Cutting Edge: CD94/NKG2 Is Expressed on Th1 But Not Th2 Cells and Costimulates Th1 Effector Functions


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Th1 and Th2 cells can be phenotypically distinguished by very few cell surface markers. To identify cell surface molecules that are specifically expressed on Th1 cells, we have generated a panel of mAbs that specifically bind the surfaces of murine Th1 but not Th2 cells. One of these Abs identified the NK cell receptor CD94 as a molecule also specifically expressed on the surface of Th1 cells. As in NK cells, CD94 is expressed on Th1 cells together with members of the NKG2 family of molecules, including NKG2A, C, and E. Cross-linking these receptors on differentiated Th1 cells in vitro costimulates proliferation and cytokine production with a potency similar to that obtained by cross-linking CD28. We propose that CD94/NKG2 heterodimers may costimulate effector functions of differentiated Th1 cells. The Journal of Immunology, 2002, 169: 5382–5386.

Activated CD4+ Th cells can be functionally divided into two major subsets, Th1 and Th2. First defined in 1986 (1), these subsets are generally distinguished by their actions, including their production of specific cytokines and involvement in different types of immune reactions. Th1 cells produce IFN-γ, IL-2, TNF, and lymphotoxin and participate in cell-mediated responses to intracellular pathogens, whereas Th2 cells produce IL-4, IL-5, IL-9, IL-10, and IL-13 and are involved in responses to large extracellular pathogens such as helminths (2, 3). The cytokines that drive helper T cell differentiation (IL-12 for Th1 and IL-4 for Th2) have been well characterized, but exactly how differentiation occurs is just beginning to be understood. In addition, a detailed surface phenotype of either subset that could unambiguously distinguish it from the other has not been defined (4). Although specific chemokine receptors are preferentially expressed on different subsets of lymphocytes, these differences are largely quantitative (4). The first steps have been taken toward the description of unique surface phenotypes for these cells by the identification of T1/ST2 as a stable marker for Th2 cells (5) and Tim-3 as a Th1 marker (6). We have chosen to seek additional Th1-specific molecules, because these would play a role in Th1 activation, differentiation, and/or effector function. They would therefore be useful not only for the detection of Th1 cells during an immune response but also for analysis of their functions.

To more clearly define the molecules that are selectively expressed on the surface of Th1 cells, we have generated a panel of mAbs that selectively recognize Th1 cells and have taken an expression cloning approach to the identification of their targets. Here we identify the NK cell receptor CD94 (7) as a molecule expressed on differentiated Th1 cells but not on Th2 cells. This receptor is coexpressed, as it is in NK cells, with the NKG2 family of proteins, and the entire array of CD94/NKG2 receptors (both activating and inhibitory varieties) appears to be expressed in Th1 cells. Preliminary functional assays suggest that these NK receptors could costimulate the expansion and cytokine production of differentiated Th1 cells.

Materials and Methods

Cells and Abs

The AE7, D10G4, and 7AS clones and DO11.10 transgenic mice have been previously used in our publications (6). The 2D2 Th1 clone is specific for MOG35–55/IAα, and Q1.4A11 and Q1.3C11 are Th2 clones specific for an altered peptide of PLP139–151, Q144, recognized in the context of IAα (all generated in our laboratory).

Abs to CD94 (18d3) and NKG2A/C/E (20d5), as well as Abs to IFN-γ (XMG1.2), IL-10 (JES5-16E3), IL-4 (11B11), CD4 (GK1.5), CD62L (MEL-14), and CD44 (IM7), and streptavidin-PE were from BD PharMingen (San Diego, CA). FITC- and PE-labeled goat anti-rat IgG Abs were from Caltag Laboratories (Burlingame, CA).

In vitro T cell polarization

For the generation of DO11.10 Th1 and Th2 cells, CD62LhighCD44low-sorted naïve DO11.10 transgenic T cells were stimulated in vitro for 7 days with OVA257-264 peptide (10 μg/ml; Quality Controlled Biochemicals, Hopkinton, MA) and irradiated BALB/c splenocytes under polarizing conditions (Th1, 5 ng/ml mIL-12 (BD PharMingen) and 10 μg/ml anti-mIL-4; Th2, 10 ng/ml mIL-4 (R&D Systems, Minneapolis, MN) and 10 μg/ml anti-mIL-12 (BD PharMingen)). Cells were subjected to four rounds of polarization and were stained for intracellular cytokine production as described previously (6) and for surface staining with 18D1 on day 10 after each round.
Generation of Th1-specific mAb

Female Lewis and Lou/m rats (Harlan Sprague Dawley; Harlan Breeder, Indianapolis, IN) were immunized three times with s.c. injections of 1 or 3 \times 10^5 Th1-polarized T cell clones and/or lines. Spleen cells from these rats were fused with myeloma cells, and supernatants thus obtained were screened by flow cytometry on Th1 and Th2 cells. Selected hybridomas that stained all Th1 but no Th2 cells were subcloned.

Expression cloning

A eukaryotic expression library was constructed using mRNA from the AE7 Th1 clone and the pAXE7 vector. Library screening was performed through expression cloning as previously described (8). Immunosellected individual plasmids were transiently transfected into COS cells followed by indirect immunofluorescence staining with the 18D1 mAb. Positive clones were sequenced. From one of these positive clones, a stable CD94 transfectant was made in HEK 293 cells using pcDNA3.1+ (Invitrogen, San Diego, CA) with 1 mg/ml G-418 (Life Technologies) as the selective agent.

RT-PCR

RNA was prepared from 10^6 cells using the Trizol reagent (Life Technologies, Gaithersburg, MD), followed by cDNA synthesis by the SuperScript protocol (Life Technologies). CD94 was amplified from the cDNA using the primers mCD94-Xho-Koz and mCD94-3′-HA-Bam (7). Primers used for specific amplification of NKG2 family members are NKG2A5, NKG2A3 (9), NKG2C3, NKG2C3/E3 (10), NKG2E5 (11), NKG2D5, and NKG2D3 (12). Thirty-five cycles of PCR were performed, using 2 μl cDNA under the following conditions: 94°C for 45 s, 60°C for 2 min (94°C for 75 s) for 35 cycles; 72°C for 10 min.

Proliferation and cytokine ELISAs

Latex beads (5.2 μm in diameter; Interfacial Dynamics, Portland, OR) were incubated at 10^7/ml in PBS with anti-CD3 (0.5 or 0.25 μg/ml) at 37°C for 30 min and then centrifuged at 3500 rpm for 15 min. The beads were then incubated as above with anti-CD94, anti-CD3, anti-NKG2A/C/E, and/or rlgG at 4, 2, 1, and 0.5 μg/ml (rlgG was used to adjust the total amount of protein in each reaction to equal that in the anti-CD94 plus anti-NKG2A/C/E sample). Beads were washed, resuspended in clone medium, and added to round-bottom 96-well plates at 10^5 or 5 \times 10^5 beads/well. DO11.10 Th1 cells were then plated at 5 \times 10^5 cells/well. After 48 h, supernatants were removed for cytokine analysis, and plates were pulsed with 1 μCi [3H]thymidine per well for 16 h. The incorporated thymidine was measured with a beta plate scintillation counter (PerkinElmer Wallac, Gaithersburg, MD). Cytokine analysis was performed by quantitative capture ELISA (BD PharMingen). Assays were developed with TMB Microwell Peroxidase Substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and read at 450 nm.

Results and Discussion

Generation and characterization of a mAb to a Th1-specific cell surface molecule

To generate Abs specific to the surface of Th1 cells, we immunized Lewis and Lou/M rats with the established murine T cell clone AE7 and a Th1 cell line derived from DO11.10 TCR-transgenic mice. A panel of ~20,000 mAbs was thus produced and screened on polarized Th1 and Th2 T cell clones and cell lines. One of these mAbs, named 18D1, selectively recognized a molecule common to all four Th1 cells tested but did not bind any Th2 cells (Fig. 1A).

To determine the expression pattern of the molecule recognized by the 18D1 mAb during Th cell differentiation, we isolated naive CD4+ T cells from DO11.10-transgenic mice and activated them in vitro under polarizing conditions to induce their differentiation into either the Th1 or Th2 subset. As shown in Fig. 1A, staining by 18D1 mAb was absent on naive helper T cells but appeared on Th1 cells by the third round of polarization and did not appear on Th2 cells through four rounds of polarization. These data suggest that the molecule recognized by 18D1 is expressed selectively on differentiated Th1 cells and not on naive T or differentiated Th2 cells.

Expression cloning of CD94 as a molecule expressed on Th1 cells

Expression cloning using the 18D1 mAb and a cDNA library from Th1 clone AE7 selected clones containing a single, ~500 bp
CD94/NKG2 receptors costimulate proliferation and cytokine production of Th1 cells

The observation that CD94/NKG2 dimers are specifically expressed on differentiated Th1 cells suggests that these molecules might play a role in Th1 cell effector function. To address this possibility, we tested the effects of cross-linking CD94 and NKG2 molecules on in vitro Th1 cell activation. Latex beads coated with anti-CD3, anti-CD94, and anti-NKG2A/C/E were used to activate Th1 cell lines. Co-cross-linking of CD3 and CD94/NKG2 receptors on the surfaces of DO11.10-polarized Th1 but not Th2 cells resulted in a marked increase in proliferation and production of IFN-γ and TNF-α over that induced by anti-CD3 stimulation alone (Fig. 4A and data not shown). To address whether this costimulatory effect was due to cross-linking of CD94, NKG2, or both, we coated beads with anti-CD3 plus either anti-CD94 or anti-NKG2A/C/E. In these experiments, beads coated with anti-CD3 plus rat IgG were used as a negative control and anti-CD3 plus anti-CD28 was used as a positive control. When DO11.10-polarized Th1 cells were stimulated with these beads in vitro, anti-CD3 plus anti-CD94 cross-linking led to a small but definite increase in proliferation and cytokine production over the anti-CD3 plus rat IgG control, and anti-CD3 plus anti-NKG2A/C/E stimulation by the mAbs led to marked increases in proliferation (Fig. 4B). The co-cross-linking by all three mAb (anti-CD3 plus anti-CD94 plus anti-NKG2)-bearing beads resulted in additional activation in terms of proliferation and cytokine production over anti-CD3 plus anti-NKG2 cross-linking, with a particularly large increase in IFN-γ production. In some experiments, however, we saw very little difference between cross-linking of CD3 plus CD94 plus NKG2 and that of CD3 plus NKG2, although both induced a significant increase in proliferation and IFN-γ production over anti-CD3 plus...
oloff et al. (22) found that CD4+ T cells induce the effector functions of their Th1 cells. Additionally, Nickerson et al. (23) found that their DAP12 knockout mouse produced lower levels of IFN-γ on Ag-specific activation and was resistant to induction of the Th1-mediated disease experimental autoimmune encephalomyelitis. In light of the present data, it seems likely that CD4+ T cell responses and cytokine production from Th1 cells. In addition, CD94/NKG2 molecules could be involved, as in NK cells and CD8+ T cells (13, 17, 23), in modulating the cytotoxic functions of Th1 cells. CD28 costimulation has been shown to be required not only for the activation and expansion of naive T cells but also for their effector functions and survival (24). As not all tissues express B7 molecules, it is possible that CD94/NKG2 could evoke effector functions and prevent Th1 cell anergy in tissues that lack B7. This possibility is supported by data showing that CD94/NKG2 expression appears on T cells as CD28 is down-regulated during activation (25). However, whether CD94/NKG2 receptors costimulate a positive signal or dampen a negative signal remains to be seen. Although these Th1 cells express the inhibitory molecule NKG2A as well as stimulatory NKG2 molecules (Fig. 3), it is possible that CD94/NKG2 could override this potential inhibition.

In summary, CD94/NKG2 receptors, which were originally thought to be important specifically to NK cell function, are also expressed on differentiated Th1 cells and may play an important role in costimulating and tuning their effector functions.

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References

FIGURE 4. CD94/NKG2 costimulate Th1 expansion and cytokine production. A, DO11.10 Th1 cells (5 × 10^5/well) were incubated with 10^5 beads/well coated with 0.5 μg/ml anti-CD3 and either rlgG or anti-CD94 (18d3) and anti-NKG2A/C/E (20d5). After 48 h, culture supernatants were removed for cytokine analysis by ELISA, and cells were pulsed with 1 μCi [3H]thymidine/well for 16 h. Ab concentrations shown on the x-axis are of anti-CD94, anti-NKG2, or one-half of total rlgG concentration. B, DO11.10 Th1 cells were incubated as above with 10^5 beads/well coated with 0.25 μg/ml anti-CD3 together with various concentrations of other Abs as shown in the legend. Similarly to A, the x-axis value is the concentration of anti-CD94, anti-NKG2, anti-CD28, or rlgG. An equal amount of rlgG was added to each sample except the anti-CD94- plus anti-NKG2-coated beads. Cytokines were analyzed by ELISA, and proliferation was measured by [3 H]thymidine incorporation.

The data presented in this paper clearly demonstrate that CD94/NKG2 receptors are expressed on Th1 cells, and cross-linking these receptors together with CD3/TCR costimulates proliferation and cytokine production from Th1 cells. In addition, CD94/NKG2 molecules could be involved, as in NK cells and CD8+ T cells (13, 17, 23), in modulating the cytotoxic functions of Th1 cells. CD28 costimulation has been shown to be required not only for the activation and expansion of naive T cells but also for their effector functions and survival (24). As not all tissues express B7 molecules, it is possible that CD94/NKG2 could evoke effector functions and prevent Th1 cell anergy in tissues that lack B7. This possibility is supported by data showing that CD94/NKG2 expression appears on T cells as CD28 is down-regulated during activation (25). However, whether CD94/NKG2 receptors costimulate a positive signal or dampen a negative signal remains to be seen. Although these Th1 cells express the inhibitory molecule NKG2A as well as stimulatory NKG2 molecules (Fig. 3), it is possible that the cumulative activation of the TCR and stimulatory CD94/NKG2/C/E receptors could override this potential inhibition.

In summary, CD94/NKG2 receptors, which were originally thought to be important specifically to NK cell function, are also expressed on differentiated Th1 cells and may play an important role in costimulating and tuning their effector functions.


