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Immunology

T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function

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ABSTRACT **T helper (Th) cells can be categorized according to their cytokine expression. The differential induction of Th cells expressing Th1 and/or Th2 cytokines is key to the regulation of both protective and pathological immune responses. Cytokines are expressed transiently and there is a lack of stably expressed surface molecules, significant for functionally different types of Th cells. Such molecules are of utmost importance for the analysis and selective functional modulation of Th subsets and will provide new therapeutic strategies for the treatment of allergic or autoimmune diseases. To this end, we have identified potential target genes preferentially expressed in Th2 cells, expressing interleukin (IL)-4, IL-5, and/or IL-10, but not interferon-γ. One such gene, T1/ST2, is expressed stably on both Th2 clones and Th2-polarized cells activated *in vivo* or *in vitro*. T1/ST2 expression is independent of induction by IL-4, IL-5, or IL-10. T1/ST2 plays a critical role in Th2 effector function. Administration of either a mAb against T1/ST2 or recombinant T1/ST2 fusion protein attenuates eosinophilic inflammation of the airways and suppresses IL-4 and IL-5 production *in vivo* following adoptive transfer of Th2 cells.**

T helper (Th) cells mediate effector functions in infectious, allergic, or autoimmune diseases through production of cytokines (1, 2). Following repeated antigenic stimulation, T cell populations preferentially producing particular combinations of cytokines can be identified *in vitro* or *ex vivo*. An operational definition by which Th1 cells produce mostly interferon (IFN)-γ, interleukin (IL)-2, or tumor necrosis factor (TNF)-β, whereas Th2 cells produce mainly IL-4 or IL-5, has proven useful in the analysis of Th cell function under physiological and pathological conditions, although such categorization is an oversimplification. Aside from their transient cytokine production, less is known about other differences between Th1 and Th2 cells. Given the importance of immunoregulation by Th cytokines, reliable surface markers distinguishing murine Th subsets from one another would be invaluable research tools.

To identify Th2-specific molecules we used differential display analysis of murine Th cells polarized into either Th1 or Th2 lines *in vitro*. One cDNA, specifically expressed in Th2 cells, was analyzed further. This cDNA had been cloned earlier by several groups investigating delayed early response genes and was called T1 (3) or ST2 (4). T1/ST2 is an orphan receptor with >25% sequence homology to the type I IL-1 receptor (4–6). However, T1/ST2 does not bind IL-1α, IL-1β, or IL-1RA (5–7) and its natural ligand is not known.

We show here at the single cell level that T1/ST2 is preferentially expressed on murine Th cells expressing predominantly IL-4, IL-5, or IL-10, but not IFN-γ or IL-2 *in vitro* and *ex vivo*. Expression of T1/ST2 is independent of IL-4, IL-5, or IL-10 because it is also present in the respective cytokine-deficient mice. Furthermore, in a murine model of Th2-dependent allergic airway inflammation, administration of either a mAb against T1/ST2 or T1/ST2 fusion protein markedly reduced the induction of a lung mucosal Th2 immune response *in vivo*.

MATERIALS AND METHODS

Animals. Mice transgenic for the ovalbumin (OVA)-specific DO11.10 T-cell antigen receptor (TCR) (8) were from D. Loh (Washington University, St. Louis) and maintained on the BALB/c background. IL-4-deficient (IL-$4^{-/-}$) BALB/c mice (9) were from R. Coffman (DNAX, Palo Alto, CA). IL-$5^{-/-}$ C57BL/6 mice (10) were from M. Kopf (Basel Institute for Immunology), and IL-$10^{-/-}$ mice (11) and their heterozygous littermates were from T. Blankenstein (MDC, Berlin). Mice were kept under pathogen-free conditions in accordance with institutional and state guidelines.

Generation of Th1 or Th2 Cells *in Vitro*. The OVA peptide $OVA_{323–339}$ was a gift of A. Kramer (Humboldt Universität, Berlin). DO11.10 TCR-transgenic $CD4^+$ T cells were cultured in complete RPMI 1640 medium with $OVA_{323–339}$ (1 μM) and irradiated BALB/c splenocytes. For Th1 phenotype development, recombinant murine IL-12 (gift of M. Gately, Hoffmann–La Roche, Nutley, NJ) and neutralizing anti-IL-4 mAb 11B11 (12) were added, and for Th2 development recombinant murine IL-4 (BioSource International, Camarillo, CA) and neutralizing anti-murine IL-12 (M. Gately) were added as described (13). Cultures were maintained for 7 days. In some experiments several rounds of antigenic stimulations under polarizing conditions were performed as indicated.

Differential Display Analysis and Cloning of T1/ST2. RNAs were harvested from DO11.10 TCR-transgenic T cell lines. Cells underwent two rounds of antigenic stimulation under Th1- or Th2-polarizing conditions. RNA was prepared 1 week after the second stimulation using RNAzol (Tel-Test, Friendswood, TX) followed by DNAse treatment (Boehringer Mannheim). Differential display analysis was performed as de-

Abbreviations: Th, T helper; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; BAL, bronchoalveolar lavage; TCR, T-cell antigen receptor; FITC, fluorescein isothiocyanate; PE, phycoerythrin; DIG, digoxigenin; wt, wild type; PMA, phorbol 12-myristate 13-acetate; MACS, magnetic cell separation system.

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scribed (14). In brief, RNA was reverse transcribed by Superscript reverse transcriptase (GIBCO) with T11GG oligos (Operon Technologies, Alameda, CA). PCR was performed with the reverse transcription reaction, dNTPs (Pharmacia), Amplitaq (Perkin–Elmer), ^{35}S-labeled dATP (Dupont/NEN), Operon oligo OP-D11 (5′-AGCGCCATTG-3′), and T11GG. The reaction mix was run on a 6% denaturing gel. Portions of the gel containing differentially expressed genes were excised and DNA was eluted, phenol extracted, ethanol precipitated, and reamplified as described (14). Amplified sequences were cloned into the TA vector (Invitrogen), sequenced, and checked against published sequences.

Generation of mAb 3E10. A DNA sequence containing the extracellular domain of T1/ST2 was PCR amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region (gift from B. Seed, Harvard Medical School, Boston). COS cells were transiently transfected by using the lipofectamine (GIBCO) protocol. Cells were cultured in Ultra-Low Ig fetal bovine serum (GIBCO) for 1 week. The recombinant protein was purified from culture supernatants by passage over a protein A column. Lou/M rats were immunized s.c. with 0.5 mg purified recombinant T1/ST2. Rats were boosted i.p. twice at 2-week intervals. Sera were analyzed for reactivity to the fusion protein by ELISA 10 days after the last boost. Four weeks later, positive animals were boosted again and sacrificed 3 days after. Splenocytes were fused with SP/2 myeloma cells and resulting clones screened for selective binding to murine Th2 clones. Serum from unimmunized Lou/M rats was used as negative control. Hybridoma 3E10 produced a rat IgG1 anti-T1/ST2 mAb.

Antibodies and Flow Cytometry. For flow cytometric analysis of various lymphocyte subsets, the following phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated mAbs were used: CD4 (GK1.5), CD8 (53–6.7), CD11b (Mac-1; M1/70), F4/80, CD11c (N418), CD45R (B220; RA3–6B2), CD62L (L-selectin; MEL-14), major histocompatibility complex class II (M5/114), and NK1.1 (PK136; PharMingen). To prevent unspecific binding of the T1/ST2-specific mAb (3E10), all samples were preincubated with blocking anti-Fcγ-R mAb 2.4G2/75 (100 μg/ml) and purified rat IgG (200 μg/ml; Nordic, Tilburg, The Netherlands) 10 min prior to and during staining with digoxigenized 3E10 (1.5 μg/ml). 3E10-labeled cells were detected by anti-digoxigenin (DIG) Fab fragments (Boehringer Mannheim) conjugated to Cy5 or magnetofluorescent liposomes (15), as indicated. Staining of 3E10 was blocked by preincubating the cells with a 100-fold excess of unconjugated 3E10. Samples were analyzed on a fluorescence-activated cell sorter (FACS)-Calibur (Becton Dickinson). Gates were set on viable cells according to forward and sideward scatter and exclusion of propidium iodide-binding particles (0.3 μg/ml).

Separation of T1/ST2-Expressing Cells *in Vitro* and *ex Vivo*. T1/ST2-expressing cells were detected among mixed Th1- and Th2-polarized DO11.10 cells *in vitro* by staining with digoxigenized 3E10, followed by anti-DIG-Fab fragments conjugated to magnetofluorescent liposomes (15). To prevent unspecific binding of the 3E10 mAb, all samples were preincubated with blocking anti-Fcγ-R mAb and purified rat IgG as described above. 3E10$^+$ cells were enriched by positive selection on VS$^+$ columns by using the high-gradient magnetic cell separation system MACS (Miltenyi Biotec, Berg.-Gladbach, Germany), as described (16). Spleen and lymph node cells from unimmunized wild-type (wt) and IL-4$^{-/-}$ BALB/c mice were stained *ex vivo* with digoxigenized 3E10, and subsequently incubated with anti-DIG-MACS microbeads for magnetic labeling and anti-DIG-Cy5 for fluorescent detection. 3E10$^+$ cells were enriched on VS$^+$ columns by MACS.

Analysis of Cytokine Production by Flow Cytometry and ELISA. Analysis of intracellular cytokines and surface markers were performed as described (17). Unsorted and separated cells (10^6/ml) were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml) and ionomycin (1 μg/ml; Sigma) for 5 hr. At 2 hr, Brefeldin A (Sigma) was added at 5 μg/ml. Before fixation, T1/ST2 was stained with digoxigenized 3E10, detected by anti-DIG-Cy5. Stained cells were fixed with 2% formaldehyde. Cells were permeabilized and incubated with biotinylated anti-CD4 (GK1.5; 10 μg/ml) and two of the following mAbs (PharMingen): anti-IL-4-PE (1D11; 3 μg/ml), anti-IL-5-PE (TRFK5; 3 μg/ml), anti-IL-10-FITC (JES5–2A5; 1 μg/ml), anti-IL-2-FITC (S4B6; 5 μg/ml), anti-IFN-γ-FITC (XMG1.2; 5 μg/ml), or anti-TNF-α-PE (MP6-XT22; 2 μg/ml), followed by streptavidin-PerCP (1 μg/ml; Becton Dickinson). FITC- or PE-labeled isotype control mAbs (PharMingen) were used at 5 and 3 μg/ml, respectively. Samples were analyzed by four-color flow cytometry and gates were set on CD4$^+$ lymphocytes. For ELISA, cells were stimulated with PMA and ionomycin as above. After 24 hr, the cytokine concentrations in the supernatants were determined as described (16).

Adoptive Transfer and Aerosol Administration of OVA. DO11.10 TCR-transgenic cells were polarized *in vitro* as described above. Viable cells were harvested and further incubated for 48 hr in IL-2 alone. Th2 cells (2×10^6) were injected i.v. into recipient BALB/c mice. Starting 24 hr later, mice were exposed daily to an aerosol of OVA (50 mg/ml; Sigma) for 20 min for 2 consecutive days. Control mice were either injected with Th2 cells and exposed to an aerosol of PBS or were exposed to OVA without cell transfer. Mice received either 20 μg of mAb against T1/ST2, 20 μg recombinant T1/ST2–IgG fusion protein, or 20 μg of either rat or human IgG as isotype control 1 hr before aerosol exposure. Mice were sacrificed on day 3.

Bronchoalveolar Lavage (BAL). Twenty-four hours after the last challenge, mice were anaesthetized with 0.3 ml of 14% urethane i.p. and the trachea cannulated. BAL was performed by injecting 0.3 ml of PBS into the lungs. The fluid was then withdrawn and stored on ice. This procedure was repeated a total of four times. The cell suspension was centrifuged and the supernatant frozen. The cell pellet was resuspended in 1 ml of PBS and total cell counts obtained. Cytospins were prepared and stained with Diff-Quik (Baxter, Deerfield, IL). Two hundred cells were counted differentially by using standard morphological criteria. Cytokine levels were measured in the BAL fluid by ELISA (PharMingen).

RESULTS

Identification and Characterization of T1/ST2. Differential display PCR identified a gene expressed in Th2 but not Th1 cells. Sequence analysis showed that this gene was identical with T1/ST2 (3, 4). Northern blot analysis using polarized TCR-transgenic T cell lines and a panel of Th1 and Th2 clones confirmed expression of T1/ST2 exclusively in Th2 cells (data not shown). Recombinant T1/ST2 was expressed and a rat IgG1 mAb 3E10, specific for T1/ST2 was produced. FACS staining with 3E10 revealed exclusive staining of Th2 clones (not shown).

Expression of T1/ST2 Correlates with Th2 Cytokine Production *in Vitro*. Spleen cells from DO11.10 TCR-transgenic mice were primed *in vitro* with peptide in the presence or absence of IL-4 and anti-IL-12, and restimulated with peptide, antigen-presenting cells, and cytokines. Ten days after the second stimulation, T cells that had or had not been exposed to IL-4 and anti-IL-12 were mixed, stained with mAb 3E10 using magnetofluorescent liposomes, and sorted by MACS. The T1/ST2$^+$ population was enriched approximately 20-fold from 4.7% in the unsorted cell population to 90% T1/ST2$^+$ cells in the MACS-sorted, enriched fraction (Fig. 1*A*). Unsorted cells and the T1/ST2-enriched or -depleted cell fractions were stimulated with PMA/ionomycin. The concentra-

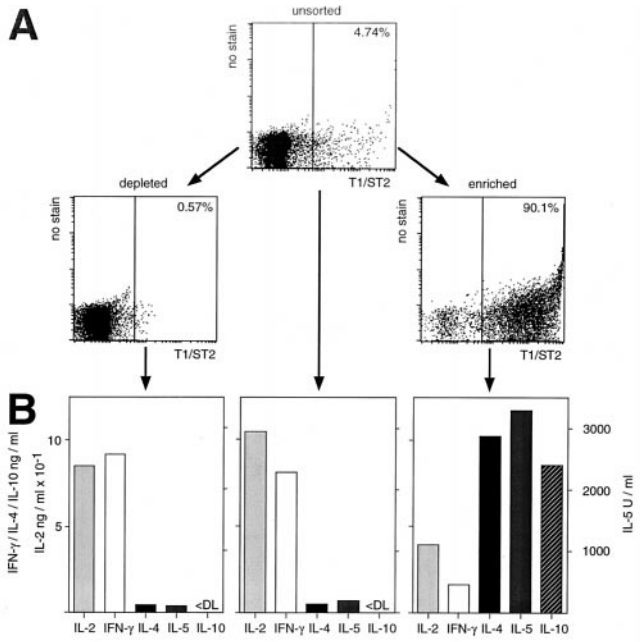

FIG. 1. Cytokine production of T1/ST2$^+$ Th cells *in vitro*. (*A*) Spleen cells from DO11.10 TCR-transgenic mice were primed *in vitro* in the presence or absence of IL-4 and anti-IL-12. One week later, the T cells were restimulated *in vitro* with peptide and antigen-presenting cells. Again, one culture received IL-4 and anti-IL-12 for further Th2 polarization, whereas the other culture did not receive additional cytokines. Ten days later, Th cells from both cultures were mixed, stained with anti-T1/ST2 mAb using magnetofluorescent liposomes, and sorted by MACS. Percentages of T1/ST2$^+$ cells are indicated. (*B*) Unsorted cells and the T1/ST2-enriched or -depleted fractions were stimulated with PMA/ionomycin. Twenty-four hours later the cytokine concentrations in the culture supernatants were determined by ELISA. Values for IL-10 in the unsorted and depleted cell fractions were below the detection limit (<DL) of 0.5 ng/ml.

tions of IL-2, IFN-γ, IL-4, IL-5, and IL-10 in the supernatants were determined by ELISA at 24 hr (Fig. 1*B*). The T1/ST2-enriched T cell fraction produced approximately 20-fold more IL-4 and IL-5 than the unsorted cells. IL-10 was only detectable in the enriched population. In contrast, the IL-2 and IFN-γ production of the T1/ST2$^+$ cells was reduced 2- and 5-fold, respectively, as compared with unsorted Th cells.

Expression of T1/ST2 on Spleen Cells *ex Vivo*. Freshly isolated spleen cells from normal BALB/c mice were stained for T1/ST2 and either CD4, CD8, CD45R, CD11b, or CD11c. For the different panels of Fig. 2 different numbers of cells (up to 10^6) were acquired such that the upper quadrants of each dot plot contain approximately 5,000 events. Approximately 1% of splenic CD4$^+$ cells expressed T1/ST2. The data shown in Fig. 2 demonstrate the specificity of this staining because it was completely blocked by excess 3E10 mAb. CD8$^+$ cells did not express T1/ST2. The CD45R staining shows that some B cells unspecifically bind the 3E10 mAb (Fig. 2*B*). Specific blocking with excess unlabeled 3E10 (Fig. 2*C*) does not alter the number of cells in the upper right quadrant, thus indicating that these cells were stained unspecifically. The number of cells in the lower right quadrant is clearly reduced in *C* as compared with *B* of the CD45R staining. Neither CD11b^{hi} macrophages, nor CD11c^{hi} dendritic cells were stained with 3E10. In addition, neither cells that were positive for the macrophage marker F4/80 nor major histocompatibility complex class II-expressing cells coexpressed T1/ST2 (data not shown). By four-color flow cytometric analysis of B6 spleens we detected T1/ST2 on the surface of only CD4$^+$ NK1.1$^-$ cells, and not on CD4$^+$ NK1.1$^+$ or CD4$^-$ NK1.1$^+$ cells. Similar patterns of T1/ST2 expression were obtained with lymph node cells (data not shown). We analyzed the differentiation status of the CD4$^+$ subpopulation expressing T1/ST2. Splenocytes were simultaneously stained for CD4, CD62L, and T1/ST2. T1/ST2 was expressed almost exclusively by antigen-experienced (CD62L^{lo}; 2.5%), but not by naive (CD62L^{hi}; <0.1%) CD4$^+$ cells (Fig. 3). 3E10 staining of CD62L^{lo} cells was completely blocked with excess unlabeled 3E10 (not shown).

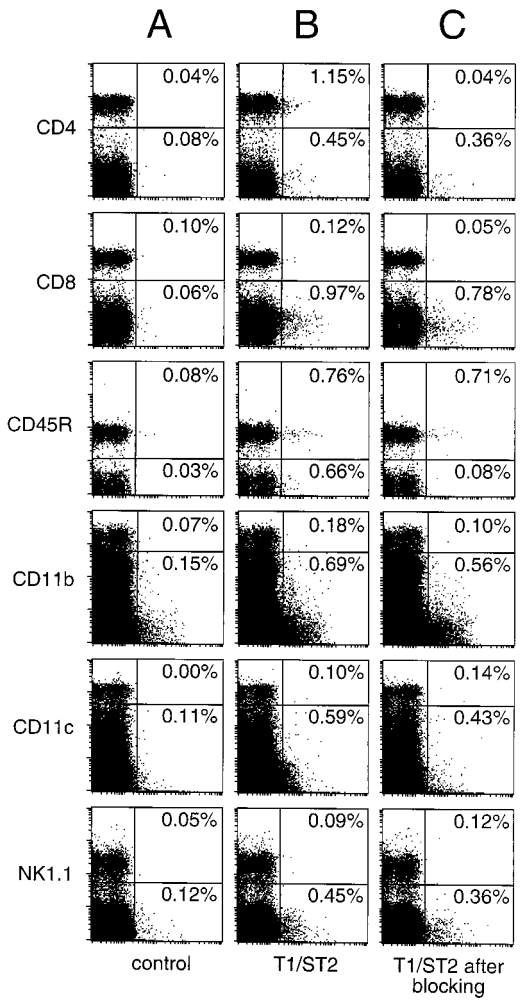

FIG. 2. Expression of T1/ST2 on spleen cells *ex vivo*. Spleen cells from BALB/c mice were stained with digoxigenized mAb against T1/ST2 (3E10), followed by Cy5-conjugated anti-DIG and PE- or FITC-conjugated mAbs against either CD4, CD8, CD45R, CD11b, or CD11c. B6 spleens were used for the detection of NK1.1$^+$ cells. Gates were set on viable cells according to forward and sideward scatter and exclusion of propidium iodide-binding particles. All samples were incubated with blocking anti-Fcγ-R mAb and purified rat IgG before and during staining with 3E10. Up to 10^6 cells were acquired to allow depiction of ≈5,000 cells of each leukocyte subset in the upper quadrants of each dot plot. The percentages shown indicate the frequency of T1/ST2$^+$ cells within the different leukocyte subpopulations. (*A*) Anti-DIG-Cy5 antibody alone. The specificity of the staining for T1/ST2 shown in *B* was controlled by preincubation of the cells with a 100-fold excess of unconjugated 3E10 as shown in *C*.

Cytokine Production by T1/ST2 Positive Th Cells *ex Vivo*. For direct evaluation of their cytokine memory, T1/ST2$^+$ cells were enriched from spleens and lymph nodes of normal unimmunized BALB/c mice by MACS and stimulated with PMA/ionomycin. Cells were stained for T1/ST2 on the surface, immediately fixed, and further stained for CD4 and intracellular TNF-α, IL-2, IFN-γ, IL-4, IL-5, or IL-10. As shown in Fig. 4, virtually all IL-5 producers and the vast majority of the IL-4-producing cells expressed T1/ST2. IL-10-producing cells were also enriched in the T1/ST2$^+$ fraction.

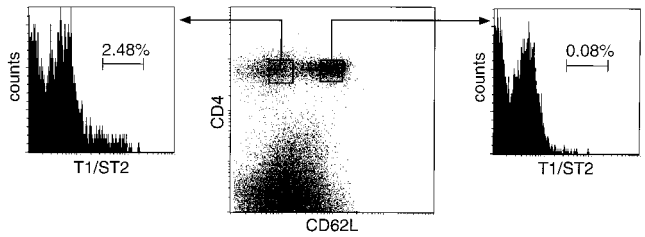

FIG. 3. Antigen-experienced but not naive Th cells express T1/ST2. Spleen cells from BALB/c mice were stained with mAbs against T1/ST2, CD4, and CD62L. Gates were set on viable cells according to forward and sideward scatter and exclusion of propidium iodide-binding particles. By gating on $CD62L^{hi}$ or $CD62L^{lo}$ $CD4^+$ cells, histograms for 3E10 staining were obtained.

Only a few of the IL-2- or IFN-γ-producing cells expressed T1/ST2, and none of them stained brightly for it. The frequency of TNF-α-producing $T1/ST2^+$ $CD4^+$ cells was reduced compared with $T1/ST2^-$ $CD4^+$ cells (Fig. 4).

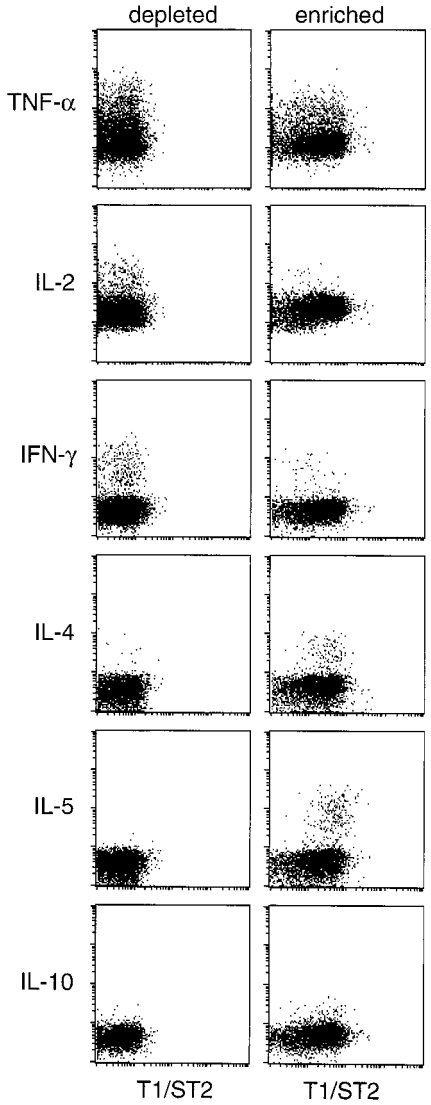

FIG. 4. Cytokine production of $T1/ST2^+$ cells *ex vivo*. $T1/ST2^+$ lymphocytes from spleens and lymph nodes of BALB/c mice were enriched by MACS. Enriched and depleted cell fractions were stimulated for 5 hr with PMA/ionomycin, fixed, and stained for CD4 and intracellular cytokines. For analysis, gates were set on $CD4^+$ lymphocytes. Equal numbers of $CD4^+$ cells are depicted in each dot plot.

Expression of T1/ST2 Is Independent of IL-4. Spleen cells from wt and IL-$4^{-/-}$ BALB/c mice were stained with mAbs against T1/ST2, CD4, and CD62L. The frequency of $CD4^+$ $T1/ST2^+$ cells was the same in BALB/c mice and BALB/c IL-$4^{-/-}$ mice; namely, approximately 1.2% of total $CD4^+$ cells and 2.5% of $CD62^{lo}$ $CD4^+$ cells (Fig. 5). Thus, expression of T1/ST2 is not dependent on IL-4.

T1/ST2 Positive Cells from IL-4-Deficient Mice Produce IL-5 and IL-10. $T1/ST2^+$ cells were enriched from the spleens of IL-$4^{-/-}$ mice by MACS, stimulated with PMA/ionomycin, and stained for surface T1/ST2, CD4, and intracellular TNF-α, IL-2, IFN-γ, IL-5, or IL-10. IL-5 was produced almost exclusively by $T1/ST2^+$ cells in the IL-$4^{-/-}$ mice (Fig. 6). The $T1/ST2^+$ population was enriched for IL-10-producing cells and contained fewer IL-2 and IFN-γ producers than the $T1/ST2^-$ cell population. The frequency of TNF-α-producing $T1/ST2^+$ $CD4^+$ cells was reduced compared with $T1/ST2^-$ $CD4^+$ cells. Thus, T1/ST2-expressing cells in IL-$4^{-/-}$ mice produce a Th2-type cytokine pattern.

Expression of T1/ST2 in IL-5- and IL-10-Deficient Mice. Spleen cells from wt C57BL/6 and IL-$5^{-/-}$ C57BL/6 mice were stained with mAbs against T1/ST2, CD4, and CD62L. Expression of T1/ST2 was detectable, albeit approximately 3-fold less in IL-$5^{-/-}$ mice (0.2% of total $CD4^+$ cells, and 0.4% of $CD62L^{lo}$ $CD4^+$ cells) compared with wt mice (0.7% of total $CD4^+$ cells and 1.3% of $CD62L^{lo}$ $CD4^+$ cells). $CD4^+$ spleen cells from both IL-$10^{-/-}$ C57BL/6 mice and their heterozygous littermates expressed equivalent levels of T1/ST2 (0.6% and 0.5% of total $CD4^+$ spleen cells, respectively). Together these data indicate that T1/ST2 is expressed independently of IL-5 and IL-10.

Attenuation of Th2-Mediated Eosinophilic Lung Inflammation and Th2 Cytokine Production by an Anti-T1/ST2 mAb or Recombinant T1/ST2 Fusion Protein. Allergen provocation of mice that had received Th2 cells resulted in marked eosinophil infiltration into the airways as assessed by BAL (3.96 ± 0.72 cells/ml $\times 10^5$, i.e., 35 ± 6% of the cellular composition of the BAL fluid, Fig. 7) compared with mice that received Th2 cells and were exposed to PBS or mice that were exposed to OVA

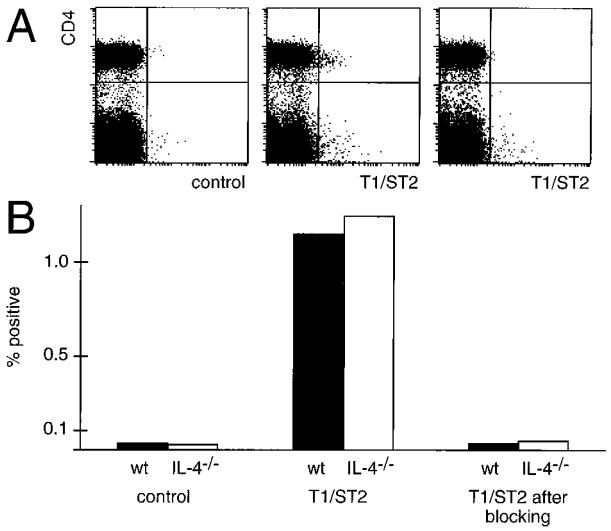

FIG. 5. $T1/ST2^+$ cells in IL-$4^{-/-}$ mice. (*A*) Spleen cells from wt and IL-$4^{-/-}$ BALB/c mice were stained with mAbs against T1/ST2, CD4, and CD62L as described for Fig. 2. The control panel shows the fluorescence signal of anti-DIG-Cy5 alone. To verify its specificity, the staining of 3E10 was blocked by preincubation of the cells with a 100-fold excess of unconjugated 3E10, which reduced the subsequent 3E10 staining on $CD4^+$ cells to control levels in both wt and IL-$4^{-/-}$ mice. The dot plots represent the results obtained with IL-$4^{-/-}$ mice. (For wt BALB/c mice see Fig. 2.) (*B*) Frequency of $T1/ST2^+$ cells among $CD4^+$ cells from wt and IL-$4^{-/-}$ mice, as determined in *A*.

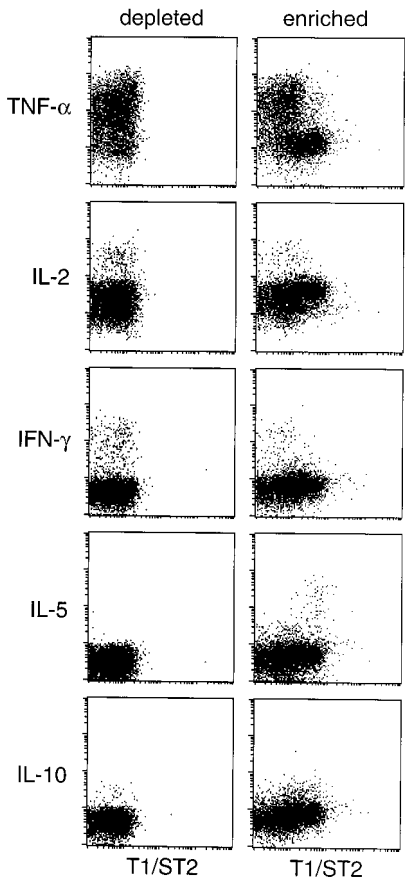

FIG. 6. Cytokine production of T1/ST2$^+$ cells from IL-4$^{-/-}$ mice *ex vivo*. Lymphocytes from spleens of IL-4$^{-/-}$ mice were stained with anti-T1/ST2 mAb and sorted by MACS. Enriched and depleted cell fractions were stimulated for 5 hr with PMA/ionomycin, fixed, and stained for CD4 and intracellular cytokines. For analysis, gates were set on CD4$^+$ lymphocytes. Equal numbers of CD4$^+$ cells are depicted in each dot plot.

alone (eosinophils $<$ 1% of the cellular composition of the BAL fluid, data not shown). Pretreatment with either 3E10 mAb (Fig. 7*A*) or T1/ST2 fusion protein (Fig. 7*B*) 1 hr before allergen provocation reduced the number of eosinophils in the airways by 70%. The BAL contained high levels of IL-4 and IL-5 in mice that received Th2 cells. There was no detectable IL-4 or IL-5 in the BAL fluid of mice that received Th2 cells and were not exposed to OVA or mice that were exposed to OVA alone (detection limit 10 pg/ml, data not shown). Pretreatment with either 3E10 mAb or T1/ST2 fusion protein resulted in a drastic reduction in IL-4 and IL-5 levels.

DISCUSSION

The development of Th effector populations that produce distinct cytokines is critical in determining the outcome of infectious, allergic, and autoimmune diseases (1, 2). To better understand the molecular basis of Th cell differentiation we attempted to identify genes that are specifically expressed in Th2 cells. Confirming and extending earlier work we found the gene coding for T1/ST2 to be expressed in Th2 but not in Th1 cell lines (18, 19). The gene coding for T1/ST2 has known homologues in rats (20) and humans (21). Alternative 3′ processing of the transcript results in two isoforms, one soluble and one membrane-bound protein (22, 23). The short mRNA was found in embryonic tissues and mammary tumors, whereas expression of the long mRNA was restricted to hematopoietic

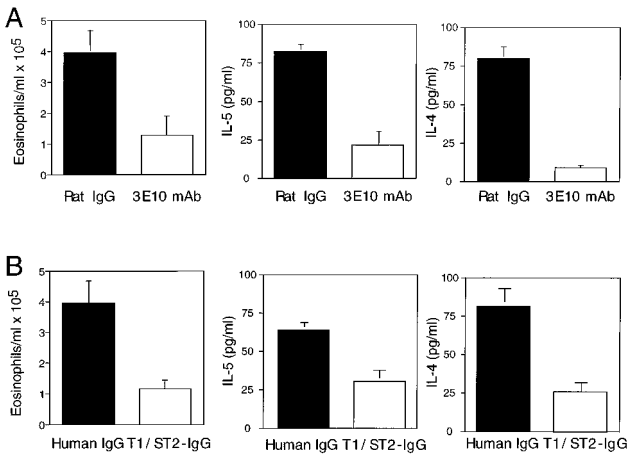

FIG. 7. Inhibition of Th2 effector function *in vivo*. Th2-recipient mice were exposed to OVA aerosols. Twenty-four hours after the last aerosol challenge BAL was performed, and differential cell counts and cytokine ELISA were done. (*A*) Mice received either T1/ST2-specific mAb 3E10 (open bars) or rat IgG as isotype control (solid bars) 1 hr before OVA challenge. (*B*) Mice received either T1/ST2–IgG fusion protein (open bars) or human IgG (solid bars). Data are shown as the mean ± SEM of 4–6 animals.

organs and the lung (7, 22, 23). To study further the role of T1/ST2, we have generated a mAb and Ig-fusion protein.

By flow cytometry, our data identify T1/ST2 as a surface molecule expressed by antigen-experienced CD4$^+$ T cells both *in vitro* and *ex vivo*, which produce high amounts of IL-4, IL-5, or IL-10, but little or no IL-2 and IFN-γ, and fewer TNF-α than T1/ST2$^-$ cells. Only a fraction of T1/ST2$^+$ cells produced type 2 cytokines. Similarly, it has been observed *in vitro* that not all cells produce cytokines simultaneously following polyclonal stimulation (17, 24). In our experiments, we used cells directly *ex vivo* rather than cells that had been stimulated *in vitro* many times. However, we cannot formally exclude the possibility that not all T1/ST2$^+$ cells are capable of producing type 2 cytokines. CD8$^+$ T cells, B cells, macrophages, dendritic cells, and NK1.1$^+$ natural killer cells, as well as NK1.1$^+$ CD4$^+$ cells do not express T1/ST2. The latter point is of interest because NK1.1$^+$ CD4$^+$ cells are potential IL-4 producers (25). It is possible that these are the T1/ST2$^-$ IL-4-producing cells detected by our *ex vivo* FACS analysis (Fig. 4).

Th2 cells specifically express the transcription factors c-Maf (26) and GATA-3 (27). Presently, however, it is not possible to use these molecules to identify live Th2 cells. Surface molecules such as CD30 (28) and the IL-1R (29), which is closely related to T1/ST2 (4–6), have been reported to be preferentially expressed on Th2 cells. However, IL-1R and CD30 are both upregulated by IL-4 (30, 31) and thus best regarded as markers for cells that have the ability to respond to IL-4. In contrast, T1/ST2 is expressed independently of IL-4 because it is expressed in IL-4$^{-/-}$ BALB/c mice to the same extent as in wt BALB/c mice. The T1/ST2-expressing CD4$^+$ cells from IL-4$^{-/-}$ mice produce IL-5 and IL-10, only a little IFN-γ and IL-2, and fewer TNF-α than T1/ST2$^-$ cells. We would, therefore, argue that the T1/ST2$^+$ cells can reasonably be called Th2 cells, even in the absence of IL-4. To our knowledge, T1/ST2 is the only Th2-specific surface molecule that is IL-4-independent. Furthermore, the expression of T1/ST2 does not depend on either IL-5 or IL-10. T1/ST2 expression was similar in IL-10$^{-/-}$ mice compared with their heterozygous littermates. Although T1/ST2 was still detectable in IL-5$^{-/-}$ mice, the expression level was reduced as compared with wt C57BL/6 mice. This could be because of a reduced number of IL-4- or IL-10-producing cells in IL-5$^{-/-}$ mice. Alternatively,

this finding could indicate coordinate regulation of IL-5 and T1/ST2.

Functional ligands for E- and P-selectin, directing T cells to sites of inflammation, have been found to be preferentially expressed on Th1 cells (32). The chemokine receptors CCR3 (33, 34) and CCR4 (34) are preferentially expressed on Th2 cells. However, CCR3 is rapidly downregulated following T cell activation and there is no clear difference between $CCR3^+$ and $CCR3^-$ T cells with respect to the production of IFN-γ, IL-2, TNF-α, or IL-10 (33). Nevertheless, these surface molecules seem to serve an important role in coordinating the migration of specific lymphocyte populations to inflamed tissues. No such function can currently be ascribed to T1/ST2.

We attempted to determine the functional significance of Th2-specific expression of T1/ST2. Therefore, we used a Th2 adoptive transfer model whereby aeroallergen provocation of Th2-recipient mice resulted in the recruitment of eosinophils into the airways and the production of IL-4 and IL-5. Pretreatment with 3E10 mAb or T1/ST2 fusion protein abrogated both the accumulation of eosinophils in the airways and the secretion of Th2 cytokines. By using a depleting anti-T1/ST2 antiserum, Xu *et al.* (19) reported resistance to *Leishmania major* infection in BALB/c mice. Our functional data suggest a possible role for T1/ST2 as a costimulatory molecule involved in the regulation of Th2 responses. Disruption of the interaction of membrane-bound T1/ST2 with its putative ligand, either by mAb (blocking T1/ST2) or soluble T1/ST2 (blocking the putative ligand), downregulated Th2 effector function.

T1/ST2 mRNA expression has been reported in mast cells (7, 23). The expression of soluble T1/ST2 could be a physiological mechanism to downregulate ongoing Th2 responses. This possibility is also supported by evidence suggesting that the expression of soluble T1/ST2 is modulated by environmental factors (5). We observed upregulation of the short mRNA coding for soluble T1/ST2 6 hr after anti-CD3/CD28 stimulation of Th2 clones (data not shown). Transcription of soluble or membrane-bound T1/ST2 depends on two different promoters with tissue-specific distribution, thus allowing for differential regulation of the two T1/ST2 isoforms (22, 23). Interestingly, the distal promoter, used in mast cells but not in fibroblasts, contains two GATA-3 binding sites (23). As GATA-3 is involved in controlling the expression of the IL-5 gene (27), it is possible that the expression of IL-5 and membrane-bound T1/ST2 are coordinately regulated in hematopoietic cells. The identification of the functional ligand(s) for T1/ST2 will provide insights in the physiological role of T1/ST2.

In summary, we have demonstrated that T cells producing type 2 cytokines express T1/ST2 on their surface both *in vitro* and *ex vivo*, independently of the characteristic Th2 cytokines IL-4, IL-5, and IL-10. Administration of mAb against T1/ST2 or of recombinant T1/ST2 block Th2 effector function *in vivo*. Thus, T1/ST2 could provide a promising target for therapeutic intervention in diseases such as allergy and asthma, which are characterized by inappropriately regulated Th2 responses.

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