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Role of CD44 in the Differentiation of Th1 and Th2 Cells: CD44-Deficiency Enhances the Development of Th2 Effectors in Response to Sheep RBC and Chicken Ovalbumin

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CD4 T cells can be primarily polarized to differentiate into Th1 or Th2 cells. CD44 is a marker of T cell activation and a property of long-lived memory cells and implicated in cell migration, activation, and differentiation. To date, whether CD44 has a role in regulating Th1-Th2 differentiation has not been determined. In this study, we compared Th1 and Th2 responses in wild-type and CD44-deficient mice in response to sheep RBC and chicken OVA, as well as examined Th1-Th2 differentiation in vivo and in vitro from CD44-sufficient and CD44-deficient naive CD4 T cells. We observed that deficiency of CD44 tended to inhibit Th1 while promoting Th2 differentiation. Furthermore, chimeric studies suggested that CD44 expression by CD4 T cells was essential for such Th2 bias. The regulation by CD44 occurred at the transcription level leading to up-regulated GATA3 and down-regulated T-bet expression in activated CD4 T cells. We also noted that CD44-deficiency could modify the state of dendritic cell subsets to induce a Th2-biased development. Results presented in this study demonstrate for the first time that CD44 participates in the regulation of Th1-Th2 differentiation. The Journal of Immunology, 2009, 183: 172–180.

CD4 helper T cells can differentiate into functionally distinct effector subsets with different cytokine expression profile and immune regulatory function based on the Ag receptor- and cytokine-mediated signals. This family is expanding. From original Th1 and Th2 lineage, it now includes Th17 and TGF-induced regulatory T cells as well as follicular helper T cells, and possibly Th9 (1–5). As an original sole paradigm, the signals that drive Th1-Th2 differentiation are clearly elucidated. Th1- or Th2-polarizing cytokines initiate signaling via IAK/STAT complexes and there are clear differences noted in these subsets. For example, STAT1, STAT4, and T-bet facilitate Th1 signaling while STAT6 and GATA3 promote Th2 differentiation. Th1 subset produces IFN-γ, Th2 subset produces IL-4, IL-5, and IL-13. Th1 response is often accompanied by the production of IgG2a while Th2 response is often accompanied by the production of IgG1 and IgE Abs. Also, Th1 and Th2 responses are often mutually exclusive. Both Th1- and Th2-specific cytokines can facilitate growth or differentiation of their own respective T cell subset, but additionally might inhibit the development of the opposing subset. At the transcription level, GATA3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells, and inhibition of Th1 cell-specific factor (6).

Polarization of naive CD4 T cells toward Th1 or Th2 mainly relies on the cytokine milieu, but it is also influenced by many other factors, such as Ag affinity for the TCR, concentration of Ag, and costimulatory molecules (6). The sources of the cells that produce the cytokines in vivo and the molecules that are involved in the regulation of Th development are still under investigation. Although Th1 and Th2 can themselves provide IFN-γ or IL-4 for the recruitment of Th1 or Th2 differentiation, respectively, the cells that initiate effector T cell differentiation in primary vs secondary responses have not yet definitively been determined (5). Many cell types may be involved in these processes, such as dendritic cells (DCs), NK, NKT cells (5, 7), or macrophages (8). There is not an exclusive conclusion so far. It is especially complicated and controversial to evaluate the relationship between DC subsets and Th development; even though the adoptive transfer of Ag-pulsed CD8α− DCs induces a Th2 response, transfer of CD8α+ DCs leads to a Th1 differentiation (9, 10).

CD44 is a widely distributed cell surface glycoprotein expressed by a variety of lymphoid and nonlymphoid cells. The CD44 family consists of a standard form and a group of isoforms resulting from the extensive alternative splicing that might attribute to its sophisticated implication in the immune responses and immune regulation. CD44 molecule participates in cell adhesion and migration, lymphocyte homing, activation and proliferation, lytic activity of T cells and NK cells, and tumor metastasis (11–14). CD44 is coupled to at least two tyrosine kinases, p185HER2 and c-Src kinase, and has broad functions in cellular signaling cascades not only by establishing specific transmembrane complexes but also by organizing signaling cascades through association with its partner proteins, which monitor changes in the extracellular matrix that influence cell growth, survival, and differentiation, as well as induction of the cytotoxicity of CTL and NK cells (15–17). CD44 is recruited to the immunological synapse during DC and T cell interactions and affects the subsequent T cell activation, IL-2 and IFN-γ production, and phosphotyrosine and protein kinase C-θ enrichment at the synapse (18). CD44 splice variant expression is

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obligatory for the migration and function of Langerhan cells (LCs) and DCs. Blockade of CD44 inhibits the emigration of LCs from the epidermis, prevents binding of activated LCs and DCs to the T cell zones of lymph nodes, and severely inhibits their capacity to induce a delayed type hypersensitivity (DTH) (19).

Th1 and Th2 lymphocytes express CD44 and use CD44 for their rolling on and adhesion to the intestinal endothelium (20, 21). However, whether CD44 regulates Th1-Th2 differentiation is not clear. In this study, we analyzed Th1 and Th2 responses in wild-type (WT) and CD44-deficient mice to particulate and soluble Ags such as sheep RBC (SRBC) and chicken OVA, respectively. In this study, we provide significant evidence that CD44 plays a role in regulation of Th1-Th2 differentiation and CD44-deficiency triggers a Th2-biased Th development.

Materials and Methods

Mice and reagents

WT C57BL/6 and CD4 T cell-deficient (CD4−/−) mice were purchased from The Jackson Laboratory. OT II mice were purchased from Taconic Farms. CD44 knockout (CD44−/−) mice were generated at Amgen Institute (Toronto, Canada) and provided to us by Dr. Tak Mak. These mice are on a C57BL/6 background and have been extensively characterized in our previous studies (22, 23). Mice were housed in the University of South Carolina Animal Facility. Animal procedures were performed according to National Institutes of Health guidelines under protocols approved by the Institute Animal Care and Use Committee of the University of South Carolina. SRBC and NycoPrep were purchased from Cedarlane Laboratories. Thioglycollate, OVA, and CFA were purchased from Sigma-Aldrich. IFA was purchased from Difco.

SRBC-induced DTH reaction

Groups of six mice each were injected s.c. with 1 × 10^8 SRBC/mouse in the abdomen. Fourteen days later, the sensitized mice were challenged s.c. with 2 × 10^7 SRBC/mouse in a volume of 20 μl in left ear. As a control, 20 μl of PBS was applied in right ear of the same mouse. The swelling of ear was measured at 0, 24, 48, and 72 h of the challenge. The magnitude of ear thickness is reported as mean thickness ± SE in each group of six mice.

Hemagglutination assay

Groups of four mice each were immunized i.p. or i.v. with 1 × 10^8 SRBC. Blood was collected and serum was prepared from each mouse on day 5 post immunization. Anti-SRBC Ab was detected by hemagglutination assay. To 20 μl of 2-fold diluted serum samples in U-shape microtitration plate, 20 μl of 2% SRBC suspension was added. The plate was incubated at 37°C for 1 h and at 4°C overnight. The hemagglutination was recorded. The highest dilution causing hemagglutination was considered as Ab titer.
resulting cells were incubated with anti-CD8. The interface was collected and washed with PBS. The strainer, cells were subjected to a gradient centrifugation in Nycodenz medium (NycoPrep). The interface was collected and washed with PBS. The strainer, cells were subjected to a gradient centrifugation in Nycodenz medium (NycoPrep). The interface was collected and washed with PBS. The strainer, cells were subjected to a gradient centrifugation in Nycodenz medium (NycoPrep).

Anti-OVA IgM, IgG1, and IgG2a Abs were from eBioscience. The staining was analyzed by flow cytometry. For cell culture, DCs were further purified by positive magnetic selection of CD11c<sup>+</sup> cells. The purity of isolated CD11c<sup>+</sup> DCs was 95–97% as determined by flow cytometric analysis.

**Th1-Th2 polarization in vitro**

Naive CD4 T cells were purified from spleens by negative selection, with minor modifications as described (25), with PE-conjugated mAb against CD8, CD19, or B220, I-<sup>A</sup>, CD16/32, CD24, NK1.1, and Gr-1 (all Abs from eBioscience) using EasySep PE Selection Kit (Stem Cell Technology) following the manufacturer’s instruction.

CD4 T cell polarization was induced by polyclonal stimulation or Ag stimulation. In the polyclonal stimulation, CD4 T cells (<95%) were primed with anti-CD3 plus anti-CD28 (3 µg/ml each, eBioscience) and irradiated (3000 rads) splenocytes (10<sup>5</sup>) from CD4<sup>+</sup> mice that were depleted of CD3 T cells by magnetic beads (Stem Cell Technology). For the Ag stimulation, CD4 T cells isolated from OT II mice were primed with OVA<sub>323–339</sub> peptide (40 µg/ml, Sigma-Aldrich) and DCs (0.2×10<sup>5</sup>). For Th1 priming, IL-12 (10 ng/ml; R&D Systems) and anti-IL-4 Abs (10 µg/ml; eBioscience) were added; for Th2 priming, IL-4 (2 ng/ml; R&D Systems), anti-IL-12 Abs (10 µg/ml; eBioscience), and anti-IFN-γ Abs (10 µg/ml; eBioscience) were added. All cultures contained IL-2 (100 U/ml; NJI). On day 4, live cells were harvested over NycoPrep medium and used for intracellular cytokine staining.

On day 4, the cultures were supplemented with 50 ng/ml PMA (Sigma-Aldrich), 1 µg/ml ionomycin (Sigma-Aldrich), and 2 µM monensin (BD Pharmingen) and incubated for another 4 h at 37°C. For the staining, cells were fixed, permeabilized, and stained intracellularly with allophycocyanin-anti-IL-4 and FITC-anti-IFN-γ Abs (eBioscience) using Cytofix/Cytoperm intracellular staining Kit (BD Pharmingen). The expression of IL-4 and IFN-γ was examined by flow cytometry.

**Intracellular staining of T-bet and GATA3**

Splenocytes were prepared on day 5 of SRBC immunization or day 10 of primary OVA immunization. T-bet and GATA3 expression were examined by intracellular staining using FoxP3 staining buffer kit (eBioscience). Cells were restimulated with PMA and ionomycin in the presence of monensin for 4 h as described above. Cells were first stained extracellularly with FITC-anti-CD4 Abs, then fixed and permeabilized and subjected to the intracellular staining with Alexa Fluor 647-anti-T-bet and PE-anti-
CD44 Abs (eBioscience). The Ab incubation was conducted for 30 min at 4°C. The staining was analyzed by flow cytometry.

**IL-12 secretion from splenic DCs**

DCs (2 x 10^6 per well) were cultured in 96-well flat-bottom plates in 200 μL RPMI 1640 containing LPS (1 μg/ml) or sonicated B16F10 tumor lysate (at indicated concentrations). Culture supernatants were collected at 24 h. The IL-12 production was examined by ELISA using OptEIA Mouse IL-12 Detection Kit (BD Pharmingen) following the manufacturer’s instruction.

**Development of chimeras**

To generate bone marrow (BM) chimeras, CD44^+/+ mice were lethally irradiated (950 rads from a 153Cs source) and reconstituted with a total of 10 x 10^6 BM cells from appropriate donor mice (described in the Results section). Mice were allowed to reconstitute for at least 6 wk before the immunization.

**Statistical analysis**

The differences between experimental groups were analyzed using the Student’s *t* test with *p* < 0.05 being considered statistically significant.

**Results**

**SRBC-induced DTH and Ab responses are decreased in CD44^-/- mice**

To measure SRBC-triggered Th1 responses, we first induced DTH reaction that is considered to be Th1-driven (26, 27). The SRBC-sensitized mice were challenged in the ear and the ear swelling reflecting the activated T cell reaction was measured. Mice challenged with PBS served as negative controls and showed a small, nonspecific increase in ear thickness. As shown in Fig. 1A, the magnitude of DTH, as determined by ear swelling, in CD44^-/- mice was dramatically reduced compared with the CD44^+/+ mice. However, the time course of DTH, namely onset and decrease in ear swelling, remained the same in both groups of mice (Fig. 1A).

We next measured SRBC-induced Ab production by hemagglutination assay. On day 5 of the primary immunization, we found that the SRBC-specific Abs were produced in both CD44^-/- and CD44^+/+ mice, but CD44^-/- mice showed a significantly decreased level in comparison to CD44^+/+ mice (Fig. 1, B and C). Immunization of i.p. (Fig. 1B) or i.v. (Fig. 1C) produced similar results. We also measured anti-SRBC Ig level on day 8 of the primary immunization, which also showed a decreased response in CD44^-/- mice when compared with CD44^+/+ mice (data not shown).

**OVA-induced Ab in CD44^-/- mice: enhanced IgG1 and decreased IgG2a**

To measure OVA-triggered response, we immunized mice with OVA and measured OVA-specific IgM on day 10 of the primary immunization and OVA-specific IgG1 and IgG2a on day 7 of the secondary immunization. As shown in Fig. 2, all three Ig subtypes could be detected in both CD44^-/- and CD44^+/+ mice. Interestingly, however, IgM and IgG1 responses were dramatically increased while IgG2a response was significantly decreased in CD44^-/- mice in comparison to CD44^+/+ mice (Fig. 2).

**Serum cytokine profile: predominant Th2-cytokines in CD44^-/- mice**

Serum cytokine productions were detected following SRBC- and OVA-immunization by the Bio-Plex assay. Immunizations were performed as described above. For SRBC-induced cytokines, mice were immunized either by i.p. or i.v. route and sera were prepared on day 5 of the primary immunization. For OVA-induced cytokines, sera were prepared on day 10 of the primary immunization or day 7 of the secondary immunization. As shown in Fig. 3, IL-4, IL-5, IL-12, IL-13, IL-17, and IFN-\(\gamma\) were produced in both CD44^-/- and CD44^+/+ mice. The level of IL-4, IL-12, and IL-17 were much lower following SRBC immunization when compared with OVA immunization. Furthermore, Th1 cytokines, IFN-\(\gamma\) and IL-12, were down-regulated in CD44^-/- mice either with SRBC or OVA immunization. In contrast, Th2 cytokines, IL-4, IL-5, and IL-13 were up-regulated in CD44^-/- mice. Overall, these results suggested a Th2-predominant cytokine phenotype in CD44^-/- mice (Fig. 3).

**Up-regulated GATA3 and down-regulated T-bet in CD44^-/- mice**

The above results clearly demonstrated that Th1 immune response was down-regulated and Th2 immune response was up-regulated.
in CD44\(^{-/-}\) mice. To determine whether this phenotype could occur at the transcriptional level, we examined the expression of two transcription factors, T-bet and GATA3. Splenocytes were isolated on day 5 of the primary SRBC immunization and day 10 of the primary OVA immunization. After restimulation with PMA and ionomycin, the expression of T-bet or GATA3 was evaluated in CD4\(^{+}\) T cells by intracellular staining. Fig. 4 shows cells that were gated for CD4\(^{+}\) T cells and analyzed for T-bet and GATA3. Our results showed that percentage of CD4\(^{+}\) T-bet cells was decreased whereas the percentage of CD4\(^{+}\) GATA3 cells was increased in CD44\(^{-/-}\)/H11002 mice when compared with CD44\(^{+/-}\)/H11001 mice using both SRBC and OVA immunization protocols (Fig. 4). These results suggested that CD44 regulation may occur at the transcriptional level.

**CD44 expression on CD4 T cells affects Th development**

To further verify that the CD44 molecule expression on CD4 T cells regulates Th1-Th2 polarization, we investigated the SRBC-triggered response using BM chimeras. The chimeras were created as described by others (28). CD4\(^{-/-}\) mice were used as recipients that lack CD4 T cells but are CD44-sufficient. The chimeras were made by reconstituting the irradiated recipients with mixture of BM that consists of three parts of BM from recipient mice and one part BM from CD44\(^{-/-}\)/H11002 mice. Hence, the chimeras contained CD4 T cells from CD44\(^{-/-}\) BM and would be CD44 deficient (marked as CD44\(^{-/-}\)/H11001 in Fig. 5), while the other hematopoietic cell types would be derived from the recipient BM itself and therefore CD44 sufficient (28). Another group of chimeras was prepared by using three parts of BM from recipient mice and one part BM from WT mice, in

**FIGURE 5.** Anti-SRBC Ab and cytokines production in chimeras. A. Phenotype of chimeras. On the eighth week of the reconstitution, splenocytes from chimeras were triple-stained with CD44-PE, CD4-FITC, or CD8-FITC, and CD3 PE-Cy7. To study CD11c\(^{+}\) cells, they were enriched from the spleen and double-stained with CD44-PE and CD11c-FITC. To analyze B220\(^{+}\) cells, splenocytes were double-stained with CD44-PE and B220-FITC. B. Mixed bone marrow chimeras in which the CD4 T cells were either CD44 sufficient or deficient were generated as described in Materials and Methods. Reconstituted mice were i.p. immunized with SRBC and anti-SRBC Ab level was measured day 5 postimmunization by hemagglutination assay. CD4\(^{-/-}\) and CD44 wild-type mice (WT) were included as controls. The data represent mean \pm SE from groups of four mice. C. Measurement of serum cytokines in the same groups of mice. The differences in each of the cytokine production between the two groups was statistically significant (p < 0.01 for each cytokine).

**FIGURE 6.** Polyclonal stimulation leading to Th1-Th2 polarization in vitro. CD62L\(^{hi}\)/CD4 T cells from CD44\(^{+/-}\) and CD44\(^{-/-}\) mice and T cell-depleted splenocytes from CD44\(^{+/-}\) mice were cocultured in Th1- or Th2- polarization condition with addition of anti-CD3 and anti-CD28 Abs as described in Materials and Methods. On day 4 of the culture, cells were restimulated with PMA and ionomycin for 4 h. IFN-\(\gamma\) and IL-4 production were then evaluated by intracellular staining. The staining was analyzed by flow cytometry. The percentage represents positive cells gated on live cells.
which all hematopoietic cells, including CD4 T cells, were CD44+ (marked as CD44+/H11001/H11001). The phenotype of these chimeras was confirmed by flow cytometry. For this, the cells were gated for CD3, CD11c, or B220, and such gated cells were analyzed for combined expression of CD4 and CD44, CD8 and CD44, CD11c and CD44, or B220 and CD44. As seen from Fig. 5, chimeras designated CD44+/H11001/H11001/CD4 had 20.2% CD4+/H11001/CD44+/H11001/CD4 T cells whereas the CD44+/H11001/H11001/CD4 chimeras had only 1.6% CD4+/H11001/CD44+/H11001/CD4 T cells. Thus, almost all of the CD4+/H11001/CD4 T cells in CD44+/H11001/H11001/CD4 chimeras were CD44-deficient. The phenotyping also showed that the generation of chimeras did not influence the expression of CD44 in other cell types; the proportion of CD44+/H11001/CD4 T cells in each group was almost identical in the two types of chimeras (Fig. 5A).

Next, the chimeras, CD44+/H11001/H11001/CD4, and WT mice were immunized i.p. with SRBC. The anti-SRBC Ab and serum cytokines were examined on day 5 of immunization by hemagglutination assay and Bio-Plex assay as described in this text. As expected, we found that the chimeras with CD44+/H11001/CD4 T cell reconstitution showed minimal level of Ab production, which was similar to the level seen in CD4−/− mice. Dramatically, the chimeras with CD44+/H11001/CD4 T cell reconstitution exhibited full restoration of the Ab production that reached similar levels as that seen in the WT mice (Fig. 5B). Again, the serum cytokine profile correlated with these data (Fig. 5C). Together, these results indicated that CD44 expression on CD4 T cells contributes to the Th1-Th2 development.

CD44+ CD4 T cells polarize toward Th2 in vitro

The in vivo Th1 to Th2 shift in immune response seen in CD44−/− mice suggested that CD44 modulates CD4 T cell differentiation. To address this further, we performed an in vitro CD4 T cell polarization experiment. CD4 T cells from CD44+/H11001/CD4 or CD44+/H11001/CD4 mice were stimulated with anti-CD3 and anti-CD28 under Th1- or Th2-polarizing conditions. Intracellular expression of IL-4 and IFN-γ were determined on day 4. As shown in Fig. 6, when compared with CD44+/H11001/CD4 T cells, CD44−/− CD4 T cells showed decreased levels of polarization to Th1; whereas they readily differentiated into Th2 cells (Fig. 6).
Involvement of DCs

Although our studies demonstrated that CD44 expression on CD4 T cells played a role in Th1-Th2 differentiation, we wondered whether CD44-deficiency on DCs could also impact the Th1-Th2 polarization. Recent studies from our laboratory demonstrated that CD44 expression on DCs is indispensable to maintain normal T cell differentiation into Th1 or Th2 lineage. To the best of our knowledge, this is the first report that demonstrates the modulatory effects of CD44 on T cell differentiation both in vivo and in vitro.

CD44-deficient mice possess normal T cell development as compared with their WT counterparts. Our previous studies have showed that there is statistically no significant difference in the proportion of T cells (CD4+, CD8+, CD3+), B cells, and macrophages in the spleen and other peripheral organs between WT and CD44-deficient mice (22, 23). These results can rule out the possibility that biased Th development in our study was caused by differential development of T cell subsets in thymus.

When we reconstituted CD44+/− mice with CD44-deficient CD4 T cells, the hemagglutination Ab production against SRBC was diminished; however, this diminished response could be completely restored with CD44-sufficient CD4 T cells (Fig. 5B). In the in vitro Th1-Th2 polarization experiments, CD44-deficient CD4 T cells showed a less degree of Th1 differentiation than the WT counterparts (Fig. 6). These results suggested that in situ expression of CD44 on CD4 T cells is indispensable to maintain normal Th1 differentiation and response.

Discussion

Consistent with the paradigm of the two types of immune responses (Th1 vs Th2), we observed a Th2-biased immune response in CD44 knockout mice. Deficiency of CD44 inhibited Th1 development and promoted Th2 differentiation. This conclusion was supported by several findings in CD44−/− mice: 1) the cytokine profile in the serum from immunized mice was dominated by Th2 cytokines; 2) decreased IgG2a production along with the enhanced IgG1 production; 3) up-regulated GATA3 but down-regulated T-bet expression; 4) decreased response to SRBC as seen using DTH reaction and hemmagglutination; 5) in the in vitro culture system, CD44−/− CD4 T cells were less polarized to Th1, but more readily polarized to Th2. Together, these results suggested that CD44 participates in the regulation of CD4 T cell differentiation into Th1 or Th2 lineage. To the best of our knowledge, this is the first report that demonstrates the modulatory effects of CD44 on Th differentiation both in vivo and in vitro.
The differential expression of GATA3 and T-bet in this study suggests that the regulatory effect of CD44 could occur at the transcription level (Fig. 4). The intracellular domain of CD44 isoforms selectively interacts with different kinases or transducer proteins and regulates specific signaling that are related to the miscellaneous functions of the CD4 molecule (15, 16). Specifically, CD44 is tightly coupled with Src kinases, such as Lck and Fyn, and such signaling cascades induce tumor cell migration (16, 31). T-bet and GATA3 are activated through the JAK/STAT signaling pathway (4, 5). Accumulating evidence indicates that STAT activation can be mediated by members of both JAK and Src family. The integration of these diverse signaling cues from active Src, JAK, and STAT (Src-JAK-STAT model) leads to cell proliferation, survival, and differentiation (32, 33). We reckon that CD44 deficiency restructures its original signaling cascades and modulates Src-JAK-STAT interaction, which leads to the differential activation and expression of T-bet and GATA3, and thereafter, Th1-Th2 differentiation.

In vitro differentiation using CD44-sufficient and –deficient DCs produced a similar pattern of Th1-Th2 differentiation (Fig. 8A). It suggested that CD44 also influences DC effect on CD4 T cell differentiation. CD44-deficient DCs showed a decreased ability to produce IL-12 in response to the stimulation of LPS and melanoma Ag (Fig. 8B). This could be one factor that contributes to the DC instruction toward Th2 differentiation. However, there are other cell sources in vivo for the IL-12 production. As cited, macrophages are another main source of IL-12 and modulators of Th1-Th2 differentiation (8, 29). Macrophage subsets, M1 and M2, initiate and regulate Th1 or Th2 response, respectively (8). Blockade of CD44 with Abs can inhibit IL-12 production from the thiglycollate-activated macrophages (34). Therefore, it is plausible that CD44-deficient macrophages may contribute to the decreased IL-12 production in the serum in our study. Nevertheless, this environment could modify functionalities of DCs that may render CD44-deficient DC a Th2-biased polarizing feature.

The pattern of DC subsets showed a higher percentage of mDC, a decreased proportion of pDC and LDC in CD44-deficient mice when compared with CD44-sufficient mice (Fig. 7). These data may be useful for further dissection of differential role of CD44-deficiency on each DC subset in Th development. Other studies have demonstrated that OVA or keyhole limpet hemocyanin-loaded LDC promote Th1 differentiation and mDC promote Th2 differentiation (9, 10). We assume that SRBC-loaded mDC and LDC may also follow the same pattern. Activation of pDC by respiratory syncytial virus and measles virus profoundly promote Th1 and suppress Th2 immune response (35). Also, Toxoplasma gondii-activated pDC enhance Th1 immune response (36). pDC is also indispensable for conventional DCs to produce IL-12 in response to Listeria monocytogenes infection (37). It is known that pDC play a more flexible role in directing either Th1 or Th2 development that is dependent on nature and dose of Ag, immunization route, differential TLR ligation, and other factors (38–40). We assume that pDC, in our system, may promote Th1 development. It seems that the DC subsets present in CD44-deficient mice, after immunization, favored an up-regulated Th2 and down-regulated Th1 differentiation. However, further functional studies using isolated CD44-deficient DC subsets are needed in extending the results and determining which subset plays critical role in instruction of Th1-Th2 differentiation.

In addition to the effect of CD44 on CD4+ T cells and DCs, the possibility that CD44 influences the B cells also remains. It should be noted that in our previous studies, CD44 expression on B cells affect their differentiation and Ig production while certain Abs against CD44 blocked B cell activation induced by agents such as LPS (41, 42).

Interestingly, in the current study, we noticed a decreased IL-17 production in CD44-deficient mice (Figs. 3 and 5B). Recent studies have suggested that IL-17 deficiency can result in both compromised DTH reaction and T-dependent humoral immune response (5, 43). In contrast, T-bet is important for continued IL-17 production in the presence of IL-23 and regulates the fate of Th1 and Th17 cells in autoimmunity (44, 45). We also found that the number of Th17 cells in SRBC-immunized CD44−/− mice was decreased, and the differentiation to Th17 lineage was also inhibited in CD44−/−CD4 T cells (data not shown). We speculate that CD44 modulation on T-bet/IL-17/Th17 interaction may form a unique mechanism in the regulation of Th development. We are currently testing this hypothesis.

Our study highlights CD44 as a player in Th development. These data may contribute toward better understanding of the pathogenesis of some immune diseases and development of a CD44-targeted modality in treatment of T cell-elicited immune diseases. For example, recently, we found that CD44-deficient mice revealed attenuated multiple sclerosis in the experimental autoimmune encephalomyelitis model (manuscript under preparation). These findings correlated with decreased myelin-specific Th1 and Th17 cells and increased myelin-specific Th2 cell response. Such a shift is known to suppress experimental autoimmune encephalomyelitis, thereby further corroborating the data presented in the current study.

Disclosures

The authors have no financial conflict of interest.

References


