Natural CD4 CD25⁺ regulatory T cells control the burst of superantigen-induced cytokine production: the role of IL-10

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Abstract
In normal mice a subpopulation of CD4 T cells constitutively expresses the IL-2 receptor α chain (CD25). This natural CD4 CD25⁺ subset is thymus-born, constitutively expresses IL-10 mRNA, does not produce IL-2 and is resistant to apoptosis. These cells behave as regulatory T cells in the control of self-tolerance, inflammatory reactions and T cell homeostasis. The mechanisms by which natural CD4 CD25⁺ cells control the immune response is unclear. We examined CD25-deficient mice, which over-express various cytokines, including proinflammatory molecules, after bacterial superantigen stimulation in vivo. We observed that this abnormal cytokine production could be controlled by the injection of natural CD4 CD25⁺ T cells and that IL-10 production is needed, as CD4 CD25⁻ T cells from IL-10 knockout mice do not correct cytokine over-production in vivo. As the circulating IL-10 produced by CD25-deficient mice was ineffective, we deduced that the key source of IL-10 was the regulatory T cell population. IL-10 is also involved in the control of cytokine production by normal T cells. However, the target of IL-10 in this control is undefined. Whether it acts directly on the effector T cells or on the regulatory CD4 CD25⁺ T cells themselves to induce their functional maturation has to be clarified.

Introduction
CD4 regulatory T cells contribute to the mechanisms that maintain self-tolerance, thereby participating in the suppression of auto-immune diseases and in the control of immune response to foreign antigens. CD4 T cells, which constitutively express the α chain of the IL-2 receptor (IL-2Rα or CD25), are one of the subpopulations which participate in this cellular regulation (1–5). Natural CD4 CD25⁺ T cells are found in the thymus and peripheral lymphoid organs, where they represent 7–10% of all CD4 T cells in normal mice (6). They are generated in the thymus and then expand in the periphery (7,8). Mice thymectomized at 3 days of life are depleted of natural CD4 CD25⁺ T cells, suggesting that the latter migrate from the thymus to the periphery after this time and cannot be generated by peripheral activation of CD4 CD25⁻ T cells. Natural CD4 CD25⁺ T cells are functionally different from the CD4 CD25⁻ T cells, even if the latter can acquire CD25 upon activation (8–10). Natural CD4 CD25⁺ T cells constitutively express IL-10 mRNA, while CD4 CD25⁻ T cells do not. The former cannot express IL-2 mRNA, even after activation, while the latter secrete IL-2 (6). The inability of natural CD4 CD25⁺ T cells to secrete IL-2 explains their dependency on IL-2 to expand in vitro and in vivo, and their absence in IL-2 gene-deficient mice (IL-2 knockout (KO)) (7). Natural CD4 CD25⁺ and CD4 CD25⁻ T cells also differ by their susceptibility to apoptosis. In vivo, viral superantigens, which can activate both cell types, induce clonal deletion of the CD4 CD25⁻ subset only, natural CD4 CD25⁺ T cells being resistant (6). Natural CD4 CD25⁺ T cells appear to be involved in the control of self-tolerance, inflammatory reactions and T cell homeostasis (1–5,11). Mice rendered deficient in natural CD4 CD25⁺ T cells by neonatal thymectomy develop auto-immune diseases in multiple organs. Onset of auto-immune diseases in these animals can be avoided by transfer of natural CD4 CD25⁺ T cells (8,9,10–12). IL-2Rα- or IL-2-gene-deficient mice, which cannot support CD4 CD25⁺ T cell expansion (7), develop lymphadenopathy and inflammatory bowel disease (IBD) (13,14). The mechanisms by which CD4 CD25⁺ T cells control the immune response are unclear and may differ according to the specific function. CD4 CD25⁺ T cells form a heterogeneous population, as they can differ in terms of...
CD44, CD45, CD69 and CD62 ligand expression (8,15). It is therefore possible that the mechanism involved may differ according to the CD4 CD25⁺ T cell subset engaged.

In this study we examined IL-2Rα-deficient mice, which are devoid of CD4 CD25⁺ T cells and over-express various cytokines after bacterial SAG (bSAG) stimulation in vivo. We found that this abnormal cytokine expression could be controlled by in vivo injection of normal CD4 CD25⁺ T cells, but not by CD4 CD25⁺ T cells from IL-10 KO mice. We deduce that IL-10 produced by natural CD4 CD25⁺ T cells is needed for this regulatory function.

**Methods**

**Mice**

The following mice were used: C57 Bl/6 (B6), their congenic counterparts C57 BA (B6 BA Thy-1.1) and IL-10 gene-deficient mice (IL-10 KO) (30) were from Iffa Credo and Transgenic Alliance (L’Arbresle, France); IL-2Rα KO mice (13) were from CDTA-CNRS (Orléans, France).

**Antibodies and immunofluorescence**

The following antibodies were used: anti-CD4 (clone GK 1.5), anti-CD8 (clone 53-6.7), anti-CD25 (clone PC 61), anti-IL-10 receptor (IL-10R) (clone 1B1) (16) and anti-CD3 (clone 145-2C11). They were used purified, uncoupled or coupled to FITC, biotin or phycoerythrin (PE). Biotinylated antibodies were revealed with streptavidin–CyChrome (Becton Dickinson). Subpopulations of labeled cells were analyzed in a FACScan cytometer (Becton Dickinson, Mountain View, CA) with the Lysys II program for data acquisition and analysis.

**Cell separation**

Peripheral lymph nodes were removed and used to prepare cell suspensions in sterile conditions. CD4 T cells were prepared by negative selection. Briefly, lymph node cells were incubated with purified anti-CD8 antibody (clone 53-6.7), and then with anti-rat antibody-coated magnetic beads to remove CD8 T cells and with anti-mouse Ig-coated beads to remove B cells (Dynabeads; Dynal, Oslo, Norway). CD8 and B cells coated with magnetic beads were then depleted with a magnet. Purified CD4 T cells were labeled with biotinylated anti-CD25 (clone PC61). CD4 CD25⁺ T cells were positively selected using MACS microbeads (Miltenyi Biotech, Paris, France). Briefly, CD4 CD25⁺ cells were first eluted from the column, then CD4 CD25⁺ T cells (retained on the column) were eluted after removing the column from the magnetic field. CD4 CD25⁺ T cells were usually 90–95% pure.

**In vivo superantigen treatment and cytokine assays**

Normal B6 and IL-2Rα KO mice were injected with 50 µg of staphylococcal enterotoxin B (SEB; Sigma, France) i.p. Serum was recovered 1, 2, 3, 4 and 5 h after the injection. In cell transfer experiments with irradiated mice, serum was recovered 2 h after SEB injection.

IL-2, IL-10, IFN-γ and tumor necrosis factor-α were assayed in serum with ELISA kits (R & D Systems Europe, Abingdon, UK) according to the manufacturer’s recommendations. Results are expressed as pg/ml serum.

**Cell transfer**

IL-2Rα KO mice 5–7 weeks old were sublethally irradiated (600 rad) and injected i.v. on the same day with 10⁶ purified CD4 CD25⁺ or CD4 CD25⁻ T cells. Control mice were irradiated but not injected. CD4 CD25⁺ and CD4 CD25⁻ T cells were from normal B6 BA mice, congeneric for the expression of Thy-1, which was used to follow donor cell reconstitution of irradiated mice.

In some experiments IL-2Rα KO mice were injected with 10⁸ purified CD4 CD25⁺ or CD4 CD25⁻ lymph node cells from IL-10 KO mice. The congenic marker was not available in this case.

Fifteen days after irradiation, mice were injected with 50 µg SEB i.p., and killed 2 h later. Serum was recovered and stored at −20°C. Lymph nodes and spleens were removed, and cell suspensions prepared. Absolute numbers of total CD4 T cells, CD8 T cells, and CD4 T cells were determined by cell counting, and anti-CD4 and anti-Thy-1 labeling when possible.

**IL-10R blockade in vivo**

Normal B6 mice were injected i.p. (3×500 µg) with purified anti-IL-10R antibody (clone 1B1) (16) or saline. The second injection was given 24 h after the first and the third 2 h later. SEB (50 µg) was injected 30 min after the last antibody injection. Mice were killed 2 h later and serum was stored at −20°C until cytokine assay.

**In vitro culture**

CD4 CD25⁻ T cells from B6 BA mice were purified and cultured (10⁵ cells/well) in 100 µl of culture medium (RPMI 1640 supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 1% sodium pyruvate, 10⁻⁵ M mercaptopoethanol and 10% FCS). CD4 CD25⁺ T cells (10⁵ or 2×10⁵) from B6 BA or IL-10 KO mice were added to CD4 CD25⁻ T cells. Cells were cultured in wells coated overnight with anti-CD3 (10 µg/ml, 50 µl/well). Phorbol myristate acetate (PMA; 50 ng/ml) was added at the initiation of the cultures. Cells were cultured for 48 h at 37°C with 5% CO₂.

After culture the absolute number of living cells per well was determined in the Trypan blue exclusion test. Pooled supernatants from several wells in each group were stored at −20°C for cytokine assays.

**Statistical analysis**

Results are expressed as means ± SEM. Comparisons were made using Student’s t-test and differences were considered significant when P < 0.05.

**Results**

SAG-induced cytokine production is enhanced in IL-2Rα KO mice

SEB was injected into normal B6 and IL-2Rα KO mice, and IL-2, IFN-γ, TNF-α and IL-10 were assayed in serum 1, 2, 3, 4 and 5 h later (Fig. 1).

Levels of the four cytokines were higher in IL-2Rα KO mice than in normal B6 mice. IL-2 levels peaked 2 h after SEB injection in normal B6 mice, but continued to increase until 4 h in IL-2Rα KO mice; cumulative IL-2 production was
the absolute number of total CD4 T cells (15 SEB and serum was collected 2 h later. At this time point mice lymph nodes. Fifteen days later they were injected with B6 or IL-2RαKO mice were injected i.p. with 50 µg of SEB and sera were collected 1–5 h later. Levels of IL-2, IFN-γ, TNF-α and IL-10 were determined in each serum sample by ELISA. Cytokine levels (pg/ml serum) were determined in four to six individual mice per time point. Results are expressed as means ± SEM.

~4 times higher in IL-2RαKO than in normal B6 mice. IFN-γ production peaked 4 h after SEB injection in both normal B6 and IL-2Rα KO mice, but was 2–3 times higher in the latter. TNF-α production peaked 2 h after SEB injection in both normal B6 and IL-R2α KO mice, but was 4 times higher in the latter. IL-10 levels were very low or undetectable in the serum of normal B6 mice. Surprisingly, despite the absence of the natural CD4 CD25+ T cell population in IL-2Rα KO mice, IL-10 was readily detectable 1 h after SEB injection and peaked at 2 h.

Thus, after SAG administration, mice lacking CD25 expression escape the normal control of cytokine production and produce much higher levels of IL-2, IFN-γ and TNF-α, as well as the immunoregulatory cytokine IL-10.

Reconstitution of IL-2Rα KO mice by natural CD4 CD25+ regulatory T cells corrects the burst of SAG-induced cytokine production

Sublethally irradiated IL-2Rα KO mice were injected with 10⁶ natural CD4 CD25+ or CD4 CD25− T cells purified from normal mice lymph nodes. Fifteen days later they were injected with SEB and serum was collected 2 h later. At this time point the absolute number of total CD4 T cells (15–20×10⁶ in the spleen plus lymph nodes) was identical whether mice were simply irradiated or both irradiated and injected with donor CD4 T cells (CD25+ or CD25−) (Fig. 2). Importantly, the absolute number of donor CD4 T cells was identical in mice injected with CD4 CD25+ or CD4 CD25− T cells (~2.5×10⁶ in the spleen plus lymph nodes). Similar numbers of CD8 T cells were also found in mice injected with CD4 CD25+ or CD4 CD25− T cells (data not shown). Injection of CD4 CD25+ T cells from normal B6 mice to IL-2Rα KO mice did not modify the elevated cytokine secretion (mean values 16298 pg IL-2/ml serum in CD4 CD25− reconstituted mice compared to 14492 pg in control mice, 2452 pg IFN-γ versus 1896 pg in control mice and 264 pg IL-10 versus 272 pg in control mice). Reconstitution with CD4 CD25+ T cells significantly reduced cytokine production compared to both control and CD4 CD25− T cell injected mice (mean values: 3676 pg IL-2/ml serum, P < 0.004; 60 pg of IFN-γ; P < 0.01; and 90 pg IL-10, P < 0.01) (Fig. 3A).

These results suggest that natural CD4 CD25+ T cells can down-regulate the elevated production of IL-2, IFN-γ and IL-10 induced by SEB in the serum of mice lacking the IL-2Rα.

Natural CD4 CD25+ T cells from IL-10, KO mice do not regulate SAG-induced cytokine production by IL-2Rα KO mice in vivo

In the model of IBD induction by transfer of CD4 CD45RBhigh cells, transforming growth factor (TGF)-β and especially IL-10 mediate the suppression of the inflammatory disease by regulatory CD45RBlow cells (17–19). As we found that IL-2Rα KO mice produced high levels of IL-10 after SEB injection and that these high levels were reduced by natural CD4 CD25+ T cell transfer, IL-10 may not have been involved in the control of cytokine production in this model. Alternatively, IL-10 produced by CD4 CD25+ cells during cell–cell interactions may have been required. IL-10 is produced by numerous cell types. Circulating IL-10 produced by cell types excluding CD4 CD25+ T cells, as it is the case in IL-2Rα KO mice (Fig. 1), may be ineffective. To test these two hypotheses, CD4 CD25+ and CD4 CD25− T cells from IL-10 KO mice were injected into irradiated IL-2Rα KO mice. Two weeks later, IL-2, IFN-γ and IL-10 levels were measured in serum
2 h after SEB injection. As shown in Fig. 3(B), CD25⁺ T cells from IL-10 KO mice did not down-regulate IL-2, IFN-γ and IL-10 production in IL-2Rx KO mice (mean values: 8143 pg IL-2/ml serum in CD4 CD25⁺ T cells compared to 9242 pg in control mice, 1364 pg IFN-γ compared to 1113 pg in control mice and 372 pg IL-10 versus 245 pg in control mice). In terms of the absolute number of T cells expressing CD25 (expressed only on donor T cells), CD4 CD25⁺ T cell numbers in recipients were similar after injection of natural CD4 CD25⁺ T cells from either normal B6 or IL-10 KO mice, showing that their reconstitution capacity was identical (not shown). Interestingly, CD4 CD25⁻ T cells from IL-10 KO mice induced enhanced production of IL-2 in the serum of their IL-2Rx KO recipient mice (27311 pg/ml serum) and of IFN-γ (8323 pg/ml serum) compared to control mice (P < 0.04 and P < 0.004 respectively). This is linked probably to the capacity of cells from IL-10 KO mice to produce high levels of these cytokines after SEB stimulation in vivo (20). Indeed, IL-10 which they did not produce was not enhanced (289 versus 245 pg in serum of control mice). However, a stimulatory effect of IL-10 KO mouse CD4 T cells on recipient cells cannot be excluded.

These results suggest that IL-10 is involved in the regulation of cytokine over-production by IL-2Rx KO mice transferred by CD4 CD25⁺ regulatory T cells. Moreover, IL-10 has to be produced by CD4 CD25⁺ regulatory T cells themselves.

IL-10 is also involved in the control of cytokine production by normal T cells both in vivo and in vitro

The results described above lead to the conclusion that normal CD4 CD25⁺ regulatory T cells producing IL-10 can correct the abnormal SAG-induced production of cytokine in mice unable to express a high-affinity IL-2R. We need to demonstrate that IL-10 and regulatory CD4 CD25⁺ T cells are also involved in the regulation of cytokine production by normal T cells.

In the first set of experiments, we blocked the IL-10R by repeated injections of anti-IL-10R antibody in normal mice, before SEB stimulation in vivo. As shown on Table 1, the in vivo production of IL-2, IFN-γ and TNF-α was enhanced by inhibiting IL-10–IL-10R interaction. It indeed suggests that IL-10 is also involved in the control of cytokine production in normal mice. Interestingly, in that experimental model, IL-10 production in the serum was not significantly modified.

In the second model we tested the effect of normal versus IL-10 KO CD4 CD25⁺ T cells on the cytokine response of normal CD4 CD25⁻ T cells to TCR triggering in vitro.

It has already been shown that regulatory CD4 CD25⁺ T cells suppress the proliferation of CD4 CD25⁻ T cells in vitro by a cell–cell interaction mechanism which involves the inhibition of IL-2 production. However, the role of IL-10 in this effect has not been demonstrated (8,10–12). We attempted to reconcile these findings with our observation of the regulatory role of IL-10 in vivo (Fig. 2 and Table 1). Purified CD4 CD25⁻ T cells were stimulated by plastic-bound anti-CD3 and PMA. CD4 CD25⁻ T cells were added at a physiologic ratio (10⁵ or 2 × 10⁵ CD25⁻ cells/10⁶ CD25⁻ cells). Production of IL-2, IFN-γ and TNF-α was assayed in the culture supernatant 48 h later. Cytokine production was
CD25+ T cells from normal B6 BA mice were isolated and cultured either alone (10^6 cells/ml) or with 10^5 or 2 \times 10^5 CD4 CD25+ T cells from B6 BA or IL-10 KO mice. Cells were stimulated with immobilized anti-CD3 and PMA. IL-2, IFN-γ and TNF-α were measured in supernatants recovered 48 h later. Results are expressed as cytokine level (pg/ml supernatant) and percent inhibition of cytokine level in mixed cultures compared to CD4 CD25+ T cells cultures alone. The absolute number of viable cells was measured at the end of the culture. Two individual experiments are shown.

<table>
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<tr>
<th>Condition</th>
<th>CD25+ Cellsa</th>
<th>IL-2 (pg/ml)</th>
<th>% Inhibition</th>
<th>IFN-γ (pg/ml)</th>
<th>% Inhibition</th>
<th>TNF-α (pg/ml)</th>
<th>% Inhibition</th>
<th>Cell no./well × 10^6</th>
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<td>–</td>
<td>–</td>
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<td>–</td>
<td>3330</td>
<td>–</td>
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<tr>
<td></td>
<td>B6 BA</td>
<td>B6 BA</td>
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<tr>
<td></td>
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<td>37</td>
<td>534</td>
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<tr>
<td></td>
<td>B6 BA</td>
<td>B6 BA</td>
<td>2 \times 10^5</td>
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<td>–</td>
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CD25+ T cells inhibit when B6 CD25+ and B6 CD25- T cells were cocultured, and the percentage inhibition was proportional to the number of CD25+ T cells. Production of both IL-2 and IFN-γ was nearly abrogated by 2 \times 10^5 CD25+ T cells. TNF-α production was less sensitive to inhibition. We then examined the involvement of IL-10 production by natural CD4 CD25+ regulatory T cells in vitro. Combinations of B6 CD25+ cells with CD25+ T cells from IL-10 KO mice were used. As shown in Table 2, natural CD4 CD25+ T cells from IL-10 KO mice can also inhibit the production of cytokine by activated normal CD4 CD25+ T cells, but this effect is poor compared to the inhibition induced by normal CD4 CD25+ T cells.

It should be noted that the absolute number of viable cells per well (Table 2) and the percentage of natural CD4 CD25+ T cells (data not shown) were similar in each experimental condition.

Collectively, these results suggested that CD4 CD25+ T cells inhibited not only IL-2 as previously shown by others (10,12) but also IFN-γ and TNF-α production by normal CD4 CD25– T cells activated by anti-CD3 in vitro. The lesser inhibition produced by CD4 CD25– T cells from IL-10 KO mice suggested that IL-10 was involved in the inhibitory process, although additional mechanisms were involved, at least in vitro.

### Discussion

Excessive immune responses to foreign antigens may lead to severe inflammatory reactions and host tissue destruction. This probably accounts for the inflammatory diseases (mainly targeting the bowel) which develop in certain gene-manipulated mice. All these mice share an impaired immune response due to disruption of either immune recognition mechanisms (TCR or MHC gene manipulation) or effector functions (cytokine or cytokine receptor gene manipulation) (13,14,31,21–24). Multiple mechanisms may be involved in these inflammatory diseases, which are often associated with other disorders such as dysregulation of homeostasis and lymphocyte accumulation in peripheral lymphoid organs. The absence of natural regulatory CD4 T cells may be one such mechanism leading to excessive inflammatory reactions. Indeed, IL-2Rα KO mice and IL-2 KO mice (13,14), which lack natural CD4 CD25+ regulatory T cells, develop IBD. We used IL-2Rα KO mice to examine whether CD4 CD25+ T cells can regulate the inflammatory response to SAG.

IL-2Rα KO mice were injected with the bSAG SEB. IL-2, IFN-γ and TNF-α production was elevated in the hour following SEB injection in IL-2Rα KO mice compared to normal B6 mice. IFN-γ and TNF-α are the main cytokines involved in the tissue damage characteristic of IBD. Indeed, injection of anti-IFN-γ and anti-TNF-α inhibits the development of IBD in SCID mice injected with pathogenic CD4 CD45RBhigh T cells as efficiently as the injection of CD4 CD45RBlow regulatory T cells (25,26).

Natural CD4 CD25+ or CD4 CD25- T cells were injected in vivo to irradiated IL-2Rα KO mice, and serum IL-2 and IFN-γ levels were then measured. Contrary to CD4 CD25- T cells, transfer of CD4 CD25+ T cells induced a reduction in IL-2 and IFN-γ production after SAG stimulation in vivo.

It has been shown that IL-10 is the most effective cytokine in controlling induced IBD, although TGF-β is also involved (3,25). Indeed, regulatory T cells from IL-10 KO mice (which themselves normally develop IBD in conventional environment) do not prevent inflammatory disease onset, while injection of anti-IL-10 or anti-IL-10R antibody in vivo abrogates the protective effect of regulatory CD4 CD45RBlow cells against IBD (19). IL-10 production by regulatory CD4 CD25+ T cells in vivo was also involved in the control of cytokine production.
production in our model. Indeed, transfer of natural CD4 CD25+ T cells from IL-10 KO mice into IL-2Rx KO mice did not down-regulate their elevated production of IL-2 and IFN-γ. Although circulating IL-10 was very low in B6 mice injected with SEB, IL-10 may also be involved in the regulation of cytokine production in normal mice. We found that IL-10R blockade by repeated injection of anti-IL-10R antibody in normal mice led to increased circulating levels of SAG-induced IL-2, IFN-γ and TNF-α, suggesting that IL-10 is currently involved in the control of cytokine production in vivo.

However, when IL-2Rx KO mice were injected with SEB, not only were IL-2, IFN-γ and TNF-α levels increased compared to normal B6 mice, but the level of circulating IL-10 also increased markedly, for up to 2 h after SEB challenge. IL-10 is produced by various cell types, including macrophages (27), which can be stimulated by SAG. The circulating IL-10 in IL-2Rx KO mice may originate from accessory cells, as CD4 regulatory T cells, which produce IL-10, are lacking in these mice. Thus, despite a high level of circulating IL-10, SAG-induced cytokine production is not properly controlled in IL-2Rx KO mice. Contacts between CD4 CD25+ and CD4 CD25− T cells are required for the suppressive effect on IL-2 production and CD4 CD25− T cell proliferation in vitro (8,10). Similar cell–cell interactions may be required for the inhibition of cytokine synthesis in vivo, between regulatory (IL-10-producing) CD4 CD25+ T cells and CD4 CD25− T cells. Interestingly, Rag-2 KO mice transferred with IL-10 KO T cells develop IBD and high-level IFN-γ production, even though the tissues of Rag-2 KO mice contain macrophages and epithelial cells capable of producing IL-10 (28). Indeed, SAG-treated Rag-2 KO mice produce high levels of IL-10 (personal unpublished data). Collectively, these results suggest that the critical source of IL-10 is the regulatory CD4 CD25+ T cell population.

Interestingly, injection of natural CD4 CD25+ T cells to IL-2Rx KO mice regulated not only circulating IL-2 and IFN-γ production induced by SAG, but also IL-10 production. One possible explanation is a feedback mechanism by which IL-10 produced by regulatory CD4 CD25+ T cells inhibits IL-10 synthesis by other IL-10-producing cells (29). Another is that CD4 CD25+ T cells use the circulating IL-10 available at the time of cell transfer for their own differentiation and/or maintenance. In that case, the target for IL-10 may be essentially the regulatory T cells themselves. Indeed, Groux and collaborators raised CD4 T cell clones by chronic antigen activation in vitro in the presence of IL-10. While they have been differentiated in vitro with IL-10, CD4 clones produced high levels of IL-10, and displayed regulatory functions both in vitro and in vivo (18). Whether IL-10 is also acting directly on the effector cells in vivo has to be demonstrated. Interestingly, we show here that IL-10 KO, CD4 CD25+ T cells transferred to IL-2Rx KO mice do not down-regulate circulating IL-10. It suggests that even when they are unable to produce IL-10, CD4 CD25+ T cells cannot use circulating ones, in order to become regulatory. It may be that autocrine production of IL-10 is needed for the maturation of CD4 CD25+ T cells in vivo.

IL-10 may not be the only factor involved in the regulatory functions of CD4 CD25+ T cells. Although IL-10 was involved in the regulation of cytokine production in vivo, other factors (such as TGF-β or IFN-γ) (3,25,30) may also play a role. These factors may be differently involved in different experimental protocols, both in vivo and in vitro. CD4 CD25+ T cells differentiated normally in vivo in IL-10 KO mice (data not shown). However, they cannot compensate their lack of IL-10 production in vivo to control SAG-induced cytokine over-production, but can partly overcome the absence of IL-10 to suppress in vitro. Indeed, we found that CD4 CD25+ T cells from IL-10 KO mice were able to inhibit CD4 CD25− T cell production of IL-2, IFN-γ and TNF-α induced by anti-CD3 and PMA in vitro. However, the suppressive activity of natural regulatory CD4 CD25+ T cells was much less effective in the absence of IL-10. Different conditions of stimulation or CD25− CD25+ T cell ratio may explain the discrepancy between our in vitro data and others, where the role of IL-10 was not obvious (8,10–12). Higher quantities of regulatory T cell may compensate for the lack of IL-10 by the production of other regulatory cytokine.

CD4 CD25+ T cells variably express markers such as CD45RB, CD44, CD69, CD54 and CD62 ligand (8,15). Further studies are required to determine whether CD4 CD25+ T cells can be divided into different functional subpopulations using different regulatory strategies.

Abbreviations

bSAG bacterial superantigen
IL-2Rx IL-2 receptor α chain
IL-10R IL-10 receptor
KO knockout
IBD inflammatory bowel disease
PMA phorbol myristate acetate
SAG superantigen
SEB staphylococcal enterotoxin B
TGF transforming growth factor
TNF tumor necrosis factor

References


