Antigen-Antibody Immune Complexes Empower Dendritic Cells to Efficiently Prime Specific CD8⁺ CTL Responses In Vivo

Danita H. Schuurhuis, Andreea Ioan-Facsinay, Bas Nagelkerken, Jolien J. van Schip, Christine Sedlik, Cornelis J. M. Melief, J. Sjef Verbeek and Ferry Ossendorp

*J Immunol* 2002; 168:2240-2246; doi: 10.4049/jimmunol.168.5.2240
http://www.jimmunol.org/content/168/5/2240

References

This article cites 39 articles, 21 of which you can access for free at:
http://www.jimmunol.org/content/168/5/2240.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Antigen-Antibody Immune Complexes Empower Dendritic Cells to Efficiently Prime Specific CD8+ CTL Responses In Vivo

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).

Class I-restricted presentation of Ags internalized by fluid-phase pinocytosis is very inefficient and requires high Ag concentrations. Presentation of exogenous Ags in MHC class I can be improved by complexing Ags to beads (8), by providing Ags in bacteria (9), or by administering Ags in apoptotic cells (10). Furthermore, DCs express several receptors that facilitate the internalization and presentation of Ags, including C-type lectin receptors such as the mannose receptor (5) and DEC205 (11), as well as receptors for the Fc domain of IgGs (FcγR and FcεR, which bind the Fc domain of IgG and IgE, respectively (12)). FcγRs are expressed on most cells of the hematopoietic lineages, including DCs, and play a pivotal role in linking the humoral and cellular arms of the immune response. Ag-IgG complexes (immune complexes (ICs)), which bind to and cross-link FcγRs, mediate a variety of responses in vitro ranging from phagocytosis to Ab-mediated cellular cytotoxicity. FcγR-mediated internalization of ICs by DCs is associated with enhanced presentation of both MHC class I- and MHC class II-binding peptides derived from the Ag present in the ICs and leads to activation of the DCs (13–15). The in vivo contribution of FcγRs and ICs to CD4\(^+\) Th cell function has been studied recently (16). In this study, mice lacking the FcγRI-, FcγRII-, and FcεRI-associated γ-chain, which is required for signaling and the cell surface expression of these receptors, showed decreased delayed-type hypersensitivity responsiveness and defective Th cell responses to a model Ag.

In this study, we have shown the in vivo CTL-priming capacity of IC-treated DCs. IC-mediated activation of DCs enables these cells to efficiently prime CD8\(^+\) CTL responses both against an exogenously loaded peptide and against endogenously processed peptide from the Ag present in the ICs. These findings underscore the relevance of cross talk through DCs between the B and T cell compartments of the immune system.

Materials and Methods

Mice

Female C57BL/6 (B6; H-2\(^d\)) 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 adenovirus type 5 (Ad5) E1-transformed B6 mouse embryo cells (MECs) and B6 MECs transected with murine CD80 (B7.1), H-2K b, and a construct expressing an endoplasmic reticulum targeting signal sequence, followed by the OVA257–264 CTL epitope SIINFEKL (SigSIINFEKL), were generated as described (17, 18). B3Z is a T cell hybridoma specific for H-2K b-restricted OVA257–264 peptide, which carries a histidine codon at the N-terminus, as provided 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 μM 2-ME. D1 cell line, a long-term growth factor-dependent immature splenic DC line derived from B6 mice, was kindly provided by P. Riccardi-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 (detected using 2 m EDTA) were collected and used. LPS of Escherichia coli (serotype 026:B6) was purchased from Sigma-Aldrich (St. Louis, MO). Synthetic peptides used were: OVA257–264, SIINFEKL; vesicular stomatitis virus nucleoprotein (VSV NP32–53), RGGYVQGL; Ad5 E1A234–243, SGPSNTTPEEI; and human papillomavirus type 16 E749–57, RARYNIVTF.

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-CD8β Ab (Ly-3.2), allophycocyanin-coupled anti-CD8a Ab (Ly-2). PE-conjugated Ad5 E1A234–243-loaded H-2D b and PE-conjugated SIINFEKL-loaded H-2K b tetramers were prepared as described (22–24). Ad5 E1A234–243- and H-2D b tetramers were kindly provided by T. Schumacher (NIK, Amsterdam, The Netherlands). Staining for H-2D b/E1A234–243 tetrameric complexes and anti-CD8α/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-CD8α 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 (rlgGro; 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 5 × 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-D-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 C15.6; 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 prepared as described in the previous paragraph, using FITC-labeled OVA (Molecular Probes, Leiden, The Netherlands), in 96-well plates. For flow cytometry, 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% (w/v) trypsin blue (Sigma-Aldrich), which quenches extracellular, but not intracellular, fluorescence (25). Flow cytometry was performed with FACSscan. 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). 

Induction of CTL responses in vivo

To induce CTL responses in vivo against exogenously loaded Ad5 E1A234–243 (SGPSNTTPEEI) or against the endogenously processed MHC class I-binding peptide OVA257–264 (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 E1A234–243 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 + Th 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 rads) B7 +, 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 51 -labeled target cells loaded with Ad5 E1A234–243 or control H-2D b-binding peptide (human papillomavirus type 16 E749–57) or with SIINFEKL or control H-2K b-binding peptide (VSV NP32–53).

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 (rlgGroOVA). After 24 h, presentation of H-2K b-restricted OVA257–264 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 rlgGroOVA 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,
IL-12 levels were independent of the concentration of OVA used, but IL-12 levels reached an optimum at about 1 μg/ml OVA for OVA-ICs (Fig. 1B and data not shown). Thus, as both LPS and ICs induce DC activation, but only ICs enhance Ag presentation, activation of the DCs by itself is not sufficient to enhance MHC class I-restricted presentation of peptides derived from exogenous Ags.

Enhanced Ag binding and uptake of OVA-ICs compared with soluble OVA

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. This indicates that uptake of OVA complexed to Abs is very efficient. Soluble OVA was taken up as well, but 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...
FIGURE 3. Increased binding and uptake of Ag provided in ICs. D1 cells were incubated with 1 μg/ml OVA-FITC in ICs (A, squares, OVA1-IC), or 1 μg/ml OVA-FITC with control rabbit IgG (B, circles, OVA1 + ctrl rIgG), or a high concentration (100 μg/ml) of soluble OVA-FITC alone (B, triangles, OVA100), at 37°C. After the indicated incubation time, cells were harvested, resuspended in the absence (closed symbols) or presence (open symbols) of trypan blue (TB), and analyzed by FACS analysis for FITC fluorescence. Trypan blue quenches extracellular FITC fluorescence. Indicated are mean fluorescence intensities (MFI) of the cell populations of six wells ± SD. Data shown are from one representative experiment of three performed. C. Representative FACS profile of D1 cells incubated for 30 min with 1 μg/ml OVA-FITC-ICs (OVA1-IC), 1 μg/ml OVA-FITC with control rabbit IgG (OVA1 + ctrl rIgG), or 100 μg/ml OVA-FITC (OVA100).

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 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.

Next to the D1 cell line, we tested the CTL-priming capacity of other DC sources. OVA-IC-treated B6 BM DCs induced high amounts of CD8+ cell 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 staining with H-2Kb-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 Kb-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-2Kb-SIINFEKL tetrameric complexes. In contrast, these cells did induce CD8+ cells specific for the exogenously loaded peptide that stained with H-2Db-E1A234-243 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

FIGURE 4. Efficient uptake of Ag provided in ICs. D1 cells grown on 3-cm petri dishes for 2 days were incubated with OVA-FITC-ICs containing 1 μg/ml OVA-FITC (A), 1 μg/ml soluble OVA-FITC (B), or 1 mg/ml soluble OVA-FITC (C). After 1 h at 37°C, cells were fixed and analyzed by confocal microscopy. Pictures showing cells representative for each condition are shown. Bar = 10 μm.
null
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 FcγRII/III, 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 FcR 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 Abs to specific FcRs on APCs can markedly reduce the concentration of Ags required for a significant immunological response. Therefore, FcR 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.

Acknowledgments

We thank P. Ricciardi-Castagnoli for the kind gift of the D1 cell line, J. Nouta for preparation of the SIINFEKL-loaded H-2Kb tetrameric complexes, P. de Lange for statistical analysis, K. Franken for providing us with the control rabbit IgG, H. Vile for purification of the rabbit IgG anti-OVA, M. Mulders and H. Krohn for animal care, and Ds. D. Roelen, R. Toes, R. Offringa, F. Claas, and S. Amigorena for critical reading of this manuscript.

References


