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Identification and Partial Characterization of a Variant of Human CXCR3 Generated by Posttranscriptional Exon Skipping

Jan Erik Ehlerter,† Christina A. Addison,† Marie D. Burdick,‡ Steven L. Kunkel,§ and Robert M. Strieter2‡

Chemokines are recognized as functionally important in many pathological disorders, which has led to increased interest in mechanisms related to the regulation of chemokine receptor (CKR) expression. Known mechanisms for regulating CKR activity are changes in gene expression or posttranslational modifications. However, little is known about CKR with respect to a third regulatory mechanism, which is observed among other seven-transmembrane receptor subfamilies, the concept of differential splicing or processing of heteronuclear RNA. We now report on the discovery of a variant human CKR, CXCR3, resulting from alternative splicing via exon skipping. The observed RNA processing entails a drastically altered C-terminal protein sequence with a predicted four- or five-transmembrane domain structure, differing from all known functional CKR. However, our data indicate that this splice variant, which we termed CXCR3-alt, despite its severe structural changes still localizes to the cell surface and mediates functional activity of CXCL11. The Journal of Immunology, 2004, 173: 6234–6240.

Chemokines are small molecules of 7–10 kDa that form a large cytokine family composed of ~50 members in the human system. These polypeptides influence very different cellular activities such as chemotaxis, adhesion, angiogenesis, and proliferation. Approximately 16 different chemokine receptors (CKR), all belonging to the large family of seven-transmembrane domain (7TMD) receptors, have been identified as mediators of chemokine activities. The fact that chemokines are recognized as functionally important for progression of many pathological phenomena such as asthma, arthritis, multiple sclerosis, or cancer, has led to increased research interest in the modes of CKR regulation. Important, relatively well-explored regulatory mechanisms of CKR activity are changes in gene expression or posttranslational modifications such as phosphorylation. However, very little is known about CKR with respect to a third regulatory mechanism that has the potential to drastically alter a receptor’s structure and that is often observed among other 7TMD receptor subfamilies (1), i.e., differential splicing or processing of heteronuclear RNA. Evidence exists that such regulation might also be important in CKR biology. Differentially processed mRNA messages have indeed been observed for CXCR4 (CXCR4 and CXCR4-Lo) (2), CCR9 (A and B) (3), and CCR2 (A and B) (4). We now report on the discovery of a posttranscriptionally altered variant of human CXCR3, the designated receptor for non-ELR-CXC-chemokines IFN-inducible protein 10/CXCL10, monokine induced by IFN-γ/CXCL9 (5), and IFN-inducible T cell α chemoattractant/CXCL11 (6). In contrast to the most recently reported CXCR3 splice variant CXCR3-B (7), we find alternative splicing of the CXCR3 message via exon skipping that entails a drastically altered C-terminal protein sequence of CXCR3, resulting in a predicted four- or five-transmembrane receptor structure. Our data indicate that this splice variant, which we termed CXCR3-alt, despite its severe structural changes still localizes to the cell surface and mediates functional activity of CXCL11.

Materials and Methods

Reagents

Human CXCL11, CXCL9, and CXCL10 were obtained from PeproTech (Rocky Hill, NJ). A mAb (clone 1C6) directed against the 37 N-terminal amino acid residues of human CXCR3 (obtained from BD Pharmingen, San Diego, CA) was used for Western blotting (1 μg/ml) and FACS (5 μg/ml) analyses.

Cell culture

CXCR3-expressing primary cells were generated by stimulation of PBMC prepared from heparinized peripheral blood from healthy donors in a manner similar to that described by Cole et al. (6). Briefly, whole blood was mixed 1:1 with physiological saline and separated by differential centrifugation using Ficoll. The cells from the PBMC-representing band were diluted in ice-cold culture medium (RPMI 1640, 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 2 mM l-glutamine) and washed three times. A total of 2 × 10^6 cells/ml was stimulated 3 days with 5 μg/ml PHA (Sigma-Aldrich, St.Louis, MO) at 37°C and 5% CO2. Subsequently, cells were stimulated for 9 days with 50 U of IL-2 (R&D Systems, Minneapolis, MN), at which point cells mainly represented T cells, expressing CD3 by >99% as assessed by FACS analyses (data not shown).

Human embryonal kidney 293 (HEK) cells (kind gift of P. Graham, Hamilton, CA) were cultured in Eagle’s MEM medium (Cambrex, Cambridge, MD) containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 2 mM l-glutamine, and Fungizone (10 μg/ml; Cambrex). HEK stably transfected with pTarget-derived plasmids were selected in the presence of 400 μg/ml G418 (Sigma-Aldrich).

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PCR, primers, cloning, Northern blotting

PCRs were performed as described previously (8). Poly(A) RNA was purified from the different cell sources by oligo(dT) Dynabeads (Dynam, Oslo, Norway) according to the manufacturer’s manual. The cDNA was prepared by reverse transcription with oligo(dT) using the Superscript kit (Invitrogen Life Technologies, Carlsbad, CA). The PCR primers (5′ to 3′), with their positions on the CXCR3 cDNA sequence (accession no. X95876), are listed as follows (the respective orientation is indicated by S for sense and A for antisense): PRI1-S (positions 8–27), GCCGATTCAACCAACAAAAGCAGGGG: PRI1-A (positions 1204–1216), CCTCTAGATGGGGGGGAGCCCGC: PRI2-S (positions 92–111), CCAAGTCGTAATAGCAGGCG: PRI2-A (positions 1052–1071), GCAGACAGCACTCACACAC: PRI3-A (positions 838–851), CAAAAGGCCACCAACACACACCA: PRI4-A (positions 692–695 and 1032–1049), CTCCCGGGACCTTTGGCCTG.

Northern blotting and DNA sequencing were performed using standard techniques. As Northern probes, PCR products generated with primers PRI1-S + A were digoxigenin (DIG) labeled. Labeling and detection of these probes was performed using the PCR DIG probe synthesis kit (Roche, Indianapolis, IN) according to the manufacturer’s manual. For sequencing of CXCR3-related inserts within the plasmid pTarget, the following primers were used: sense, ptrget2, CGGCCAGAGATTATATAATACGACT- pri-1802, CGTACGATGCTGACGCGT: anti-2053, AGCTGTTGTCGCTTCTGT: antisense, ptrget1, TAACCAGGTCTTATTAGGGTACA. Sequencing reaction was performed using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Biosciences, Piscataway, NJ).

Cloning of CXCR3 variants coding sequences was performed from PCR-products using PRI1-S and PRI1-A as primers. PCR products were ligated into the T-overhang plasmid pTarget (Promega, Madison, WI) according to the manufacturer’s directions. pTarget provides the human CMV promoter to allow insert transcription and bears the neomycin resistance gene. Resulting plasmids pTar-CXCR3-s and pTar-CXCR3-alt were insert-specifically sequenced and subsequently used for transfection experiments of HEK cells. Transfections were performed by standard calcium phosphate precipitation using 20 µg of plasmid DNA. Transfection efficiencies were about 90–100%. Subsequently, cells were either analyzed 48 h later for CXCR3 expression by flow cytometry or cultured under G418 selection to allow insert transcription and bear the neomycin resistance gene. Results indicated that we were not seeing a PCR product from a 980-bp signal, which was expected from the published CXCR3 cDNA sequence (accession no. X95876), we observed another signal at ~650 bp. Both PCR products were observed at very stringent annealing temperatures (up to 67°C), using different open reading frame-enclosing primers (e.g., PRI1-S and PRI1-A, Fig. 1), and was absent when using genomic DNA as template (data not shown), indicating that we were not seeing a PCR artifact.

Cloning of the whole open reading frame of the novel CXCR3 message into the TA-overhang plasmid pTarget and subsequent sequencing revealed a variant in which the nucleotide sequence was identical with that of CXCR3 except for missing bases 696-1032. We called this variant CXCR3-alt.

Subsequently, constructed PCR antisense primers 3-A and 4-A allowed for isoform-specific detection of the two CXCR3 messages. Primer 3-A was designed complementary to the sequence missing in the CXCR3-alt message and should thus specifically detect cDNA coding for full-size CXCR3. In contrast, primer 4-A was designed to hybridize to the CXCR3-alt-specific sequence that arises from joining bases on positions 695 and 1032. As shown in Fig. 1, both primers 3-A and 4-A in combination with the common sense primer 2-S yielded only a single band of the expected size of 650 bp. Both PCR products were observed at very stringent annealing temperatures (up to 67°C), using different open reading frame-enclosing primers (e.g., PRI1-S and PRI1-A, Fig. 1), and was absent when using genomic DNA as template (data not shown), indicating that we were not seeing a PCR artifact.

Results

Identification of a novel splice variant of CXCR3

Analyzing the expression of CXCR3 by PHA/IL-2-stimulated PBMC by RT-PCR, we surprisingly observed two instead of one product using the primer set PRI2-S and PRI2-A (Fig. 1). Apart from a 980-bp signal, which was expected from the published CXCR3 cDNA sequence (accession no. X95876), we observed another signal at ~650 bp. Both PCR products were observed at very stringent annealing temperatures (up to 67°C), using different open reading frame-enclosing primers (e.g., PRI1-S and PRI1-A, Fig. 1), and was absent when using genomic DNA as template (data not shown), indicating that we were not seeing a PCR artifact.

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Analyses of cells transfected with CXCR3-alt

Posttranscriptional excision of bases 696-1032 within the CXCR3 message entails severe changes for the receptor’s predicted protein structure. Because such splicing not only removes a coding mRNA fragment but also causes a frame shift, CXCR3-alt on a protein level is identical with full-size CXCR3 only up to the amino acid...
glutamine (Q) on position 209 within the second extracellular loop (Fig. 2). The following stretch of 58 amino acid residues in CXCR3-alt has no sequence homology to the remaining 158 residues in the original CXCR3 (Fig. 2), and no or one TMD (TMD prediction programs SPLIT and TMpred, respectively; see Materials and Methods). These analyses indicate that, with CXCR3-alt, we have discovered an isoform of CXCR3 bearing a structure that has not been associated with a functional CKR so far. Therefore, we were interested in the functional and biochemical properties of CXCR3-alt compared with full-size CXCR3. Hence, we stably transfected human embryonic kidney cells with pTar-CXCR3-alt (HEK-alt), or with pTar-CXCR3-full-size (HEK-fs).

**Analysis of mRNA expression by Northern blotting and RT-PCR**

Northern blot analyses of RNA from HEK-alt cells showed only a single band that was migrating faster than the major band observed with RNA from HEK-fs cells (Fig. 3A). Additionally, analyses of the latter RNA revealed a fainter band comigrating with the single band seen with HEK-alt RNA, which might represent the alternatively spliced message (compare arrow-marked band in lane 3 in Fig. 3A). Interestingly, such an additional fainter, faster-migrating band could also be observed in analyses of RNA obtained from stimulated PBMC (Fig. 3A, lane 8). These observations indicate that cells expressing full-size CXCR3, i.e., HEK-fs and stimulated PBMC, also bear the splice variant’s message, although at a lower
level. We also noticed that the CXCR3-specific signal intensity obtained from HEK-alt mRNA was generally ~10-fold lower than that obtained from HEK-fs (compare Fig. 3A). This held true for different HEK-fs and HEK-alt clones (data not shown). Because the probe was generated to bind RNA from both isoforms, this observation suggested that, under similar conditions, lower message levels were reached with the splice variant compared with the full-size variant, possibly due to reduced mRNA stability. Moreover, conventional RT-PCR of the CXCR3-alt using specific primer sets are shown in Fig. 3B. The full-size-specific primer combination PRI 1-S/3-A reacts only with the HEK fs-cDNA, whereas the splice-specific primer combination PRI 1-S/4-A generates a signal from the cDNA of both, HEK-fs as well as HEK-alt. This supports the view that the splice product is always present in cells expressing the full-size product.

FIGURE 2. Comparison of amino acid sequences of splice variant CXCR3-alt and CXCR3-full-size (fs). Dashes indicate sequence identical with CXCR3-fs. Bold letters show the novel sequence in CXCR3-alt. Gray boxes surround predicted transmembrane regions according to the program TMpred.

FIGURE 3. Biochemical analyses of HEK cells transfected with pTar-CXCR3-alt (HEK-alt), pTar-CXCR3-fs (HEK-fs), and the pTarget-plasmid alone (HEK-c). A, Analyses of stably transfected HEK cells on mRNA level. Shown is a Northern blot probed with a DIG-labeled PCR product complementary to both CXCR3 variants. Indicated amounts of RNA were applied. For comparison, RNA from unstimulated or PHA/IL-2-stimulated PBMC (refer to Materials and Methods) was analyzed. Arrows point out potential splice-variant RNA in Northern blot of cells expressing full-size-CXCR3. B, Conventional RT-PCR of the CXCR3-alt using specific primer sets. The full-size-specific primer combination PRI 1-S/3-A only reacts with the HEK fs-cDNA, whereas the splice-specific primer combination PRI 1-S/4-A generates a signal from the cDNA of both, HEK-fs as well as HEK-alt. C, Analyses of stably transfected HEK cells on protein level. Lysates of transfectants and, for comparison, of PHA/IL-2-stimulated PBMC, were separated by 10% SDS-PAGE and blotted. Blots were developed using anti-human CXCR3 mAb 1C6. D, Analyses of surface-expressed CXCR3 isoforms on transfected HEK cells. Cells were transfected with pTarget (pTar), pTar-CXCR3-alt, and pTar-CXCR3-fs, and after 48 h, were harvested using citric saline. Subsequently, surface-expressed receptor was detected with mAb 1C6. Via biotinylated secondary antiserum and RPE-labeled streptavidin, CXCR3-expressing cells are labeled with RPE. One of two experiments with similar outcome is shown.
Analysis of protein expression by Western blotting

We were interested in whether the dramatically altered CXCR3-alt isoform was expressed and stable on the protein level. For that purpose, we analyzed lysates of HEK-alt and HEK-fs cells by SDS-PAGE and subsequent Western blotting for the presence of the respective isoform. Using an Ab directed against the N terminus of CXCR3, we found CXCR3-alt protein indeed being expressed (Fig. 3C, lane 3). However, CXCR3-alt was detected at much lower levels than full-size CXCR3 (Fig. 3C, compare lanes 1 and 3), although the Ab’s epitope should not be affected by the alternative splicing. These data indicate that CXCR3-alt protein is in fact being expressed albeit in lower amounts than full-size CXCR3. It appears noteworthy that both molecules differed strikingly in their electrophoretic migration behavior. Blot analyses of HEK-alt lysates revealed a well-focusing band at ~33 kDa, corresponding quite well with the predicted size of 28,715 Da for the 267 residues containing CXCR3-alt, taking into account potential N-glycosylation on the extracellular parts of the receptor. In contrast, the 368 residues containing full-size CXCR3 with a predicted molecular mass of 40,659 Da did not give a well-focusing band. Instead, it appeared as a broad signal between 33 and 75 kDa when SDS-PAGE samples were mildly reduced at 37°C. Boiling, in contrast, led to shift of the CXCR3-fs but not the CXCR3-alt signal to an apparent molecular mass of ~250 kDa. These observations indicate that alternative splicing entails further biochemical differences besides reduction in size.

Analysis of surface expression of CXCR3 isoforms by immunofluorescence

The drastic structural changes observed with the CXCR3 splice variant could result in defects in intracellular trafficking and surface expression. To elucidate this question, we performed CXCR3-specific FACS analysis of stably transfected HEK cell lines. Although full-size CXCR3-expressing HEK-fs cells displayed 7-fold increased median fluorescence compared with the mock-transfected HEK cells, HEK-alt cells expressing the splice variant showed only slightly increased surface immunoreactivity that was not significant (data not shown). This observation indicated that more sensitive detection could be required for CXCR3-alt detection. Therefore, we analyzed HEK cells after transient transfection with CXCR3 isoform constructs, which would result in high uptake of plasmid DNA and entail enhanced expression of the respective CXCR3 isoform. In fact, cells transiently transfected with CXCR3 full-size encoding plasmid pTar-CXCR3-fs showed 19-fold higher CXCR3-specific median fluorescence (e.g., 365 arbitrary units (a.u.)) than the background signal of mock-transfected cells (e.g., 19 a.u.) (compare Fig. 3D). Most interestingly, cells transfected with the splice variant encoding construct pTar-CXCR3-alt also showed clearly detectable CXCR3-alt expression with a median fluorescence (e.g., 36 a.u.) 2-fold above background (compare Fig. 3D). These data show that CXCR3-alt does indeed localize to the cell surface and might thus be functional as ligand receptor.

Analysis of chemotactic responsiveness of CXCR3-alt-expressing cells toward CXCL11, CXCL10, and CXCL9

To assess whether the CXCR3 splice variant was functional as receptor, we compared HEK cells stably transfected with the CXCR3 isoforms with respect to their capacity to chemotactically migrate in response to CXCR3 ligands IFN-inducible T cell α chemoattractant/CXCL11, IFN-inducible protein 10/CXCL10, and monokine induced by IFN-γ/CXCL9. As shown in Fig. 4, we found that HEK-fs cells expressing the full-size receptor showed a pronounced chemotactic response toward CXCL11 already at a concentration of 1 mM (CI, ~6), whereas higher CXCL11 concentrations hardly increased the CI. In contrast, whereas CXCL10 and CXCL9 caused chemotactic activation of the HEK-fs cells, the effect was less than CXCL11 at 100 mM (Fig. 4A). Mock-transfected cells HEK-c, on the contrary, did not respond to the CXCR3 ligands. The observation that, on transfected HEK cells, CXCL11 was functionally more potent than CXCL10 or CXCL9 corresponds to data obtained for CXCR3 expressed on other cell types (6, 12). Most interestingly, we found that HEK-alt cells expressing the CXCR3 splice variant also showed chemotactic responses toward CXCL11. However, the CIs of HEK-alt cells were considerably lower. The strongest and most significant response (CI, 3.3) was observed at the highest CXCL11 concentration tested (100 mM) (Fig. 4A). We did not observe induction of chemotactic activity in HEK-alt cells by either CXCL10 or CXCL9, which could be due to lack of sensitivity of the assay. To further assess the proximal G-protein coupling of CXCR3-alt, we next assessed the effect of Ptx on the chemotactic response of HEK-fs and HEK-alt cells to CXCR3 ligands (Fig. 4B). We found that Ptx inhibited the chemotactic response of HEK-fs to all three CXCR3 ligands (Fig. 4B). Furthermore, we determined that Ptx inhibited the chemotactic response of CXCL11 on HEK-alt cells.

Discussion

We report on the discovery, identification, and partial characterization of an alternatively spliced isoform of CXCR3, which we termed CXCR3-alt. Differentially processed mRNA messages have previously been observed for CKR such as CXCR4 (2), CCR9 (3), and CCR2 (4). CXCR4 polymorphism has been shown to be due to intron retention, whereas alternative splicing is responsible for the different CKR transcripts. Recently, first data have been published, indicating that differential mRNA processing is an important issue for CXCR3 biology as well. Lasagni et al. (7) identified a novel exon region within the CXCR3 gene, which is included into the CXCR3 mRNA in certain cell types only. Such alternative splicing produces the CXCR3 isoform CXCR3-B, which not only binds CXCL9, CXCL10, and CXCL11, but also PF-4/CXCL4. CXCR3-B is structurally very similar to the original CXCR3, differing only in the presence of an extended N terminus.

We now present a completely different CXCR3 splice product, CXCR3-alt. Our data show that the coding sequence for this splice variant is the result of exon skipping in the context of alternative splicing, where within the original CXCR3 open reading frame (composed of at least three exons: I = . . .-bp 695; II = bp 696-1032; III = bp 1033- . . .), exon II is left out (Fig. 1). This observation implies that, whenever the message for the original full-size CXCR3 is recombinantly constructed, introduced into cells, and then transcribed, the CXCR3-alt message is also generated. In fact, in HEK cells recombinantly expressing full-size CXCR3, by PCR, we always observe a message for CXCR3-alt (data not shown). Hence, functional effects observed with cells recombinantly expressing full-size CXCR3 may reflect a mixture of effects caused by the different splice variants CXCR3-fs and CXCR3-alt.

Alternative splicing is associated with drastic structural alterations of the receptor protein. Due to a frameshift, the splicing process leads to a change in sequence after amino acid Q209 within the second extracellular loop of the full-size CXCR3 protein, from which, instead of 159 aa, only 58 aa follow (Fig. 2). Due to this changed sequence, CXCR3-alt is predicted to have only four or five TMDs, depending on the TMD prediction program used (see Data bank analyses in Materials and Methods). This structural impact is more severe than those observed with so-far-known CKR
and splice variants that showed rather moderate N- or C-terminal modulations (2–4, 7). The CKR splice variant that, with respect to structure, most closely resembles CXCR3-alt is the genomic mutant of CCR5, CCR5Δ32 (13). CCR5Δ32 lacks the final three TMDs of the original 7TMD receptor and is considered either not to be translated into protein (13), or, if so, not to be transported to the cell surface (14), resulting in lack of biological activity. Using sensitive detection methods, we could show that these properties are not true for CXCR3-alt. Although several lines of evidence show that CXCR3-alt is expressed at considerably (~15-fold) lower levels than the full-size receptor, CXCR3-alt is clearly expressed as protein and appears to traffic as functional receptor to the cell surface (Fig. 3). Possibly the same is the case for the CCR5 mutant CCR5Δ32, and more sensitive methods might be required for its detection.

Facing the low mRNA and protein expression levels of CXCR3-alt in comparison to full-size CXCR3 (Fig. 3), one may doubt a functional relevance of the presence of the CXCR3-alt splice variant. However, it has to be kept in mind that the CXCR3-B isoform shows low expression levels as well (7), indicating that reduced expression may be a general phenomenon of CXCR3 splice variants and also low amounts of such variants can well be of functional importance. In fact, we could show that, despite its structural changes, CXCR3-alt surprisingly still possesses, albeit reduced, functional activity mediating chemotactic effects induced by CXCL11 (Fig. 4). In addition, the chemotactic activity of CXCL11 through CXCR3-alt is Ptx sensitive, supporting the notion that proximal G-protein coupling is intact and Gi dependent. Apparently, CXCL11 can still interact with the CXCR3 variant lacking the third and most of the second extracellular loop. This finding is corroborated by literature data indicating that especially the N terminus and first extracellular loop of CXCR3 contribute to the interaction of CXCL11 with CXCR3 (12). A major reason for reduced chemotactic activity of CXCL11 may be the reduced surface expression of CXCR3-alt protein compared with that of the full-size receptor (Fig. 3). Due to the lack of receptor binding experiments, which were not within the scope of this study, we cannot estimate how far reduced CXCL11 activity is also due to reduced ligand affinity. The questions of affinity and quality of interaction will have to be addressed in an independent study. Such a study may also clarify the way in which glycosaminoglycans are involved in the binding of IFN-inducible non-ELR chemokines to CXCR3. Interestingly, full-size CXCR3 as the only CKR contains a potential recognition site for the attachment of glycosaminoglycans (S304-G307) (15), which has been conserved in slightly modified form in the CXCR3-alt protein (S213-G215). It remains...

**FIGURE 4.** A. Functional analyses of HEK cells stably transfected with pTar-CXCR3-alt (HEK-alt), pTar-CXCR3-fs (HEK-fs), and the pTarget-plasmid alone (HEK-c). Cells were analyzed in a modified Boyden chamber for their chemotactic response toward indicated concentrations of chemokines CXCL11, CXCL9, and CXCL10. B, The effect of Ptx on the chemotactic response of HEK-fs and HEK-alt cells to CXCR3 ligands. The cellular responses are shown as CIs indicating the ratio of migrated cells in the presence of a stimulus compared with migrated cells in the absence of a stimulus. Given are the mean values of six independent experiments ± SD. Statistical significances with an error of p < 0.05, 0.01, and 0.001, are indicated with *, **, and ***, respectively.
to be shown, however, whether these sites have any functional relevance.

The physiological function of CXCR3-alt, however, remains unclear. Possibly alternative splicing of CXCR3 is just a way to regulate the number of the full-size and fully active receptor on the transcriptional level. In contrast, CXCR3-alt might regulate the number of the full-size and fully active receptor on the cotranslational level, e.g., by heterodimerization, a mechanism by which CCR5-32 was postulated to modulate the full-size CCR5’s function (14). Under different PBMC stimulation conditions using PHA, IL-2, IL-4, IL-12, or IFN-γ, we could, however, not see a regulation in the ratio of the CXCR3-alt to the CXCR3-fs message. Whenever we observe a CXCR3-fs message, we also detect the splice variant’s message (data not shown). Perhaps the CXCR3-alt message is an always-occurring message, we also detect the splice variant’s message (data not shown). The physiological function of CXCR3-alt, however, remains unexplored. It has been shown thatCXCR3Alt functions in inflammation (5), diseases of lung (16), kidney (17), and CNS (18), and angiostasis and cell proliferation (19, 20), will have to scrutinize which functional relevance this novel CXCR3 isoform indeed has.

References