

CXCR3-mediated opposite effects of CXCL10 and CXCL4 on T_H1 or T_H2 cytokine production

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Background: Two variants of the CXCR3 receptor exist, one (CXCR3-A) reactive with CXCL9, CXCL10, and CXCL11 and the other (CXCR3-B) also reactive with CXCL4. Both variants are contemporarily expressed by human T cells.

Objective: We sought to investigate the *in vitro* effects of CXCL10 and CXCL4 on the production of T_H1 or T_H2 cytokines.

Methods: The cytokine profile of antigen-specific human CD4⁺ T-cell lines obtained in the absence or presence of CXCL10 or CXCL4 was evaluated by means of quantitative RT-PCR, flow cytometry, and ELISA.

Results: CXCL10 upregulated IFN- γ and downregulated IL-4, IL-5, and IL-13 production, whereas CXCL4 downregulated IFN- γ and upregulated T_H2 cytokines. Similar effects were also observed on polyclonally activated pure naive CD4⁺ T cells. The opposite effects of CXCL10 and CXCL4 on T_H1 and T_H2 cytokine production were inhibited by an anti-CXCR3 antibody able to neutralize both CXCR3-A and CXCR3-B and were apparently related to the activation of distinct signal transduction pathways. Moreover, CXCL10 upregulated mRNA levels of T-box expressed in T cells and downregulated GATA-3 expression, whereas CXCL4 downregulated T-box expressed in T cells and upregulated GATA-3. Finally, CXCL4, but not CXCL10, induced direct activation of IL-5 and IL-13 promoters.

Conclusion: CXCL10 and CXCL4 exert opposite effects on the production of human T_H1 and T_H2 cytokines, likely through their respective interaction with CXCR3-A or CXCR3-B and the consequent activation of different signal transduction pathways. This might represent an internal regulatory pathway of T_H cell responses and might contribute to the modulation of chronic inflammatory reactions, including allergy. (*J Allergy Clin Immunol* 2005;116:1372-9.)

Key words: CXCR3, T_H1, T_H2, CXCL10, CXCL4, GATA-3, T-bet, immune deviation

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Abbreviations used

cAMP: Cyclic adenosine monophosphate

PKA: Protein kinase A

PMA: Phorbol 12-myristate 13-acetate

SK: Streptokinase

STAT: Signal transducer and activator of transcription

T-bet: T-box expressed in T cells

UCB: Umbilical cord blood

It is increasingly clear that chemokines regulate inflammation by exhibiting biologic activities in addition to their chemotactic properties described initially.^{1,2} For example, the chemokines CXCL9, CXCL10, and CXCL11 are able to exert chemotactic activity on T_H1 cells and natural killer cells,^{3,4} induce chemotaxis and proliferation of human mesangial cells,⁵ and inhibit the growth of human microvascular endothelial cells.^{6,7} We recently showed that these different, and even opposite, functions of CXCL9, CXCL10, and CXCL11 could be explained, at least in part, by the existence of a previously unrecognized alternatively spliced variant of the CXCR3 receptor, which was named CXCR3-B, whereas the already known variant (CXCR3) was renamed CXCR3-A.⁷ Of note, CXCR3-B was also found to act as a functional receptor for CXCL4, another angiostatic chemokine.⁷ CXCL9, CXCL10, and CXCL11 interact with both receptor variants, although with higher affinity for CXCR3-A than for CXCR3-B, whereas CXCL4 exhibits almost selective affinity for CXCR3-B.⁷

Both CXCR3-A and CXCR3-B receptor variants were found to be contemporarily expressed by either resting or activated human T cells, with the expression of CXCR3-A being consistently higher than the expression of CXCR3-B.⁷ Previous studies have shown that CXCL10 not only acts on human T cells by inducing the recruitment of T_H1 cells into inflamed tissues,³ but it also selectively promotes the dominance of environmental antigen-driven IFN- γ responses in healthy nonallergic human subjects.⁸ By contrast, CXCL4 was found to exert inhibitory effects on both T-cell proliferation and IFN- γ production by antigen-stimulated T cells.⁹

In this study we demonstrate that CXCL10 and CXCL4 exert opposite effects on the production of human T_H1 and T_H2 cytokines and suggest that these effects might be

related to their respective interaction with CXCR3-A or CXCR3-B and the subsequent activation of distinct signal transduction pathways.

METHODS

Reagents and antibodies

For information on reagents and antibodies, see the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Subjects

Human samples used in this study were obtained in accordance with the ethical standards of the responsible regional committee on human experimentation.

Generation of antigen-specific short-term CD4⁺ T-cell lines

To generate antigen-specific short-term CD4⁺ T-cell lines, PBMCs (1×10^6 /mL) obtained from 10 atopic Der p 1–sensitive and 10 nonatopic healthy volunteers were stimulated with streptokinase (SK; 1000 IU/mL) or Der p 1 (10 μ g/mL) in the absence or presence of CXCL10 (1 μ M) or CXCL4 (1 μ M). On day 6, antigen-activated T cells were expanded for an additional 8 days with IL-2 (20 U/mL). Under these conditions, more than 90% of cells recovered on day 14 were CD4⁺ T lymphocytes, with the other being CD8⁺ T cells.^{10,11}

Generation of T-cell lines from umbilical cord blood–derived naive CD4⁺ T cells

For information on generation of T-cell lines from umbilical cord blood (UCB)-derived naive CD4⁺ T cells, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Real-time quantitative RT-PCR (Taqman)

TaqMan RT-PCR was performed as described elsewhere.⁷ For probes used, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Flow cytometric analysis of intracellular cytokines

T blasts (1×10^6) from antigen-specific short-term CD4⁺ T-cell lines were stimulated with phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) plus ionomycin (1 μ M) for 6 hours, the last 4 in the presence of brefeldin A (5 μ g/mL), as previously described.¹¹

Quantitation of cytokine concentrations in supernatants

For information on quantitation of cytokine concentrations in supernatants, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Measurement of intracellular cyclic adenosine monophosphate levels

T blasts (1×10^6) from Der p 1–specific short-term CD4⁺ T-cell lines were cultured for 2 hours in the absence or presence of CXCL10 (1 μ M), CXCL4 (1 μ M), or forskolin (10 μ M). Cells were then lysed, and intracellular cyclic adenosine monophosphate (cAMP) levels were measured with a commercially available colorimetric kit, as previously described.⁷

Confocal microscopy

For information on confocal microscopy, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Transient transfections and luciferase assay

For information on transient transfections and luciferase assay, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

Statistical analyses

For information on statistical analyses, refer to the [Methods](#) section in the Online Repository in the online version of this article at www.jacionline.org.

RESULTS

CXCL10 and CXCL4 exert opposite effects on cytokine production by antigen-specific short-term CD4⁺ T-cell lines

SK- (T_H1-like) or Der p 1–specific (T_H2-like) short-term CD4⁺ T-cell lines were generated in the absence or presence of CXCL10 or CXCL4 from 10 atopic Der p 1–sensitive donors, and on day 14 of culture, mRNA levels for IFN- γ , IL-4, IL-5, and IL-13 were measured. As expected, SK-specific short-term CD4⁺ T-cell lines expressed mRNA for IFN- γ but low levels of IL-4, IL-5, or IL-13, whereas Der p 1–specific short-term CD4⁺ T-cell lines expressed mRNA for IL-4, IL-5, and IL-13 in addition to IFN- γ . The presence of CXCL10 increased the levels of IFN- γ mRNA in either SK- or Der p 1–specific short-term CD4⁺ T-cell lines, whereas IL-4, IL-5, and IL-13 mRNA levels were downregulated. By contrast, CXCL4 downregulated IFN- γ and increased IL-4, IL-5, and IL-13 mRNA levels in both SK- and Der p 1–specific CD4⁺ T-cell lines ([Fig 1](#)).

The same antigen-specific T-cell lines were also investigated by means of flow cytometry on day 14, after polyclonal stimulation, for IFN- γ and IL-13 production. As shown in [Fig E1, A](#), in the Online Repository in the online version of this article at www.jacionline.org, in SK-stimulated cultures, CXCL10 reduced the proportions of IL-13–producing cells, whereas in Der p 1–stimulated cultures, it increased the proportions of IFN- γ –producing T cells and significantly reduced the proportions of IL-13–producing T cells. By contrast, CXCL4 increased IL-13–producing cells, whereas it decreased the proportions of IFN- γ –producing cells in either SK- or Der p 1–specific short-term CD4⁺ T-cell lines ([Fig E1, A](#)). Both CXCL10 and CXCL4 exerted the same opposite effects on the production of IFN- γ and IL-13, even on CD4⁺ T cells from nonatopic donors (see [Fig E1, B](#), in the Online Repository in the online version of this article at www.jacionline.org). Similar results were obtained when concentrations of IFN- γ and IL-13, released into cell-free supernatants on stimulation with PMA plus anti-CD3 mAb of T-cell blasts from either atopic or nonatopic donors, were measured by using appropriate ELISAs ([Table E1](#) in the Online Repository in the online version of this article at www.jacionline.org).

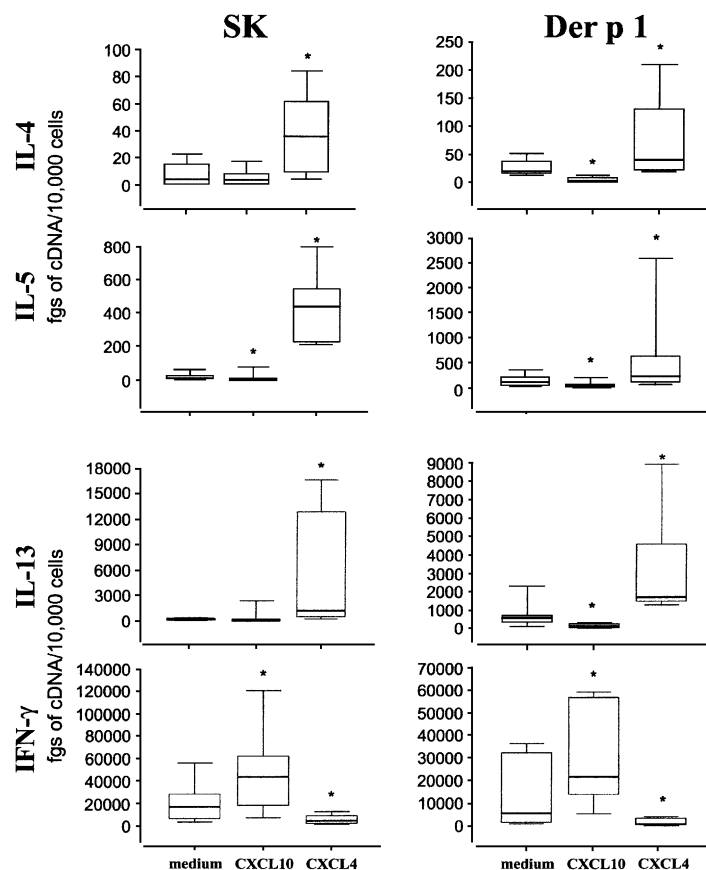


FIG 1. Effects of CXCL10 or CXCL4 on cytokine mRNA expression by antigen-specific T-cell lines. mRNA levels of each cytokine were measured by means of quantitative RT-PCR and compared with those obtained with medium alone. Medians and 25th and 75th percentiles are shown in boxes, and minimum and maximum values are shown as whiskers ($n = 10$). * $P < .05$.

Opposite effects of CXCL10 and CXCL4 on production of T_H1/T_H2 cytokines are likely mediated by their respective interaction with CXCR3-A or CXCR3-B

The expression of mRNA for both CXCR3-A and CXCR3-B was then examined in PBMCs from 10 atopic donors before their stimulation with SK or Der p 1, as well as on antigen-specific short-term $CD4^+$ T-cell lines generated from the same donors. Consistent with previous results,⁷ mRNA for both CXCR3-A and CXCR3-B was expressed by either resting or activated T cells. However, CXCR3-A mRNA levels were consistently higher than CXCR3-B levels, and the expression of both receptor variants was significantly increased by antigen stimulation, with CXCR3-A being 5 times more expressed than CXCR3-B in unstimulated and at least 20 times in either SK- or Der p 1-stimulated cultures assessed on day 14 (see Fig E2, A, in the Online Repository in the online version of this article at www.jacionline.org). The antigen-induced increase of mRNA levels for either CXCR3-A or CXCR3-B was not significantly different in parallel cultures containing CXCL10 or CXCL4 (data not shown). Of note, surface CXCR3 protein was found to be expressed

at comparable levels in both SK- and Der p 1-specific T-cell lines, and there were no differences between CXCR3⁺ and CXCR3⁻ Der p 1-specific $CD4^+$ T cells in the ability to produce IL-4 or IFN- γ (Fig E2, B and C, in the Online Repository in the online version of this article at www.jacionline.org).

We therefore sought to determine whether the opposite effects of CXCL10 and CXCL4 on the production of T_H1/T_H2 cytokines could be explained by their respective interaction with CXCR3-A or CXCR3-B. To this end, the effect of an anti-CXCR3 mAb capable of neutralizing the activity of both CXCR3-A and CXCR3-B⁷ was assessed on SK- or Der p 1-specific short-term $CD4^+$ T-cell lines from 7 different atopic donors. The addition of anti-CXCR3, when compared with the isotype-matched control mAb, strongly inhibited either CXCL10- or CXCL4-related effects on the proportions of IL-13- and IFN- γ -producing cells in both SK- and Der p 1-specific short-term $CD4^+$ T-cell lines (Fig 2). To provide additional, even if indirect, evidence that the T_H1 shifting induced by CXCL10 was mediated by its interaction with CXCR3-A, whereas the T_H2 shifting promoted by CXCL4 could be due to its interaction with CXCR3-B, CXCL10 and CXCL4 were assessed for their ability to

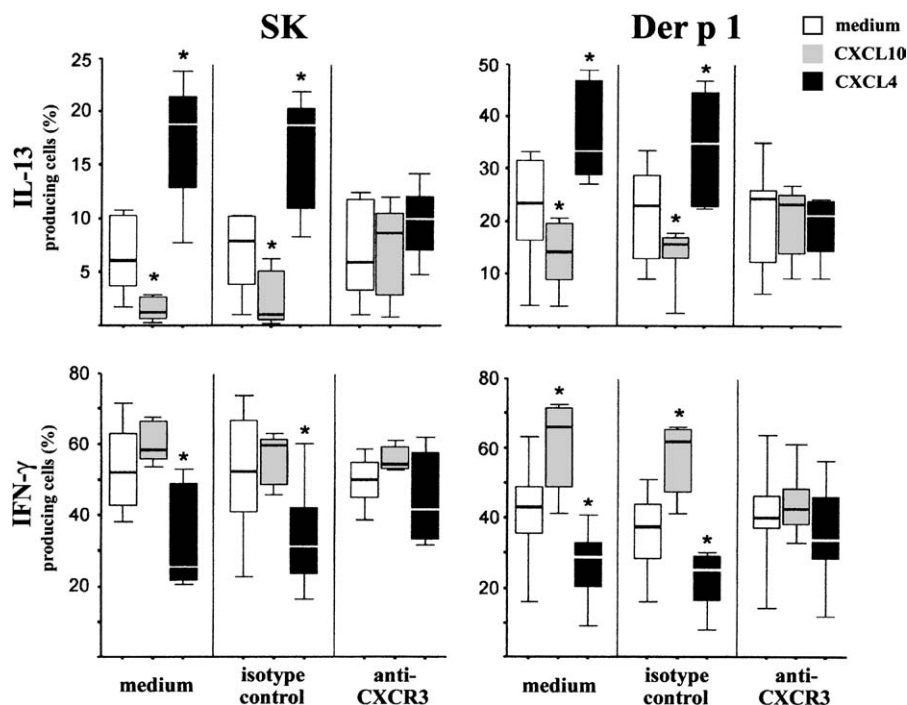


FIG 2. Inhibition of CXCL10- or CXCL4-mediated effects by an anti-CXCR3 mAb. Percentages of IL-13- or IFN- γ -producing cells under the indicated culture conditions are shown. Medians and 25th and 75th percentiles are shown in *boxes*, and minimum and maximum values are shown as *whiskers* ($n = 7$). * $P < .05$.

induce an increase of intracellular cAMP levels in Der p 1-specific short-term CD4⁺ T-cell lines derived from 7 different atopic donors. As shown in Fig 3, A, CXCL4 increased intracellular cAMP up to levels almost comparable with those induced by forskolin, whereas CXCL10 did not exhibit any significant effect. To establish whether the CXCL4 activity was related to its capacity to increase cAMP production, the effect of cAMP inhibitors on the production of IL-13 or IFN- γ by Der p 1-specific short-term CD4⁺ T-cell lines derived from 7 different atopic donors was assessed by means of flow cytometry. As shown in Fig 3, B, the addition of either RP-8-Br-cAMPS (a protein kinase A [PKA] inhibitor) or MDL-12 (a cAMP antagonist) strongly inhibited the CXCL4-induced upregulation of IL-13, whereas pertussis toxin (a G_i inhibitor) did not exert any effect (data not shown). Of note, only MDL-12, but not RP-8-Br-cAMPS, reverted the CXCL4-mediated inhibition of IFN- γ production. By contrast, both inhibitors did not exhibit any relevant effect on the activity of CXCL10 on the production of the 2 cytokines (Fig 3, B). Comparable effects were induced by both inhibitors on SK-specific short-term CD4⁺ T-cell lines (data not shown).

CXCL10 and CXCL4 have opposite effects on T-box expressed in T cells and GATA-3 expression

The mechanisms by which CXCL10 and CXCL4 exert opposite effects on the production of T_H1/T_H2 cytokines were then investigated. To this end, PBMCs from 7 atopic

donors were stimulated with anti-CD3 plus anti-CD28 mAbs in the absence or presence of CXCL10 or CXCL4, and mRNA levels for signal transducer and activator of transcription (STAT) 4, T-box expressed in T cells (T-bet), STAT6, c-maf, and GATA-3 were measured at different times after cell activation. As shown in Fig 4, CXCL10 induced a strong, although transient, upregulation of T-bet mRNA expression, as well as a persistent downregulation of GATA-3 mRNA expression. By contrast, CXCL4 downregulated the expression of T-bet starting at 48 hours, which was followed by a strong increase of GATA-3 transcription at 96 hours. Similar effects of CXCL4 and CXCL10 on GATA-3 transcription could also be observed in SK- or Der p 1-specific short-term CD4⁺ T-cell lines derived from 7 different atopic donors (see Fig E3 in the Online Repository in the online version of this article at www.jacionline.org). Moreover, in Der p 1-specific short-term CD4⁺ T-cell lines generated in the presence of CXCL4, the proportions of CD3⁺ T cells showing nuclear localization of GATA-3 protein were significantly increased (median, 351.5% min-max, 250.0% to 500.0%) in comparison with cultures performed in the absence of either CXCL10 or CXCL4 (median, 278.8% min-max, 58.8% to 444.4% $P < .05$), whereas GATA-3 nuclear expression was virtually absent in cultures stimulated in the presence of CXCL10 (median, 43.7% min-max, 0% to 88.2% $P < .05$). Of note, in CXCL10-free cultures, T cells expressed GATA-3 mainly in the cytoplasm, whereas in CXCL4-containing cultures GATA-3 was consistently detected at the nuclear level,

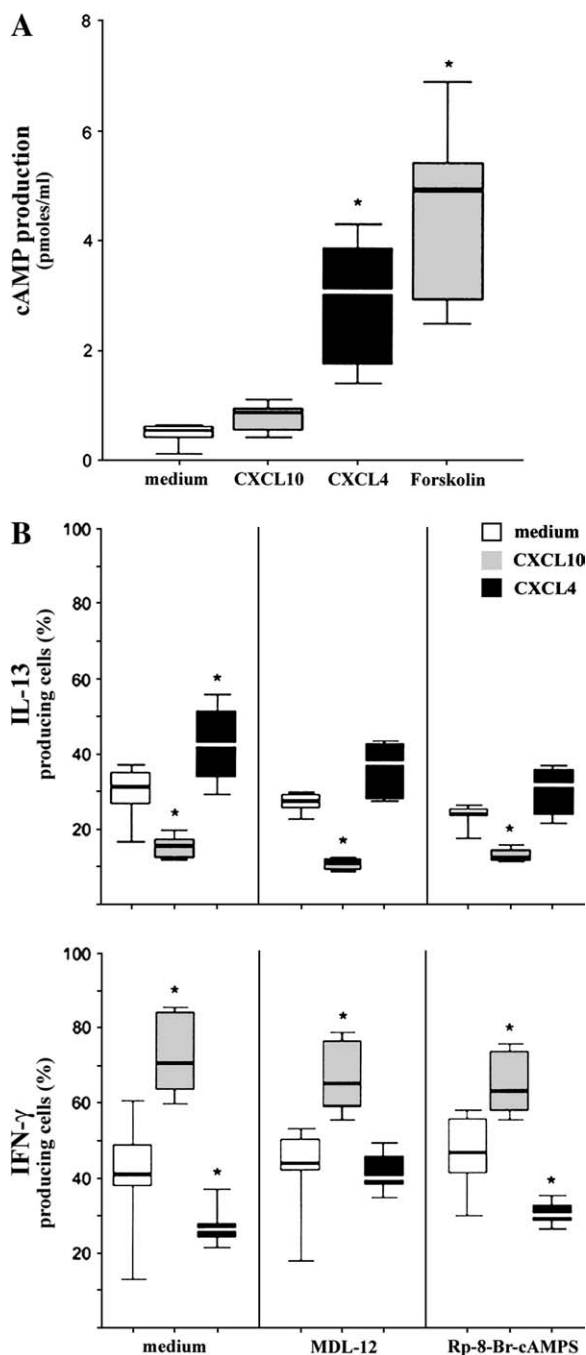


FIG 3. cAMP mediates CXCL4 effects on cytokine synthesis. **A**, cAMP assessment in Der p 1-specific T-cell lines ($n = 7$) in the absence or presence of CXCL10, CXCL4, or forskolin. **B**, Percentages of cells producing the given cytokine under the indicated culture conditions. Medians and 25th and 75th percentiles are shown in boxes, and minimum and maximum percentiles are shown as whiskers ($n = 7$). * $P < .05$.

as shown by costaining T cells with To-pro-3 (see Fig E4 in the Online Repository in the online version of this article at www.jacionline.org). Neither of the 2 chemokines exhibited any relevant effect on the transcription

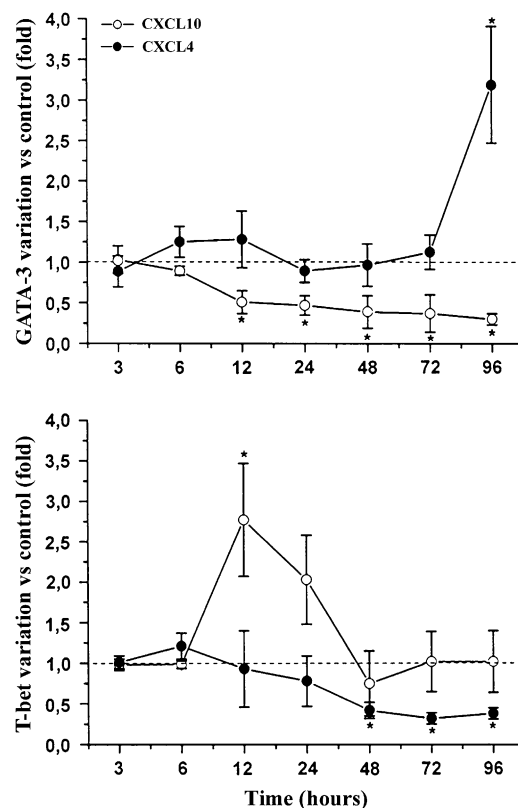


FIG 4. Opposite effects of CXCL10 and CXCL4 on T-bet and GATA-3 expression. T-bet and GATA-3 mRNA levels in polyclonally stimulated PBMCs are shown. Mean values ($\pm$ SD) of fold variations of mRNA levels are shown ($n = 7$). * $P < .05$.

of STAT4 or STAT6 either in 96-hour polyclonally activated PBMCs or in antigen-specific short-term CD4⁺ T-cell lines, with a CXCL4-related increase of c-maf being observed only in the latter (data not shown).

To provide additional evidence that the T_H2-promoting effect of CXCL4 was due to the increased transcription of GATA-3, transient transfection of the pGL3 vector carrying a luciferase construct driven by the IL-5 and IL-13 promoters was performed in an unstimulated human T-cell clone, as well as in the same clone stimulated with anti-CD3 plus anti-CD28 mAbs. This clone was a T_H2 clone that expressed CXCR3, including the CXCR3-B variant, and its cAMP production was upregulated by CXCL4 (see Fig E5 in the Online Repository in the online version of this article at www.jacionline.org). CXCL4 increased the activation of both IL-5 and IL-13 promoters only in the stimulated T_H2 clone, whereas the addition of CXCL10 did not exhibit any activating effect (Fig 5). Of note, when the same clone was transfected with a pGL3 vector carrying a luciferase construct driven by the IL-5 promoter, which contained a defective GATA-3 binding site, evidence of IL-5 activation was never observed (data not shown). No activating effects by either CXCL10 or CXCL4 on IL-4 and IFN- γ proximal promoters were found (data not shown).

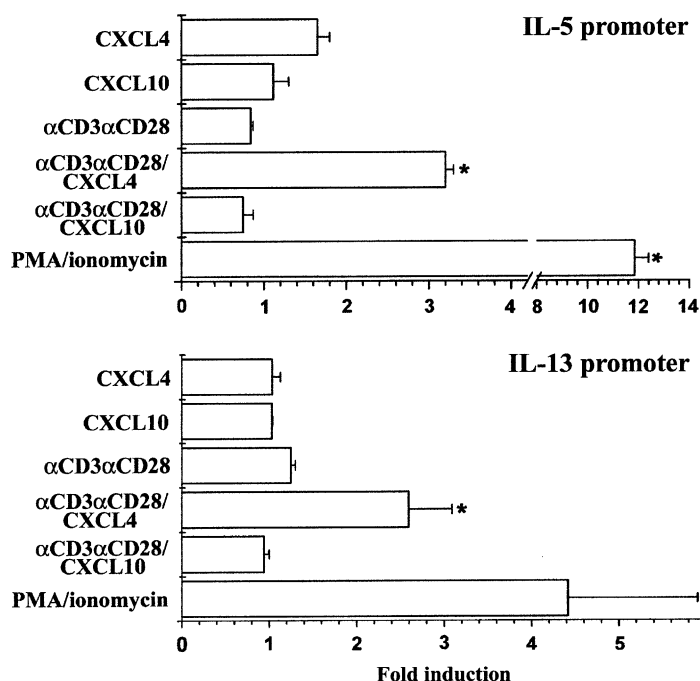


FIG 5. CXCL4 induces activation of IL-5 and IL-13 promoters. T blasts from a T_H2 clone were transiently transfected with the IL-5 and IL-13 promoter firefly luciferase reported plasmid and cultured for 4 hours as indicated. Bars represent mean values ($\pm$ SD) of fold induction in comparison with cells cultured in medium alone found in 3 separate experiments. * $P < .05$.

CXCL10 and CXCL4 exert opposite effects on cytokine production by naive $CD4^+$ T cells

Naive $CD4^+$ T cells were purified from UCB mononuclear cells of 5 newborns and stimulated with PHA in the presence or absence of CXCL10 or CXCL4 to provide more direct evidence that the 2 chemokines exerted their regulatory activity on T_H cell polarization by directly interacting with T cells. On day 6, the production of IFN- γ and IL-13 was evaluated by means of flow cytometry. As shown in Fig 6, CXCL10 significantly increased the proportions of IFN- γ -producing cells, whereas proportions of IL-13 $^+$ cells were significantly reduced. By contrast, CXCL4 downregulated the proportions of IFN- γ -producing cells and increased those of IL-13-producing cells.

DISCUSSION

The results of this study demonstrate that the chemokines CXCL10 and CXCL4 exert opposite effects on the production by human T cells of the T_H1 cytokine IFN- γ or the T_H2 -type cytokines IL-4, IL-5, and IL-13, respectively. CXCL10 upregulated, at both the mRNA and protein levels, the production of IFN- γ , whereas it downregulated the production of IL-4, IL-5, and IL-13. CXCL4 not only downregulated the production of IFN- γ on the same cells but also strongly upregulated the production of T_H2 -type cytokines. The opposite effects of CXCL10 and CXCL4 on the production of T_H1 and T_H2 cytokines

were neither restricted to a given antigen nor limited to or influenced by the atopic condition. In addition, the same effects were observed on polyclonally stimulated pure naive $CD4^+$ T cells, suggesting that the polarizing activity of CXCL4 or CXCL10 was due to their direct interaction with T lymphocytes.

We have recently described the existence of a previously unrecognized alternatively spliced variant of the CXCR3 receptor, which was named CXCR3-B, whereas the already known variant was renamed CXCR3-A.⁷ CXCL9, CXCL10, and CXCL11 interact with both receptor variants, although with higher affinity for CXCR3-A than for CXCR3-B, whereas CXCL4 exhibits almost selective affinity for CXCR3-B.⁷ The demonstration that both CXCR3-A and CXCR3-B were expressed in human T cells prompted us to investigate the possibility that the opposite effects of CXCL10 and CXCL4 on T_H1/T_H2 cells could be due to their interaction with CXCR3-A or CXCR3-B, respectively. Unfortunately, we could not provide a direct demonstration for the validity of this hypothesis. Indeed, mAbs selectively neutralizing each of the 2 variants in functional assays are not available. Moreover, attempts to evaluate the effects of silencing each of the 2 genes were unsuccessful because living cells that could be recovered after transfection were not sufficient to allow us to establish antigen-specific T-cell lines. However, indirect evidence on the respective role of CXCR3-A and CXCR3-B was suggested by the results of 2 different experimental approaches. First, the addition of an anti-CXCR3 mAb able to neutralize both CXCR3-A

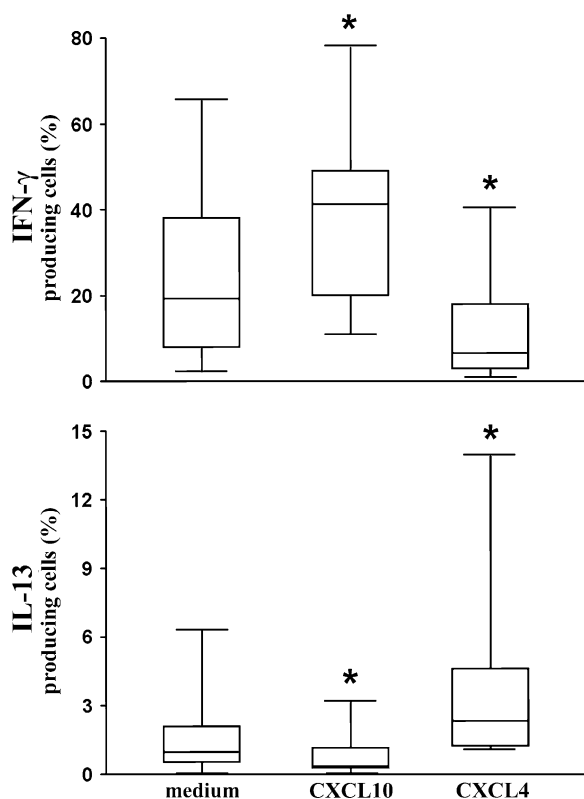


FIG 6. Effects of CXCL10 or CXCL4 on intracellular cytokine production by pure naive CD4⁺ T cells obtained from UCB mono-nuclear cells of 5 newborns. Percentages of cells producing the given cytokine in cultures without or with CXCL4 or CXCL10 are shown. Medians and 25th and 75th percentiles are shown in boxes, and minimum and maximum percentiles are shown as whiskers. **P* < .05.

and CXCR3-B⁷ inhibited both the opposite effects of CXCL10 and CXCL4 on IL-13 and IFN- γ production in either SK- or Der p 1-specific short-term CD4⁺ T-cell lines. Second, and more importantly, we took advantage from our previous demonstration that CXCR3-B exhibits coupling to G_s proteins and induces increase of intracellular cAMP levels, whereas CXCR3-A does not.⁷ Accordingly, even in T cells, CXCL4, but not CXCL10, induced an increase of intracellular cAMP levels. Furthermore, a PKA inhibitor or a cAMP antagonist strongly inhibited the CXCL4-induced upregulation of IL-13 in Der p 1-stimulated cultures, whereas a G_i inhibitor, such as pertussis toxin, did not exert any effect. Of note, only the cAMP antagonist, but not the PKA inhibitor, reverted the CXCL4-mediated inhibition of IFN- γ production, which is consistent with the observation that the cAMP-induced inhibition of IFN- γ synthesis is PKA independent.^{12,13}

The results of this study also demonstrate that CXCL10 was able to induce an increase of T-bet mRNA levels and a downregulation of GATA-3 transcription in PBMCs polyclonally activated for short periods of time, as well as in SK- or Der p 1-specific T-cell lines. In both systems CXCL4 downregulated T-bet and also induced the

increase of GATA-3 mRNA levels. These findings are in agreement with the results of a previous study showing that the increase of intracellular cAMP levels results in GATA-3 phosphorylation, followed by its nuclear translocation and subsequent mRNA transcription.¹⁴ Furthermore, in the same culture systems, GATA-3 mRNA levels were downregulated by CXCL10, and there was virtually no expression of GATA-3 in CXCL10-containing, Der p 1-specific short-term CD4⁺ T-cell lines. More importantly, CXCL4 was able to activate the transcription of IL-5 and IL-13 through direct binding to their proximal promoters, whereas in the same system neither effects by CXCL4 on IL-4 nor by CXCL10 on IFN- γ promoter were observed. Thus our data are consistent with the recent observation demonstrating that GATA-3 induces IL-4-independent T_H2 cell differentiation by both opening the *IL4-IL13* locus and directly activating the IL-5 and IL-13 promoters.¹⁵⁻¹⁷ On the other hand, the lack of a direct effect of CXCL10 on IFN- γ promoter can be explained by the fact that T-bet drives modest activation of luciferase reporter transgenes containing the proximal human IFN- γ promoter, and these elements are insufficient to direct robust T_H1-specific expression of IFN- γ activation, as are transgene constructs containing up to 3.4 kb of 5' flank.¹⁸

An interesting question emerging from this study is the possible *in vivo* physiologic role of the opposite regulatory effects of CXCL10 and CXCL4 and, consequently, of their receptor variants CXCR3-A and CXCR3-B on human T_H1/T_H2 polarization. The T_H1-polarizing activity of CXCL10 clearly provides a positive amplification loop with IFN- γ , which is its major inducer.¹⁹ Thus the production of CXCL9, CXCL10, and CXCL11 might protect nonatopic subjects from T_H2-mediated allergic inflammation by amplifying the T_H1-mediated response against common environmental allergens.⁸ Consistent with this hypothesis is the recent demonstration that allergic subjects are hyporesponsive to the T_H1-type loop promoted by CXCL9, CXCL10, and CXCL11.²⁰ By contrast, the *in vivo* physiologic role of CXCL4 and of the expression in T cells of its receptor CXCR3-B on the function of T_H2 cells is still unclear. At present, CXCL4 is known to be a cationic protein that is stored in platelet α granules and secreted on platelet activation.²¹ Evidence of platelet activation has been found in patients with bronchial asthma, and increased expression of CXCL4 has been reported in human asthmatic subjects after allergen bronchial provocation testing or during symptomatic periods,^{22,23} suggesting the existence of some link between the platelet product CXCL4 and T_H2 responses. Taken together, the results of this study suggest the existence of a regulatory pathway that might be critical in determining clinical tolerance to allergens or the induction, maintenance, and amplification of allergen-specific T_H2 responses.

REFERENCES

- Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;12:121-7.
- Baggiolini M, Loetscher P. Chemokines in inflammation and immunity. *Immunol Today* 2000;21:418-20.

3. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsetti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129-34.
4. Inngjerdingen M, Damaj B, Maghazachi AA. Expression and regulation of chemokine receptors in human natural killer cells. *Blood* 2001;97:367-75.
5. Romagnani P, Beltrame C, Annunziato F, Lasagni L, Luconi M, Galli G, et al. Role for interactions between IP-10/Mig and CXCR3 in proliferative glomerulonephritis. *J Am Soc Nephrol* 1999;10:2518-26.
6. Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest* 2001;107:53-63.
7. Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med* 2003;197:1537-49.
8. Gangur V, Simons ER, Hayglass K. Human IP-10 selectively promotes dominance of polyclonally activated and environmental antigen-driven IFN- γ over IL-4 responses. *FASEB J* 1998;12:705-13.
9. Fleischer J, Grage-Griebenow E, Kasper B, Heine HG, Ernst M, Brandt E, et al. Platelet factor 4 inhibits proliferation and cytokine release of activated human T cells. *J Immunol* 2002;169:770-7.
10. Annunziato F, Cosmi L, Galli G, Beltrame C, Romagnani P, Manetti R, et al. Assessment of chemokine receptor expression by human Th1 and Th2 cells in vitro and in vivo. *J Leukoc Biol* 1999;65:691-9.
11. Brugnolo F, Sampognaro S, Liotta F, Cosmi L, Annunziato F, Manuelli C, et al. The novel synthetic immune response modifier R-848 (Resiquimod) shifts human allergen-specific CD4⁺ TH2 lymphocytes into IFN- γ -producing cells. *J Allergy Clin Immunol* 2003;111:380-8.
12. Wang J, Barkel RA, Charboneau R, Loh HH, Roy S. Morphine negatively regulates interferon- γ promoter activity in activated murine T cells through two distinct cyclic AMP-dependent pathways. *J Biol Chem* 2003;278:37622-31.
13. Bryce PJ, Dascombe MJ, Hutchinson IV. Immunomodulatory effects of pharmacological elevation of cyclic AMP in T lymphocytes proceed via a protein kinase A independent mechanism. *Immunopharmacology* 1999; 41:139-46.
14. Chen C-H, Zhang D-H, LaPorte JM, Ray A. Cyclic AMP activates p38 mitogen-activated protein kinase in Th2 cells: phosphorylation of GATA-3 and stimulation of Th2 cytokine gene expression. *J Immunol* 2000;165:5597-605.
15. Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th1/Th2 polarization. *Immunol Today* 2000;21:479-83.
16. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat Immunol* 2004;5:1157-65.
17. Lanteleme E, Mantovani S, Palermo B, Campanelli R, Sallusto F, Giachino C. Kinetics of GATA-3 gene expression in early polarizing and committed human T cells. *Immunology* 2001;102: 123-30.
18. Zhu H, Yang J, Murphy TL, Ouyang W, Wagner F, Saparov A, et al. Unexpected characteristics of the IFN- γ reporters in nontrans-formed T cells. *J Immunol* 2001;167:855-65.
19. Luster AD, Ravetch JV. Genomic characterization of a gamma-interferon-inducible gene (IP-10) and identification of an interferon-inducible hypersensitive site. *Mol Cell Biol* 1987;7:3723-31.
20. Campbell JD, Gangur V, Simons ER, Hayglass KT. Allergic humans are hyporesponsive to a CXCR3 ligand-mediated Th1 immunity-promoting loop. *FASEB J* 2004;18:329-31.
21. McLaren KM, Holloway L, Pepper DS. Human platelet factor-4 and tissue mast cells. *Thromb Res* 1980;19:293-7.
22. Yamamoto H, Nagata M, Tabe K, Kimura I, Kiuchi H, Sakamoto Y, et al. The evidence of platelet activation in bronchial asthma. *J Allergy Clin Immunol* 1993;91:79-87.
23. Averill FJ, Hubbard WC, Proud D, Gleich GJ, Liu MC. Platelet activation in the lung after antigen challenge in a model of allergic asthma. *Am Rev Respir Dis* 1992;145:571-6.

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