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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.

**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

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3 Abbreviations used in this paper: LN, lymph node; dLN, draining LN; WT, wild type.

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FIGURE 1. C57BL/6-derived T cells 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.

stained with PE-conjugated anti-mouse CXCR3 (R&D Systems) and either FITC-labeled anti-CD4 or anti-CD8 Abs (Biolegend). Flow cytometric analysis of LN and lesion cells was performed with a BD FACSCalibur.

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-labeled 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 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.
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 levels (B).

**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 by real-time PCR (D and E, respectively). Data are presented as the mean results from two to three independent experiments (\(\pm\)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 (\(\pm\)SEM). * , p-value \(< 0.05\).

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- and BALB/c-derived T cells rapidly induced CXCR3 surface protein (Fig. 5A) while their BALB/c-derived counterparts expressed only marginal levels of CXCR3-PE.

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 ng/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 (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.

**FIGURE 6.** High levels of IL-4 are not responsible for reduced CXCR3 expression in BALB/c-derived T cells. Neutralizing IL-4 with mAbs (clone 11B.11, 10–40 μg/ml) during and post stimulation did not affect CXCR3 surface expression by BALB/c- 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.

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
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 dLNs 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, intralesional 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+CD3+ 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 naive 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

**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 dLNs of IL-10−/− BALB/c mice infected with *L. major* were analyzed by flow cytometry 3 wk post infection. 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.

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<tr>
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<th>BALB/c</th>
<th>C57BL/6</th>
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<tbody>
<tr>
<td>Control Ab</td>
<td>79.23 (8.78)</td>
<td>494.03 (76.95)</td>
</tr>
<tr>
<td>Anti-IL10R Ab</td>
<td>164.73 (11.64)*</td>
<td>531.65 (85.39)</td>
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<tr>
<td>Rat IgG2a-PE</td>
<td>15.52 (2.72)</td>
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*P<0.05
CXCR3 production in keratinocytes and induces migration of Th1 cells. Albanesi et al. (18) found that IL-4 enhances CXCL9 and CXCL10, 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-γ, previous studies have shown that IFN-γ, STAT1, and the transcription factor T-bet are required for efficient induction of CXCR3 on certain T cell subsets (14–16). IFN-γ, which signals via IFN-γR/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-γ production, we measured levels of IFN-γ as well as Th2-associated IL-4 and IL-10 in culture supernatant of activated T cells from BALB/c and C57BL/6 mice. In addition, we quantified mRNA levels of T-bet as well as IFN-γR 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-γ, 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-γR mRNA were also comparable in T cells from BALB/c and C57BL/6 mice and correlated with surface expression of IFN-γR as assessed by flow cytometry (data not shown). Collectively, these findings show that lack of IFN-γ production or impaired expression of T-bet or IFN-γR 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-γ 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 restimulated. 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-γ) 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-10R 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 given that C57BL/6 leukocytes show increased expression of CXCR3 upon activation. Anti-CD3/CD28-activated T cells from BALB/c mice show an impaired T helper 2 polarization in response to Leishmania major infection. 6 Immunol. Lett. 57: 41–44.


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.

![Figure 7](image-url)
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*-acetyleneuraminic 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.

