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A Mandatory Role for STAT4 in IL-12 Induction of Mouse T Cell CCR5

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IL-12 was recently shown to induce CCR5 on TCR-triggered mouse T cells. Considering that STAT4 is the most critical of IL-12 signaling molecules, this study investigated the role for STAT4 in the induction of CCR5 expression. IL-12R was induced by stimulation with anti-CD3 plus anti-CD28 mAb similarly on T cells from wild-type (WT) and STAT4-deficient (STAT4−/−) mice, but the levels of IL-12R induced on IFN-γ-deficient (IFN-γ−/−) T cells were lower compared with WT T cells. Exposure of TCR-triggered WT T cells to IL-12 induced CCR5 expression. In contrast, TCR-triggered STAT4−/− T cells failed to express CCR5 in response to IL-12. IL-12 stimulation induced detectable albeit reduced levels of CCR5 expression on IFN-γ−/− T cells. Addition of rIFN-γ to cultures of IFN-γ−/− T cells, particularly to cultures during TCR triggering resulted in restoration of CCR5 expression. However, CCR5 expression was not induced in STAT4−/− T cells by supplementation of rIFN-γ. These results indicate that for the induction of CCR5 on T cells, 1) STAT4 plays an indispensable role; 2) such a role is not substituted by simply supplementing rIFN-γ; and 3) IFN-γ amplifies CCR5 induction depending on the presence of STAT4.


Interleukin-12 plays a central role in the initiation and control of cell-mediated immune responses through its many actions on NK and T cells (1, 2). For example, IL-12 induces the production of a representative inflammatory cytokine, IFN-γ (3, 4) and enhances the cytolytic activity of NK and T cells (3, 5). Most notably, IL-12 promotes the differentiation of naive CD4+ T cells toward the Th1 phenotype by priming them for IFN-γ production (1, 6–9) and inducing the expression of functional Th1-specific surface molecules such as the IL-18R (10–14).

A considerable body of evidence highlights the role of chemokine receptors in the regulation of leukocyte migration from the vascular compartment to inflammatory sites (15–17). The chemokine receptors CCR5 and CXCR3 have been regarded as characteristic of CD4+ Th1 lymphocytes (18–20) and implicated in the recruitment of Th1 cells to inflammatory sites. Our recent study has shown that IL-12 is capable of inducing the expression of CCR5 on TCR-triggered CD4+ and CD8+ T cells (21), providing an aspect of explanation of how IL-12 contributes to promoting an inflammatory Th1 response. However, the molecular mechanism by which IL-12 regulates the expression of this chemokine receptor is unknown.

IL-12 exerts its effects on IFN-γ expression/Th1 development depending on signaling through STAT4 (22, 23). In the present study, we investigated the role for this transcription factor in the induction of CCR5 expression. Considering the involvement of STAT4 in the production of IFN-γ with the capacity to influence the expression of various surface molecules, experiments were performed using STAT4-deficient (STAT4−/−) as well as IFN-γ-deficient (IFN-γ−/−) mice. The results demonstrate that TCR-triggered wild-type (WT) T cells expressed CCR5 in response to IL-12 whereas STAT4−/− T cells failed. IFN-γ−/− T cells expressed detectable but reduced levels of CCR5, and the levels were restored to those observed for WT T cells by supplementing rIFN-γ particularly during TCR triggering. However, CCR5 expression was not induced on STAT4−/− T cells even in the presence of exogenous IFN-γ. These results indicate that STAT4 plays an indispensable role in CCR5 induction on T cells and that while IFN-γ amplifies CCR5 expression, the role mediated by STAT4 involves the function for which IFN-γ fails to substitute.

Materials and Methods

Mice

BALB/c mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). STAT4−/− BALB/c mice (BALB/c-Stat4tm1Ts ) (22) and IFN-γ−/− BALB/c mice (BALB/c-Ifngtm1Ts ) (24) were obtained from The Jackson Laboratory (Bar Harbor, MA). These knockout mice were bred in our laboratory and used at 6–9 wk of age.

Reagents

Mouse rIL-12 and human rIL-12 were provided by Genetics Institute (Cambridge, MA). Mouse rIL-2, human rIL-2, and mouse rIFN-γ were kindly provided by Shionogi (Osaka, Japan). Mouse MIP-1α was purchased from R&D Systems (Minneapolis, MN). Anti-CD3 (145-2C11) (25), anti-mouse CD28 (Pv-1) (26), anti-I-Aβ (34–5–53S) (27), anti-mouse IL-12 (C17.8) (28), and anti-human CD3 (OKT3) (American Type Culture Collection, Manassas, VA) mAbs were purified from culture supernatants or ascitic fluids of respective hybridomas. Allophycocyanin-conjugated anti-mouse CD8 or anti-mouse CD4 (BD PharMingen, San Diego, CA), PE-conjugated anti-mouse CCR5 mAb, mouse anti-human CD28 (BD PharMingen, San Diego, CA), PE-conjugated anti-human CCR5 (2D7) mAbs, and PE-conjugated streptavidin were purchased from BD PharMingen. FITC-conjugated anti-human CD4 (B-F5) and FITC-conjugated anti-human CD8 (MCD8) mAbs were obtained from IQ Products (Groningen, The Netherlands). Biotinylated antibodies were kindly provided by Shionogi (Osaka, Japan). Mouse MIP-1α was purposed from Shizuka Laboratory Animal Center (Hamamatsu, Japan). Mouse MIP-1α was purchased from R&D Systems (Minneapolis, MN). Anti-CD3 (145-2C11) (25), anti-mouse CD28 (Pv-1) (26), anti-I-Aβ (34–5–53S) (27), anti-mouse IL-12 (C17.8) (28), and anti-human CD3 (OKT3) (American Type Culture Collection, Manassas, VA) mAbs were purified from culture supernatants or ascitic fluids of respective hybridomas. Allophycocyanin-conjugated anti-mouse CD8 or anti-mouse CD4 (BD PharMingen, San Diego, CA), PE-conjugated anti-mouse CCR5 mAb, mouse anti-human CD28 (BD PharMingen, San Diego, CA), PE-conjugated anti-human CCR5 (2D7) mAbs, and PE-conjugated streptavidin were purchased from BD PharMingen. FITC-conjugated anti-human CD4 (B-F5) and FITC-conjugated anti-human CD8 (MCD8) mAbs were obtained from IQ Products (Groningen, The Netherlands). Biotinylated antibodies were kindly provided by Shionogi (Osaka, Japan). Mouse MIP-1α was purposed from Shizuka Laboratory Animal Center (Hamamatsu, Japan). Mouse MIP-1α was purchased from R&D Systems (Minneapolis, MN). Anti-CD3 (145-2C11) (25), anti-mouse CD28 (Pv-1) (26), anti-I-Aβ (34–5–53S) (27), anti-mouse IL-12 (C17.8) (28), and anti-human CD3 (OKT3) (American Type Culture Collection, Manassas, VA) mAbs were purified from culture supernatants or ascitic fluids of respective hybridomas. Allophycocyanin-conjugated anti-mouse CD8 or anti-mouse CD4 (BD PharMingen, San Diego, CA), PE-conjugated anti-mouse CCR5 mAb, mouse anti-human CD28 (BD PharMingen, San Diego, CA), PE-conjugated anti-human CCR5 (2D7) mAbs, and PE-conjugated streptavidin were purchased from BD PharMingen. FITC-conjugated anti-human CD4 (B-F5) and FITC-conjugated anti-human CD8 (MCD8) mAbs were obtained from IQ Products (Groningen, The Netherlands). Biotinylated antibodies were kindly provided by Shionogi (Osaka, Japan).
mouse anti-I-Ad/b mAb and then incubated with magnetic particles bound to goat anti-mouse Ig (Advanced Magnetics, Cambridge, MA). A T cell population depleted of anti-I-Ad/b-labeled and surface Ig^+^ cells was obtained by removing cell-bound magnetic particles with a rare earth magnet (Advanced Magnetics). Purity of the resulting T cell populations was examined by flow cytometry and found to be consistently >95%.

For the preparation of human T cell populations, human peripheral blood leukocyte (PBL) samples were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation of heparinized venous blood from healthy volunteers in the Department of Oncology, Osaka University Graduate School of Medicine (Osaka, Japan). The cells recovered at the interface were washed, suspended in medium, and used as a PBL population. A T cell population was prepared from PBL by positive selection as previously described (21). Briefly, PBL were incubated with anti-CD4 (OKT4) plus anti-CD8 (OKT8) mAb, followed by labeling supraperparamagnetic microbeads conjugated to goat anti-mouse Ig mAb (Miltenyi Biotec, Sunnyvale, CA). Labeled cells were separated from unlabeled cells by magnetic cell sorting using the MiniMACS (Miltenyi Biotec). The magnetic cells were retained in a MiniMACS magnet inserted into a MiniMACS magnet while the nonmagnetic cells passed through. Labeled cells were eluted after the column was removed from the magnet. Purity of the resulting populations was checked by flow cytometry. The purity of T cells (CD4- or CD8-positive) was >95%.

**Stimulation of T cells with anti-CD3 plus anti-CD28 mAb**

For the stimulation of the T cells, 5 μg/ml anti-CD3 (2C11) and 2 μg/ml anti-CD28 (Pv-1) were immobilized to individuawells of 24-well culture plates (Corning 25820; Corning Glass, Corning, NY) in a volume of 0.5 ml. After 3 h, solutions were discarded and plates were washed with PBS twice. Purified T cells were cultured in 2 ml of RPMI 1640 medium supplemented with 10% FBS and 2-ME at 37 °C in 5% CO₂ for 48 h in the presence of 100 U/ml rIL-2 or 1000 pg/ml rIL-12 (Fig. 1, left panels). TCR-triggered T cells were then cultured for an additional 48 h in the presence of [α-32P]UTP. The protected fragment (172 bp) was separated on a denaturing sequencing gel. As an internal control for the amount of RNA loaded onto the gel, RNA was simultaneously hybridized to a total volume of 20 μl using random primers and SuperScript II RNase H^- Reverse Transcriptase (Life Technologies, Rockville, MD). PCR amplification was conducted in a total volume of 50 μl 1× PCR buffer (TaKaRa, Otsu, Japan) containing 1.0 μl of the first strand cDNA, 0.2 mM of each dNTP, 0.5 μM of each primer and 1.25 U Taq DNA polymerase (TaKaRa). The magnetic cells were retained in a MiniMACS column inserted from each cDNA batch with SYBR Green staining. Sequences of the IL-12Rβ1, IL-12Rβ2, and β2m (for standardization), were amplified from each cDNA batch with 28, 28, and 20 amplification cycles, respectively.

**Calcium mobilization assay**

T cells were suspended at 1×10⁶/ml in 2% FBS/PBS containing 3 μM fura 2-AM (Dojindo, Kumamoto, Japan) and incubated at 37°C for 30 min. Fura 2-loaded cells were pelleted and washed twice and then resuspended at 5×10⁵/ml in PBS containing 0.5 mM CaCl₂. The calcium response was initiated by the addition of 1 nM MIP-1α. Cells were analyzed for free calcium ion by measurement of fura 2 fluorescence emission on fluorescence photometer Hitachi F-3000 (Tokyo, Japan).

**Results**

The capacity of IL-12 to induce CCR5 expression on both TCR-triggered human and mouse T cells

Our recent study demonstrated that resting and TCR-triggered human T cells express negligible and very low levels of CCR5, and enhanced levels of CCR5 expression are induced on TCR-triggered T cells following stimulation with IL-12 or IL-2 (21). We confirmed part of these observations (Fig. 1, left panels). We examined whether this is also the case with mouse T cells. Purified T cells were prepared from normal BALB/c mouse lymph nodes and stimulated in vitro with immobilized anti-CD3 plus anti-CD28 mAbs for 48 h. Like human T cells, resting and TCR-triggered mouse T cells expressed marginal levels of CCR5 (data not shown). TCR-triggered T cells were then cultured for an additional 48 h in the presence of 100 U/ml rIL-2 or 1000 pg/ml rIL-2 (Fig. 1, right panels). The results show that exposure of TCR-triggered mouse T cells to IL-12 resulted in CCR5 induction, whereas unlike human T cells, they failed to induce CCR5 expression in response to IL-2. These results indicate that IL-12 plays a critical role in CCR5 induction in mouse T cells.

A critical requirement for STAT4 in CCR5 induction

STAT4 is a representative and probably most critical of various IL-12 signaling molecules (22, 23, 32, 33). We determined whether STAT4 deficiency affects IL-12-mediated CCR5 induction. This was done using T cells from STAT4⁻/⁻ mice as well as those from IFN-γ⁻/⁻ mice because a representative of IL-12 bioactivities is the capacity to stimulate IFN-γ production (1, 2), and this capacity depends upon STAT4 activation (22, 23). Resting and TCR-triggered T cells from STAT4⁻/⁻ and IFN-γ⁻/⁻ mice, like
those from WT mice, expressed only marginal levels of CCR5 (data not shown). TCR-triggering of WT T cells induced both IL-12Rβ1 and β2 mRNA (Fig. 2A) along with the expression of IL-12R detected as the IL-12-binding site (Fig. 2B). The levels of mRNA and surface protein expression were comparable between WT and STAT4−/− T cells (Fig. 2, A and B). However, stimulation of TCR-triggered STAT4−/− T cells with IL-12 only marginally induced CCR5 expression (Fig. 3, middle panels). Unlike STAT4−/− T cells, TCR-triggered IFN-γ−/− T cells expressed reduced levels of IL-12Rβ1 and β2 mRNA (Fig. 2A). IL-12R expression on these cells was also lower than that on WT T cells (Fig. 2B). Although IFN-γ−/− T cells expressed CCR5 after IL-12 exposure, the levels were substantially reduced in these T cells as compared with those observed in WT T cells (Fig. 3). Consistent with this, neutralization of IFN-γ produced during TCR-triggering of WT T cells resulted in the reduction of CCR5 induction (Fig. 4).

We examined whether differential surface CCR5 levels induced on WT, STAT4−/−, and IFN-γ−/− T cells correlate with those for mRNA expression and the responsiveness to the relevant chemokine stimulation. Fig. 5A shows the time course of CCR5 mRNA induction in TCR-triggered WT T cells following IL-12 stimulation. TCR-triggered WT T cells express strikingly enhanced levels of CCR5 mRNA at relatively later time points (>24 h after IL-12 stimulation). Therefore, a comparison of CCR5 mRNA expression was made between WT, STAT4−/−, and IFN-γ−/− T cells 24 h after IL-12 exposure (Fig. 5B). In contrast to a striking enhancement in WT T cells, CCR5 up-regulation after IL-12 stimulation

FIGURE 1. Requirement for IL-12 in the induction of CCR5 expression on TCR-triggered mouse T cells. Left panels, Purified human PBL T cells (10⁶/well) were stimulated with 3 μg/ml immobilized anti-human CD3 (OKT3) plus 1 μg/ml anti-human CD28 mAb for 2 days in 24-well culture plates. These stimulated T cells were recultured in the presence of 15 U/ml human rIL-2 or 1000 pg/ml human rIL-12 for an additional 2 days. Right panels, Purified mouse lymph node T cells (1.5 × 10⁶/well) were similarly stimulated with 5 μg/ml immobilized anti-mouse CD3 plus 2 μg/ml anti-mouse CD28 mAb and recultured in the presence of 100 U/ml mouse rIL-2 or 1000 pg/ml mouse rIL-12. Both human and mouse cytokine-stimulated T cells were stained doubly with PE-conjugated anti-CCR5 mAb and a mixture of anti-CD4 and anti-CD8 (FITC-conjugated mAbs for human and allophycocyanin-conjugated mAbs for mouse). The numbers on the upper right corners on each panel are the percentages of CCR5-positive cells of CD4⁺/CD8⁻ T cells.

FIGURE 2. Induction of IL-12R in WT, STAT4−/− and IFN-γ−/− T cells following anti-CD3/anti-CD28 stimulation. Purified T cells prepared from WT, STAT4−/−, and IFN-γ−/− mouse lymph nodes were stimulated with anti-CD3 plus anti-CD28. The cells were subjected to RT-PCR for the determination of IL-12Rβ1 and β2 mRNA expression (A) and stained for IL-12R (IL-12-binding site) as described in Materials and Methods (B).
was not observed in STAT4−/− T cells or was apparently weaker in IFN-γ−/− T cells than that of WT T cells. It should be noted that CCR5 mRNA levels were significantly lower in IL-12-unstimulated STAT4−/− and IFN-γ−/− T cells than WT T cells. Moreover, IL-12 exposure failed to influence such a reduced level of CCR5 mRNA in IL-12-unstimulated STAT4−/− T cells.

To compare the functional status of CCR5 expressed on the surface of IL-12-stimulated T cells, we examined whether a CCR5-reactive chemokine, MIP-1α, induces Ca2+ mobilization as a result of surface CCR5 stimulation. MIP-1α is reactive with CCR1 as well as CCR5 (15–17). Although CCR1 was induced on splenic macrophages, this chemokine receptor was not found on TCR-triggered, IL-12-exposed T cells (data not shown). Fig. 6 shows that stimulation with MIP-1α results in high levels of Ca2+ influx in IL-12-stimulated WT T cells following TCR-triggering. This contrasted with negligible and low levels of Ca2+ influx in IL-12-stimulated STAT4−/− and IFN-γ−/− T cells. Together, the results indicate that differential levels of CCR5 expression on IL-12-exposed WT, STAT4−/− and IFN-γ−/− T cells are supported by the data obtained for the expression of CCR5 mRNA as well as the induction Ca2+ mobilization representing the functional status of CCR5.

The failure of IFN-γ to correct the defect in STAT4−/− T cells
To determine whether the defective CCR5 induction in STAT4−/− T cells results mainly or partly from their failure to produce IFN-γ,

FIGURE 4. Neutralization of IFN-γ produced by WT T cells during TCR triggering reduces CCR5 induction. WT T cells were stimulated with anti-CD3 plus anti-CD28 in the presence of anti-IFN-γ mAb (20 µg/ml) or control rat Ig for 2 days. Cells were restimulated with IL-12 for 2 days.

FIGURE 5. TCR-triggered STAT4−/− T cells fail to up-regulate CCR5 mRNA expression following IL-12 stimulation. A, WT T cells were similarly stimulated with anti-CD3/anti-CD28 mAbs and then treated with IL-12 for the indicated hour. B, TCR-triggered WT, STAT4−/−, and IFN-γ−/− T cells were exposed to IL-12 for 24 h. Total RNA was isolated from these IL-12-treated and untreated cells and subjected to the RNase protection assay to evaluate CCR5 mRNA expression.

FIGURE 6. Differential MIP-1α responsiveness in WT, STAT4−/−, and IFN-γ−/− T cells. WT, STAT4−/− and IFN-γ−/− T cells exposed to IL-12 following anti-CD3/anti-28 triggering were stimulated with 1 nM rMIP-1α.

FIGURE 7. Addition of IFN-γ to IL-12-exposed culture of IFN-γ−/− but not of STAT4−/− T cells up-regulates IL-12-mediated CCR5 induction. WT, STAT4−/−, and IFN-γ−/− T cells stimulated with anti-CD3/anti-CD28 (1st culture) were cultured in the presence of 100 U/ml rIFN-γ and/or 1000 pg/ml rIL-12 (2nd culture).
WT T cells stimulated with IL-12 in the absence or presence of anti-CD3 were substantially lower as compared with those observed in selective CCR5 induction in the mouse system, we investigated the expression on mouse T cells after TCR-triggered STAT4 deactivation. The present study demonstrates that STAT4 is also critical for the regulation of CCR5 induction. It is well known that STAT4 up-regulates or induces CCR5 expression. However, the mechanism for STAT4-induced up-regulation of CCR5 induction is not simply via IFN-γ production because exogenous IFN-γ failed to correct the defect observed in STAT4−/− T cells. neutralization of IFN-γ produced by WT T cells during TCR triggering reduced CCR5 induction considerably by the subsequent IL-12 stimulation. CCR5 induction was also decreased in IFN-γ−/− T cells. Consistent with the reports that STAT4 has the capacity to up-regulate the expression of IL-12, IL-12 expression was lower in IFN-γ−/− than in WT T cells. Thus, this may partially explain reduced levels of CCR5 induction in IFN-γ−/− T cells. Although IFN-γ amplifies CCR5 expression either directly or indirectly via up-regulation of IL-12, the effect of IFN-γ is manifested depending on the presence of STAT4. Taken together, STAT4 plays a mandatory role in CCR5 induction. It is assumed that STAT4 acts as a direct transcription factor for CCR5 gene expression or induces the expression of other genes whose products contribute to the expression of CCR5 genes. Further studies are required to characterize the regulatory element for CCR5 gene and to determine whether there exist elements with which STAT4 interacts directly or indirectly.

An important aspect of the present study concerns the question of why IL-2 fails to up-regulate CCR5 expression on mouse T cells, whereas IL-2 as well as IL-12 induce the expression of this gene.

Discussion

Stimulation of IL-12R results in a sequence of IL-12 signaling that includes the activation of Janus kinases and phosphorylation of transcription factors as targets for these kinases (22, 23, 32, 33). Among such signaling molecules, STAT4 is the most critical and the activation of this transcription factor leads to the manifestation of various IL-12 bioactivities such as IFN-γ expression and Th1 development (22, 23).

Our previous report demonstrated that IL-12 as well as IL-2 up-regulates CCR5 expression on TCR-triggered human T cells (21). The present study confirmed this and also showed that unlike human T cells, the expression on mouse T cells after TCR-triggering is induced by IL-12 but not by IL-2. Based on IL-12-selective CCR5 induction in the mouse system, we investigated the role for STAT4 in IL-12 induction of CCR5 on mouse T cells. The results demonstrated that the participation of STAT4 is mandatory for CCR5 induction and that IFN-γ, as a cytokine produced through STAT4 activation, amplifies the expression of this chemokine receptor. Because the defect in CCR5 induction due to STAT4 deficiency was not corrected by exogenous IFN-γ, the requirement of STAT4 is for the induction not only of IFN-γ expression, but also of additional unknown mechanisms.

Resting T cells do not express IL-12R, and they acquire IL-12 responsiveness including IL-12R expression upon TCR-triggering (11, 34-36). IL-12R was similarly induced on anti-CD3/anti-CD28-triggered T cells from WT and STAT4−/− mice. Lawless et al. (13) reported that the induction of IL-12Rβ1 and β2 mRNA was slightly reduced in STAT4−/− T cells. In their study, unfractionated splenocytes were stimulated with 2 μg/ml anti-CD3 alone (13). In ours, purified T cells were stimulated with higher doses (5 μg/ml) anti-CD3 plus anti-CD28 mAb. The induction of IL-12R, particularly of the IL-12Rβ2 subunit requires CD28 costimulation (36). Both TCR and CD28 would be stimulated more strongly in our system than in theirs. Thus, while Lawless et al. (13) observed a minor decrease in IL-12R chain expression in STAT4−/− T cells following anti-CD3 stimulation, using anti-CD3 plus anti-CD28 we found no significant difference in IL-12R expression between WT and STAT4−/− T cells. Because TCR triggering in the present condition can induce IL-12R in STAT4−/− T cells, the defect in IL-12-mediated CCR5 induction observed in STAT4−/− T cells is not due to their failure to express IL-12R, but ascribed to the problem in IL-12 signaling downstream of this cytokine receptor. Although IFN-γ expression is a representative of IL-12 bioactivities, IL-12 has been reported to induce the expression of other genes involved in Th1 development/function. These include genes for IRF-1 as transcription factors (37, 38) and IL-18R as a surface molecule (10–14). Moreover, Lawless et al. (13) showed that STAT4 regulates multiple components of signaling pathways to the expression of these genes. Similarly, the present study demonstrates that STAT4 is also critical for the regulation of CCR5 induction.
chemokine receptor in human T cells (Ref. 21 and this study). Initially, two independent groups have shown the up-regulatory effect of IL-2 (19, 20) as well as the down-regulatory effect of IL-12 on CCR5 expression in human PBL T cells (20). However, in these studies, freshly prepared PBL T cells were used as the target cell. As shown in our previous study (21), these resting T cells do not express IL-12R, which may account for the observation that IL-12 fails to induce CCR5 expression. Although IL-2 was capable of inducing CCR5 expression on resting T cells (19, 20), the IL-2 effect was observed only after long-term (>8-day) exposure to this cytokine (19, 20). One of the earlier studies (19) also showed that the phenotype of responding T cells was CD45RO+ (memory phenotype). It may be assumed that native T cells not expressing IL-2R cannot respond to IL-2, whereas CD45RO+ T cells present as a component of T cells in PBL have the capacity to respond to IL-2. Moreover, long term (more than an 8-day) IL-2 stimulation might contribute not only to inducing CCR5 expression on CD45RO+ T cells but also to expanding CCR5+ cells generated as a minor population.

The capacity of IL-12 to induce CCR5 expression on human T cells was demonstrated by exposing this cytokine to TCR-triggered T cells that have expressed sufficient levels of IL-12R (21). The stimulation of similarly activated human T cells with IL-2 also up-regulated CCR5 expression. It should be noted that this was achieved within 48 h without requiring long-term exposure, which differed from the time course of CCR5 induction observed for memory T cells directly stimulated with IL-2. However, exposure of TCR-triggered mouse T cells to IL-2 failed to induce CCR5 expression (this study). Moreover, it was found in our most recent study that IL-2 not only fails to induce CCR5 expression per se but also down-regulates IL-12 induction of CCR5 expression in the presence of both cytokines (M. Iwasaki, T. Mukai, H. Fujiwara, and T. Hamaoka, manuscript in preparation). Thus, there exists a fundamental difference in the cytokine-mediated regulation of CCR5 expression between human and mouse T cells.

IL-12 plays a critical role in Th1 differentiation (1, 6–9) and this cytokine exerts its function through activating STAT4 (22, 23). A representative of Th1 functions, IFN-γ expression is induced in a STAT4-dependent way (22, 23). Th1 cells are activated in lymphoid organs and have to migrate from there to inflammatory sites to exhibit its anti-inflammatory responses. Therefore, the acquisition of the migratory capacity is crucial in the implementation of Th1 function. In this context, our present results illustrate that IL-12 confers TCR-triggered T cells with an capacity to migrate to inflammatory sites and that the acquisition of such a capacity is achieved depending on STAT4 activation. Thus, the present results add to a growing list of knowledge regarding multiple requirements of signaling molecules and pathways to Th1 development and function.

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