Up-Regulating CXCR3 upon Activation

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T Cells from *Leishmania major*-Susceptible BALB/c Mice Have a Defect in Efficiently Up-Regulating CXCR3 upon Activation

Joseph Barbi,* Frank Brombacher,† and Abhay R. Satoskar2*

Genetic background influences the outcome of *Leishmania major* infection. C57BL/6 mice mount a Th1 response and resolve infection. In contrast, BALB/c mice mount a Th2 response and develop chronic lesions. This susceptible phenotype is seen even though BALB/c mice generate IFN-γ-producing T cells at proportions similar to C57BL/6 mice in their lymph nodes (LN) early after infection. We had previously shown that chemokine receptor CXCR3 mediates immunity against *L. major* by recruiting IFN-γ-producing T cells to the lesions of C57BL/6 mice. Therefore, we hypothesized that IFN-γ-secreting T cells in BALB/c mice are unable to confer protection because they may be defective in up-regulating CXCR3. To test this hypothesis, we analyzed kinetics of CXCR3-expressing T cells in the LN and lesions of BALB/c and C57BL/6 mice during *L. major* infection. Additionally, we compared the ability of T cells from BALB/c and C57BL/6 mice to up-regulate CXCR3 upon activation. We found that resolution of *L. major* infection in C57BL/6 mice was associated with an increase in the proportion of CXCR3+ T cells in regional LN and lesions, whereas disease progression in BALB/c mice was associated with a decrease in these populations. Anti-CD3/CD28-activated T cells from naive BALB/c but not C57BL/6 mice were defective in up-regulating CXCR3. Impaired induction of CXCR3 on BALB/c T cells was not due to lack of IFN-γ and was mediated partially by IL-10 but not IL-4 or IL-13. We propose that defective CXCR3 up-regulation on T cells in BALB/c mice may contribute to *L. major* susceptibility. The Journal of Immunology, 2008, 181: 4613–4620.

The *Leishmania* are obligate intracellular parasites that cause a wide range of diseases such as cutaneous, mucocutaneous, and visceral leishmaniasis. In humans, cutaneous leishmaniasis caused by *Leishmania major* usually manifests as a localized, self-resolving lesion associated with development of long-term immunity (1, 2). The murine model of cutaneous *L. major* infection has been well characterized and frequently has been used as a functional model of Th1 and Th2 cell responses (3). Most inbred mice such as C57BL/6, C3H, and CBA/J are genetically resistant to *L. major* and spontaneously resolve infection (4) because they mount a protective IL-12-induced Th1-type response. In contrast, susceptible BALB/c mice develop large nonhealing lesions and mount a Th2 response that is associated with the production of the cytokines IL-4 and IL-10. Nevertheless, previous studies have shown that BALB/c mice are capable of mounting a Th1 response and contain a significant number of IFN-γ-producing T cells in their lymph nodes (LN)5 comparable to C57BL/6 mice during the early phase of *L. major* infection (5, 6). C57BL/6 mice also produce high levels of IL-4 similar to BALB/c mice early after infection. Furthermore, some studies have found that deletion of the IL-4 or IL-4R gene in BALB/c mice has no effect on the outcome of *L. major* infection (7, 8). Taken together, these findings suggest that genetically regulated mechanisms other than Th1/Th2 cytokine production may also control the outcome of *L. major* infection in BALB/c mice.

We had previously found that the CXCR3, which binds CXCL9, CXCL10, and CXCL11, plays a critical role in immunity against *L. major* in C57BL/6 mice by mediating recruitment of IFN-γ-producing T cells from the LN to lesion (9). This was perhaps not surprising since CD4+ Th1 cells have been shown to preferentially express CXCR3 (10). Because CXCR3 is critical for Th1 cell migration and *L. major*-infected BALB/c mice fail to restrict early parasite growth in the skin despite containing a high number of IFN-γ-producing T cells in their LN, we hypothesized that BALB/c mice may have a defect in up-regulating CXCR3 on T cells. Such a defect may prevent recruitment of Th1 cells to the lesion and, therefore, contribute to *L. major* susceptibility. To test this hypothesis, we analyzed CXCR3-expressing T cells in the lesions and LN from BALB/c and C57BL/6 mice during *L. major* infection. In addition, we compared the ability of naive CD4+ and CD8+ T cells from BALB/c and C57BL/6 mice to up-regulate CXCR3 upon anti-CD3/anti-CD28 activation in vitro. Our results indicate that both CD4+ and CD8+ T cells from susceptible BALB/c, but not resistant C57BL/6, mice have an intrinsic defect in efficiently up-regulating CXCR3 upon activation, which may contribute to susceptibility to *L. major*.

**Materials and Methods**

**Mice and parasites**

Sex- and age-matched C57BL/6 and BALB/c mice were purchased from Harlan Sprague Dawley and maintained at The Ohio State University according to institutional regulations. IL-4Ra−/− mice were provided by Alison Finnegan, Chicago, IL. For intradermal infections, 1 × 10⁶ *L. major* promastigotes (LV39) were injected into the ears of C57BL/6 and BALB/c mice.

**Leukocyte isolation and flow cytometry**

At time points post infection, three to four C57BL/6 and BALB/c mice were sacrificed. The draining LN (dLN) were excised and single-cell suspensions of LN cells were obtained by teasing through a 70-μm mesh. Lesion leukocytes were isolated as described previously (9). Cells were
infection, CXCR3 deficiency results in more severe nonresolving disease compared with WT mice. This heightened susceptibility coincided with deficient T cell mobilization and local IFN-γ production (9). Others have reported that resistant C57BL/6 mice produce high levels of CXCR3 ligands in response to L. major infection, whereas susceptible BALB/c mice do not (13). These findings indicate that CXCR3 plays a critical role in immunity against L. major in a resistant mouse and that L. major-susceptible and -resistant mice differ in their ability to produce CXCR3 ligands. However, whether CXCR3 is differentially expressed by susceptible and resistant mice remains to be investigated.

Therefore, we infected L. major-susceptible BALB/c and -resistant C57BL/6 mice intradermally by inoculating 1 × 10⁴ L. major promastigotes into ear dermis and frequency of CXCR3 expressing T cells in the dLN and lesions temporally using flow cytometry. Throughout the course of infection, L. major-infected C57BL/6 mice displayed higher frequencies of CXCR3 expressing CD4⁺ and CD8⁺ lymphocytes in their dLNs compared with BALB/c mice (Fig. 1A). Similar trends in CXCR3⁺ T cell frequencies were also noted between the lesions of BALB/c and C57BL/6 mice (Fig. 1B). Furthermore, analysis of the dLN and lesion revealed that CXCR3⁺ T cells comprised greater percentages of the CD4⁺ and CD8⁺ compartments of infected C57BL/6 mice than BALB/c mice (Fig. 1C, and data not shown). Also, C57BL/6 mice contained more CXCR3⁺ CD4⁺ and CD8⁺ T cells in their dLNs (Fig. 2, bottom panels) and lesions than BALB/c mice (Fig. 2, top panels), although L. major-infected C57BL/6 mice showed a decline in CXCR3-expressing T cells in the dLNs and lesions as they resolved the infection (data not shown). These results demonstrate that resolution of L. major infection in C57BL/6 mice is associated with an increase in CXCR3⁺ T cells in their dLNs and lesions, whereas disease progression in BALB/c mice is associated with no or only modest increase in these cell populations.

**Results**

L. major-infected BALB/c mice contain lower frequencies of CXCR3-expressing T cells in their regional nodes and lesions than C57BL/6 mice

CXCR3 is preferentially expressed on activated Th1-promoting cells (10). Previously, our group found that during L. major infection, CXCR3-expressing T cells in their dLN and lesions were an-

**In vitro stimulation and treatment of T cells**

Cell suspensions were obtained from the excised LNs and spleens of uninfected mice. Then, 90–95% pure CD3⁺ T cell or CD4⁺ and CD8⁺ T cell populations were obtained via nylon wool columns and immunomagnetic isolation (Mitenyi Biotec) from either wild-type (WT) C57BL/6, BALB/c, IL-4R−/−, or IL-10−/− BALB/c mice (11). T cells were incubated 48 h at 0.5–2.5 × 10⁶ cells/well in a 24-well plate precoated with 3 and 4 μg/ml anti-CD3 (clone 145–2C11) and anti-CD28 (clone 37.51) Abs (Biolegend), respectively. Following in vitro activation, cells were transferred to uncoated wells and rested in their conditioned media for 24 h before flow cytometry analysis. In brief, in vitro activated cells were recovered and washed in cold PBS before staining with PE-conjugated anti-mouse CXCR3 Abs or a rat IgG2a-PE control (R&D Systems). Stained cells were either analyzed immediately or fixed in 1% paraformaldehyde for short-term storage.

**Cytokine ELISA**

Culture supernatants were sampled after 48 h of incubation at 37°C, 5% CO₂, and then analyzed for the presence of IFN-γ, IL-4, and IL-10 by sandwich ELISA as described previously (9).

**Semi-quantitative real-time PCR**

Total RNA was extracted from T cells using TRIzol Reagent (Invitrogen), and cDNA was synthesized and amplified as described previously (12). Primer sequences were found using the Harvard’s PRIMER BANK website, and oligonucleotides were purchased.

**Statistical analysis**

Statistically significant differences were determined by an unpaired Student’s t test. A p-value <0.05 was considered significant.

**Results**

L. major-infected BALB/c mice contain lower frequencies of CXCR3-expressing T cells in their regional nodes and lesions than C57BL/6 mice

CXCR3 is preferentially expressed on activated Th1-promoting cells (10). Previously, our group found that during L. major infection, CXCR3-deficient mice express CXCR3 more readily than those of BALB/c mice during L. major infection. Cells of the draining LN (A) and (B) lesions of C57BL/6 mice infected with L. major were analyzed by flow cytometry and were found to contain higher percentages of CXCR3⁺/CD4⁺ and CXCR3⁺/CD8⁺ lymphocytes than those of infected BALB/c mice after 3 wk of L. major infection. Also CXCR3⁺ cells comprised a larger percentage of the CD4⁺ and CD8⁺ T cell compartments in the C57BL/6 dLN throughout the observed course of infection (C). A and B, Representative results from one of five independent experiments. Numbers indicate the percent of lymphocytes. C, Mean percentage of either CD4⁺ or CD8⁺ T cells that also express CXCR3⁺ (±SEM) from three to five independent trials. * p-value <0.05.
Naive T cells from BALB/c but not C57BL/6 mice are less efficient in up-regulating CXCR3 upon activation

To determine whether T cells from C57BL/6 and BALB/c mice inherently differ in their ability to up-regulate CXCR3, naive T cells were isolated from the spleens of naive mice and stimulated with plate-bound anti-CD3/anti-CD28 Abs as described previously (11). Expression of CXCR3 on these activated T cells was compared by flow cytometry. Following in vitro stimulation, T cells from C57BL/6 mice efficiently up-regulated CXCR3. In contrast, the increase in CXCR3 was minimal in similarly stimulated T cells from BALB/c mice (Fig. 3A). Moreover, low CXCR3 expression on activated BALB/c T cells also correlated with low CXCR3 mRNA (Fig. 3B).

FIGURE 2. The draining LN and lesions of C57BL/6 mice contain greater numbers of CXCR3+ T cells than those of BALB/c mice during L. major infection. From flow cytometry analysis, the absolute number of CXCR3+CD4+ and CXCR3+CD8+ T cells were calculated for C57BL/6 and BALB/c mice infected with L. major. Higher numbers of C57BL/6 dLN (bottom panels) and lesion cells (top panels) express CXCR3 compared with the cells of BALB/c mice. Shown are the mean results (±SEM) from at least two independent trials. *, p-value <0.05.

FIGURE 3. Upon in vitro mitogenic stimulation, C57BL/6-derived T cells dramatically up-regulate CXCR3 while those of BALB/c mice do not. In brief, T cells purified from uninfected BALB/c and C57BL/6 mice were stimulated in vitro by plate-bound anti-CD3/anti-CD28 Abs for 48 h before removal from stimulation and a 24-h rest period. Flow cytometric staining of these activated cells revealed that C57BL/6-derived T cells (solid peak) but not BALB/c T cells (hollow gray peak) stained intensely for surface CXCR3 (A). Isotype controls were denoted by dotted hollow peaks. Real-time PCR measurement of CXCR3 mRNA showed that this trend extended to the transcript level since C57BL/6- and BALB/c-derived T cells induced CXCR3 mRNA at comparable levels (B). A, Representative result from one of five trials is shown. B, Represents the averaged result of three independent trials (±SEM). *, p-value <0.05.
mRNA levels suggesting that suppression of CXCR3 expression was at the transcript level (Fig. 3B). This defect in CXCR3 expression was observed in CD4\(^+\)/H11001 and CD8\(^+\)/H11001 T cells of BALB/c mice purified and activated separately (data not shown). Baseline levels of CXCR3 were low on prestimulated T cells from both BALB/c and C57BL/6 mice (data not shown) as previously reported (12, 14).

Reduced CXCR3 expression on T cells of BALB/c mice is not due to lack of IFN-\(\gamma\)

It has been shown that IFN-\(\gamma\) and the transcription factor T-bet are required for efficient induction of CXCR3 on certain T cell subsets (14–16). To determine whether failure of BALB/c T cells to up-regulate CXCR3 was associated with reduced production of IFN-\(\gamma\), we analyzed levels of Th1-associated IFN-\(\gamma\) as well as Th2-associated IL-4 and IL-10 in the above culture supernatants by ELISA. Also, we quantified mRNA levels of T-bet and IFN-\(\gamma\)R (Fig. 4D and E) as well as surface levels of IFN-\(\gamma\)R (data not shown) were comparable in activated T cells of both groups by RT-PCR (D and E, respectively). Data are presented as the mean results from two to three independent experiments (±SEM). *, p-value <0.05.

**FIGURE 4.** Despite higher IL-4 and IL-10 production by BALB/c-derived T cells, both strains are similarly capable of IFN-\(\gamma\), IFN-\(\gamma\)R, and T-bet expression. At various time points after stimulation was ceased, the culture supernatants of activated T cells were analyzed by ELISA, and, at each sampling, BALB/c-derived T cells activated in vitro with anti-CD3/anti-CD28 make levels of IFN-\(\gamma\) similar to C57BL/6/ (A) but T cells of BALB/c mice made significantly more IL-10 and IL-4 (B and C). Additionally, T-bet and IFN-\(\gamma\)R mRNA levels were found to be comparable in activated T cells of both groups by RT-PCR (D and E, respectively). Data are presented as the mean results from two to three independent experiments (±SEM).

Blockade of IL-4 or IL-4Ra deficiency does not restore expression of CXCR3 on activated T cells from BALB/c mice

Between 10 and 48 h post stimulation, C57BL/6/6- and BALB/c-derived T cells produced high and comparable levels of IFN-\(\gamma\) upon activation (Fig. 4A). However, activated T cells from BALB/c mice produced significantly more IL-4 and IL-10 as compared with similarly activated T cells from C57BL/6 mice (Fig. 4B, C). Also, mRNA levels of T-bet and IFN-\(\gamma\)R (Fig. 4D, E) as well as surface levels of IFN-\(\gamma\)R (data not shown) were comparable in T cells from both groups. These results show that even though BALB/c T cells produce higher levels of Th2-associated cytokine, they are not deficient in IFN-\(\gamma\) production, T-bet induction, or IFN-\(\gamma\)R expression compared with C57BL/6-derived cells. Furthermore, they suggest that neither lack of IFN-\(\gamma\) production or deficient expression of IFN-\(\gamma\)R is responsible for the inability of BALB/c-derived T cells to up-regulate CXCR3.

**FIGURE 5.** The kinetics of CXCR3 up-regulation by C57BL/6- and BALB/c-derived T cells. At the time points specified in Fig. 4, T cells were also sampled and CXCR3 expression was evaluated by flow cytometry. Although at 10 h poststimulation, both groups expressed low levels of CXCR3, by 24–48 h after stimulation, the majority of C57BL/6/ (A) but not BALB/c-derived T cells (B) expressed high levels of CXCR3. The histograms shown are the representative results of two independent experiments with similar results.
CXCR3 after 48 h (Fig. 5B). Interestingly, the slight increase in CXCR3 staining of BALB/c-derived T cells coincided with reduction in IL-4 concentrations at later time points poststimulation (Fig. 4, B and C).

To determine whether IL-4 was involved in preventing up-regulation of CXCR3 on BALB/c T cells, we stimulated BALB/c and C57BL/6 T cells in the presence of 10 μg/ml anti-IL-4 or an isotype-matched control Ab and examined the expression of CXCR3 by flow cytometry. In addition, we analyzed CXCR3 expression on T cells from WT BALB/c and IL-4R−/− BALB/c mice following in vitro activation with anti-CD3/CD28. Blockade of IL-4 (Fig. 6A) or lack of IL-4Rα−/− BALB/c mice following in vitro activation with anti-CD3/CD28. Blockade of IL-4 (Fig. 6A) or lack of IL-4Rα−/− BALB/c mice (gray hollow peak) failed to increase expression of CXCR3 on BALB/c-derived T cells, indicating that IL-4 and IL-13 were not responsible for suppression of CXCR3. As expected, expression of CXCR3 on the T cells of C57BL/6 mice were unaffected by IL-4 neutralization (Fig. 6C).

Blockade of IL-10R partially restores expression of CXCR3 on T cells from BALB/c mice

Because T cells from BALB/c mice also produced significantly more IL-10 than C57BL/6 T cells after stimulation, we investigated whether the cytokine was involved in preventing up-regulation of CXCR3 on BALB/c T cells. To this end, we blocked IL-10 signaling by including anti-IL-10R Abs in the culture media of BALB/c- and C57BL/6-derived T cells and examined its effect on CXCR3 expression. Additionally, we activated T cells from WT BALB/c and IL-10−/− BALB/c mice in vitro with anti-CD3/CD8 and compared levels of CXCR3. Flow cytometric analysis showed that CXCR3 expression by anti-IL-10-treated BALB/c T cells was greater than that of isotype control-treated cells yet still lower than C57BL/6 T cells (Fig. 7A). Not surprisingly, C57BL/6-derived T cells were unaffected by IL-10R blockade (data not shown). Furthermore, activated T cells from IL-10−/− BALB/c mice expressed more CXCR3 than WT controls (Fig. 7B). Additionally, increased CXCR3 expression in the absence of IL-10 was also noted on T cells in the dLN of IL-10−/− mice infected with L. major (Fig. 7C). Collectively, these results indicate that IL-10 is partially responsible for inhibiting CXCR3 expression on T cells in BALB/c mice.

T cells from C57BL/6 mice are resistant to IL-10-mediated CXCR3 suppression and express significantly lower levels of IL-10R

Since we found that IL-10 was partially involved in preventing up-regulation of CXCR3 on BALB/c T cells, we determined whether IL-10 can block induction of CXCR3 on T cells in resistant mice. Naive T cells from C57BL/6 mice were stimulated in vitro with anti-CD3/CD28 as described above in the presence of rIL-10 and expression of CXCR3 was analyzed by flow cytometry. Interestingly, rIL-10 failed to suppress expression of CXCR3 on C57BL/6 T cells (Fig. 4C) suggesting that these T cells were refractory to IL-10-induced suppression of CXCR3 expression. We therefore compared levels of IL-10R on activated T cells from BALB/c and C57BL/6 mice using real-time RT-PCR as well as flow cytometry. Anti-CD3/CD28-activated CD4+ and CD8+ T cells from C57BL/6 mice displayed less induction of IL-10R

![Figure 6](http://www.jimmunol.org/.../11B.11, 10–40 μg/ml) during and post stimulation did not affect CXCR3 surface expression by BALB/c- (A) derived T cells as measured by flow cytometry. Solid black peaks represent control Ab treated cells, while gray hollow peaks and dotted lines represent anti-IL-4-treated cells and staining isotype controls, respectively. Likewise IL-4Rα−/− BALB/c T cells (black hollow peak) did not up-regulate CXCR3 more efficiently than WT BALB/c T cells (gray hollow peak) (B). Isotype controls are represented by a solid black peak. Also, C57BL/6-derived T cells were unaffected by IL-4 neutralization (C). Shown are representative histograms from one of three independent trials and mean fluorescence intensity values for CXCR3-PE staining from at least three experiments are indicated as are the averaged result for isotype control treated cells.
mRNA and expressed less IL-10R as compared with BALB/c-derived T cells (Fig. 8, A and B). These data suggest that low levels of IL-10R on C57BL/6 mice may be responsible for their refractoriness to IL-10-mediated down-regulation of CXCR3.

Discussion

We had previously shown that CXCR3 plays a critical role in immunity against L. major by controlling T cell recruitment and IFN-γ levels at the site of infection. The novel finding in the present study is that CD4+ and CD8+ T cells from L. major-susceptible BALB/c, but not -resistant C57BL/6, mice have a defect in efficient up-regulation of CXCR3 upon activation. It is well documented that Th1-associated cytokines IL-12 and IFN-γ are crucial for resolution of L. major infection in C57BL/6 mice, whereas Th2-associated cytokines IL-4, IL-10, and IL-13 have been implicated as susceptibility factors in BALB/c mice. However, a previous study has documented that both BALB/c and C57BL/6 mice contain comparable frequencies of IFN-γ-producing T cells in their dLNAs during early phase of L. major infection, yet BALB/c mice fail to control infection. One explanation for this observation could be that IFN-γ-producing T cells in the LN of BALB/c mice migrate less efficiently to the infected skin because BALB/c mice produce less CXCR3 ligand CXCL10 than C57BL/6 mice during L. major infection (13). However, intranasal administration of rIL-10 (CXCL10) to BALB/c mice shortly after L. major infection increases NK cell cytotoxicity in the LN but fails to limit disease progression (13). These data suggest that IFN-γ-producing T cells in BALB/c mice may not be responsive to CXCL10 and fail to migrate to lesion. In the present study, we found that resolution of L. major infection in C57BL/6 mice was associated with an influx CXCR3+ T cells in the LN as well as lesions, whereas disease progression in BALB/c mice was associated with reduced frequency of CXCR3+ T cells at both these sites. We also found that naïve T cells from BALB/c mice up-regulated CXCR3 less efficiently than C57BL/6 T cells following in vitro activation with anti-CD3/CD28. Furthermore, low CXCR3 expression on BALB/c T cells also correlated with low CXCR3 mRNA levels suggesting that suppression of CXCR3 was at the
level of transcription. In vitro stimulation studies performed using purified CD4\(^+\) and CD8\(^+\) T cells revealed that both these cell types in BALB/c mice had a defect in up-regulating CXCR3 (data not shown). Taken together, these findings suggest that a defect in CXCR3 up-regulation on T cells could contribute to disease progression in BALB/c mice. In addition, they indicate that the inability of activated T cells from BALB/c mice to up-regulate CXCR3 is not due to lack of IFN-\(\gamma\).

Previous studies have shown that IFN-\(\gamma\), STAT1, and the transcription factor T-bet are required for efficient induction of CXCR3 on certain T cell subsets (14–16). IFN-\(\gamma\), which signals via IFN-\(\gamma\)R/STAT1 pathway, induces expression of T-bet (17), which in turn enhances expression of CXCR3 on T cells (15, 16). To determine whether reduced expression of CXCR3 on BALB/c T cells is due to lack of IFN-\(\gamma\)-production, we measured levels of IFN-\(\gamma\) as well as Th2-associated IL-4 and IL-10 in culture supernatants of activated T cells from BALB/c and C57BL/6 mice. In addition, we quantified mRNA levels of T-bet as well as IFN-\(\gamma\)R by real-time RT-PCR. Upon activation with anti-CD3/CD28, T cells from C57BL/6 and BALB/c mice produced significant and comparable amounts of IFN-\(\gamma\), but later produced significantly more IL-4 and IL-10. Both groups showed significant induction of T-bet and comparable levels of T-bet mRNA. Levels of IFN-\(\gamma\)R mRNA were also comparable in T cells from BALB/c and C57BL/6 mice and correlated with surface expression of IFN-\(\gamma\)R as assessed by flow cytometry (data not shown). Collectively, these findings show that lack of IFN-\(\gamma\)-production or impaired expression of T-bet or IFN-\(\gamma\)R are not responsible for failure of CXCR3 up-regulation in T cells from BALB/c mice. In addition, they suggest that Th2-associated cytokines may be involved in antagonizing IFN-\(\gamma\)- and T-bet-induced expression of CXCR3 in BALB/c T cells.

Th2-associated cytokines IL-4 and IL-10 that mediate susceptibility to L. major, also regulate expression of several chemokine receptors and their ligands. However, previous studies have reported that IL-4 and IL-10 play distinct roles in regulation of expression of CXCR3 and its ligands CXCL9 and CXCL10. For example, Albanesi et al. (18) found that IL-4 enhances CXCL9 and CXCL10 production in keratinocytes and induces migration of CXCR3\(^+\) T cells in murine model of allergic contact dermatitis. IL-4 treatment also exacerbated disease in a Th1 cells transfer model of colitis, which was associated with up-regulation of CXCR3 and its ligands in the colon (19). In contrast, IL-10 was found to inhibit expression of CXCR3 in microglial cells as well as human eosinophils (20). In the present study, a decrease in the levels of IL-4 in the culture supernatants from BALB/c mice was associated with a modest increase in levels of CXCR3 suggesting that IL-4 may be involved in suppressing expression of CXCR3. However, blockade of IL-4 using neutralizing Abs failed to restore expression of CXCR3 to levels observed on C57BL/6 T cells. Additionally, T cells from IL-4Ra\(--\)BALB/c mice were deficient in up-regulating CXCR3 upon activation. In contrast, blockade of IL-10R using anti-IL-10R Ab enhanced expression of CXCR3 on BALB/c-derived T cells. Furthermore, in vitro-activated T cells from IL-10\(--\)BALB/c mice expressed CXCR3 more readily than WT controls. Expression of CXCR3 on the T cells of C57BL/6 mice were unaffected by IL-4 neutralization or IL-10R blockade or rIL-4 treatment. Together, these results demonstrate that IL-10 is partly responsible for preventing up-regulation of CXCR3 on T cells in BALB/c mice. Furthermore, they show that IL-4 is not involved in inducing CXCR3 expression on T cells. It is interesting to note that levels of IL-4 in culture supernatants dropped rapidly when activated T cells of BALB/c mice were rested. This may be due to rapid consumption of IL-4 by these cells or simply due to degradation of this cytokine. Nonetheless, despite the presence of abundant “pro-Th1 factors” (i.e., T-bet and IFN-\(\gamma\)) and decrease in IL-4 levels, BALB/c-derived T cells failed to up-regulate CXCR3 levels. Furthermore, blockade of IL-10R or lack of IL-10 only partially restored expression of CXCR3 on BALB/c T cells indicating that other molecular mechanisms may be involved in negatively regulating induction of CXCR3 on these cells. One such potential mechanism is that a Th2-associated transcription factor GATA3, which directly binds to T-bet, may be involved in preventing T-bet-induced induction of CXCR3 in BALB/c T cells. We are currently investigating this possibility in ongoing studies in our laboratory.

Because IL-10/IL-10R pathway was found to be involved in suppressing CXCR3 expression on BALB/c T cells, we investigated whether IL-10 can block induction of CXCR3 on T cells in L. major-resistant mice. In addition, we analyzed expression of IL-10R and measured IL-10 mRNA levels in activated T cells from both strains. We found that rIL-10 failed to prevent up-regulation of CXCR3 on
C57BL/6 T cells, suggesting that these cells were resistant to IL-10-mediated CXCR3 suppression. Furthermore, BALB/c T cells expressed higher levels of IL-10R and contained more IL-10R mRNA than C57BL/6 T cells. Taken together, these results suggest that lower levels of IL-10R on C57BL/6 T cells may render them resistant to IL-10-mediated CXCR3 suppression and aid in up-regulation of CXCR3. Our finding are supportive of a previous study which showed that C57BL/6 leukocytes are more resistant to the IL-10-mediated immune-suppressive effect of regulatory T cells than those of BALB/c mice (21).

Although IL-10 has been observed to inhibit CXCR3 expression by mouse microglia and human eosinophils (22), no such role is known for T cells. Interestingly, in the murine colitis model, forced expression of IL-10 results in amelioration of this Th1- (and CXCL10-) mediated disease that is associated with reduced expression of CXCR3 and its ligands (23). Our observations are particularly interesting in light of recent studies revealing the IL-10-producing capacity of Th1 cells as a means of limiting their own inflammatory potential (24). One such consequence of T cell-derived IL-10 may be to limit CXCR3-mediated cell homing – a recruitment axis critical for Th1 trafficking in numerous disease models.

In conclusion, resolution of L. major infections by resistant C57BL/6 mice is associated with an increase in CXCR3 expressing T cells in their LN and whereas disease progression in susceptible BALB/c mice correlates with fewer CXCR3-expressing T cells in their LN and lesions whereas disease progression in susceptible BALB/c mice is associated with an increase in CXCR3 expressing T cells in experimental murine leishmaniasis in resistant and susceptible mice. Int. Immunol. 15: 1853–1861.


Corrections


In Results, Fig. 7B was mislabeled and a sentence explaining the data in Fig. 7C was omitted. Under the heading Blockade of IL-10R partially restores expression of CXCR3 on T cells from BALB/c mice, the corrected sentences should read: “Flow cytometric analysis showed that CXCR3 expression by anti-IL-10R-treated BALB/c T cells was greater than that of isotype control-treated cells yet still lower than C57BL/6 T cells (Fig. 7A). Exogenously added recombinant IL-10 failed to suppress CXCR3 levels on C57BL/6 T cells (Fig. 7C).” The figure callout after “L. major” in the next sentence should read “(Fig. 7D)” instead of “(Fig. 7C).”

Also in Results under the heading T cells from C57BL/6 mice are resistant to IL-10-mediated CXCR3 suppression and express significantly lower levels of IL-10R, the figure callout after the segment “Interestingly, rIL-10 failed to suppress expression of CXCR3 on C57BL/6 T cells” should read “(Fig. 7C)” instead of “(Fig. 4C).”

The revised Fig. 7 is shown below. The published legend is correct but is shown again for reference.

![Flow cytometric analysis showing CXCR3 expression on T cells from BALB/c and C57BL/6 mice](image)

**FIGURE 7.** IL-10 suppresses CXCR3 expression by BALB/c T cells, but not those of C57BL/6. Blocking IL-10R with mAbs (clone 1B1.3A, 10 μg/ml) allowed moderate surface CXCR3 expression by BALB/c T cells activated in vitro (hollow gray peak). Control Ig-treated cells are represented by a solid black peak (A). Likewise, IL-10−/− BALB/c T cells (black hollow peak) induce CXCR3 more readily than WT controls (gray hollow peak). A solid black peak represents isotype controls (B). Treatment of C57BL/6 T cells with rIL-10 (10 μg/ml) (hollow gray peak) did not suppress CXCR3 expression compared with untreated controls (solid black peak) (C). IL-10 deficiency also results in higher CXCR3 expression in vitro. The dLN of IL-10−/− BALB/c mice infected with L. major were analyzed by flow cytometry 3 wk postinfection. IL-10−/− mice contained more CXCR3+/CD3+ cells in their dLN than WT BALB/c mice (D). Shown are representative results from at least three independent experiments. Shown are mean fluorescence intensity values for CXCR3-PE staining (±SEM) from at least three experiments and the averaged result for isotype control treated cells. *, p value <0.05.
In Fig. 4, the wrong labels were assigned to Fig. 4, B and C, during the final formatting of the figure. The data and conclusions in the manuscript are not affected. The correct Fig. 4 is shown below. The published legend is correct but is shown again for reference.

**FIGURE 4.** Cell proliferation and cytokine production. **A**, At the peak of the disease (days 18–22 p.i.), a proliferation assay was performed. Stimulation index (SI) was the ratio of MOG35–55-stimulated MNC proliferation/spontaneous MNC proliferation. Quantification of cytokine mRNA expression in (B) spleen and (C) spinal cord from WT mice or GAT-1−/− mice with EAE was accomplished using real-time PCR. Results are shown as means ± SD. Data are representative of three individual experiments (n = 5 mice/group). *p < 0.05; **p < 0.01; ***p < 0.001.


The authors thank Ajit Varki for bringing to their attention that CD83 does not share any sequence homology with CD33 and therefore cannot be classified as a Siglec.
In the second paragraph of the Introduction, on page 3865, the authors wrote: “We now present data suggesting that CD83 mediates adhesion of DC to circulating monocytes and to a fraction of activated T cells or stressed T cells by a specific binding of CD83 to a 72-kDa counterreceptor (ligand). We further show that CD83Ig binding to its ligand is eliminated by neuraminidase, an enzyme specific for the most common sialic acid, N-acetylneuraminic acid. Thus, CD83Ig binds to a carbohydrate epitope that depends on sialic acid residues. This classifies CD83 as a sialic acid-binding Ig-like lectin.”

In the next-to-last paragraph of the Discussion, on page 3871, the authors wrote: “CD83 is structurally related to the B7 ancestral gene family, and its closest homology is 23% of identity with the myelin protein Po, which is an I-type lectin that recognizes a sulfated carbohydrate. Therefore, it is highly interesting that the CD83 ligand contains a sialic acid, classifying CD83 as a siglec i.e., it belongs to a subfamily of I-type lectins that can bind sialic acids and presently includes nine members.”

The authors correct the inaccurate classification of CD83 as a Siglec molecule, yet confirm their findings. CD83 has a single extracellular V-type Ig-like domain that binds to sialic acid residues. This classifies CD83 as an I-type lectin (1–3) but not as a Siglec, due to CD83 lack of homology with CD33.

Accordingly, the title of the article should have been: “CD83 is an I-type lectin adhesion receptor that binds monocytes and a subset of activated CD8+ T cells.”

Additions to the References are listed below.

