Delicate Balance among Three Types of T Cells in Concurrent Regulation of Tumor Immunity

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Abstract

The nature of the regulatory cell types that dominate in any given tumor is not understood at present. Here, we addressed this question for regulatory T cells (Treg) and type II natural killer T (NKT) cells in syngeneic models of colorectal and renal cancer. In mice with both type I and II NKT cells, or in mice with neither type of NKT cell, Treg depletion was sufficient to protect against tumor outgrowth. Surprisingly, in mice lacking only type I NKT cells, Treg blockade was insufficient for protection. Thus, we hypothesized that type II NKT cells may be neutralized by type I NKT cells, leaving Tregs as the primary suppressor, whereas in mice lacking type I NKT cells, unopposed type II NKT cells could suppress tumor immunity even when Tregs were blocked. We confirmed this hypothesis in 3 ways by reconstituting type I NKT cells as well as selectively blocking or activating type II NKT cells with antibody or the agonist sulfatide, respectively. In this manner, we showed that blockade of both type II NKT cells and Tregs is necessary to abrogate suppression of tumor immunity, but a third cell, the type I NKT cell, determines the balance between these regulatory mechanisms. As patients with cancer often have deficient type I NKT cell function, managing this delicate balance among 3 T-cell subsets may be critical for the success of immunotherapy for human cancer.

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Introduction

There is increasing evidence suggesting that the immune system plays an important role in eliminating or controlling cancer and that failure of or escape from this mechanism allows tumors to expand (1). Tumors escape from the immune system by using different regulatory molecules and regulatory cells. At the cellular level, tumors can induce T-cell anergy and T-cell suppression and recruit regulatory cells such as CD4+ regulatory T cells (Treg; ref 2), myeloid suppressor cells (3), M2 macrophages (4), and natural killer T (NKT) cells (5, 6). One of the most extensively studied negative regulators is the CD4+ Treg, characterized by the expression of interleukin (IL)-2 receptor α, known as CD25, and the intracellular expression of transcription factor forkhead box p3 (Foxp3). A role for Tregs in tumor immunity was first discovered when antitumor T-cell immune responses were enhanced in mice inhibited for the function of this T-cell subpopulation in vivo by anti-CD25 monoclonal antibody (mAb), clone PC61. The blockade of Tregs was found to induce tumor immunity in many tumor models, including leukemia, myelomas, and sarcomas (7). Blockade of Tregs by using other reagents such as denileukin diftitox (immunotoxin-conjugated IL-2, Ontak) and cyclophosphamide also inhibited tumor growth (8, 9) and enhanced vaccine-induced immunity (10, 11).

Another kind of regulator is the NKT cell. NKT cells are a unique subset of T cells capable of recognizing lipid antigens presented by the MHC-like molecule CD1d. They can be divided into at least 2 subsets. Type I NKT cells express an invariant T-cell receptor (TCR)-α chain using the Vα14-Jα18 segment. These cells can be activated by the prototypic lipid antigen α-galactosylceramide (α-GalCer). Type II NKT cells express a diverse TCR repertoire, distinct from Vα14-Jα18 and can be activated by other lipids, such as sulfatide (12). Each subset of NKT cells can be activated by a specific group of lipids that cannot activate the other subset. There are 2 strains of NKT cell–deficient mice: CD1d−/− mice that lack both type I and type II NKT cells, and Jα18−/− mice that lack type I NKT cells but still retain type II NKT cells. By using these strains, it has been shown that type I NKT cells promote tumor immunity (13–15), whereas type II NKT cells can mediate suppression of tumor immunosurveillance in multiple mouse tumor models (16). Previously, we found that these 2 subsets counteracted each other to regulate tumor immunity when they were simultaneously stimulated, suggesting a new immunoregulatory axis (5, 17, 18).

In some tumor models, Tregs were found to play a critical role in the suppression of tumor immunity, whereas in other models type II NKT cells were found to be the key suppressive cells. It is unclear why different regulatory cells suppress tumor immunity in different models and what determines which cells control the immune response to tumors. The answers to these questions are still elusive.
Here, by using a widely studied subcutaneous CT26 syngeneic colon tumor model, in which tumor immunity was found to be regulated by Tregs in wild-type (WT) mice, as well as the R331 renocarcinoma cell line, we investigated the relative role of 2 kinds of suppressors—Tregs and type II NKT cells—and the mechanism determining the balance between them. We found that in the absence of both type I and II NKT cells (CD1d<sup>-/-</sup> mice), Tregs regulate tumor immunity, similar to the situation in WT mice. However, in the absence of just type I NKT cells (Jcl18<sup>+</sup> cells; mice), eliminating or blocking Tregs is not sufficient to overcome immune suppression. Also, by blocking Tregs or type II NKT cells in Jcl18<sup>-/-</sup> mice, we discovered that having either one of the suppressors is sufficient to suppress the immune response against tumor formation. Which of these suppressors plays a predominant role in the regulation of tumor immunity depends on the presence of type I NKT cells, as type I NKT cells were found to counteract type II NKT cells. In this study, for the first time, we revealed the relative role of Tregs and type II NKT cells in controlling immunity to the same tumor, and discovered that the balance between these regulatory cells is determined by a third cell, the type I NKT cell. This finding may be critical in the therapy for human patients with cancer, because they often are deficient in type I NKT cell functions (6, 19–21).

**Materials and Methods**

**Mice**

Female BALB/c mice were purchased from Animal Production Colonies, Frederick Cancer Research Facility, National Cancer Institute (NCI; Frederick, MD). BALB/c CD1d<sup>-/-</sup> mice (The Jackson Laboratory, Bar Harbor, ME) and BALB/c Jcl18<sup>+/+</sup> mice (provided by M. Taniguchi, RIKEN Institute, Yokohama, Japan, and by D. Umetsu, Harvard Medical School, Boston, MA) were bred at the NCI under specific pathogen-free and *Helicobacter*-free conditions. Female mice (at least 6–8 weeks of age) were used for all experiments. All experimental protocols were approved by and carried out under the guidelines of the NCI’s Animal Care and Use Committee.

**Cell lines**

A CT26 colon carcinoma cell line was maintained in RPMI-1640 supplemented with 10% fetal calf serum, l-glutamine (2 mmol/L), sodium pyruvate (1 mmol/L), nonessential amino acids, and 2-mercaptoethanol (5 × 10<sup>-5</sup> mol/L). R331, a subline of the RENCA BALB/c renal carcinoma line, was a kind gift of Dr. Thomas Sayers (NCI, Frederick, MD) and was maintained in RPMI-1640 supplemented with 5% fetal calf serum and all of the above supplements.

**In vivo tumor assay and antibody treatment**

A single cell suspension of 5 × 10<sup>6</sup> CT26 cells or 5 × 10<sup>5</sup> R331 cells in 0.1 mL of PBS was injected subcutaneously on day 0. Mice were treated on day –5 with 0.5 mg anti-mCD25 (PC61, Harlan Laboratories) and in some experiments with 0.2 mg of anti-mCD1d (IB1, Harlan Laboratories) on days 1, 4, and 7. Rat immunoglobulin G (IgG) was purchased from Sigma-Aldrich and injected as a control for antibody treatments. Tumor size was measured periodically, starting day 7, by caliper gauge.

**Sulfatide treatment**

3′-Sulfo-C24:1 galactosylceramide (sulfatide; Avanti Polar Lipids) was dissolved in PBS + 0.5% Tween 20, and 30 μg/mouse were injected subcutaneously 1 hour after tumor challenge.

**In vitro Treg suppression assay**

The Treg suppression assay was conducted as previously described (22). Briefly, magnetic bead sort depleted CD4<sup>+</sup> CD25<sup>+</sup> T cells (5 × 10<sup>6</sup>, responders) and varying numbers of CD4<sup>+</sup> CD25<sup>+</sup> T cells (Tregs) from WT and Jcl18<sup>+</sup> lymph nodes (brachial, axillary, inguinal, mesenteric, popliteal, and lumbar) were incubated in the presence of CD90.2-depleted splenocytes (5 × 10<sup>6</sup>, accessory cells) and 0.5 μg/mL anti-mouse CD3 (145-2C11; BD Biosciences). The cells were cultured in a 96-well flat-bottom plate (Corning) for 72 hours in triplicate. Suppression was evaluated by cell proliferation, measured by [<sup>3</sup>H]-thymidine incorporation (1 μCi/well, added at the last 16 hours of incubation). Percentage of suppression was calculated according to: 100 × [1 – (CPM of Treg culture/CPM of non-Treg culture)].

**Isolation of liver lymphocytes**

Liver lymphocytes were prepared as previously described (23, 24). Livers were perfused with liver perfusion medium (Invitrogen), minced, and digested with liver digest medium (Invitrogen) at 37°C for 15 minutes. Hepatocytes were removed by centrifugation (500 rpm for 1 minute), and liver lymphocytes were then purified by a 40%/80% gradient of Percoll (Sigma-Aldrich).

**Adoptive transfer of liver type I NKT cells**

Lymphocytes from 40 naive BALB/c livers were isolated and stained with phycoerythrin (PE)-PBS57/CD1d tetramer (The NIH Tetramer Facility, Bethesda, MD) for 40 minutes at 4°C, followed by staining with anti-PE beads (Miltenyi). PBS57/CD1d-tetramer−positive cells (type I NKT cells) were separated by using AutoMACS (Miltenyi). Efficacy of the separation was evaluated by measuring PBS57/CD1d-tetramer<sup>+</sup> CD3<sup>+</sup> cells by FACSCalibur (BD Biosciences), and the data were analyzed using FlowJo (Tree Star). A total of 4 × 10<sup>5</sup> type I NKT cells in 0.2 mL PBS were injected intravenously a day before tumor challenge into Jcl18<sup>-/-</sup> recipients.

**Visualizing liver type II NKT cells with sulfatide-loaded CD1d dimers**

Sulfatide was loaded on mCD1d-Ig fusion protein (mCD1d dimer; mouse CD1d dimerX; BD Biosciences) as described in Parish and colleagues (in preparation), modified from the sulfatide/CD1d tetramer method of (12). Briefly, mCD1d dimer was loaded with sulfatide (Avanti Polar Lipids) or PBS at 37°C overnight. Buffer was replaced with PBS using Amicon Ultra Centrifugal Filters 30K (Millipore), followed by the addition of PE−anti-mouse IgG antibodies (BD Biosciences) and incubated for 1 hour at room temperature. Mouse IgG (BD Biosciences) was added for an additional 30 minutes at room temperature to saturate unbound excess anti-IgG antibodies. Liver lymphocytes were stained with CD1d dimers for 1 hour at 4°C, followed by cell surface staining.
Flow cytometry

Total spleen or lymph node cells were incubated with anti-CD16/CD32 (clone 93, Biolegend) and stained with anti-CD3 (145-2C11, Biolegend), anti-CD4 (RM4-5, BD), anti-CD25 (PC61, eBioscience), and anti-Foxp3 (FJK-16S, eBioscience) for the evaluation of Treg frequency. Enriched liver lymphocytes were incubated with anti-CD16/CD32 and stained with anti-CD3, yellow viability dye (LIVE/DEAD Fixable dead cell stain kit; Invitrogen), and PBS57/CD1d-tetramers (NIH Tetramer Facility) or sulfatide/CD1d dimers (prepared as described earlier). Enriched liver type I NKT cells were also stained with anti-CD16/CD32 and stained with anti-CD3, yellow viability dye (LIVE/DEAD Fixable dead cell stain kit; Invitrogen), and PBS57/CD1d-tetramers (NIH Tetramer Facility) or sulfatide/CD1d dimers (prepared as described earlier). Enriched liver type I NKT cells were also stained with anti-CD25. The cells were visualized on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences) and LSRII using DIVA software (BD Biosciences). Data were analyzed by FlowJo (Tree Star).

Statistics

The data were analyzed using the nonparametric Mann–Whitney test, Wilcoxon rank sum test, t test with conservative variance estimation, or repeated measures ANOVA test using JMP software (version 8, SAS Institute). Significance was determined at \( P < 0.05 \).

Results

Tregs are not necessary for the suppression of tumor immunity in Jc18–/– mice

In an attempt to understand the relative roles of Tregs and type II NKT cells in the regulation of tumor immunity, we first addressed the necessity of the 2 suppressors in NKT cell–deficient mice. We used a CT26 subcutaneous tumor model with 3 strains of mice, all on the BALB/c background: WT that have both type I and II NKT cells, CD1d–/– that lack both types of NKT cells, and Jc18–/– that lack type I NKT cells but retain type II NKT cells. In this tumor model, the suppression of tumor immunity in WT mice has been shown to be regulated by Tregs (25). Consistent with this previous report, we found that blocking Tregs by anti-CD25 protected WT mice (Fig. 1A). Similarly, protection was also observed by blocking Tregs in CD1d–/– mice, although without treatment, tumors grew...
Thus, the fact that Tregs are not necessary for immune suppression is mediated by both type II NKT cells and Tregs in Jα18⁻/⁻ mice

It has been shown that type I and II NKT cells counteract each other’s functions (17). Thus, to explain the surprising difference in the effect of anti-CD25 in WT, CD1d⁻/⁻ and Jα18⁻/⁻ mice, we hypothesized that in WT mice, the 2 types of NKT cells cancel each other’s effects on tumor immunity, leaving Tregs as the dominant suppressor. On the other hand, in CD1d⁻/⁻ mice, both types of NKT cells are absent, again leaving Tregs as the dominant suppressor. In both circumstances in which Tregs dominate, anti-CD25 treatment is effective to induce tumor rejection, whereas we hypothesize that in Jα18⁻/⁻ mice anti-CD25 treatment alone is not effective because the lack of type I NKT cells allows unopposed type II NKT cells to suppress tumor immunity as well (Fig. 2A). Consistent with this hypothesis, we found a higher frequency of type II NKT cells in livers of Jα18⁻/⁻ mice than in those of WT mice (Fig. 2B). The difference is not due to a simple dilution effect in the absence of type I NKT cells because we found that the actual numbers of type II NKT cells were significantly higher in Jα18⁻/⁻ mice (WT vs. Jα18⁻/⁻, 1.1 ± 0.6 × 10⁵ vs. 3.2 ± 1.3 × 10⁵; P = 0.0082). To further test our hypothesis, we significantly more slowly than in WT mice (P = 0.007). Surprisingly, however, anti-CD25 treatment did not protect Jα18⁻/⁻ mice (Fig. 1A). These results suggest that Tregs are necessary for the regulation of tumor immunity in WT and CD1d⁻/⁻ mice but not in Jα18⁻/⁻ mice.

Although lack of Treg function in Jα18⁻/⁻ mice would be expected to have an effect opposite to what we observed, we nevertheless wanted to rule out any differences in frequency and/or function of Tregs among the strains. Therefore, we compared the proportion (Fig. 1B) and suppressive activity (Fig. 1C) of Tregs among the strains of mice. Gating on CD3⁺ CD4⁺ CD127⁻ Foxp3⁺ cells in spleens and lymph nodes of mice from each strain, there was no difference in the frequency (Fig. 1B). Also, there was no difference in the suppressive activity of Tregs between Jα18⁻/⁻ and WT against responders from the same strain (Jα18⁻/⁻ and WT responders, respectively; Fig. 1C), and both WT and Jα18⁻/⁻ responders were susceptible to a similar degree to Tregs from different strains. Thus, the fact that Tregs are not necessary for immune regulation in Jα18⁻/⁻ mice is not due to differences in frequency and/or function of Tregs or in susceptibility of conventional CD4⁺ T cells to suppression by Tregs.
examined the necessity of both types of suppressors in Jα18−/− mice. A prediction of our hypothesis is that it is necessary to remove both type II NKT cells and Tregs to remove immune suppression in Jα18−/− mice, as removing Tregs alone was not sufficient to induce tumor protection (Fig. 1A).

Because the activation of type II NKT cells is CD1d-dependent, and in Jα18−/− mice, the only cells dependent on CD1d are type II NKT cells, we used CD1d-blocking antibody to prevent NKT cell activation in vivo. We found that blocking NKT cell activation using anti-CD1d alone did not protect WT or CD1d−/− mice from tumor development, suggesting that blockade of NKT cell activation is not sufficient to induce tumor rejection (Fig. 3). In Jα18−/− mice, neither blocking the activation of type II NKT cells alone with anti-CD1d, nor depletion of Tregs alone with anti-CD25, was sufficient to affect the tumor growth. However, when mice were treated with both anti-CD1d and anti-CD25, protection was achieved. This result suggested that either type II NKT cells alone or Tregs alone are sufficient for immune suppression in Jα18−/− mice and that blockade of both types of regulatory cells is necessary to induce protective tumor immunity.

**Adoptive transfer of type I NKT cells induces tumor protection in Treg-deficient Jα18−/− mice**

The results earlier confirm our hypothesis that in the Jα18−/− mice, both types of regulatory cells are active, so both need to be blocked, whereas in the WT mice, only Tregs are active and need to be blocked. The second part of our hypothesis is the explanation for this, namely that in the WT mice that have both type I and II NKT cells, the type II NKT cells are inhibited by type I, leaving only Tregs active. The only difference between Jα18−/− mice and WT mice is the lack of type I NKT cells, so their absence now reveals the suppressive nature of type II NKT cells. Thus, we suggest that the protective effect of Treg blockade is dependent on the balance between 2 types of NKT cells. A prediction of this hypothesis is that adoptive transfer of type I NKT cells that counteract type II NKT cells should restore the balance between the NKT cell subsets, neutralizing type II NKT cells and making Treg blockade protective in type I NKT cell–deficient Jα18−/− mice. Therefore, to test this hypothesis, we adoptively transferred enriched type I NKT cells from WT livers into Jα18−/− mice.

In our hands, type I NKT cells comprise approximately 10% of total liver lymphocytes (Fig. 4A). These lymphocytes were enriched for type I NKT cells by using PBS57/CD1d-tetramer with magnetic beads. After the enrichment, we found that 80% of the cells were PBS57/CD1d-tetramer reactive (Fig. 4B).

Next, we examined the enriched population of type I NKT cells for possible contamination by Tregs. This is important as the cells are transferred into mice already treated with anti-CD25. If transferred Tregs were to contaminate the enriched type I NKT cells, this could result in the suppression of tumor immunity regardless of the activity of type II NKT cells. We found that approximately 0.5% of the enriched type I NKT cells are CD4+ CD25+. Among those cells, some of them were CD1d-tetramer-reactive NKT cells that may have been activated by the tetramer staining during the purification process. Thus, less than 75% of the gated double positive population was presumably Tregs, which represents less than 0.4% of the enriched type I NKT cells (Fig. 4C). Therefore, the vast majority of the cells that were transferred into Jα18−/− mice were type I NKT cells, and these cells contained very few Tregs.

To evaluate the efficacy of the adoptive transfer of type I NKT cells into Jα18−/− mice, we examined the frequency of type I NKT cells in the spleens and livers 6 days after the intravenous injection of 4 × 10⁵ PBS57/CD1d-tetramer–reactive cells. There was 20% and 10% type I NKT cell reconstitution in livers and spleens, respectively, of recipient mice compared with WT mice (Fig. 4D), suggesting that the adoptive transfer was efficient, but reconstitution was incomplete. Twenty-four hours after the transfer, mice were challenged subcutaneously with CT26 cells and monitored for tumor growth (Fig. 4E). The adoptive transfer of type I NKT cells into Jα18−/− mice by itself did not affect tumor burden. However, in anti-CD25–treated Jα18−/− mice that were adoptively transferred with type I NKT cells, protection was achieved despite only partial

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**Figure 3.** A combination of Treg blockade and type II NKT cell blockade reduces tumor burden in Jα18−/− mice. CT26 cells (5 × 10⁵) and anti-CD25 (0.5 mg) were injected into WT, CD1d−/−, and Jα18−/− mice (5 mice/group) as described in Fig. 1A. Some mice were also treated with 0.2 mg of anti-CD1d mAb or Rat IgG on days 1, 4, and 7. Tumor size was measured twice a week. Jα18−/− mice that received the combined treatment developed significantly smaller tumors (P = 0.0079 against Jα18−/− mice that were treated with anti-CD25 alone or anti-CD1d alone). Mice that received the combined treatment (anti-CD25+anti-CD1d) and mice that received only anti-CD25 treatment developed similar tumor size in WT and CD1d−/− groups. Data are presented as mean ± SD. The experiment was repeated 3 times with comparable results.
reconstitution. This result showed that even with only 10% reconstitution in the spleen and 20% in the liver (that could be achieved with cells from 40 donor livers), the presence of type I NKT cells made Treg blockade effective to reduce tumor burden in Jα18−/− mice. We infer that suppression of type II NKT cells by type I NKT cells left Tregs as the major
increased tumor size in anti-CD25 injection. Tumor size was measured twice a week. Sulfatide significantly suppressed tumor growth, the sulfatide treatment in the anti-CD25-treated mice significantly reduced the percentage of type I NKT cells (mean 41% reduction; range, 32%–46%; P < 0.01) in livers of tumor-challenged animals 72 hours after injections (Fig. 5B). These results show that shifting the balance between type I and II NKT cells at the level of their activity and numbers by stimulating type II NKT cells can overcome the neutralizing effect of type I NKT cells on type II, and thereby reveal the immunoregulatory potential of type II NKT cells even in the presence of type I NKT cells. Thus, overall, we conclude that the protective effect of Treg blockade in WT mice relies on the absence of suppression by type II NKT cells, and that either selectively stimulating type II NKT cells or removing their natural inhibitor, the type I NKT cells, unmask the presence of a second immunoregulatory T cell, the type II NKT cell, that acts in parallel with Tregs to control tumor immunity.

Figure 5. Sulfatide treatment after Treg blockade suppresses tumor immunity in WT mice. A, CT26 cells (5 x 10^6) and anti-CD25 mAb (0.5 mg) were injected into WT mice as described in Fig. 1A (5 mice/group). One hour after tumor challenge, mice were injected subcutaneously with 30 μg sulfatide or control vehicle at a site adjacent to that of the tumor injection. Tumor size was measured twice a week. Sulfatide significantly increased tumor size in anti-CD25-treated mice (P = 0.0079 vs. vehicle + anti-CD25–treated mice). Data are mean ± SD. The experiment was repeated 3 times. B, anti-CD25–treated mice were challenged with CT26 cells and injected with vehicle or 30 μg/mouse sulfatide. Seventy-two hours after the injections, livers from the mice were perfused and processed to enrich lymphocytes. Total liver lymphocytes were stained with PerCP-Cy5.5-anti-CD3 and PE-PBS57/CD1d tetramer to enumerate type I NKT cells. The proportion of type I NKT cells in the mice with anti-CD25 + sulfatide was significantly lower than in the mice with anti-CD25 alone (mean 41% reduction; range, 32%–46%; P < 0.01 by t test with conservative variance estimation). Presented density plots are representative of 4 independent experiments.

Relative Role of Tregs and NKT Cells

Type II NKT cell activation abrogates the protective effect of Treg blockade in WT mice

A further prediction of our hypothesis is that activating regulatory type II NKT cells after Treg blockade should shift the balance of NKT cell subsets in WT mice toward immunosuppression, changing the outcome of tumor growth. We previously reported that activation of a subset of type II NKT cells by sulfatide can suppress the protective effect of type I NKT cells (17). Thus, as a further test of the hypothesis, we treated tumor-bearing mice with or without anti-CD25 with NKT cells (17). Thus, as a further test of the hypothesis, we treated tumor-bearing mice with or without anti-CD25 with sulfatide (Fig. 5).

Treating Treg-intact WT mice with sulfatide (30 μg/mouse) did not affect tumor growth compared with mice treated with vehicle or untreated mice (Fig. 5A). However, in mice treated with anti-CD25, sulfatide treatment abrogated the protective effect of anti-CD25. Consistent with the effect on tumor growth, the sulfatide treatment in the anti-CD25–treated mice significantly reduced the percentage of type I NKT cells (mean 41% reduction; range, 32%–46%; P < 0.01) in livers of tumor-challenged animals 72 hours after injections (Fig. 5B).

In some settings, type II NKT cells were found to dominate the immunoregulatory T cell population in CD1d−/− mice or blockade of their activation does not result in tumor rejection. Therefore, we hypothesized that a similar mechanism might apply, and so asked whether anti-CD25 treatment induces rejection of tumors in Jc18−/− mice, and if not, whether a combination of anti-CD1d and anti-CD25 can protect. Consistent with the previous report, anti-CD25 treatment induced tumor rejection in WT mice. In contrast, the same treatment did not affect tumor growth in Jc18−/− mice (Fig. 6A).

Discussion

In this study, for the first time, we revealed the relative role for the 2 suppressors, Tregs and type II NKT cells, in the same tumor model and showed that the balance between them is determined by a third cell, the type I NKT cell, that counterbalances type II NKT cells. The role of each suppressor alone has been documented in the past in a variety of tumor models. In some settings, type II NKT cells were found to dominate the suppression of tumor immunity (16, 17, 26, 27), whereas in other settings Tregs were found to be the primary regulatory cell (7, 16, 25). In contrast to these studies, we decided to focus on the relative roles of both suppressors in the same tumor model, the subcutaneous CT26 colon carcinoma. We found that each one of the regulators is sufficient to induce suppression of tumor immunity in the absence of type I NKT cells (Jc18−/− mice) and to achieve protection the effects of both regulators need to be abrogated.
We showed that adoptive transfer of type I NKT cells into Jc18−/− mice partially protected the mice from tumor growth only after Treg blockade, suggesting that blockade of both suppressors is needed (Fig. 4E). This result strengthens our hypothesis that the protective effect of Treg blockade relies on the balance between the 2 types of NKT cells as we saw in Fig. 5. However, the protection was only partial. Analyzing the efficacy of the adoptive transfer revealed that only 20% reconstitution was observed in the liver and 10% in the spleen. It could be that the reason for the partial protection is the low percentage reconstitution of type I NKT cells in the spleen. It could be that the reason for the partial protection is the low percentage reconstitution of type I NKT cells in the spleen. It could be that the reason for the partial protection is the low percentage reconstitution of type I NKT cells in the spleen. 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II NKT cells by sulfatide in vivo abrogates the protective effect of anti-CD25 in WT mice. Both results strongly suggest cross-regulation between the 2 types of NKT cells. These results are consistent with our previous reports (32) and with our current findings that type II NKT cells are more frequent in type I-deficient Jz18-/- mice and that conversely, stimulation of type II NKT cells diminishes the frequency of type I NKT cells. The mechanism by which the 2 types of NKT cells regulate each other remains elusive. The cross-regulation can potentially occur in 2 different ways. One is by direct interaction between the 2 types of NKT cells, either through soluble factors or by cell-to-cell contact. Although de...
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Concurrent Regulation of Tumor Immunity
Delicate Balance among Three Types of T Cells in

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