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Invariant NKT Cells Promote CD8$^+$ Cytotoxic T Cell Responses by Inducing CD70 Expression on Dendritic Cells[1]

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Activation of invariant NK T (iNKT) cells with the glycolipid α-galactosylceramide promotes CD8$^+$ cytotoxic T cell responses, a property that has been used to enhance the efficacy of antitumor vaccines. Using chimeric mice, we now show that the adjuvant properties of iNKT cells require that CD40 triggering and Ag presentation to CD8$^+$ T cells occur on the same APCs. We demonstrate that injection of α-galactosylceramide triggers CD70 expression on splenic T cell zone dendritic cells and that this is dependent on CD40 signaling. Importantly, we show that blocking the interaction between CD70 and CD27, its costimulatory receptor on T cells, abrogates the ability of iNKT cells to promote a CD8$^+$ T cell response and abolishes the efficacy of α-GalCer as an adjuvant for antitumor vaccines. These results define a key role for CD70 in linking the innate response of iNKT cells to the activation of CD8$^+$ T cells. *The Journal of Immunology,* **2008, 180: 4615–4620.**

Invariant NK T (iNKT)[3] cells represent a unique T lymphocyte sublineage (1). They express both $\alpha\beta$ TCR and NK cell receptors and recognize glycolipids presented by CD1d molecules. Most mouse iNKT cells express the Vα4-Ja18 TCR rearrangement, whereas the equivalent population in humans express the Vα24-Jα18 TCR rearrangement (1). Activation of iNKT cells can lead to beneficial immune responses, including antitumor responses, protection from a variety of infectious agents, prevention of autoimmunity, and maintenance of self-tolerance (1).

Stimulation of iNKT cells has been shown to have profound effects on the priming of conventional T cells. Thus, injection of α-galactosylceramide (αGalCer), a strong agonist for iNKT cells, triggers a powerful CD8$^+$ cytotoxic T cell response to a coadministered protein Ag (2–4). Consequently, iNKT cell agonists have been shown to act as adjuvants for antitumor T cell vaccines (5, 6). The precise mechanism responsible for the adjuvant effects of iNKT cell agonists is not fully understood. Injection of αGalCer leads to dendritic cell (DC) maturation as evident by increased expression of the costimulatory proteins CD80 and CD86 as well as enhanced production of TNF-α, IFN-γ, and IL-12 (2–4). Analysis of mice that are made deficient in the production of these cytokines or are unable to respond to IFN-γ, along with studies involving in vivo administration of recombinant TNF-α and IFN-γ, demonstrate that these cytokines do not mediate the adjuvant effects of αGalCer on conventional CD8$^+$ T cells (2, 4). In contrast, T cell immunity following administration of αGalCer was abrogated in CD40 ligand (CD40L) or CD40-deficient mice (2, 4). Two hours following activation, iNKT cells up-regulate CD40L and thus acquire the capability to activate DCs via CD40 (4). DCs obtained from αGalCer-injected normal, but not CD40-deficient, mice were able to initiate T cell immunity after transfer into naive mice (4). Thus, taken together the available evidence indicates that maturation of DCs by iNKT cells via the CD40L-CD40 interaction is critical for promoting priming of conventional T cells. The maturation signals triggered by ligation of CD40 on DCs in the context of iNKT cell-mediated induction of adaptive immunity are unknown. Previous work has shown that presentation of peptide-MHC I complexes by DCs to CD8$^+$ T cells was unaffected by the absence of CD40 (4). In addition, CD40 signaling was dispensable for the αGalCer-mediated increase in CD80 and CD86 on DCs (4). Up-regulation of CD80 and CD86 on DCs after injection of αGalCer was shown to be dependent on TNF-α and IFN-γ. However, administration of TNF-α and IFN-γ together with a protein Ag did not lead to T cell priming, despite the ability of these cytokines to increase CD80 and CD86 expression on DCs (4). Together these data indicate that increased expression of CD80 and CD86 per se is not responsible for the adjuvant properties of αGalCer on conventional T cells. In this study, we address the mechanism responsible for the enhanced CD8$^+$ T response following activation of iNKT cells in vivo.

CD70 is a member of the TNF family and the ligand for the T cell costimulatory receptor CD27 (7–9). In previous studies, we have shown that the CD70-CD27 interaction is required for CD8$^+$ T cell priming when agonistic CD40 mAb or the TLR3 ligand poly(I:C) are used as adjuvants and also during CD4$^+$ Th cell-dependent CD8$^+$ T cell responses (10–12). In this study, we demonstrate that expression of CD70 is induced on DCs within the T cell areas of the spleen following activation of iNKT cells in vivo. We provide evidence that CD70 expression is essential for mediating the adjuvant effects of αGalCer on conventional CD8$^+$ T cells.

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[3] Abbreviations used in this paper: iNKT, invariant NKT cell; DC, dendritic cell; αGalCer, α-galactosylceramide; CD40L, CD40 ligand; OVAp, OVA peptide; WT, wild type; MR, mannose receptor.

Materials and Methods

Abs and reagents

Anti-CD70 (TAN1.6), a nondepleting mAb that blocks the CD70-CD27 interaction, anti-A31 lymphoma Id control mAb (Mc39-16, rat IgG2a), anti-CD40L mAb (MR1, hamster IgG), and anti-BCL1 lymphoma Id control mAb (AT65, hamster IgG) were described previously (10–13). Biotin anti-CD70 (FR70) was purchased from BD Pharmingen. αGalCer was synthesized using published procedures (14–16). OCH, a derivative of αGalCer that has a truncation of two hydrocarbons in the fatty acyl chain and of nine hydrocarbons in the sphingosine chain (17), was a gift from Prof. Stephan Gadola (University of Southampton, Southampton, U.K.). OVA was obtained from Sigma-Aldrich and OVA peptide 257–264 (OVAp) with the sequence SIINFEKL was obtained from Peptide Protein Research. PE H-2K^b/OVA $_{257–264}$ tetramer was purchased from Beckman Coulter. Allophycocyanin-conjugated CD1d tetramer (ProImmune) was loaded with αGalCer according to the manufacturer's protocol. Briefly, 1 mg/ml stock solution of αGalCer in DMSO was diluted 5-fold with PBS/0.5% Tween 20, sonicated for 30 min at room temperature, added to the tetramer at a 12 M excess of the lipid, and incubated at room temperature overnight in the dark.

Mice and in vivo experiments

BALB/c, C57BL/6 (B6), OT-I, bm1, and CD40$^{-/-}$ mice were bred and maintained in a pathogen-free environment and were used at 8–12 wk of age. To generate bone marrow chimeras, 6-wk-old wild-type (WT) B6 recipients were irradiated with 10 Gy from a ^{137}Cs source and reconstituted with 2×10^6 WT (control), bm1, CD40$^{-/-}$, or a 1:1 mixture of bm1/CD40$^{-/-}$ bone marrow cells. Chimeric mice were maintained on neomycin-containing water for 3 days before and 2 wk after irradiation. Six to 8 wk after injection of bone marrow cells, the efficiency of reconstitution was confirmed by flow cytometry analysis of PBL labeled with combinations of PE anti-CD4/FITC anti-CD8 or PE anti-CD19/FITC anti-CD40 mAbs. OVA-specific T cell responses were primed in WT or chimeric B6 mice by i.v. administration (day 0) of OVA (0.5 mg) in combination with 2 μg of αGalCer or OCH. In some experiments, mice were injected i.v. with OVAp (30 nmol) with or without αGalCer (2 μg). For measurement of OT-I T cell responses, CD8$^+$ OT-I T cells were purified by negative selection and 7×10^5 cells were injected i.v. into chimeric mice. OVA-specific CD8$^+$ T lymphocytes were identified as CD8$^+$H-2K^b/OVA$_{257–264}$ tetramer$^+$ cells. Anti-J558L T cell responses were generated in BALB/c mice by injecting irradiated (75 Gy) J558L cells (2×10^7 cells i.v.) with or without αGalCer. Depletion of CD8$^+$ T cells was achieved by injecting a depleting anti-CD8 mAb (YTS 169.4.2.1; 1 mg) i.p. on days −3, −1, and 0 and was maintained by three additional weekly administrations of 1 mg of the Ab. Abs to CD40L, CD70, or Id (control Ig) were administered i.p. at the dose of 0.5 mg on days 0 and 1. For the tumor protection assays, 2×10^6 live E.G7 or 5×10^6 J558L cells were inoculated s.c. 7 days after priming. Tumor growth was regularly monitored and the experiments were terminated when tumor size exceeded 150 mm^2. Animal experiments were conducted in accordance with the U.K. Home Office guidelines and approved by the University of Southampton Ethical Committee.

Detection of iNKT cells

PBL were incubated with αGalCer-loaded allophycocyanin-conjugated CD1d tetramer, FITC anti-CD3, and PerCP anti-B220 to exclude B220$^+$ B cells capable of nonspecific binding to the CD1d tetramer.

Confocal microscopy

Ten-micrometer frozen sections were fixed in acetone, blocked with 5% normal goat serum, and incubated with rat anti-CD19 (in-house), rat anti-interdigitating DC Ag (MIDC-8; Serotec), followed by Alexa Fluor 488-conjugated goat anti-rat Ab (Molecular Probes). Sections were washed with PBS and incubated with purified rat IgG (in-house, 10 μg/ml, 1 h) before incubating with biotinylated rat anti-CD70 (FR70) overnight at 4°C. Tyramide signal amplification was used to enhance the CD70 staining (TSA kit no.22; Invitrogen Life Technologies) followed by streptavidin-conjugated Alexa Fluor 546 (Molecular Probes). In some experiments, CD70 was detected using the anti-CD70 mAb TAN 1.6 and Alexa Fluor 488-conjugated goat anti-rat polyclonal Ab. This protocol gave identical results to staining with the biotin-labeled anti-CD70 mAb FR70 (data not shown). Sections were counterstained with TOPRO-3 (Molecular Probes) and mounted in Vectashield (Vector Laboratories). Negative controls included omission of primary Abs, replacement of anti-CD70 with an irrelevant rat mAb (anti-Id), and, for colocalization, sections were labeled with MIDC-8 followed by biotinylated rat anti-F4/80 in place of anti-CD70 (data not shown). Images were collected sequentially on a Leica TCS SP2 confocal laser scanning microscope using argon (488 nm), Green helium/neon (543 nm) and RedHeNe (633 nm) lasers and a pinhole equivalent to 1 Airy disc. Image files (TIFF) were transferred to Adobe Photoshop CS2 and contrast stretched to use the whole gray scale.

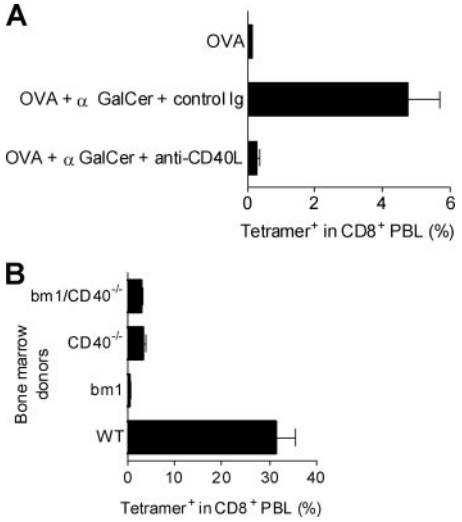

FIGURE 1. iNKT cell-dependent priming of CD8$^+$ T cells requires CD40 signaling and peptide/MHC I presentation by the same APCs. *A*, Mice were injected with OVA or OVA with αGalCer and a control Ig. Another group of mice received OVA and αGalCer along with a blocking anti-CD40L mAb. Seven days later, polyclonal OVA-specific CD8$^+$ T cells were enumerated in peripheral blood by staining with H-2K^b/OVA$_{257–264}$ tetramer. *B*, Four types of bone marrow chimeras were generated. Lethally irradiated WT B6 mice were reconstituted with WT, bm1, CD40$^{-/-}$, or a 1:1 mixture of bm1/CD40$^{-/-}$ bone marrow cells. CD8$^+$ OT-I T cells were then adoptively transferred into each group of chimeric mice. Mice were then challenged i.v. with OVA and αGalCer and 7 days later OVA-specific CD8$^+$ T cells were enumerated in peripheral blood as in *A*. Each bar represents the mean ± SE (n = 3–6 mice/group). The data shown are representative of two independent experiments.

Results

iNKT cell-dependent priming of CD8$^+$ T cells requires CD40 signaling and peptide/MHC I presentation by the same APCs

We initially confirmed that αGalCer-mediated priming of Ag-specific CD8$^+$ T cells was dependent on the CD40L-CD40 interaction (2, 4). Thus, injection of αGalCer along with soluble OVA resulted in the priming of OVA-specific CD8$^+$ T cells, whereas injection of OVA alone failed to prime OVA-specific T cells (Fig. 1*A*). Furthermore, administration of a CD40L mAb (MR-1) that blocks the CD40L-CD40 interaction abolished the T cell response (Fig. 1*A*). To further probe the role of CD40 in iNKT cell-mediated priming of CD8$^+$ T cells, we examined whether CD40 triggering by activated iNKT cells and Ag presentation to CD8$^+$ T cells occur on the same APCs. Groups of lethally irradiated mice were reconstituted with bone marrow from CD40$^{-/-}$, bm1, or WT mice. A separate group of mice was reconstituted with a 1:1 mixture of bone marrow cells from CD40$^{-/-}$ and bm1 mice. APCs from bm1 mice (K^{bm1}), unlike those from WT C57BL/6 mice (K^b), cannot present OVA peptides to CD8$^+$ T cells (18). Thus, in the mixed CD40$^{-/-}$/bm1 chimera there are two types of APCs: ~ 50% of the APCs are capable of being activated via CD40, but these cells lack the ability to present Ag to CD8$^+$ T cells and the remaining APCs are capable of presenting Ag, but cannot be activated via CD40. CD8$^+$ OT-I TCR-transgenic T cells (19) were then adoptively transferred into mice before they were challenged with OVA and

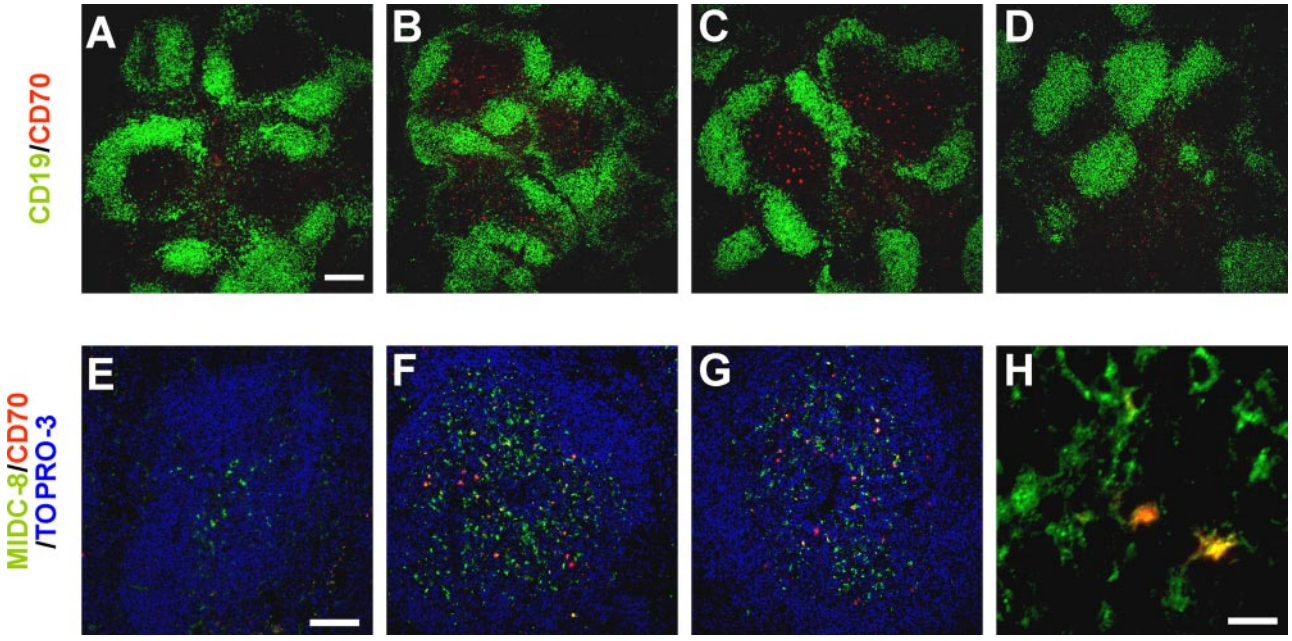

FIGURE 2. Administration of αGalCer triggers CD70 on DCs in the T cell areas of the spleen. Ten-micrometer frozen spleen sections obtained from mice treated with αGalCer (2 μg/animal, given i.v.) for the times indicated and stained with anti-CD70 (red) and CD19 (B cell specific, green; *A–D*) or anti-CD70 (red) and MIDC-8 (T cell zone DC specific, green; *E–H*). CD70 expression, which is undetectable in spleen sections of naive mice (*A*), is evident in the T cell areas 20 h (*B*) and 48 h (*C*) after αGalCer treatment. Coadministration of anti-CD40L mAb blocked αGalCer-induced expression of CD70 (*D*; data from 48-h time point). Expression of CD70 following the administration of αGalCer is confined to DCs in the T cell areas (colocalization signal, yellow) (*E*, naive; *F*, 20 h; *G* and *H*, 48 h). Sections were counterstained with TOPRO-3 (blue; *E–G*). Bars, 200 μm (*A*), 100 μm (*E*), and 10 μm (*H*). The data shown are representative of at least two independent experiments.

αGalCer as an adjuvant and the $CD8^+$/OT-I T cell response was measured on day 7. The ability of αGalCer to promote priming of OVA-specific $CD8^+$ T cells was inhibited substantially in mice reconstituted with the mixture of $CD40^{-/-}$/bm1 bone marrow (Fig. 1*B*). These results demonstrate that the adjuvant effects of αGalCer require that CD40 signaling and Ag presentation to $CD8^+$ T cells occur on the same APCs and are indicative of a role for a CD40-induced membrane protein(s) in promoting $CD8^+$ T cell responses.

αGalCer injection triggers CD70 expression on DCs in the T cell areas of the spleen

To assess the requirement of CD70 in iNKT cell-driven responses of $CD8^+$ T cells, we first examined whether activation of iNKT cells in vivo leads to induction of CD70 expression. Expression of CD70 was absent in the spleens of naive mice. However, 20 and 48 h following i.v. injection of αGalCer, specific staining with anti-CD70 mAb was observed on a subset of cells within the T cell

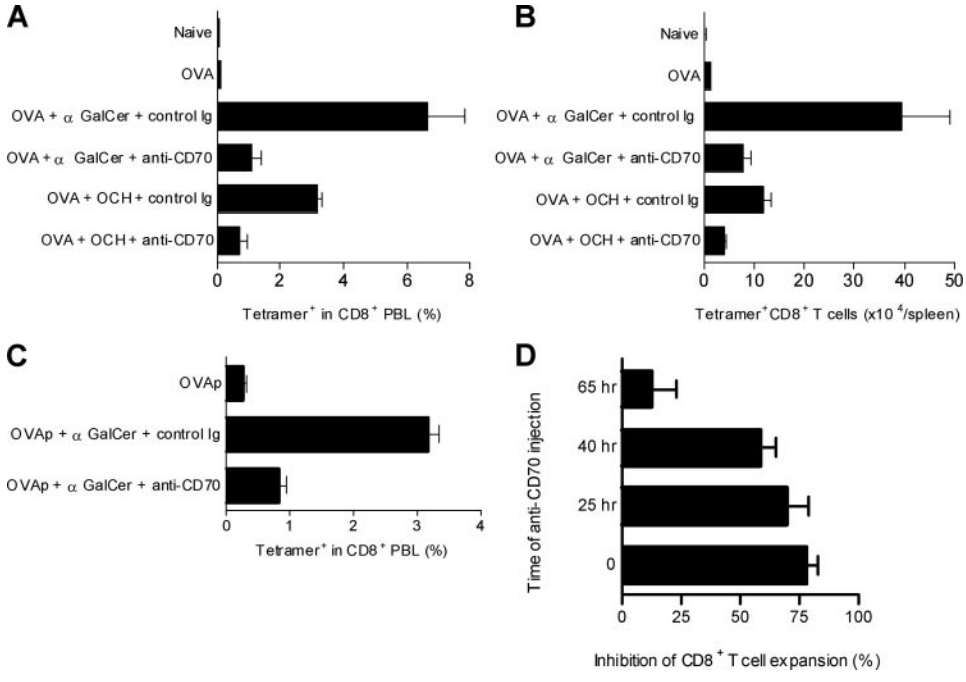

FIGURE 3. CD70 is required for *i*NKT cell-mediated $CD8^+$ T cell priming. B6 mice were injected i.v. with OVA or OVA and iNKT cell-specific agonists (αGalCer or OCH). In some experiments, OVA was replaced with OVAp (*C*). Seven days after immunization, $CD8^+$ T cells in peripheral blood (*A*, *C*, and *D*) and spleens (*B*) were evaluated for binding to H-$2K^b$/$OVA_{257–264}$ tetramer. (*A–C*) Following immunization mice were treated with neutralizing anti-CD70 mAb or a control Ig on days 0 and 1. *D*, Anti-CD70 mAb was administered concurrently with OVA and αGalCer or 25, 40, and 65 h after administration of OVA and αGalCer. Each bar represents the mean ± SE ($n = 4$ mice/group). Similar data were obtained in four other independent experiments.

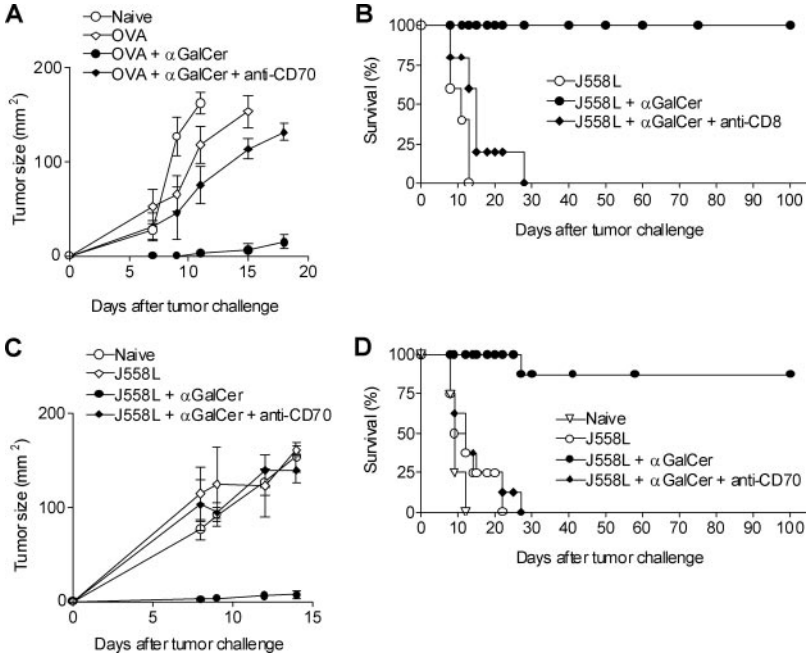

FIGURE 4. CD70 is required for the generation of antitumor immunity by αGalCer. *A,* B6 mice were injected with OVA, with or without αGalCer, on day 0. One group of mice received neutralizing anti-CD70 mAb on days 0 and 1. On day 7, all mice received 2 million E.G7 cells s.c. and tumor growth was then monitored. *B–D,* BALB/c mice were injected i.v. with 20 million irradiated J558L cells with or without αGalCer. In some experiments, CD8$^+$ T cells were depleted to assess their role in the generation of antitumor immunity (*B*). On days 0 and 1, some αGalCer-treated mice also received anti-CD70 mAb (*C* and *D*). Seven days after immunization, mice were challenged s.c. with 5 million J558L cells and tumor growth was monitored at regular intervals. *A* and *C,* The mean tumor sizes ± SE ($n = 5$). *B* and *D,* Survival of mice inoculated with J588L tumor cells. J558L-bearing mice were sacrificed as soon as the tumor size exceeded 150 mm^2, and the survival was monitored up to day 100 ($n = 5$ for *B* and $n = 10$ for *D*). The data shown are representative of at least two independent experiments.

areas of the spleen (Fig. 2, *A–C*). Expression of CD70 was completely abolished if mice were also administered an anti-CD40L mAb that blocks the interaction of CD40L with CD40 (Fig. 2*D*). These data suggested that i.v. injection of αGalCer results in CD70 expression on DCs within the T cell areas of the spleen. This was confirmed by costaining spleen sections with MIDC-8 (20, 21), a mAb that reacts specifically with T cell zone DCs (Fig. 2, *E–H*). We did not observe any significant staining with anti-CD70 mAb of other cells within the spleen after injection of αGalCer. We also noticed that after injection of αGalCer there was a significant increase in MIDC-8$^+$ cells in the T cell areas of the spleen (Fig. 2, *E–G*). This is likely to represent an increase in DC numbers caused by mobilization of T cell zone DC precursors (22, 23). Our data, therefore, demonstrate that activation of iNKT cells in vivo leads to a remarkable increase in CD70-expressing DCs in the T cell areas of the spleen.

The adjuvant properties of iNKT cells toward CD8$^+$ T cells are dependent on CD70

Since expression of CD70 is induced on DCs following injection of αGalCer, we examined whether CD70 is required for mediating the adjuvant effects of αGalCer on CD8$^+$ T cells. As shown in Fig. 3, blocking the interaction between CD70 and CD27 in vivo with a nondepleting mAb (10–12) abolished the ability of αGalCer to promote priming of OVA-specific CD8$^+$ T cells. The effect of the anti-CD70 mAb was observed both in peripheral blood as well as in the spleen (Fig. 3, *A* and *B*). A similar decrease in CD8$^+$ T cell priming was observed when OCH, an alternative iNKT cell ligand that is less effective in sustaining TCR stimulation than αGalCer (17, 24), was used as an adjuvant (Fig. 3, *A* and *B*). Injection of the H-2K (K^b)-restricted immunodominant OVAp (SIINFEKL) with αGalCer also resulted in priming of OVA-specific CD8$^+$ T cells and this was dependent on CD70 (Fig. 3*C*). To address when CD70 mediated its effects, we injected the anti-CD70 mAb immediately, 25 h, 40 h, or 65 h after administration of OVA and αGalCer and measured the CD8$^+$ T cell response on day 7. Injection of the anti-CD70 mAb 65 h after administration of Ag and αGalCer caused little if any inhibition of CD8$^+$ T cell priming, whereas all other treatments resulted in significant inhibition (Fig. 3*D*). These data show that the CD70-CD27 interaction is required during the first 2 days after injection of Ag and αGalCer.

To assess the role of CD70 in the adjuvant effects of αGalCer in an antitumor vaccine setting, we used two different vaccination models. In the first model, we immunized mice with OVA in the presence of αGalCer and administered anti-CD70 mAb to assess the role of CD70 in the generation of a T cell response against OVA-expressing tumors. Seven days after immunization, we challenged the mice with E.G7 cells, a T cell lymphoma that expresses OVA (25). Immunization with OVA in the presence of αGalCer triggered antitumor immunity, resulting in a significant delay in tumor growth. Administration of the anti-CD70 mAb concurrently with the vaccine abolished this protective effect, consistent with its ability to block the generation of an OVA-specific CD8$^+$ T cell response (Fig. 4*A*). In the second model, we immunized mice with irradiated J588L cells, a plasmacytoma that expresses the P1A cancer rejection Ag that is recognized by CD8$^+$ T cells (26, 27). Immunization with irradiated J588L cells and αGalCer, but not irradiated tumor cells alone, generated long-term protective immunity against a

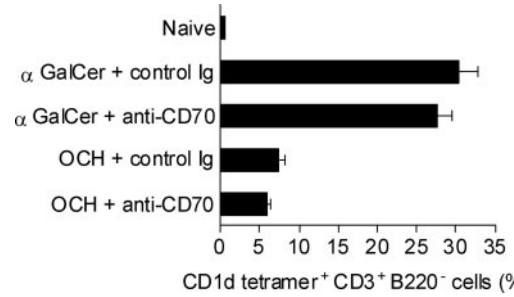

FIGURE 5. iNKT cell expansion is independent of CD70. Activation of iNKT cells in vivo was achieved by injecting αGalCer or OCH. Expansion of iNKT cells was measured on day 3 by evaluating CD1d tetramer$^+$CD3$^+$B220$^-$ cells in peripheral blood. The data shown are representative of four independent experiments.

challenge with live tumor cells and this protection was dependent on $CD8^+$ T cells (Fig. 4*B*). When mice were immunized with irradiated J588L cells and αGalCer and were given anti-CD70 mAb with the vaccine, the protective effect of αGalCer was completely abolished (Fig. 4, *C* and *D*). These data demonstrate the essential role of CD70 in the generation of antitumor T cell responses following administration of vaccines that incorporate αGalCer as an adjuvant.

iNKT cell expansion is independent of CD70

We determined whether CD70 played a role in the expansion of iNKT cells following their activation in vivo. Injection of αGalCer or OCH resulted in ~60 and 12-fold expansion of peripheral blood iNKT cells, respectively, and this expansion was not affected by administration of the neutralizing anti-CD70 mAb (Fig. 5). Similarly, neutralization of CD70 did not affect the expansion of iNKT cells within the spleen (data not shown). Taken together, these data demonstrate that iNKT cell expansion proceeds independently of the CD70-CD27 interaction.

Discussion

The ability of αGalCer to stimulate conventional T cell responses to a coadministered protein Ag has been attributed to the induction of CD40L expression on iNKT cells and the subsequent activation of DCs via CD40 (2, 4). Activation of DCs by iNKT cells triggers an increase in the expression of the costimulatory proteins CD80 and CD86 as well as enhanced secretion of TNF-α, IFN-γ, and IL-12, but none of these changes is sufficient for the expansion of conventional $CD8^+$ T cells. In this study, we provide evidence for a novel mechanism for enhanced $CD8^+$ T cell immunity following activation of iNKT cells. We show that injection of αGalCer triggers CD70 expression on DCs in the T cell areas of the spleen (Fig. 2). CD70 was absent on DCs in unstimulated mice and its induction by αGalCer was entirely dependent on CD40 signaling (Fig. 2). Importantly, we found that the ability of αGalCer to promote $CD8^+$ T cell responses is highly dependent on CD70 (Fig. 3). Accordingly, neutralization of CD70 in vivo abolishes the adjuvant effect of αGalCer and negates the antitumor immunity generated by vaccination with a model tumor Ag or apoptotic tumor cells (Fig. 4).

That CD70 is required during the first 2 days after injection of αGalCer (Fig. 3*C*) is consistent with the pattern of CD70 expression on DCs (Fig. 2) and the reported temporal association between DCs and $CD8^+$ T cells in secondary lymphoid tissue (28). Naive $CD8^+$ T cells interact with Ag-loaded activated DCs in three distinct phases. Phase 1 consists of brief contacts and lasts for up to 8 h. In phase 2, $CD8^+$ T cells and Ag-bearing activated DCs engage in more stable interaction that lasts for ~12 h. On the second day (phase 3), T cells resume their motile behavior, making only transient contacts with DCs. The functional significance of the three different interaction phases is not fully understood. Establishment of stable T cell-DC interactions appears to coincide with the ability of T cells to differentiate into cytokine-secreting cells, whereas T cell proliferation ensues during phase 3 (28). In our study, CD70 was detected on DCs at 20 and 48 h after injection of αGalCer (Fig. 2), and administration of the neutralizing anti-CD70 mAb as late as 40 h after immunization still resulted in significant inhibition of $CD8^+$ T cell responses (Fig. 3*C*). It should be noted, however, that the magnitude of inhibition achieved by neutralizing CD70 at 40 h was not as high as that seen following Ab administration at earlier time points (Fig. 3*C*). This, therefore, suggests that appropriate expansion of polyclonal $CD8^+$ T cells requires CD70 costimulation throughout the first 2 days after immunization with soluble protein and αGalCer.

In the spleen, there are two distinct DC subsets distinguished by expression of different markers and localization to a discrete anatomic location (21, 29–32). T cell zone DCs express CD8α, DEC205, and the Ag recognized by mAb MIDC-8, whereas DCs that reside in the red pulp and marginal zone are $CD8\alpha^-$ and express the Ag recognized by mAb 33D1. Recently, cross-presentation of soluble OVA to $CD8^+$ T cells was shown to be dependent on the expression of the mannose receptor (MR), an endocytic receptor present on some $CD8\alpha^+$ DCs (33, 34). OVA endocytosed via the MR enters an early endocytic compartment distinct from lysosomes that facilitate cross-presentation of antigenic peptides to $CD8^+$ T cells (33). $CD8\alpha^+$ DCs have also been shown to cross-present Ags to $CD8^+$ T cells following capture of dying cells (35). In this study, we showed that iNKT cell-dependent priming of $CD8^+$ T cells after immunization with soluble OVA and αGalCer requires CD40 signaling and peptide/MHC I presentation by the same APCs (Fig. 1*B*). Because in vivo cross-presentation is primarily a function of $CD8\alpha^+$ DCs (32, 33, 35–37), our data suggest that iNKT cells trigger direct activation or "licensing" of this DC subset by inducing the expression of CD70, thus providing the critical costimulatory signal required for $CD8^+$ T cell expansion.

Recently, Soares et al. (38) demonstrated preferential expression of CD70 on $DEC205^+$ DCs upon coculture with activated $CD4^+$ T cells and this was essential for driving $CD4^+$ T cell proliferation and differentiation into IFN-γ-producing effector cells (38). Consistent with this observation, administration of Ag targeted to $DEC205^+$ DCs along with a combination of agonistic CD40 mAb and poly(I:C) as an adjuvant, resulted in the priming of $CD4^+$ T cells in a CD70-dependent manner. Conversely, Ag targeting to the $33D1^+$ DC subset triggered $CD4^+$ T cell priming independent of CD70. Furthermore, priming of $CD4^+$ T cells by nontargeted Ag that was presented by both DC subsets was only partially dependent on CD70. In light of the recent findings by Soares et al. (38), we investigated whether the requirement for CD70 in the αGalCer-mediated priming of $CD8^+$ T cells was affected by the type of the Ag-presenting DCs. We administered Ag in the form of the immunodominant OVAp SIINFEKL along with αGalCer and investigated the effect of CD70 blockade on the priming of OVA-specific $CD8^+$ T cells. Unlike whole OVA, OVAp is not targeted to a particular APC and should therefore be presented by $DEC205^+$ and $33D1^+$ DCs. Administration of the anti-CD70 mAb inhibited $CD8^+$ T cell priming by OVAp and αGalCer to levels comparable to those seen when whole OVA was used as Ag. Thus, irrespective of whether Ag is presented only by $DEC205^+$ DCs or is more widely presented, the adjuvant properties of αGalCer toward $CD8^+$ T cells are critically reliant on CD70. It is possible that $CD8\alpha^+DEC205^+$ DCs, which express higher levels of CD1d than $CD8\alpha^-$ DCs (39), are the main APCs that prime $CD8^+$ T cells following activation of iNKT cells. This notion is consistent with our data demonstrating that CD70 is expressed in T cell zone and not marginal zone or red pulp-associated DCs after αGalCer administration (Fig. 2).

By revealing the mechanism responsible for the adjuvant effect of iNKT cells toward $CD8^+$ CTLs, it might be possible in the future to develop adjuvants that stimulate effective immunity without the inflammation associated with activation of the innate immune response.

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Disclosures

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