Inducing Tumor Immunity through the Selective Engagement of Activating Fcγ Receptors on Dendritic Cells

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Abstract

Induction of tumor-specific immunity requires that dendritic cells (DCs) efficiently capture and present tumor antigens to result in the expansion and activation of tumor-specific cytotoxic T cells. The transition from antigen capture to T cell stimulation requires a maturation signal; in its absence tolerance, rather than immunity may develop. While immune complexes (ICs) are able to enhance antigen capture, they can be poor at inducing DC maturation, naive T cell activation and protective immunity. We now demonstrate that interfering with the inhibitory signal delivered by FcγRIIB on DCs converts ICs to potent maturation agents and results in T cell activation. Applying this approach to immunization with DCs pulsed ex-vivo with ICs, we have generated antigen-specific CD8+ T cells in vivo and achieved efficient protective immunity in a murine melanoma model. These data imply that ICs may normally function to maintain tolerance through the binding to inhibitory FcγRs on DCs, but they can be converted to potent immunogenic stimuli by selective engagement of activating FcγRs. This mechanism suggests a novel approach to the development of tumor vaccines.

Key words: immune complexes • Fcγ receptors • DC maturation • inhibitory/activating receptor pairs • T cell immunity

Introduction

Elucidation of the mechanisms responsible for protective immunity against tumors is a prerequisite to the development of tumor-specific vaccines and effective immunotherapy for cancer. Because of their ability to be specifically captured by dendritic cells (DCs), enhance antigen presentation, and elicit tumor-specific killer T cells, immune complexes (ICs) are emerging as an attractive means of delivering tumor-specific antigens to stimulate protective immunity (1, 2). DCs have been shown to be a key cell in the pathway of antigen capture and presentation to T cells, having the unique ability to directly prime naive CD4+ and CD8+ T cells (3–5), through their ability to efficiently uptake, process, and present antigen on MHC class I and II molecules, together with costimulatory molecules such as B7 and CD40 (5, 7, 14). Furthermore, DCs have been shown to efficiently present exogenously derived antigens (e.g., ICs) on MHC class I molecules to naive CD8+ T cells (9–12), accounting for the phenomenon of cross-priming (13). However, for DCs to efficiently stimulate T cells, a process known as maturation is required. Immature DCs, specialized for antigen capture, undergo maturation in response to inflammatory cytokines and bacterial products, leading to the upregulation of costimulatory molecules such as B7 and CD40 (3, 5, 7, 14). This requirement for maturation insures that activation of the immune response be coupled to a mechanism capable of discriminating between endogenous antigens and those derived from exogenous sources, typically microbial pathogens. Thus, in the absence of receiving a maturation signal, the interaction of antigen–presenting immature DCs with T cells can result in peripheral tolerance (14, 15). The challenge of tumor vaccination is to find means of overcoming this protective mechanism and eliciting effective T cell stimulation to endogenous antigens expressed by tumor cells (16–19).

ICs are capable of either enhancing or suppressing the immune response, resulting from their ability to interact with Fcγ receptors on B cells, macrophages, or DCs, thus triggering different cellular responses (20, 21). Engagement of Fcγ receptors by ICs can lead to either activation or inhibitory signaling depending on the specific FcγR being engaged. Activating Fcγ receptors I and III associate with the immunoreceptor tyrosine–based activation motif (ITAM)–containing ɣ-chain and their engagement by ICs results in src and syk kinase–mediated activation responses. In contrast, the inhibitory Fcγ receptor IIB is a single subunit receptor containing a cytoplasmic immunoreceptor tyrosine–based inhibition motif (ITIM) domain that inhibits
Materials and Methods

Analysis of FcγR Expression on DCs and T Cell Priming Assays. Bone marrow–derived DCs were prepared as described previously (24). DCs were grown from bone marrow progenitors in RPMI 1640 containing 5% FCS supplemented at 3% vol/vol with supernatant from J558L cells transduced with murine (n) GM-CSF. Day 6 DC cultures derived from bone marrows of wild-type (WT) mice (all in the C57BL/6 background) were double-stained with anti–CD11c-PE (HL3; BD PharmMingen) and tetramers. Tetramer staining was done at 4°C, for 1 h with 1 μg of anti–CD8α and tetramers per 10^6 cells. H-2K^b/ova tetrarmers carried the immunodominant OVA peptide SIINFEKL and were designed as described previously (31, 32).

Results and Discussion

To evaluate whether the balance between activation and inhibitory FcγRs on DCs is critical to induce maturation and effective tumor immunity in vivo in response to ICs, DCs were generated from bone marrow–derived from C57BL/6 WT and FcγR-deficient mice and characterized for FcγR surface expression. The relative contributions of the low-affinity activating (FcγRII) and inhibitory (FcγRIIB) receptors on the surface of immature DCs was determined by FACS® analysis of DC isolated from WT and FcγR-deficient animals. FcγRIIB plus RIIB expression was determined by 2.4G2 staining, a mAb that binds equivalently to both receptors (33, 34). The FcγRIIB component was determined by 2.4G2 staining on DCs obtained from mice deficient in the common γ chain (R1/RIII^−/−; reference 35) which only express FcγRIIB. The FcγRIII component was determined by staining DCs obtained from FcγRIIB-deficient mice with 2.4G2, which on these cells would only bind to FcγRIII. As seen in Fig. 1A, DCs pre-
CD11c

One representative histogram for 2.4G2 staining is shown, gating on the WT, cells and is not required for IC-mediated enhancement of antigen presentation. As seen from the FACS® analysis, this subset of DCs dominantly express inhibitory FcγRIIB, which accounts for \(\sim 75\%\) of total FcγR expression. Consistently, the mean fluorescence intensity on WT DCs observed with the RIIB-specific Ly17.2 mAb (27) also accounted for \(\sim 75\%\) of 2.4G2 staining (data not shown). A similar pattern of FcγR expression was observed in DCs purified from spleen (data not shown). These results indicate that resting DCs express mainly inhibitory FcγRIIB on their surface.

Both WT and FcγRIIB\(^{-/-}\) immature DCs are equally able to mediate IC presentation to T cells, as shown in Fig. 1 B. DCs isolated from either WT or FcγRIIB-deficient mice were incubated with ICs composed of rabbit IgG anti-OVA and OVA, and then tested for their ability to mediate antigen presentation to MHC class I- and class II-restricted OVA-specific T cells (OT-I and OT-II, respectively) (25, 26). IC-mediated enhancement of MHC class I and II presentation was equally efficient for both WT and FcγRIIB\(^{-/-}\) DCs, indicating that there are no differences between these cells in their ability to capture and present IC-derived antigens to class I- or class II-restricted T cells (Fig. 1 B).

In contrast, DC maturation by ICs was enhanced when activating FcγRs were selectively ligated through the use of FcγRIIB-deficient mice (Fig. 2). In comparison to LPS-induced maturation, WT DCs are inefficiently induced to mature in response to ICs, consistent with previous reports (2, 10, 12). However, a subset of DCs derived from FcγRIIB-deficient mice display a maturation phenotype comparable to that seen for LPS, with upregulation of MHC class II (Fig. 2 A) and B7.2 (Fig. 2 B) surface expression. As seen from the FACS® analysis, this subset of DCs incubated with ICs show a marked increase in MHC class II and B7.2 expression, indicative of maturation of these cells. This effect was not the result of perturbation of DC development by FcγRIIB deletion, as demonstrated by the ability of WT DCs to enhance their maturation in response to ICs when FcγRIIB is blocked by the FcγRIIB-specific mAb Ly17.2 (Fig. 2 A and B). Furthermore, FcγRIIB-deficient DCs show equivalent maturation in response to LPS as compared with WT DCs (Fig. 2 A and B). Conversely, and consistent with previous reports (10), IC engagement of DCs derived from \(\gamma\) chain–deficient mice lacking activation FcγRs does not result in DC maturation (data not shown). These results indicate that the ability of ICs to induce DC maturation is determined by the balance of activation and inhibitory FcγRs. Selective engagement of the ITAM-containing FcγRs by ICs in the absence of coligation of ITIM-containing inhibitory FcγR results in enhanced maturation.

The functional consequence of IC-induced maturation of DCs in vitro was tested in vivo in a murine melanoma model to determine if DCs induced to mature by selectively engaging activating FcγRs resulted in enhanced antitumor immunity. FcγRIIB\(^{-/-}\) DCs were pulsed with OVA-IgG ICs as before and then used to immunize C57BL/6 mice. As a control, C57BL/6 mice were immunized with WT DCs treated under the same conditions. 2 wk after a single DC immunization, mice were challenged...
either with an OVA-expressing clone of the melanoma line B16 (28, 30) (Fig. 3 A) or with control B16 cells not expressing OVA. The animals were scored for the kinetics of tumor appearance as well as the absolute size of the tumor mass. As shown in Fig. 3, mice immunized with FcγRIIIB-deficient DCs pulsed with OVA–IC developed protective immunity to the tumor challenge, with no detectable tumor appearance, while animals immunized with WT-DC are statistically different (P < 0.0001), naive and WT-DC are not (P < 0.079).
WT DCs pulsed with OVA-ICs developed tumors (Fig. 3 B and C), although with a significant delay in appearance (Fig. 3 B and C). No tumor protection was observed when the challenging tumor was the parental B16 line (OVA-negative). Similarly, neither WT nor FcγRIIB−/− unpulsed DCs protected against B16 or B16-OVA. These results indicate that the antitumor response elicited by immunization with OVA–IC-pulsed DCs was antigen (OVA)-specific (Table I).

To determine the mechanism of this protection by DC immunization, we tested peripheral blood of mice immunized with FcγRIIB−/− unpulsed DCs or WT DCs for the presence of OVA-specific CD8+ T cells. Peripheral blood CD8+ T cells from DC-immunized and tumor-challenged mice were stained for H-2Kb/OVA tetramers (H-2Kb/SIINFEKL) (31, 32). OVA-specific CD8+ T cells were only expanded in peripheral blood of mice immunized with FcγRIIB−/− DCs pulsed with OVA-IgG ICs (Fig. 4). These results indicate that FcγRIIB−/− DCs have an enhanced ability to prime antigen-specific CD8+ T lymphocytes in vivo, thus contributing to an effective antigen-specific antitumor immune response.

We conclude by suggesting that effective antitumor immunization by ICs requires that selective Fc receptor engagement be achieved to induce DC maturation and thus efficient CD8+ T cell priming. This selectivity may be accomplished by blocking the inhibitory Fc receptor or by engineering the Fc region of the IgG molecule of the IC to preferentially engage activation and not inhibitory FcγR (36). Conversely, the preferential expression of the

Table I.  Tumor Appearance at 3 wk

| Treatment | Challenging tumor | Tumor+ mice/ | challenged mice |
|-----------|-------------------|--------------|----------------|
| Naive     | B16-OVA           | 8/8          |                |
| WT unpulsed | B16-OVA           | 4/4          |                |
| FcγRIIB−/− unpulsed | B16-OVA           | 4/4          |                |
| WT plus OVA-IC | B16-OVA           | 6/8          |                |
| FcγRIIB−/− plus OVA-IC | B16-OVA           | 1/8          |                |
| Naive     | B16               | 4/4          |                |
| WT unpulsed | B16               | 4/4          |                |
| FcγRIIB−/− unpulsed | B16               | 4/4          |                |
| WT plus OVA-IC | B16               | 4/4          |                |
| FcγRIIB−/− plus OVA-IC | B16               | 4/4          |                |

DC cultures derived from bone marrows of WT or FcγRIIB−/− mice (C57BL/6 background) were pulsed with OVA-IgG ICs and used to immunize naive syngeneic C57BL/6 mice. 2 wk after this single immunization, mice were challenged subcutaneously with a variant of the melanoma B16 tumor line that expresses OVA as a neo-antigen (MO4). Data shown are fraction of mice with palpable tumors at 3 wk after tumor challenge. As antigen specificity controls, some mice were injected with untreated DCs and others were challenged with the parental (OVA negative) B16 tumor line.

Figure 4. FcγRIIB−/− DCs efficiently induce expansion of antigen-specific CD8+ T cells. Peripheral blood cells obtained 2 wk after challenging mice with B16-OVA tumor, were double stained with anti–CD8α–FITC (53–6.7; BD PharMingen) and H-2Kb/OVA-PE tetramers. Tetramer staining was done at 4°C, for 1 h with 1 μg of anti-CD8α and tetramers per 10^6 cells. H-2Kb/OVA tetramers carried the immunodominant OVA peptide SIINFEKL and were designed as described previously (references 31 and 32). (Left) Naive C57BL/6 mice; (middle) C57BL/6 mice immunized with WT DCs pulsed with OVA-IgG ICs; (right) C57BL/6 mice immunized with FcγRIIB−/− DC pulsed with OVA-IgG ICs. *P < 0.02.
inhibitory FcγR on immature DCs suggests that maturation and activation of immune responses may not normally occur in response to IC cross-linking of immature DCs, and may even provide a mechanism to maintain peripheral tolerance to self-antigens. Chronic inflammatory states or reduced inhibitory receptor expression, as has been documented in autoimmune susceptible mouse strains (37, 38), might contribute to the loss of tolerance and expansion of autoreactive lymphocytes by inappropriate activation of DC maturation and subsequent T cell stimulation. Restoring FcγRIIB expression may thus offer an approach to reestablishing tolerance.

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