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Enforced Expression of GATA-3 in Transgenic Mice Inhibits Th1 Differentiation and Induces the Formation of a T1/ST2-Expressing Th2-Committed T Cell Compartment In Vivo

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The transcription factor GATA-3 is essential for early T cell development and differentiation of naive CD4+ T cells into Th2 effector cells. To study the function of GATA-3 during T cell-mediated immune responses in vivo, we investigated CD2-GATA3 transgenic mice in which GATA-3 expression is driven by the CD2 locus control region. Both in the CD4+ and the CD8+ T cell population the proportion of cells exhibiting a CD44highCD45RBlowCD62Llow Ag-experienced phenotype was increased. In CD2-GATA3-transgenic mice, large fractions of peripheral CD4+ T cells expressed the IL-1 receptor family member T1/ST2, indicative of advanced Th2 differentiation. Upon in vitro T cell stimulation, the ability to produce IL-2 and IFN-γ was decreased. Moreover, CD4+ T cells manifested rapid secretion of the Th2 cytokines IL-4, IL-5, and IL-10, reminiscent of Th2 memory cells. In contrast to wild-type CD4+ cells, which lost GATA-3 expression when cultured under Th1-polarizing conditions, CD2-GATA3-transgenic CD4+ cells maintained expression of GATA-3 protein. Under Th1 conditions, cellular proliferation of CD2-GATA3-transgenic CD4+ cells was severely hampered, IFN-γ production was decreased and Th2 cytokine production was increased. Enforced GATA-3 expression inhibited Th1-mediated in vivo responses, such as Ag-specific IgG2a production or a delayed-type hypersensitivity response to keyhole limpet hemocyanin. Collectively, these observations indicate that enforced GATA-3 expression selectively inhibits Th1 differentiation and induces Th2 differentiation. The increased functional capacity to secrete Th2 cytokines, along with the increased expression of surface markers for Ag-experienced Th2-committed cells, would argue for a role of GATA-3 in Th2 memory formation. The Journal of Immunology, 2001, 167: 724–732.

The CD4+ Th lymphocytes develop into two functionally distinct subsets that can be distinguished on the basis of their cytokine production profile (1, 2). Th1 cells are characterized by the production of IFN-γ and TNF-β, whereas Th2 cells typically produce IL-4, IL-5, IL-10, and IL-13. Each subset mediates distinct effector functions in vivo. Th1 cells are predominantly involved in immune responses against intracellular pathogens and are associated with autoimmune disease. Th2 cells are of importance in the defense against extracellular pathogens and are implicated in atopy and allergic diseases (3–5).

Both Th1 and Th2 cells are derived from a common naive precursor (4–6). Signaling pathways initiated by cytokines play a dominant role in driving the differentiation of activated naive CD4+ T cells into either effector phenotype (2, 7). For instance, IL-12 induces the differentiation of naive Th cells to the Th1 effector phenotype (8–11) by activation of the transcription factor Stat4 (12–14). On the other hand, Th2 differentiation is mediated by Stat6 activation through IL-4 receptor engagement (15–21). In response to chronic antigenic stimulation in vivo, progressive polarization of the cytokine responses ultimately leads to the commitment of Th cells to mutually exclusive Th phenotypes, which are thought to be maintained independently of extrinsic factors (22, 23).

Stat6 induces the expression of the transcription factors GATA-3 and c-Maf (24), which have been shown to be selectively expressed in a Th2-specific fashion (25–27). Using Stat6-deficient cells it has been shown that, although IL-4 and Stat6 signaling may initially direct Th2 development, GATA-3 and c-Maf are capable of inducing the development of stable Th2 commitment, independent of Stat6 (28). In vitro differentiation into Th1 cells induces chromatin remodeling of the IFN-γ locus and, conversely, the differentiation into Th2 cells induces remodeling of the IL-4/IL-5/IL-13 locus (29, 30). Recently, GATA-3 has been shown to play an instructive role in directing Th2 differentiation (31).

During early T cell development, GATA-3 gene expression is required for the development of the earliest T cell progenitors (32–34). GATA-3 levels are low during the two phases of TCR gene rearrangement, but are high in the fraction of rapidly proliferating cells that insulates these two periods of TCR rearrangement (33). GATA-3 expression remains high in CD4+ thymocytes, but progressively declines in CD8+ thymocytes (see accompanying paper). GATA-3 is detected in naive CD4+ T cells and expression levels increase substantially during Th2 differentiation (26, 27). GATA-3 expression has been shown to be indispensable for Th2 development and is down-regulated in response to IL-12-mediated Stat4 activation (27, 35). GATA-3 strongly transactivates the IL-5 promoter, but appears to have only limited effects on IL-4 gene transcription (27, 36, 37). Retroviral introduction of GATA-3 during in vitro Th1 differentiation of naive CD4+ T cells resulted in an inhibition of IFN-γ production, independently of IL-4 (35, 38),
and a down-regulation of IL-12Rβ2 (35), which normally accompanies Th2 differentiation (39).

The manipulation of Stat6 and GATA-3 expression in Th1 and Th2 polarization cultures of wild-type or specific cytokine-deficient cells in vitro have added significantly to our understanding of the molecular basis of Th1/Th2 differentiation. However, limited data are available on the role of GATA-3 during immune responses in animal models, partly because the embryonic lethality of GATA-3 deficiency in mice precluded in vivo studies (40). Analysis of transgenic mice with T cell-specific expression of a dominant-negative mutant of GATA-3 indicated that inhibition of GATA-3 activity reduced the key features of asthma, including Th2 cytokine levels, eosinophilia, and IgE production (41).

To study the function of GATA-3 during T cell differentiation, we generated transgenic mice in which the expression of GATA-3 is under the control of the human CD2 locus control region (see accompanying paper). In these mice, the enforced GATA-3 expression induced the development of thymic lymphomas of CD4−CD8+ T cells and inhibited the maturation of CD8 single-positive (SP)3 cells in the thymus. Within the CD8 SP population, sensitivity; KLH, keyhole limpet hemocyanin; TNP, trinitrophenol; HA, hemagglutinin.

mature CD69low HSA low cells was significantly reduced. The number of anti-CD3 mAb were purchased from BD PharMingen (San Diego, CA): FITC-conjugated anti-CD2-GATA3 mAb were used to detect GATA3 activity in vitro. Furthermore, the expression of surface markers specific for Ag-experienced Th2 cells, along with the increased functional capacity to secrete the Th2 cytokines, indicated that transgenic GATA-3 expression induced Th2 commitment and pointed at a role for GATA-3 in Th2 memory formation.

Materials and Methods

Mice

The CD2-GATA3 mice are described in the accompanying paper and were crossed on a uniform FVB background. To determine the genotype of the mice, tail DNA was analyzed by Southern blotting as described in the accompanying paper.

Flow cytometric analyses

The preparation of single-cell suspensions, mAb incubations and three- or four-color cytometry have been described previously (42). The mAb were purchased from BD PharMingen (San Diego, CA): FITC-conjugated anti-CD3ε, PE-conjugated anti-CD4 (L3T4), anti-CD24/heat-stable Ag, anti-CD25 (clone 3C7), anti-CD62L and anti-CD69, CyChrome-conjugated anti-CD4 (L3T4), anti-CD8 and anti-CD44, biotinylated anti-CD4 (L3T4), and anti-CD8, APC-labeled anti-CD3ε and anti-CD4. Anti-CD45RB (M1/70), CD11b/Mac-1 (M1/70), CD40 (FGK-45.5), B220 (RA3-6B2), and IgM (M41), followed by streptavidin-conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Using a VarioMACS, CD4+ T cells were purified according to the manufacturer’s instruction to purity >95%. The CD4+ T cells were cultured for up to 5 days in the presence of IL-2 (50 U/ml) on 96-well plates precoated with 10 μg/ml anti-CD3 (145 2C11) mAb.

Purification of CD4+ T cells and in vitro cultures

Single-cell suspensions from spleen were incubated with biotinylated mAb to CD8 (YTS-169), CD11b/Mac-1 (M1/70), CD40 (FGK-45.5), B220 (RA3-6B2), and IgM (M41), followed by streptavidin-conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Using a VarioMACS, CD4+ T cells were purified according to the manufacturer’s instruction to purity >95%. The CD4+ T cells were polarized into Th1 and Th2 effector cells in a total volume of 200 μl for 4 days in the presence of 5 μg/ml anti-CD28 (37.51) and 50 μIU/ml IL-2 on 96-well plates, which were precoated with 10 μg/ml anti-CD3ε (145 2C11) (33). Th1-polarizing cultures included 5 ng/ml rIL-12 (R&D Systems, Minneapolis, MN) and 10 μg/ml neutralizing mAbs to IL-4 (11B11). Th2-polarized cells were cultured in the presence of 10–50 ng/ml rIL-4 and 10 μg/ml neutralizing mAbs to IFN-γ (XMG1.2). After 4 days of culture, the cells were thoroughly washed and transferred to new anti-CD3-coated 96-well plates and cultured in the presence of IL-2, without addition of further cytokines or neutralizing Abs.

To measure DNA synthesis during T cell cultures, cells were pulsed with [3H]thymidine for ~16 h, harvested, and counted using standard methods. Cytokine levels in culture supernatants were determined by ELISA using the Opteia kit for IL-4, IL-5, IL-10, and IFN-γ (BD Pharmingen) according to the manufacturer’s instructions. Expression of GATA-3 protein was evaluated using a Western blotting procedure as described in the accompanying paper.

DTH responses

DTH responses were performed essentially as described by Cua et al. (47). In short, mice were immunized i.p. with 100 μg KLH in 250 μl PBS and on day 6, they were challenged with 150 μg KLH in 25 μl PBS in the left hind footpad. The right hind footpad was injected with a vehicle control (25 μl PBS). Responses were quantified 24 and 48 h after the challenge by measuring the difference in footprint thickness between the KLH- and the PBS-injected footpads.

Immunohistochemistry

Tissue samples were embedded in OCT compound and frozen 5-μm cryostat sections were acetone fixed and single labelings were performed using standard procedures (45). The mAbs biotinylated anti-IL-4 (11B11) and alkaline phosphatase-conjugated anti-IL-5 (TRFK5) were purified hybridoma supernatants and conjugated according to standard procedures. Biotinylated anti-IL-10 (SXC1) was purchased from BD PharMingen.

Results

Increased expression of Ag-experienced T cell surface markers in CD2-GATA3-transgenic mice

In two independent CD2-GATA3-transgenic lines expression of hemagglutinin (HA)-tagged GATA-3 was under the control of the CD2 locus control region. Due to the presence of this transgene, SUNNYVALE, CA). For four-color analysis, 105–2 × 105 events were scored using a FACSCalibur dual laser instrument (BD Biosciences).

For intracellular detection of cytokines, cells were stimulated for 40 h in the presence of mAb to CD25 (37.51; 5 μg/ml) in 96-well plates (106 cells/well) precoated with mAb to CD3ε (145 2C11; 10 μg/ml in PBS). Subsequently, cells were stimulated by adding PMA (50 ng/ml; Sigma, St. Louis, MO) and calcium ionophore (500 ng/ml; Sigma) for 5 h. For the last 3 h of the culture, brefeldin A (10 μg/ml; Sigma) was added to the cells. Finally, cells were harvested and stained with CyChrome-labeled mAb to CD4 or CD8 (BD Pharmingen). Cells were fixed using 2% paraformaldehyde and stored up to 5 days at 4°C. Intracellular cytokine staining was performed using PE-labeled mAb to IL-2, IL-4, and IL-10 (BD Pharmingen) and APC-labeled mAb to IL-2, IL-5, and IFN-γ (BD Pharmingen) according to the manufacturer’s instructions.

Serum Ig detection and in vivo immunizations

Total serum Ig levels were determined by subclass-specific sandwich ELISA as described previously (45). Immunizations were done i.p. with 100 μg trinitrophenoylelchpt hemocyanin (TNP-KLH) precipitated on alum. Serum levels of TNP-specific Ig subclasses were determined by ELISA, using TNP-specific standards (IgG1, IgG2a, and IgG2b) or TNP-specific reference serum samples (IgM and IgG3), as described elsewhere (46).

Purification of CD4+ T cells and in vitro cultures

Single-cell suspensions from spleen were incubated with biotinylated mAb to CD8 (YTS-169), CD11b/Mac-1 (M1/70), CD40 (FGK-45.5), B220 (RA3-6B2), and IgM (M41), followed by streptavidin-conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Using a VarioMACS, CD4+ T cells were purified according to the manufacturer’s instruction to purity >95%. The CD4+ T cells were cultured for up to 5 days in the presence of IL-2 (50 U/ml) on 96-well plates precoated with 10 μg/ml anti-CD3 (145 2C11) mAb.

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GATA-3 expression was significantly enhanced in the thymus, especially in the DP fraction. In these mice, the expression of transgenic GATA-3 in peripheral lymphoid organs was low (see accompanying paper). In the experiments described below, we did not detect any differences between the two independent lines TgA and TgB.

To determine the effect of enforced GATA-3 expression on the development of the peripheral T cell compartments, we investigated the sizes of the CD4+ and CD8+ populations in CD2-GATA3-transgenic mice and their nontransgenic littermates at different ages (Fig. 1A). In spleen and mesenteric lymph nodes, the numbers of CD4+ T cells in CD2-GATA3 mice were either increased (3 wk of age) or within normal ranges (12 wk of age). In contrast, the CD8+ T cell populations were consistently reduced in number, to ~50% of normal, irrespective of the age analyzed (Fig. 1A). Despite the observed increased apoptosis in the fraction of mature SP cells in the thymus (see accompanying paper), the CD2-GATA3-transgenic mice were not found to be lymphopenic at any of the ages analyzed.

Because GATA-3 is involved in the stabilization of the Th2 phenotype and the maintenance of Th2 cytokine expression, which are important features of Th memory, we wanted to assess whether the enforced expression of GATA-3 had an effect on the development of memory T cells. Ag activation induces the expression of CD44 and decreases CD45RB and L-selectin (CD62L) expression on the cell surface of T cells. As this profile of cell surface marker expression is maintained, even after cells have reverted to a quiescent state, it can be used to define Ag-experienced T cells (48). As shown in Fig. 1, A and B, in CD2-GATA3-transgenic mice both the CD4+ and the CD8+ T cell populations contained increased proportions of CD44high, CD45RBlow, and CD62Llow cells. This was found both in spleen and in lymph nodes, and pointed to the presence of elevated numbers of either recently activated or Ag-experienced T cells (48). The expression levels of CD69 and IL-2Rα (CD25), which are markers of recently activated T cells (49), were low in CD2-GATA3 mice and nontransgenic littermates (shown for CD25 in Fig. 1, A and B), arguing against the presence of major fractions of recently activated T cells in CD2-GATA3 mice. It has been shown that the proportions of T cells with an Ag-experienced phenotype increases with age, probably as a result of progressive Ag experience (50, 51). When we compared CD44 and CD62L expression at two different ages, 3 and 7 mo, we indeed found that the conversion to the CD44highCD62Llow Ag-experienced T cell phenotype increased with age, both in CD2-GATA3-transgenic mice and in control mice (shown for CD62L in Fig. 1C).

Taken together, these results indicated that in the CD2-GATA3 mice both the CD4+ and the CD8+ T cell population contained increased proportions of cells with an Ag-experienced phenotype.

**Enforced expression of GATA-3 results in increased numbers of T1/ST2-positive CD4+ T cells in the periphery**

The IL-1R family member T1/ST2 is preferentially expressed on the surface of murine Th2 cells (43, 52–54). It was recently shown that CD4+ cells become T1/ST2 positive after repeated antigenic stimulation under Th2-polarizing conditions and that Th2 cytokine production precedes T1/ST2 expression (55). Therefore, T1/ST2 expression appears to be a late event in Th2 commitment.

To investigate whether CD4+ T cells in CD2-GATA3 mice exhibited preferential Th2 polarization and advanced Th2 commitment in vivo, we evaluated surface expression of T1/ST2 (Fig. 2). In nontransgenic controls, we found T1/ST2 expression on 5.1% ±

![FIGURE 1](http://www.jimmunol.org/)

*FIGURE 1. Enforced GATA-3 expression is associated with increased numbers of T cells with a memory surface phenotype. A, Single-cell suspensions from spleen and mesenteric lymph nodes from 3- and 12-wk-old CD2-GATA3-transgenic mice and nontransgenic littermates were analyzed for CD4 and CD8 expression. Surface CD4/CD8 profiles are shown as dot plots in which the percentages of total lymphocytes within the indicated CD4+ and CD8+ gates are given. B, Single-cell suspensions from spleen and mesenteric lymph nodes from 12-wk-old CD2-GATA3-transgenic mice and nontransgenic littermates were stained for CD4 and CD8, along with CD44, CD45RB, or CD25. Total CD4+ and CD8+ T cell populations were analyzed for the indicated markers. Results are displayed as histograms of CD2-GATA3-transgenic mice (bold lines) and those of nontransgenic littermates (thin lines). The percentages of activated/Ag-experienced cells (CD44high, CD45RBlow, or CD25+) are indicated above the marker line in bold (CD2-GATA3-transgenic mice) and below the marker line (nontransgenic littermates). Data shown are representative of at least six mice examined in each group. C, The numbers of activated/Ag-experienced (CD62Llow) cells in CD2-GATA3-transgenic and control mice at two different ages, 12 and 30 wk. The percentages are indicated as in B.*
1.3 and 1.1% ± 0.1 (n = 5) of CD4<sup>+</sup> T cells in spleen and mesenteric lymph nodes, respectively. In four-color labelings with CD4, CD8, CD44, and T1/ST2, it was shown that in nontransgenic mice T1/ST2 expression was largely confined to the CD44 high fraction of activated/memory CD4<sup>+</sup> T cells (Fig. 2).

The CD2-GATA3-transgenic animals showed a significant increase in the proportions of T1/ST2<sup>+</sup> cells: 31% ± 1 and 23% ± 2 (n = 3) in spleen and mesenteric lymph nodes, respectively. This increase could not be attributed solely to the increased proportion of CD4<sup>+</sup> T cells with a CD44 high activated/memory phenotype, since T1/ST2 was also found to be expressed on naive CD4<sup>+</sup> T cells with a CD44 low surface profile (Fig. 2).

When we analyzed T1/ST2 expression in the thymic subpopulations, we found induction of T1/ST2 on a small fraction of the CD4<sup>+</sup> SP cells in CD2-GATA3-transgenic mice: 6.0% ± 1.0 (n = 3) compared with 0.4% ± 0.05 in nontransgenic mice (n = 5). In contrast, T1/ST2 expression was not significantly induced on CD8<sup>+</sup> SP thymocytes (<0.5%). Consistent with the reported absence of T1/ST2 on the surface of CD8<sup>+</sup> cells (43, 52), we found very low numbers of T1/ST2-positive cells in nontransgenic mice. However, some T1/ST2 expression was observed on CD8<sup>+</sup> T cells in spleen (3.0% ± 0.8) and lymph node (1.7% ± 0.6) from CD2-GATA3-transgenic mice (Fig. 2).

In summary, these data indicate that enforced expression of GATA-3 resulted in significantly increased numbers of peripheral CD4<sup>+</sup> T cells with an advanced Th2-committed T1/ST2 phenotype, not only in the CD44<sup>high</sup> activated/memory T cell compartment but also in CD44<sup>low</sup> naive T cells.

**Increased ability to secrete Th2 cytokines in CD2-GATA3-transgenic T cells**

As one hallmark of a memory cell population is the ability to secrete a wider diversity of cytokines (48), we investigated the ability of peripheral T cells to synthesize various cytokines. After polyclonal in vitro stimulation of mesenteric lymph node cells, significant differences in the cytokine production profiles between wild-type and CD2-GATA3-transgenic mice were observed. When intracellular cytokine expression was analyzed by flow cytometry, CD2-GATA3-transgenic CD4<sup>+</sup> and CD8<sup>+</sup> T cells manifested decreased expression of IL-2 and IFN-γ (Fig. 3A). In addition, the...

**FIGURE 2.** Aberrant T1/ST2 surface expression in CD2-GATA3-transgenic mice. Single-cell suspensions from spleen (A) and mesenteric lymph nodes (B) from 2–3-mo-old CD2-GATA3-transgenic mice and nontransgenic littermates were stained for CD4, CD8, CD44, and T1/ST2. CD4<sup>+</sup> and CD8<sup>+</sup> T cells were gated and analyzed for CD44 and T1/ST2 expression. Data are displayed as dot plots, and the percentages of gated cells within the indicated T1/ST2<sup>+</sup> quadrants are shown.

**FIGURE 3.** Peripheral T cells from CD2-GATA3-transgenic mice produce Th2 cytokines upon activation. A, Decreased synthesis of IL-2 and IFN-γ in CD2-GATA3-transgenic T cells. Mesenteric lymph node T cells were stimulated with anti-CD3ε for 40 h, and intracellular IL-2 and IFN-γ were determined by flow cytometry, in conjunction with membrane expression of CD4 and CD8. Results are displayed as histograms of CD2-GATA3-transgenic mice (bold lines) and those of nontransgenic littermates (thin lines). The percentages of cytokine-positive cells are indicated below the marker line in bold (CD2-GATA3-transgenic mice) and above the marker line (nontransgenic littermates). B, Th2 cytokine profiles of CD2-GATA3-transgenic and control T cells. Mesenteric lymph node T cells were stimulated with anti-CD3ε for either 40 or 60 h as indicated. Wild-type mice; CD2-GATA3-transgenic mice. The levels of the indicated cytokines were measured in the culture supernatants by ELISA. C, Proliferation, determined by [3H]thymidine incorporation, in response to anti-CD3ε stimulation of purified CD4<sup>+</sup> T cells. Data are given as mean values ± SD.
production of the Th2 cytokines IL-4, IL-5, and IL-10 was increased in CD2-GATA3-transgenic T cells after 40 and 60 h of culture (Fig. 3B).

The effect of enforced GATA-3 expression on T cell proliferation was studied in in vitro stimulations of purified CD4⁺ T cells. When [³H]thymidine incorporation was assessed at day 1 or day 2, the CD2-GATA3-transgenic T cells manifested significantly reduced proliferation, as compared with nontransgenic T cells (Fig. 3C).

Collectively, the CD2-GATA3-transgenic T cells were characterized by an increased production of Th2 cytokines and reduced production of IL-2 and IFN-γ upon stimulation, suggesting the presence of an increased memory compartment within the peripheral T cell population in vivo.

Expression of GATA-3 in CD2-GATA3-transgenic T cell cultures under Th1-polarizing conditions

Next, we performed in vitro Th1/Th2 polarization culture experiments on purified CD4⁺ T cells from spleen and lymph nodes to investigate whether the differentiation potential into T effector phenotypes was altered in the CD2-GATA3 mice. In these experiments, cells were stimulated with anti-CD3ε mAbs, either under “default” conditions (without additional cytokines or Abs), Th1-polarizing conditions (in the presence of IL-12 and anti-IL-4 mAbs), or under Th2-polarizing conditions (in the presence of IL-4 and anti-IFN-γ mAbs) for 4 days. Subsequently, the cells were washed and restimulated with anti-CD3ε mAbs for 3 days, without additional Abs or cytokines.

First, we evaluated the GATA-3 expression in lymph node-derived T cell cultures by Western blotting and intracellular flow cytometric analyses using a mouse monoclonal antiserum specific for GATA-3 (Fig. 4, A and B). In nontransgenic and CD2-GATA3-transgenic CD4⁺ T cell cultures, GATA-3 protein was abundantly expressed both under default and Th2-polarizing conditions. GATA-3 protein could not be detected in nontransgenic Th1 cultures (Fig. 4A). In contrast, GATA-3 was expressed in CD2-GATA3-transgenic Th1 cultures, although the levels of expression were lower than in the corresponding Th2 or default cultures (Fig. 4A). The GATA-3 protein present had the apparent molecular mass of endogenous GATA-3, and no transgene encoded GATA-3 bands could be detected using Abs specific for the HA tag present in the transgenic GATA-3 protein.

Intracellular GATA-3 protein flow cytometric assays allowed a comparison of mean fluorescence intensity values in histogram overlays. Although these analyses are limited by a background signal of the GATA-3 Ab, they showed that GATA-3 protein levels were higher in the cultures of CD2-GATA3-transgenic mice when compared with those of the nontransgenic littermates, irrespective of the culture conditions (shown for Th1 and Th2 conditions in Fig. 4B).

FIGURE 4. The effect of enforced GATA-3 expression in CD4⁺ T cell cultures under Th1- and Th2-polarizing conditions. A, Western blotting analysis showing the expression of GATA-3 in purified CD4⁺ T cells from the indicated nontransgenic (wt) and CD2-GATA3 (tgA and tgB) mice cultured for 7 days under default (D), Th1-, and Th2-polarizing conditions. B, Expression of GATA-3 in CD2-GATA3-transgenic (bold lines)- and nontransgenic (thin lines)-purified CD4⁺ T cells that were activated under Th1 or Th2 conditions as indicated. Cell suspensions were stained for surface CD4 and CD8 and subsequently for intracellular GATA-3 protein. Flow cytometry results are displayed as histograms of CD4⁺CD8⁻ cells. C, Cytokine production in supernatants of 7-day cultures of purified CD4⁺ T cells under default, Th1, and Th2 conditions as indicated. D, Wild-type mice; CD2-GATA3-transgenic mice. D, Cell viability after 7 days of culture under Th1- or Th2-priming conditions of purified CD4⁺ T cells of the indicated mice. Cell suspensions were stained for surface CD4 and CD8 and propidium iodide (PI). Results are displayed as histograms of CD4⁺CD8⁻ cells. E, Proliferation, determined by [³H]thymidine incorporation, in response to stimulation with anti-CD3ε of purified CD4⁺ cells under Th1 and Th2 conditions. Data are given as mean values ± SD. □, Wild-type mice; CD2-GATA3-transgenic mice. Data are representative of five repeat experiments.
Therefore, we conclude that due to the presence of the CD2-GATA3 transgene, GATA-3 protein expression is maintained in CD4+ T cells that are cultured under Th1-polarizing conditions.

The Th1/Th2 differentiation potential of CD2-GATA3-transgenic T cells in vitro

Cytokine production was evaluated in the Th1/Th2-polarized CD4+ T cell cultures from lymph nodes or spleen. CD2-GATA3-transgenic CD4+ T cells produced normal amounts of IL-4, but higher levels of IL-5 and IL-10 in the default or Th2-polarized cultures when compared with nontransgenic CD4+ T cells (Fig. 4C). In addition, when CD2-GATA3-transgenic CD4+ T cells were cultured under Th1-polarizing conditions, they produced significantly increased amounts of IL-4, IL-5, and IL-10. Irrespective of the culture conditions, the production of IFN-γ was significantly reduced in the CD2-GATA3-transgenic CD4+ T cells when compared with those from nontransgenic littermates (Fig. 4C).

The reduced IFN-γ production by CD2-GATA3-transgenic CD4+ T cells cultured under Th1-polarizing conditions could either result from an inhibitory effect of GATA-3 on the differentiation of naive cells into Th1 cells, or by an inhibition of the amount of IFN-γ produced by differentiated Th1 effector cells. To distinguish between these possibilities, we assessed cell viability and proliferation in the T cell cultures. When analyzed by flow cytometry using propidium iodide, CD4+ T cells from CD2-GATA3-transgenic mice showed increased cell death under Th1 culture conditions at day 7, as compared with nontransgenic littermates (Fig. 4D). When [3H]thymidine incorporation was assessed at day 7, we observed a specific inhibitory effect of enforced GATA-3 expression on cell proliferation in Th1 cultures (Fig. 4E). By contrast, the presence of the CD2-GATA3 transgene enhanced viability and proliferation of CD4+ T cells in the Th2 cultures (Fig. 4, D and E).

Collectively, these observations demonstrate that although the presence of the CD2-GATA3 transgene inhibited the proliferation of Th cells under Th1-polarizing conditions, considerable production of Th2 cytokines was still present. Furthermore, the enforced GATA-3 expression significantly supported proliferation and differentiation of Th2 effector cells in a Th2 environment.

GATA-3-expressing lymphoblastoid tumors express Th2 cytokines

The coordinate expression of IL-4, IL-5, and IL-13 is thought to be under the direct control of GATA-3, as GATA-3 specifically interacts with an intergenic DNase I hypersensitivity site in the Th2 cytokine locus that contains the IL-4/IL-5/IL-13 gene cluster (30). However, the mechanism by which GATA-3 would regulate IL-10 expression is unknown. The rapid production of IL-10 after anti-CD3 stimulation in vitro (Fig. 3C) would suggest that GATA-3 is directly involved in the regulation of IL-10 gene expression. To further address this question, we examined lymphoblastoid tumor samples from CD2-GATA3-transgenic mice. At the age of 9 mo, ~50% of these mice developed thymic lymphomas that were CD4−CD8+CD45− and expressed high levels of GATA-3 (see accompanying paper). Immunohistochemical analyses of thymic tumor tissues showed that most of the tumors contained areas where the lymphoblastoid cells had lost expression of CD8 and sometimes also CD4 (data not shown). Particularly in such areas, very high expression of the Th2 cytokines IL-4, IL-5, or IL-10 was found (Fig. 5, A–C). Moreover, when tumor cells were cultured in the presence of anti-CD3ε, an extremely high production of Th2 cytokines, including IL-10, was observed (an example is shown in Fig. 5D).

These findings show that high expression of GATA-3 in lymphoblastoid tumor cells is associated with high level production of Th2 cytokine production, suggesting that like the IL4/IL-5/IL-13 locus, the IL-10 gene may be a direct target of GATA-3.

Enforced GATA-3 expression inhibits switching to IgG2a in an Ag-specific immune response

Serum levels of individual Ig isotypes are generally dependent on the Th1/Th2 balance. IL-4 primes mouse B lymphocytes for switching to IgG1 and IgE, while IgG2a responses are induced by IFN-γ (56). When total serum Ig levels were determined in 2–3-mo-old CD2-GATA3-transgenic mice and nontransgenic littermates by ELISA, a selective increase in IgG1 was found in the CD2-GATA3-transgenic animals (Fig. 6A). The levels of all other isotypes, including IgE, were similar in the two groups of mice (Fig. 6, A and C, IgE preimmune values).

To analyze Ig class switching in a T cell-dependent response in vivo, mice were immunized i.p. with 10 µg TNP-KLH, which was precipitated on alum. After 2 mo a booster dose of 100 µg TNP-KLH in PBS was given. CD2-GATA3-transgenic mice showed a significantly decreased secondary TNP response for the IFN-γ-dependent isotype IgG2a on day 7 after the booster injection, as measured in a TNP-specific ELISA (Fig. 6B). The TNP-specific levels of the other Ig isotypes elicited in this response were comparable between transgenic animals and wild-type littermates (shown for IgG1 in Fig. 6B). Also the primary IgG2a response to TNP-KLH in vivo was specifically decreased in the CD2-GATA3-transgenic animals (to ~15% of normal), as determined in the serum at day 7 and 14 after an i.p. immunization with 100 µg TNP-KLH precipitated on alum (data not shown). The Th2-dependent induction of heavy chain class switch to IgE was determined at day 11 after i.p. injection of 10 µg TNP-KLH precipitated on alum. CD2-GATA3-transgenic mice manifested elevated total serum IgE levels in this response (Fig. 6C).

Next, we directly tested the ability of CD2-GATA3-transgenic T cells to respond to Ag after a previous i.p. immunization with TNP-KLH. CD4+ T cells were purified from spleens of immunized CD2-GATA3-transgenic mice and control littermates 4 wk
Enforced GATA-3 expression diminishes the DTH response to KLH

To directly test whether enforced GATA-3 expression suppresses Th1-dependent immune responses in vivo, we assayed DTH responses to the protein Ag KLH. Two-month-old mice were primed by i.p. injection of 100 μg KLH and challenged on day 6 by injection of 25 μl PBS alone and 25 μl PBS containing 150 μg KLH in the left and right hind footpad, respectively. Twenty-four hours after the injections, footpad thickness was measured and the difference between the two footpads was calculated (Fig. 7). The KLH-induced footpad swelling was significantly reduced in CD2-GATA3-transgenic animals, as compared with the wild-type littermates. This reduction of footpad swelling did not reflect delayed kinetics of the DTH response, since also at 48 h after the injections footpad swellings were still essentially absent in the CD2-GATA3-transgenic mice.

These observations demonstrate that GATA-3 expression has a severe inhibitory effect on the Th1-mediated DTH response in vivo.

Discussion

Previous studies using Th1 and Th2 polarization cultures of wild-type and specific cytokine-deficient cells in vitro have identified GATA-3 as a master switch in Th2 development (26–28, 30, 31, 35, 38). GATA-3 does not only induce the expression of Th2-specific cytokines, but also acts as a repressor of Th1 differentiation. Introduction of GATA-3 by retroviral infection into naive T cells strongly inhibited IFN-γ production, independently of IL-4 expression (35, 38).

To analyze the function of GATA-3 in an in vivo system, we investigated transgenic mice that expressed GATA-3 under the control of the CD2 locus control region. In these mice, the expression of transgenic GATA-3 in the peripheral T cells of the spleen or lymph nodes was very low (see accompanying paper). However, in contrast to wild-type CD4+ cells, which lost GATA-3 expression when cultured under Th1-polarizing conditions, CD2-GATA3-transgenic CD4+ cells maintained expression of endogenous GATA-3. This could explain our observation of increased GATA-3 protein levels in T cell cultures, while transgenic GATA-3 could not be detected in Western blotting analyses using Abs to GATA-3 or the HA tag (Fig. 4, A and B).

FIGURE 7. Diminished DTH responses in CD2-GATA3-transgenic mice. Ratios of footpad swelling of KLH-injected over PBS-injected footpads in nontransgenic mice (○, n = 9) and CD2-GATA3-transgenic mice (●, n = 6) 24 h after the injections.
Our analyses of the CD2-GATA3-transgenic mice support the findings that GATA-3 expression inhibits Th1 development. The enforced GATA-3 expression inhibited Th1-mediated responses in vivo, including Ag-specific IgG2a production and DTH responses to protein Ag. In our Th1/Th2 polarization cultures, enforced GATA-3 expression under Th1-inducing culture conditions resulted in a reduction of cell survival, proliferation, and IFN-γ production. The additional findings of increased T1/ST2 expression in CD4+ T cells and elevated total level of IgG1 serum levels suggest that the presence of the CD2-GATA3 gene drives T cells preferentially toward differentiation along the Th2 pathway. Therefore, we conclude that GATA-3 plays a dual role in vivo in the differentiation of naive Th cells into Th2 cells, since it both represses Th1 differentiation and induced Th2 differentiation.

Various lines of evidence indicate that the enforced expression of GATA-3 may enhance Th2 memory cell formation. First, in CD2-GATA3-transgenic mice, the peripheral T cell compartment contained a high proportion of cells with an Ag-experienced cell surface profile, defined as CD44hiCD45RBlowCD62Llow and negative for CD25 and CD69. The ratio of naive vs memory phenotype cells decreased with age, as normally seen in wild-type mice. The possibility that peripheral T cells obtained the Ag-experienced surface phenotype because of a homoeostatic proliferation mechanism (57) in the CD2-GATA3-transgenic mice is unlikely, because these mice were not lymphopenic at any of the ages analyzed. Second, in CD2-GATA3-transgenic mice the expression of the Th2-specific T1/ST2 marker within the CD44hi memory Th cell population in spleen and lymph nodes was increased by a factor ~6 and ~20, respectively. T1/ST2 marker expression is associated with advanced Th2 commitment, as it was shown to be expressed only after repeated antigenic stimulation under Th2-polarizing conditions in vitro, with delayed kinetics compared with the kinetics of Th2 cytokines (55). Third, CD2-GATA3-transgenic T cells were rapidly induced to synthesize IL-4, IL-5, and IL-10 in vitro, whereas production of IL-2 was low, which is typical for memory Th2 cells (48). Fourth, T cells from CD2-GATA3 mice exhibited an increased recall response to TNP-KLH Ag in vitro. Finally, the selective increase of the total levels of the IL-4-dependent isotype IgG1 in the serum would also be consistent with increased Th2 memory formation.

It is presently not clear how GATA-3 would affect Th2 memory formation. GATA-3 may regulate the cell fate decision of activated CD4+ T cells, by reducing activation-induced cell death, in favor of Th2 memory cell formation. Alternatively, GATA-3 may facilitate the differentiation process of dividing effector T cells that are already committed to the memory cell fate. A third possibility is that GATA-3 would act as a survival factor for Th2 memory cells. This is not very likely, because survival alone does not appear to be sufficient for memory cell formation, as was shown by the absence of increased memory cell formation in Bel-2-transgenic mice (58). Additional experiments are required to define GATA-3 targets that are involved in Th2 memory cell formation.

One of the molecules involved in Th2 memory development may be T1/ST2, as it is normally specifically expressed in the Th2 lineage within the compartment of CD44hi activated/memory T cells (Fig. 2). Because cross-linking of T1/ST2 enhanced proliferation of Th2 cells that were stimulated with suboptimal concentrations of anti-CD3 mAb (55), it is possible that the increased proliferation and cell survival of CD2-GATA3-transgenic CD4+ T cells in the in vitro Th2 polarization cultures originates from increased T1/ST2 expression. The increased expression of T1/ST2 in CD2-GATA3-transgenic mice would argue for a direct regulation of T1/ST2 transcription in T cells by GATA-3. The identification of three GATA elements in the minimal GATA-responsive T1/ST2 promoter in mast cells (59) would support this hypothesis of a direct regulation of T1/ST2 expression by GATA-3, independent of Th2-specific cytokines. Therefore, we hypothesize that GATA-3 is not only essential for instructive differentiation of naive Th cells into committed Th2 cells (26–28, 31, 35), but can also affect proliferation and survival of GATA-3-expressing CD4+ T cells through the induction of T1/ST2.

In conclusion, this study shows that enforced expression of GATA-3 inhibits Th1 function and induces Th2 commitment in vivo. Moreover, the increased expression of T1/ST2, the enhanced production of Th2 cytokines in response to T cell activation, and the elevated serum levels of IgG1 in CD2-GATA3-transgenic mice argue for a role of GATA-3 in the formation of Th2 memory.

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