^6 cells/well) with irradiated (10,000 rad) Ad5-transformed MECs (5 × 10^6/well) to restimulate responses to the exogenously loaded Ad5 peptide or with irradiated (10,000 rad) B7.1-, H-2K^b-, and SigSIINFEKL-transformed MECs (5 × 10^6/well) to restimulate responses to the SIINFEKL peptide, in 1.5-ml cultures in 24-well plates in the absence of additional cytokines. After 7 days, lymphocyte cultures were tested for staining with tetrameric complexes and for cytotoxicity against Eu^3+ -labeled target cells loaded with Ad5 E1A_{234-243} or control H-2D^b-binding peptide (human papillomavirus type 16 E7_{49-57}) or with SIINFEKL or control H-2K^b-binding peptide (VSV NP_{52-59}).

Results

ICs improve MHC class I-restricted Ag presentation by DCs and induce maturation of these cells

To establish and optimize the treatment of DCs with ICs in our laboratory, the efficiency of MHC class I-restricted Ag presentation of OVA-derived epitopes upon incubation of the well-established immature murine DC line D1 (20) with OVA-ICs was analyzed. D1 cells were incubated with OVA-ICs containing titrated amounts of OVA protein and a fixed concentration of OVA-specific rabbit IgG (rlgGroVA). After 24 h, presentation of H-2K^b-restricted OVA_{257-264} CTL epitope (SIINFEKL) was analyzed by measuring the activation of B3Z T cell hybridoma cells. OVA-ICs induced very efficient presentation of the SIINFEKL epitope. Optimal IC formation was achieved at about 1 μg/ml OVA at a fixed Ab concentration of 25 μg/ml (Fig. 1A). In contrast, detectable processing and presentation of SIINFEKL by D1 cells after incubation with soluble OVA or OVA with control rabbit IgG were only observed at OVA concentrations of 30 μg/ml or higher. At higher concentrations of OVA, no optimal IC formation takes place (see below); therefore, the level of T cell activation follows the curve of soluble OVA protein above 10 μg/ml.

Induction of IC-mediated maturation of D1 cells was analyzed by measurement of IL-12 production as well as surface expression of costimulatory molecules and MHC molecules. LPS was used as a positive control for optimal induction of maturation of D1 cells. IL-12 production (Fig. 1B) and increased surface expression of CD86 (B7.2) (Fig. 1C), CD40, and MHC class I (K^b) (data not shown) were induced by OVA-ICs, but not by rlgGroVA alone, soluble OVA, or OVA with control rabbit IgG. These data show that ICs induce functional activation of DCs by up-regulation of relevant surface molecules, induction of IL-12 production, as well as improved efficiency of MHC class I-restricted Ag presentation.

Activation of DCs is not sufficient to improve MHC class I-restricted Ag presentation

ICs can activate DCs. To test the possibility that DC activation as such enhances presentation of OVA-derived epitopes, we incubated D1 cells with soluble OVA protein in the presence or absence of the potent DC-activating compound LPS. D1 cells incubated with soluble OVA and stimulated with LPS did not exhibit improved presentation of SIINFEKL to B3Z T cell hybridoma cells. In contrast, OVA-ICs strongly improved presentation of this CTL epitope (Fig. 2A). IL-12 production by D1 cells was induced by both LPS and OVA-ICs (Fig. 2B). In the case of LPS treatment,
Enhanced MHC class I-restricted presentation of Ag present in ICs can be due to more efficient internalization of complexed Ag, mediated by FcRs expressed on DCs, compared with soluble Ag. To investigate this, D1 cells were incubated with 1 μg/ml OVA-FITC in ICs, 1 μg/ml OVA-FITC with control rabbit IgG, or 100 μg/ml soluble OVA. After different incubation times, cells were harvested and resuspended in the absence or presence of trypan blue. Trypan blue quenches extracellular, but not intracellular, fluorescence (25). D1 cells incubated with OVA-FITC-ICs were strongly fluorescent, already after 10-min incubation (Fig. 3A). At this time point, one-quarter of the fluorescence was not quenched by trypan blue; therefore, a major portion of the OVA appeared to be internalized. After 3 h, most of the fluorescent OVA was internalized. This indicates that uptake of OVA complexed to Abs is very efficient. Soluble OVA was taken up as well, but far less efficiently. Uptake could only be observed at high concentrations of OVA (100 μg/ml) in contrast to 1 μg/ml OVA, which is the corresponding concentration used in ICs (Fig. 3B). Soluble OVA-FITC could not be quenched by trypan blue, indicating that there is hardly any binding to the cell surface. FACs profiles of cells incubated for 30 min with OVA-FITC-ICs, OVA-FITC with control rabbit IgG, or a high concentration of soluble OVA-FITC are shown in Fig. 3C. Confocal microscopy analysis of D1 cells incubated with 1 μg/ml OVA-FITC in ICs or 1 μg/ml soluble OVA-FITC showed strongly fluorescent cells for ICs (Fig. 4A), but not for soluble OVA (Fig. 4B). Strongly fluorescent D1 cells were observed, however, when 1 mg/ml soluble OVA was used (Fig. 4C). Z-scan analysis of optical slices from the top to the bottom of the cells indicated that the OVA-FITC in ICs was indeed largely internalized (data not shown). Thus, the enhanced Ag presentation observed after incubation of DCs with ICs compared with soluble Ag is at least partially due to increased uptake of Ag.

IC-mediated activation enables DCs to efficiently prime peptide-specific CD8+ CTLs in vivo

We have recently shown that activation of D1 cells by LPS treatment or CD40 triggering induces efficient CD8+ CTL-priming capacity in vivo (4). To investigate whether IC-mediated activation of D1 cells reflects functional activation and leads to efficient CTL priming in vivo, D1 cells were treated with LPS, soluble OVA, OVA with control rabbit IgG, or OVA-ICs. Cells were washed, exogenously loaded with an Ad5 CTL epitope, and injected i.v. into mice depleted for CD4+ cells. Ten days after immunization, spleens were harvested and splenocytes were analyzed directly ex vivo for the presence of Ad5-specific CD8+ cells by staining with PE-conjugated H-2Db-E1A234-243 tetrameric complexes. Both LPS- and OVA-IC-activated D1 cells induced considerable
amounts of CD8\(^+\) cells staining with tetrameric complexes in 6 of 10 and 13 of 16 mice, respectively. In contrast, none of the mice injected with peptide-loaded D1 cells that were preincubated with soluble OVA or OVA with control rabbit IgG had significant numbers of CD8\(^+\) cells staining with tetrameric complexes (Fig. 5). Staining of splenocytes with tetrameric complexes 7 days after in vitro restimulation with Ad5-transformed stimulator cells showed similar results, although the percentages of tetramer-positive cells were higher due to the restimulation (data not shown). Measurement of Ad5-specific cytotoxic activities of these bulk cultures paralleled the results obtained by staining with tetrameric complexes (data not shown). Thus, IC-induced activation of D1 cells enables these cells to prime efficient CTL responses in vivo in mice depleted for CD4\(^+\) cells, and therefore bypasses CD4\(^+\) T cell help.

**DCs incubated with OVA-ICs acquire the capacity to induce OVA-specific CTLs in vivo**

Incubation of D1 cells with OVA-ICs results in up-regulation of the expression of costimulatory and MHC molecules, induction of IL-12 production, increased OVA uptake, and efficient MHC class I-restricted presentation of OVA-derived peptides. We investigated whether OVA-IC-activated D1 cells are capable of inducing OVA-specific CTLs in vivo. Splenocytes from the same mice described in the previous paragraph, harvested 10 days after immunization, were analyzed directly ex vivo for the presence of OVA-specific CD8\(^+\) cells by staining with H-2K\(^b\)-SIINFEKL tetrameric complexes. In contrast to the Ad5-specific CTLs, staining of splenocytes directly ex vivo did not show significant staining of CD8\(^+\) cells for tetrameric complexes in any of the mice (data not shown). However, 7 days after in vitro restimulation with K\(^b\)-SIINFEKL-expressing stimulator cells, cultures showed high numbers of CD8\(^+\) cells staining with tetrameric complexes in mice injected with D1 cells incubated with OVA-ICs. No detectable staining of CD8\(^+\) cells for tetrameric complexes was detected in cultures from mice injected with D1 cells incubated with soluble OVA, OVA with control rabbit IgG, or LPS (Fig. 6A). D1 cells incubated with soluble OVA and LPS did not induce CD8\(^+\) cell staining with H-2K\(^b\)-SIINFEKL tetrameric complexes. In contrast, these cells did induce CD8\(^+\) cells specific for the exogenously loaded peptide that stained with H-2D\(^b\)-E1A\(_{234–243}\) tetrameric complexes (data not shown). Furthermore, 16 of 16 mice injected with OVA-IC-treated D1 cells showed strong CTL reactivity against SIINFEKL (Fig. 7A)-loaded, but not against control peptide-loaded target cells (Fig. 7B) after restimulation in vitro. In contrast, mice that had been injected with D1 cells incubated with LPS, soluble OVA, or OVA with control rabbit IgG did not show any specific CTL responses against SIINFEKL-loaded target cells (Fig. 7A).

Next to the D1 cell line, we tested the CTL-priming capacity of other DC sources. OVA-IC-treated B6 BM DCs induced high...
Significance of differences (two-tailed Mann-Whitney test): D1 vs D1/OVA.

Induction of peptide-specific CD8<sup>+</sup> T cells in vivo by OVA-IC-activated D1 cells. D1 cells incubated with soluble OVA, control rabbit IgG with OVA, LPS, or OVA-ICs were exogenously loaded with an Ad5 CTL epitope nonrelated to the IC and injected into B6 mice depleted for CD4<sup>+</sup> cells. After 10 days, spleen cells were analyzed freshly ex vivo for the presence of Ad5-specific CD8<sup>+</sup> cells capable of interacting with H-2D<sup>d</sup>-E1A<sub>234-243</sub> tetrameric complexes. Indicated are percentages of the CD8<sup>+</sup> cells staining with tetrameric complexes. Each circle represents one mouse. The mean percentage of CD8<sup>+</sup> cells staining for tetrameric complexes per experimental group is indicated. Results shown are the combined results of three experiments performed (n = 5), D1 + OVA (n = 11), D1 + OVA + ctrl rIgG (n = 5), D1 + LPS (n = 10), D1 + OVA-IC (n = 16). Significance of differences (two-tailed Mann-Whitney test): D1 + OVA vs D1 + LPS, p = 0.0011; and D1 + OVA vs D1 + OVA-IC, p < 0.0001.

Discussion
This study shows that administration of Ag to DCs in the form of ICs endows these cells with the capacity to prime efficient CD8<sup>+</sup> CTL responses in vivo. These DCs can prime CTLs against peptides derived from the Ag present in the ICs, in a CD4<sup>+</sup> Th-independent way. At least two mechanisms contribute to this phenomenon. First, uptake of Ag in ICs is improved compared with uptake of soluble Ag, leading to enhanced presentation of Ag, i.e., peptides derived from the ICs. Second, ICs directly activate the DCs, thereby bypassing the need for CD4<sup>+</sup> T cell help to prime CTL responses in vivo.

In the “license to kill” model (26, 27), CD4<sup>+</sup> Th cells activate APCs, thereby licensing them to directly activate CTLs. Previously, we reported that LPS- and CD40 triggering-induced maturation of DCs licenses these cells to induce CD8<sup>+</sup> CTL responses in vivo, in the absence of CD4<sup>+</sup> Th cells (4). In this study, we show that IC-induced DC maturation enables these cells to prime efficient peptide-specific CD8<sup>+</sup> CTLs in vivo in the absence of CD4<sup>+</sup> Th cells. Furthermore, it has been reported recently that ICs augment Ag presentation for in vivo CD4<sup>+</sup> Th cell responses (16). These studies implicate that DCs play a central role in the cross talk between humoral and cellular immune responses. DCs present MHC class I- and II-binding peptides more efficiently when Ag is provided in the form of ICs compared with soluble Ag (Fig. 1) (14). In addition, IC-treated DCs are activated, which enables them to prime T cell responses in vivo. We suggest the following model for IC-mediated enhancement of immune responses. During an ongoing immune response, IgG Abs may initially enhance CD4<sup>+</sup> Th cell responses. CD4<sup>+</sup> Th cells are activated and improve B cell activation, Ab production, and class switch, leading to formation of more ICs. CD8<sup>+</sup> CTLs may be primed more efficiently, especially during a secondary response, when Abs are produced quickly, leading to quicker clearance of the pathogen. In the lymphocytic choriomeningitis virus model for example, interaction between the cellular and humoral immune response is required for efficient virus control (28–30). In this system, a strong specific CD8<sup>+</sup> CTL response initially controls the infection. Neutralizing Abs play an important role in the long-term control of the virus. Ab-mediated viral control is observed in CTL-deficient mice, but virus variants emerge that escape the Ab response. The presence of Abs may, by formation of ICs, lead to improved CTL priming and,

FIGURE 5. Induction of peptide-specific CD8<sup>+</sup> T cells in vivo by OVA-IC-activated D1 cells. D1 cells incubated with soluble OVA, control rabbit IgG with OVA, LPS, or OVA-ICs were exogenously loaded with an Ad5 CTL epitope nonrelated to the IC and injected into B6 mice depleted for CD4<sup>+</sup> cells. After 10 days, spleen cells were analyzed freshly ex vivo for the presence of Ad5-specific CD8<sup>+</sup> cells capable of interacting with H-2D<sup>d</sup>-E1A<sub>234-243</sub> tetrameric complexes. Indicated are percentages of the CD8<sup>+</sup> cells staining with tetrameric complexes. Each circle represents one mouse. The mean percentage of CD8<sup>+</sup> cells staining for tetrameric complexes per experimental group is indicated. Results shown are the combined results of three experiments performed (n = 5), D1 + OVA (n = 11), D1 + OVA + ctrl rIgG (n = 5), D1 + LPS (n = 10), D1 + OVA-IC (n = 16). Significance of differences (two-tailed Mann-Whitney test): D1 + OVA vs D1 + LPS, p = 0.0011; and D1 + OVA vs D1 + OVA-IC, p < 0.0001.

FIGURE 6. DCs incubated with OVA-ICs induce CD8<sup>+</sup> T cells in vivo, specific for the OVA-Ag present in the ICs. Spleen cells from mice described in Fig. 4 (A), or from mice injected with OVA-IC- or OVA with control rabbit IgG-treated BM DCs (B) were restimulated in vitro using H-2K<sup>b</sup>-SIINFEKL-expressing cells. After 7 days, cells were analyzed for the presence of CD8<sup>+</sup> cells capable of interacting with H-2K<sup>b</sup>-SIINFEKL tetrameric complexes. Indicated are percentages of the CD8<sup>+</sup> cells staining with tetrameric complexes. Each circle represents one mouse. The mean percentage of CD8<sup>+</sup> cells staining for tetrameric complexes per experimental group is indicated. Results shown are the combined results of three experiments performed (A) (see Fig. 4). No staining was observed with control peptide-loaded H-2K<sup>b</sup> tetrameric complexes in any of the cultures (data not shown).
FIGURE 7. OVA-IC-treated D1 cells induce strong CTL responses in vivo, specific for the OVA-Ag present in the ICs. In addition to staining with tetrameric complexes, cells restimulated for 7 days in vitro, as described in Fig. 5, were tested in a cytotoxicity assay using target cells loaded with SIINFEKL or control peptide (VSV NP52–65). For each mouse, the percentage of specific lysis was calculated as the mean of triplicate wells. Data shown are mean percentage of specific lysis ± SD, averaged from mice injected with D1 cells incubated with soluble OVA (n = 11), control rabbit IgG with OVA (n = 5), LPS (n = 10), and OVA-ICs (n = 16). Results shown are the combined results of three experiments performed.

therefore, improved virus control, even if the Abs have no virus-neutralizing capacity.

In this study, we show that IC-activated DCs can efficiently prime peptide-specific CD8+ CTLs in vivo, and do so at least as efficiently as LPS-activated DCs (Fig. 4). This might be related to triggering of different signaling pathways by LPS and ICs. LPS binds to and signals via Toll-like receptor 4, ultimately resulting in activation of NF-κB (31, 32). The IgG-ICs used in this study can bind to receptors for the Fc region of IgG Abs (FcγRs). D1 cells and fresh DCs cultured from murine bone marrow precursors have been shown to express all three FcγRs: FcγRI, FcγRII, and FcγRIII (15). In addition to these data, we measured efficient binding of monomeric IgG2a to D1 cells (data not shown). Monomeric IgG2a binds exclusively to FcγRI. Furthermore, we showed that the 2.4G2 Ab, recognizing FcγRII/III, stains D1 cells, indicating the expression on these cells of FcγRII/III (data not shown). FcγRI and III are composed of a ligand-binding module and a transducing module (the γ-chain), which contains immunoreceptor tyrosine-based activation motifs (33, 34). Signal transduction via FcγRs might activate transcription factors that are not activated by Toll-like receptor 4-mediated signal transduction, thereby inducing additional factors playing an important role in CTL priming in vivo.

FcγRs mediate the enhanced internalization and MHC class I-restricted presentation of peptides derived from ICs. In vitro data support that the enhancement of MHC class I-restricted Ag presentation is FcγR mediated, as γ-chain−/− DCs, which lack surface expression of FcγRI, FcγRII, and FcεRI, are not activated by ICs, and do not present IC-derived MHC class I-binding peptides more efficiently than peptides derived from soluble Ags (Ref. 15 and our unpublished data).

Different IgG subclasses have different affinities for the three FcγRs. In our studies, we have used purified OVA-specific polyclonal rabbit IgG, which binds to all three murine FcγRs (35–37). In vivo, depending on the type of pathogen and the local cytokine environment, different subclasses of IgG Abs are produced. Because both activating and inhibitory FcγRs exist, the final outcome of FcγR cross-linking is determined by the balance between positive and negative signals, which depends on the IgG subclasses locally present. We are currently investigating the relative role of the different Ab subclasses and Fcγ types on the presentation of peptides derived from ICs and the cross-priming of CTLs by DCs. Furthermore, the affinity of the Ab and the relative location of B and T cell epitope in the Ag have been shown to influence the efficiency of presentation to CD4+ T cells (38, 39).

Targeting Ags to specific FcγRs on APCs can markedly reduce the concentration of Ags required for a significant immunological response. Therefore, FcγR targeting may be particularly useful for immunization against low dose Ag. Pretreatment of DCs with ICs in vitro, injecting preformed ICs in vivo, or aiming to vaccinate for both Ab and CTL responses are rational possibilities to design successful novel strategies for immunotherapy.

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