IL-2 Regulates CD103 Expression on CD4+ T Cells in Scurfy Mice that Display Both CD103-Dependent and Independent Inflammation

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J Immunol 2009; 183:1065-1073; Prepublished online 24 June 2009;
doi: 10.4049/jimmunol.0804354
http://www.jimmunol.org/content/183/2/1065
IL-2 Regulates CD103 Expression on CD4+ T Cells in Scurfy Mice that Display Both CD103-Dependent and Independent Inflammation

Rahul Sharma,* Sun-sang Joe Sung,* Christian E. Abaya,* Angela Chiao-Ying Ju,* Shu Man Fu,2,3* and Shyr-Te Ju2,3*†

Scurfy (Sf) mice lack CD4+Foxp3+ regulatory T cells and develop fatal multiorgan inflammation (MOI) mediated by CD4+ T cells. Introducing Il2−/− gene into Sf mice (Sf.II2−/−) inhibited inflammation in skin and lung. As a major integrin receptor for the organs, we compared CD103 expression on the CD4+ T cells of B6, II2−/−, Sf, and Sf.II2−/− mice. CD103+CD4+ T cells, but not CD8+ T cells or CD11c+ dendritic cells, were significantly up-regulated only in Sf mice, indicating Il2−/− dominantly and specifically inhibited CD103 up-regulation in Sf CD4+ T cells. In addition, CD4+Foxp3+ regulatory T cell CD103 expression was not reduced in Il2−/− mice. Introducing CD103−/− into Sf mice inhibited inflammation in skin and lung as compared with age-matched Sf mice, but they died at ~7 wk old with inflammation developed in skin, lungs, and colon, demonstrating fatal MOI induced by CD103-independent mechanism. Transfer of Sf CD4+ T cells induced MOI more rapidly than CD103+ CD4+ T cells, indicating the presence of CD103-dependent mechanism for inflammation. In vitro stimulation with anti-CD3 plus anti-CD28 beads confirmed that CD103 induction in the CD4+Foxp3− T cells in Il2−/− and Sf.II2−/− is defective and cannot be restored by rIL-2 or rIL-15. The data indicate that IL-2 is required for optimal CD103 induction on CD4+ T cells in Sf mice and this effect contributes to inflammation in an organ-specific manner. IL-2 also has additional roles because the protection of skin and lung inflammation in Sf.II2−/−, but not Sf.CD103−/− mice is lifelong and Sf.II2−/− mice have longer lifespan than Sf.CD103−/− mice. The Journal of Immunology, 2009, 183: 1065–1073.

Thymocyte differentiation and selection impart distinct functions with different surface marker phenotypes into different T cell populations. In the CD4+ T cell compartment, two subsets are generated, as follows: a CD25+ regulatory T cells or CD11c+ dendritic cells, were significantly up-regulated only in Sf mice, indicating Il2−/− dominantly and specifically inhibited CD103 up-regulation in Sf CD4+ T cells. In addition, CD4+Foxp3+ regulatory T cell CD103 expression was not reduced in Il2−/− mice. Introducing CD103−/− into Sf mice inhibited inflammation in skin and lung as compared with age-matched Sf mice, but they died at ~7 wk old with inflammation developed in skin, lungs, and colon, demonstrating fatal MOI induced by CD103-independent mechanism. Transfer of Sf CD4+ T cells induced MOI more rapidly than CD103+ CD4+ T cells, indicating the presence of CD103-dependent mechanism for inflammation. In vitro stimulation with anti-CD3 plus anti-CD28 beads confirmed that CD103 induction in the CD4+Foxp3− T cells in Il2−/− and Sf.II2−/− is defective and cannot be restored by rIL-2 or rIL-15. The data indicate that IL-2 is required for optimal CD103 induction on CD4+ T cells in Sf mice and this effect contributes to inflammation in an organ-specific manner. IL-2 also has additional roles because the protection of skin and lung inflammation in Sf.II2−/−, but not Sf.CD103−/− mice is lifelong and Sf.II2−/− mice have longer lifespan than Sf.CD103−/− mice. The Journal of Immunology, 2009, 183: 1065–1073.

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Received for publication December 29, 2008. Accepted for publication May 12, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institutes of Health Grants DE-017579 and AR-051203 (to S.-T.J.), AR-047988 and AR-049449 (to S.M.F.), and AI-079906 (to S.-s.J.S.), and a grand-in-aid from the Beirne B. Carter Center of Immunology (to R.S.).

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4 Abbreviations used in this paper: Treg, CD4+Foxp3+ regulatory T cell; LN, lymph node; MOL, multiorgan inflammation; Sf, scurfy.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.0804354
Currently, it is unclear whether the phenotype associated with II2−/− mice is due to complete absence of Treg cells or to lack of functional Treg cells.

Materials and Methods

Mice

C57BL/6 (B6), B6.Fas+Ipr, B6.JIl2−/−, B6.129S2(C)-Itgaxoeicopgj (CD103−/−), B6.Cg-Foxp3gY1/J, and B6.129S7-Rag+Mmog1/J (Rag1−/−) mice were obtained from The Jackson Laboratory. B6.JIl2−/− mice were bred with male B6 mice to produce Sf mice (CD103−/−). Male B6.Cg-Foxp3gY1/J mice were bred with female B6 mice to generate Sf mice (CD103−/−). These hypotheses, predictions, and some novel findings are presented in this study.

Cell purification and in vitro stimulation

CD4+ T cells were purified using the CD4+ T cell negative purification kit (Miltenyi Biotec). The CD4+ CD103+ T cells were purified by using PE-labeled anti-CD103 mAb and anti-PE beads (Miltenyi Biotec), according to the manufacturer’s protocols. Purified CD4+ T cells (106) or unfractionated LN T cells (2 × 106) from age- and sex-matched B6, II2−/−, and Sf mice were cultured with anti-CD3/anti-CD28 beads (Invitrogen) at a cell/bead ratio of 1 in a 48-well culture plate for 72 h. IL-2 (50 IU/ml), IL-15 (100 ng/ml), and TGF-β (2.5 ng/ml), obtained from PeproTech, were added either alone or in combination during activation.

Adoptive transfer

LN cells (15 × 106) from Sf II2−/−, Sf Foxp3Ipr, or Sf CD103−/− mice were injected i.v. into adult Rag1−/− male mice. Histological examination was conducted at 4–7 wk after transfer on H&E-stained sections of various organs.

Histology

Tissues/Organs from age-matched males of various strains were fixed with 10% neutral buffered formalin (Fisher Scientific), and sections of paraffin-embedded tissue were stained with H&E. Tissues/Organs examined included skin, ear, lung, colon, and liver.

Results

Sf, but not II2−/− and Sf II2−/− mice display severe inflammation in skin, tail, and lung

We have recently reported a detailed comparison of inflammation among various organs of Sf, II2−/−, and Sf II2−/− mice (22). The major organs that displayed severe inflammation in Sf, but not II2−/− and Sf II2−/− mice were skin, tail, and lung, whereas all mice were found to have a moderate level of inflammation in the liver. In contrast, colitis was observed in II2−/− and Sf II2−/− that lived beyond weaning, but not in Sf mice that rarely lived beyond weaning. Although these observations indicate that the phenotype associated with II2−/− mutation is dominant over Sf, the mechanism(s) responsible was not addressed in that study.

CD103−/− CD4+ T cells are increased in Sf, but not II2−/− and Sf II2−/− mice

Because the inflammation-inducing cells in Sf mice are CD4+ T lymphocytes and CD103 plays a critical role in the homing of cells to cutaneous and mucosal organs, we determined the expression of CD103−/− CD4+ T cells among B6, Sf, II2−/−, and Sf II2−/− mice (Fig. 1). As shown in Fig. 1A, the fraction of CD4+ T cells that expressed CD103 was low, and ranged between 3 ± 1% in the LN of normal B6 mice. In contrast, the fraction of CD103−/− CD4+ T cells in Sf mice was 15 ± 2%, presumably due to the spontaneous activation resulting from the loss of Treg. Interestingly, CD103−/− expression on the CD4+ T cells of II2−/− mice was not increased and remained in the range of 4 ± 1%. Importantly, the lack of increase in CD103 expression on CD4+ T cells was also observed in Sf II2−/− mice (2 ± 1%), indicating that this is not due to the presence in II2−/− mice the residual Treg that suppress T cell activation and CD103 expression, but rather a real dominant

was filtered through a 100-mesh nylon filter (BD Biosciences), and the lymphocytes were harvested by centrifugation through a 45–70% discontinuous Percoll gradient. Cells were suspended in 100 μl of PBS solution (containing 4 mg of BSA and 1 μg of anti-FeR mAb 2.4G2) and incubated with 0.2 μg of various fluorescent Abs for 30 min at 4°C. FITC-, PE-, or PE-Cy5-conjugated Abs for 30 min at 4°C. FITC-, PE-, or PE-Cy5-conjugated Abs for 30 min at 4°C. FITC-, PE-, or PE-Cy5-conjugated Abs for 30 min at 4°C. FITC-, PE-, or PE-Cy5-conjugated Abs for 30 min at 4°C.
CD4, as described in calculated. Three to five mice per group were used.

and lung inflammation in these mice. The total CD103 from that observed in the skin and lungs of B6 mice (Fig. 1, mice. In contrast, the percentage of CD103 expression on Treg (40), inflammation did not down-
effect of IL2/−/− operated at a stage after the Treg checkpoint (Fig. 1A). It should be emphasized that the IL-2 requirement for the up-regulation of CD103+CD4+ T cells was not observed by comparing IL2/−/− with B6 mice. Only by comparing Sf with Sf.IL2/−/− mice was this dominant phenotype observed, indicating that IL-2 requirement for CD103 expression is associated with spontaneous CD4+ T cell activation in Sf mice. Although inflammation signal has been implicated in the down-regulation of CD103 expression on Treg (40), inflammation did not down-regulate CD103 expression in our system because high CD103 expression on CD4+ T cells was observed in the highly inflamed tissues of Sf mice.

Skin and lungs in IL2/−/− and Sf.IL2/−/−, but not Sf mice are deficient in CD103+CD4+ T cells

Because CD103 expression is not increased in the LN of IL2/−/− and Sf.IL2/−/− mice, we determined the expression of CD103+CD4+ T cells in the skin and lungs. Like LN cells, the percentage of CD103+CD4+ T cells was increased in the skin and lungs of Sf mice. In contrast, the percentage of CD103+CD4+ T cells was low in IL2/−/− and Sf.IL2/−/− mice and was not significantly different from that observed in the skin and lungs of B6 mice (Fig. 1, B and C). Table I summarized the total number of T cells, CD4+ T cells, and CD103+CD4+ T cells in the LN, lungs, and skin. As a result of lymphoproliferation, LN cell numbers in IL2/−/− and Sf mice were high, but significantly higher numbers of total T cells were observed in the LN of Sf.IL2/−/− mice. Similar findings were observed for CD4+ T cells. In contrast to LN, high numbers of total T cells and total CD4+ T cells were observed in the skin and lungs of Sf mice, but not IL2/−/− and Sf.IL2/−/−, reflecting the lack of skin and lung inflammation in these mice. The total CD103+CD4+ T cells in the LN of Sf mice are very high, but are moderate in the LN of IL2/−/− and Sf.IL2/−/− mice, even though the latter contained twice as many CD4+ T cells (Table I). Thus, only Sf mice contained high CD103+CD4+ T cells in their LN, skin, and lungs, and this increase correlated with organ inflammation. The data suggest that CD103+CD4+ T cells are the critical inflammation-inducing T cells in Sf mice, and the lack of these cells in IL2/−/− and Sf.IL2/−/− mice is one reason that these mice failed to develop inflammation in the skin and lungs.

IL-2-dependent regulation of CD103 is specific to CD4+Foxp3+ T cells

Because a significant portion of CD4+Foxp3+ Treg expresses CD103 and because IL2/−/− mice have a significant level of CD4+Foxp3+ Treg in the periphery, it becomes important to determine whether the Treg in IL2/−/− mice express low CD103 like their CD4+Foxp3+ T cell counterparts. We determined the total number of Treg and the CD103 expression on Treg isolated from LN, lung, liver, skin, and colon (Fig. 2A). In agreement with a previous report, the percentage of CD4+Foxp3+ Treg in the LN CD4+ T cells in IL2/−/− mice (4%) is ~33% of that present in B6 counterpart (9%). However, the total CD4+ T cells in the LN of IL2/−/− mice are 2–3 times more than B6 (22) (this study). Thus, the total number of Treg was only slightly reduced in the IL2/−/− mice. Moreover, the ratio of CD103+ Treg to CD103− Treg was higher in IL2/−/− mice than B6 control. Similar findings and trends were observed in the other organs examined (Fig. 2A). The CD103+ Treg in IL2/−/− colon appear to have lower Foxp3 expression than B6 control, but somewhat higher CD103 expression. In addition, CD103− Treg was almost absent, suggesting a strong local environmental influence on CD103 expression on Treg (40). The presence of CD103+ Treg in IL2/−/− mice should provide additional force to protect the skin and lungs from inflammation amid the reduced levels of CD103+CD4+ inflammation-inducing T cells.

The preferential regulation of CD103 expression by IL-2 on CD4+ T cells can only be observed by comparing CD103+CD4+ T cell expression levels among B6, Sf, IL2/−/−, and Sf.IL2/−/− mice. This activation-dependent expression of CD103 is in contrast to the constitutive expression of CD103 on CD8+ T cells observed in normal mice. The percentages of CD103+CD8+ T cells in the LN of B6, Sf, IL2/−/−, and Sf.IL2/−/− mice are 73, 49, 19, and 18%, respectively (Fig. 2B). Thus, unlike CD4+ T cells, CD103 expression on CD8+ T cells is down-regulated in Sf, IL2/−/− mice, and Sf.IL2/−/− mice.

Because CD103 is also expressed on CD11c+ DC cells, we determined whether they were reduced in IL2/−/− and Sf.IL2/−/− mice as compared with B6 and Sf mice, respectively. No significant

Table 1. IL2−/− inhibits the preferential expression of CD103+CD4+ T cells in the LN, skin, and lungs of Sf mice*

<table>
<thead>
<tr>
<th>Mice</th>
<th>Total Cells</th>
<th>CD4+ T Cells</th>
<th>CD103+CD4+ T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN (number of cells × 10⁶)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>17 ± 2</td>
<td>6 ± 1</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Sf</td>
<td>71 ± 14</td>
<td>24 ± 7</td>
<td>0.9 ± 0.02</td>
</tr>
<tr>
<td>Sf.II2−/−</td>
<td>131 ± 35</td>
<td>43 ± 14</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Lung (number of cells × 10⁶)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>21 ± 2</td>
<td>4 ± 1</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Sf</td>
<td>22 ± 2</td>
<td>5 ± 2</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Sf.II2−/−</td>
<td>26 ± 4</td>
<td>19 ± 4</td>
<td>5 ± 0.7</td>
</tr>
<tr>
<td>Skin (number of cells × 10⁶)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>26 ± 4</td>
<td>0.9 ± 0.3</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>Sf</td>
<td>18 ± 3</td>
<td>0.8 ± 0.2</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>Sf.II2−/−</td>
<td>13 ± 9</td>
<td>1.6 ± 1.2</td>
<td>3 ± 0.08</td>
</tr>
</tbody>
</table>

*Lymphocytes were obtained from LN, lungs, and skin of indicated mice and counted. Cells were stained for CD4 and CD103, and the total number of CD4+ and CD103+CD4+ T cells was calculated. The data were obtained from four to five mice for each group.
There was no change in the expression of CD103 on the CD11c+ DC cells as compared with the Sf counterpart. These observations indicate that the IL-2-dependent up-regulation of CD103 expression is specific to CD4+ T cells in Sf mice.

**CD103 is a major target of IL-2-regulated receptor for Sf T cell trafficking**

We examined several cell surface receptors involved in T cell trafficking using available mAb to determine whether CD103 expression on Sf CD4+ T cells is a major target of IL-2-dependent regulation (Fig. 3). None of the receptors examined were significantly different in the LN CD4+ T cells between Sf and SfIIFas+/+ mice as compared with B6 control. LN cells from these mice were stained for CD11c and CD103, as described in Materials and Methods. Representative data from at least three experiments are presented for B and C.

**Effect of CD103−/− on MOI in Sf mice**

If CD103+CD4+ T cells are required for inflammation in the skin and lungs of Sf mice, targeted knockout of CD103 should inhibit such inflammation. This interpretation is tested by breeding the CD103−/− mutation into Sf mice (Fig. 5). The result showed a considerable inhibition of inflammation in the ears, skin, tail, and lungs as compared with age-matched Sf control (Fig. 5). Moreover, inflammation in the liver remained in the SfCD103−/− mice (Fig. 5B). Despite initial inhibition of MOI, significant inflammation in the liver, skin, and lungs and moderate colitis and inflammation in tail were observed in SfCD103−/− mice that lived beyond weaning. As a result, the lifespan of SfCD103−/−...
mice was only slightly prolonged to ~7–8 wk, which is significantly shorter than Sf.II2−/− mice, suggesting that IL-2 plays additional roles in the inflammation response other than regulating CD103 expression on CD4+ T cells. Interestingly, leukocyte infiltration was weak around epithelial cell areas, most notably in the ears as compared with that in the Sf mice (Fig. 5B, top panels). Both skin and lung showed mild inflammation in 3-wk-old Sf.CD103−/− mice, but severe inflammation developed in the 7-wk-old Sf.CD103−/− mice. Leukocyte infiltration was observed in the sinusoids of the liver of Sf.CD103−/− mice in addition to perivascular infiltration that was mainly observed in Sf mice (Fig. 5B, fourth row panels). The moderate colitis in Sf.CD103−/− mice suggests that CD103−CD4+ T cells could induce colitis, but the short lifespan prevented a full-blown inflammation in the colon (Fig. 5B, bottom panels).

It is important to note that the total peripheral lymphocytes of B6.CD103−/− mice are inherently less than B6 by ~30%. The total peripheral lymphocytes in Sf.CD103−/− mice are also significantly less than Sf mice by ~40–50%, and this could have impacted the extent of organ-specific inflammation as well. In contrast, this reduction of cell number underscores the power of CD103-independent mechanisms for MOI. To address this issue, we transferred equal numbers of LN cells from Sf and Sf.II2−/− mice into adult Rag1−/− males and examined various organs for inflammation 4 and 7 wk later (Fig. 5B, columns 4–6). At 4 wk posttransfer, all the organs examined for the Rag1−/− mice that received LN cells from Sf mice showed leukocyte infiltration and destruction of organ architecture (Fig. 5B, column 4). In contrast, at the 4-wk time point, the inflammation in the ear and skin was undetectable for the mice that received LN cells from Sf.CD103−/− mice, although mild to moderate inflammation could be seen in the liver, lung, and colon. The data demonstrate that CD103+CD4+ T cells are more effective in inducing skin and lung inflammation through CD103-dependent mechanism. However, at 7 wk posttransfer, both groups displayed external signs of inflammation on the ears and skin. Upon histological examination, inflammation was observed in ear, skin, lungs, liver, and colitis with various degrees of intensity (Fig. 5B, column 6). Taken together, these observations demonstrate that MOI mediated totally by CD103-independent mechanisms exist, as demonstrated in Sf.CD103−/− mice. Thus, additional regulatory mechanisms must be used in Sf.II2−/− mice to account for their lifelong protection of skin and lung inflammation and their longer lifespan than Sf.CD103−/− mice.

In vitro regulation of CD103 expression on CD4+ T cells

To better understand the role of IL-2 in the regulation of CD103 expression on CD4+ T cells, we conducted in vitro stimulation assays. LN CD4+ T cells were purified from B6, II2−/−, Sf, and Sf.II2−/− mice and activated with anti-CD3/anti-CD28 beads in...
the presence or absence of IL-2, IL-15, and TGF-β1. As shown in Fig. 6, TCR stimulation alone resulted in a robust net increase in CD103 expression on Sf CD4+ T cells (27 ± 6%), whereas a weak net increase was observed for B6 (6 ± 4%), Il2−/− (5 ± 3%), and Sf.II2−/− (8 ± 2%) samples. Addition of IL-2 and IL-15 did not further increase CD103 expression (rows 3 and 4), suggesting the culture system is limited by TGF-β1. Indeed, addition of TGF-β1 dramatically increased CD103+CD4+ T cells to 53 ± 6% and 72 ± 12% for B6 and Sf mice, but only 17 ± 5% and 17 ± 7% for II2−/− and Sf.II2−/− samples, respectively. Addition of IL-2 together with TGF-β1 also failed to increase CD103 expression on Sf.II2−/− samples, but only 20 ± 4%. Similar treatment of age- and sex-matched II2−/− mice also failed to completely restore CD103 expression to the same level as B6 control, and this failure is statistically significant (Fig. 6B). Furthermore, addition of IL-15, either alone or together with TGF-β1, did not influence the

FIGURE 4. Sf.II2−/− mice transfer inflammation in the colon, but not in stomach and small intestine like Sf.Faslpr/lpr mice do. LN cells from 8- to 12-wk-old Sf.Faslpr/lpr and Sf.II2−/− mice were transferred i.v. into adult Rag1−/− recipients, and tissue inflammation was determined 8 wk after. Stomach, small intestine, and colon from the donors and the recipients were processed, H&E stained, and examined under microscope. Sf.II2−/− mice, but not Sf.Faslpr/lpr, also failed to transfer inflammation in the skin and lung (data not shown).

FIGURE 5. Effect of CD103−/− on tissue inflammation in Sf mice. Sf (3-wk-old), Sf.CD103−/− (3-wk-old), and Sf.CD103−/− (7-wk-old) mice were photographed (A), and various tissues were processed for histological analysis (B). LN cells from 3-wk-old Sf and Sf.CD103−/− mice (15 × 10⁶ cells) were transferred to adult Rag1−/− male, and various tissues were processed for histological analysis 4 and 7 wk later (B, right panels). The data are representative of two independent experiments; each contained two mice in each group.
expression of CD103 on these CD4⁺ T cells (last row). Taken together, these observations indicate that the CD103 expression defect in Sf.IL2−/− mice can be recapitulated under the in vitro stimulation condition, but the defect cannot be restored by the exogenous IL-2 and IL-15.

**Discussion**

A major point of the study is the observation that CD103 expression on CD4⁺ T cells was significantly up-regulated in Sf mice, and this up-regulation was not observed in IL2−/− mice and was inhibited in Sf.IL2−/− mice. The in vivo regulation of CD103 expression on CD4⁺ T cells and its dependence on IL-2 are novel because CD103 expression on CD4⁺ T cells has not been appreciated even though they are the critical cells directly responsible for the induction of many autoimmune and inflammation responses. In addition, other than TGF-β1, few activators for CD103 expression are known, and our identification that IL-2 is critically required for CD103 up-regulation in CD4⁺ T cells in Sf mice represents an important breakthrough and opens up new avenues in this area of research. It is significant that not only have we demonstrated IL-2-dependent regulation of CD103 expression in CD4⁺ T cells in Sf mice, but also we have correlated this regulation with the development of inflammation in skin and lungs. Because CD4⁺ T cells of Sf mice transferred MOI, the link between IL-2-dependent CD103 expression on CD4⁺ T cells and inhibition of inflammation in Sf.IL2−/− mice in an organ-specific manner suggests that the interaction between CD103⁺ CD4⁺ T cells and E-cadherin target organs plays a role in the inflammation process.

An exception is the observation that colons are strongly inflamed in IL2−/− mice. Annacker et al. (41) have shown that CD4⁺CD45RBhigh T cells from CD103−/− mice could transfer colitis to BALB/c.scid/scid recipients. In another study, transfer of CD4⁺CD45RBhigh T cells into BALB/c.scid/scid recipients induced both colitis and psoriasis, but only skin-infiltrating T cells predominantly expressed the P-selectin ligand and E-selectin ligand (cutaneous lymphocyte Ag) cells, correlating organ-specific inflammation with specific T cell subsets (42). It is interesting that colitis is preferentially or selectively induced in many animal models in which a common defect affecting general immune regulation appears to be the culprit. Also, colon may be more sensitive to inflammation-inducing T cells because it anatomically and environmentally subjects to constant immune stimulation from different immunogens and microbiota (43, 44). Given the high sensitivity of colon to inflammation, the residual CD103⁺ CD4⁺ T cells in IL2−/− and Sf.IL2−/− may be sufficient to induce inflammation in the colon. This appears not to be the case because the colonic CD4⁺ T cells from Sf.IL2−/− mice (5 wk old) were low in CD103 expression, whereas extremely high CD103⁺ CD4⁺ T cells were observed in the colon of Sf mice that survived a few days after weaning (data not shown). Thus, mechanism for colitis independent from CD103⁺ CD4⁺ T cells must exist, as indicated by our study of Sf.CD103−/− mice and the study by Annacker et al. (41). The molecular mechanism by which IL-2 induces CD103 up-regulation in CD4⁺ T cells is under investigation. The first question to be addressed is whether IL-2 regulates CD103 expression through the induction of TGF-β1 and/or its receptors. TGF-β1-specific ELISA kit with a sensitivity of detecting ~100 pg/ml (Promega) failed to detect active TGF-β1 in the sera of B6, IL2−/−, Sf, and Sf.IL2−/− mice. In addition, there were no differences in the expression of total TGF-β1 in these samples after acid treatment for latent TGF-β1 (data not shown). Thus, IL-2 may be required for the induction of TGF-β receptors, as suggested by TGF-β1 mRNA analysis that showed a 4-fold increase in Sf CD4⁺ T cells as compared with Sf.IL2−/− T cells (our unpublished observation). We also activated T cells with anti-CD3/anti-CD28 beads and observed increased CD103 expression on CD4⁺ T cells from Sf.
mice, but not other mice tested. Addition of excess active TGF-β1 (2.5 ng/ml vs <120 pg/ml latent form in culture supernatants) strongly increased CD103 expression on the CD4+ T cells in Sf and B6 mice, but not much in IL2−/− and Sf/IL2−/− samples. Importantly, IL-2 plus TGF-β1 also failed to completely restore the CD103 expression on IL2−/− and Sf/IL2−/− CD4+ T cells. It is likely that the inflammation has driven these cells beyond the point in such a way that response to IL-2 and TGF-β1 cannot be totally restored under the in vitro system.

Introducing CD103−/− into Sf mice inhibited the inflammation in skin, tail, and lungs initially, but the CD103-independent inflammation eventually developed at ~3 wk after weaning, at which time the mice became moribund. In contrast, IL2−/− mutation-mediated inhibition of skin and lung is long-lasting throughout the lifespan of the mice. In addition, CD103−/− mutation prolongs the lifespan of Sf mice significantly shorter than IL2−/− mutation. Thus, IL2−/− must play other roles in addition to regulating CD103 expression on CD4+ T cells in the inflammatory process. The results obtained with Sf/CD103−/− mice suggest that other integrins and chemokine receptors for cell trafficking and retaining are involved in the MOI. Selective and preferential use of specific chemokine/ receptor systems has been described for skin, lungs, and gut (42, 45, 46). We compared the expression of some of these receptors on target organs between Sf and Sf/IL2−/− mice using limited number of available mAb (Fig. 3). The data suggest that IL-2 regulates several receptors, but CD103 remains to be one of the major targets of regulation. Given the limited target receptors examined and the fact that CD103-independent mechanisms exist for both skin and lung inflammation, we compared the mRNA levels of various chemokine receptors between the CD4+ T cells of Sf and Sf/IL2−/− mice. Our preliminary data identified superexpression of cysteiny1 leukotriene receptor 1, leukotriene B4 receptor 1, integrin αE, CCR1, CXCL2, and CCR8 in Sf CD4+ T cells as compared with Sf/IL2−/− CD4+ T cells. Thus, IL-2 appears to be a master regulator for several trafficking/retention receptors that can account for the CD103-independent mechanisms observed in Sf/CD103−/− mice.

As a receptor for E-cadherin and signal transduction, CD103 controls the trafficking and retaining of lymphocytes in target organs. Thus, CD103/E-cadherin interaction is the final stage of cell trafficking and the beginning of immune reaction in the target organs. Many studies have suggested the importance of CD103 expression in the mucosal and cutaneous inflammation process. The present study demonstrates that IL-2, by regulating such a critical molecule involved in target organ inflammation, facilitates organ-specific inflammation in a manner opposing its role as a positive regulator of Treg that inhibit inflammation. Most importantly, our studies using IL2−/− mice consider its effects on not only Treg, but also on IL-2-dependent mechanisms beyond Treg checkpoint and beyond CD103-dependent mechanism. In this regard, it is of great significance that we have established that CD103-independent inflammation-inducing mechanism is capable of fully inducing the fatal MOI in the absence of Treg, implying that CD103-targeted therapy will be unlikely to produce long-lasting protection against inflammation of E-cadherin+ organs. Our study further accentuates the role of IL-2 in regulating multiple mechanisms of T cells such that lifelong protection from spontaneous skin and lung inflammation from inflammation-inducing CD4+ T cells can be succeeded in Sf/IL2−/− mice.

Disclosures

The authors have no financial conflict of interest.


