Cutting Edge: CXCR4-Lo: Molecular Cloning and Functional Expression of a Novel Human CXCR4 Splice Variant

Shalley K. Gupta and Kodandaram Pillarisetti

*J Immunol* 1999; 163:2368-2372; 
http://www.jimmunol.org/content/163/5/2368

References

This article cites 27 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/163/5/2368.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Human CXCR4 is a specific receptor for the CXC chemokine stromal cell-derived factor-1 (SDF-1) and a coreceptor for T cell line tropic strains of HIV-1. Genetic knockouts of CXCR4 and SDF-1 have delineated their critical role during embryonic cardiogenesis, leukopoiesis, and vasculogenesis. Herein, we used bioinformatics and differential strategies like isoform-specific RT-PCR and Northern blots to identify and clone a novel unspliced isoform of human CXCR4, termed CXCR4-Lo. CXCR4-Lo corresponds to a larger ~4.0-kb mRNA transcript and differs from the known human CXCR4 by the first 9 aa in the functionally important NH2-terminal extracellular domain of the receptor. CXCR4-Lo-transfected rat basophil leukemia-2IH3 cells responded to SDF-1 with a transient rise of intracellular Ca2+ concentration and by undergoing chemotaxis. Expression of CXCR4-Lo is noteworthy, as it may have differential affinity as a coreceptor for HIV strains in comparison with CXCR4. Furthermore, CXCR4-Lo may also provide a functional backup to CXCR4 during embryogenesis. The Journal of Immunology, 1999, 163: 2368–2372.

Shalley K. Gupta1 and Kodandaram Pillarisetti

Cutting Edge: CXCR4-Lo: Molecular Cloning and Functional Expression of a Novel Human CXCR4 Splice Variant

Department of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Received for publication March 17, 1999. Accepted for publication July 6, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Address correspondence and reprint requests to Dr. Shalley K. Gupta, Mail Code UW2511, Department of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406. E-mail address: Shalley_kgupta@sbphrd.com

2 Abbreviations used in this paper: SDF-1, stromal cell-derived factor-1; EC, extracellular; EST, expressed sequence tag; FLIPR, fluorometric imaging plate reader; ORF, open reading frame; RBL, rat basophil leukemia cells; [Ca2+]i, intracellular Ca2+ concentration.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
Transfection of rat basophil leukemia (RBL)-2H3 cells

Full-length CXCR4 and CXCR4-Lo cDNAs were subcloned into pCR3.1 (Invitrogen, San Diego, CA) eukaryotic expression vectors, and their orientation was confirmed by sequencing. The resultant expression constructs were transfected into RBL cells (American Type Culture Collection, Manassas, VA) by electroporation, and transfected cells were selected by using RPMI 1640 media containing 800 μg/ml G-418 (Life Technologies, Rockville, MD). CXCR4 and CXCR4-Lo specific mRNA expression was confirmed by Northern blot analysis. FACS analysis to compare surface expression of CXCR4 and CXCR4-Lo in transfected RBL cell lines was done with the CXCR4-specific 12G5 mAb as described before for endothelial cells (7).

Functional characterization of CXCR4-Lo in stably transfected RBL cells

Mobilization of [Ca\textsuperscript{2+}] in transfected RBL cells was measured by fluorometric imaging plate reader (FLIPR; Molecular Devices) analysis (23). Briefly, 6 × 10\textsuperscript{4} cells were added per well of a 96-well plate and grown for 24 h. Cells were loaded with Fluo-3 AM dissolved in dye-loading buffer (Eagle’s minimum essential medium with 0.1% BSA and 15 mM sulfinpyrazone) for 60 min at 37°C. Following this, the dye-loading buffer was removed, and 100 μl hydrolysis buffer (15 mM sulfinpyrazone in Eagle’s minimum essential medium) was added to each well and incubated for 10 min at 37°C. Relative changes in fluorescence counts as a response to [Ca\textsuperscript{2+}] flux after addition of SDF-1α (R&D Systems, Minneapolis, MN) are expressed as FLIPR units.

SDF-1α-induced migration of transfected RBL cells was done as described for HUVECs (7). Briefly, 5 × 10\textsuperscript{5} cells in serum-free RPMI 1640 medium (with 0.25% BSA) were added in the top chamber of a 6.5-mm diameter, 5-μm pore poly carbonate transwell culture insert (Costar, Cambridge, MA). Incubation was conducted at 37°C in a 5% CO\textsubscript{2} incubator for 18 h. Migrated cells in
the lower chamber were counted with a ZM Coulter counter (Coulter Diagnostics, Hialeah, FL). Percent migration was calculated based on the total initial input of cells per well.

Results and Discussion

Molecular cloning and analysis of the novel un-spliced CXCR4-Lo variant cDNA

We previously demonstrated the expression of two distinct mRNAs, a 1.7-kb and a 4.0-kb transcripts for CXCR4 in HUVECs, and had speculated on the putative existence of an alternatively spliced isoform (7). Subsequently, we used bioinformatics to identify homologous ESTs with sequence variation in the NH2-terminal region of the EC domain of CXCR4. Two variant cDNA clones, designated as CXCR4-Lo, were isolated from a human neutrophil cDNA library and sequenced. Unlike CXCR4, the 1071-bp ORF of CXCR4-Lo cDNA clone is encoded by a single exon. The coding region is initiated from an alternate in-frame ATG start codon found within the intron sequence characterized in the recently published genomic structure for CXCR4 (20, 21) and 25-bp upstream of the known AG acceptor site used for intron splicing (Fig. 1, A and B). Significantly, the predicted full-length 357-aa CXCR4-Lo receptor (not shown, GenBank accession no. AF147204) contains a longer neo-NH2-terminal EC domain that is different from the corresponding CXCR4 sequence by the first 9 aa residues M-S-I-P-L-P-L-L-Q (Fig. 1, A and B), while the remaining sequence is identical. The 3′ untranslated region of CXCR4-Lo (not shown) has an AATAAA polyadenylation consensus signal and is identical with the published genomic sequence of human CXCR4 (20).

Although alternative splicing was recently demonstrated for murine CXCR4 (18, 19, 22), the human CXCR4-Lo we have discovered is structurally distinct and generated by a unique mechanism. In the case of murine CXCR4b (18, 19, 22), a different GT splice donor site is used to yield an isoform that is shorter by 2 aa, while the overall functional activity, genomic structure, and intron splicing mechanism are conserved (18, 22). In contrast, in human CXCR4-Lo, the known intron remains unspliced and the entire gene is expressed as a single exon to yield the longer 4.0-kb transcript (Fig. 1B). Other cases of retention and read through of “coding introns” have also been observed, most recently with the generation of a new CTL Ag from a tyrosinase-related protein mRNA (24).

To further test this hypothesis, and also address the question whether the two human CXCR4 isoforms are products of a single gene, RT-PCR primers (Lo-346 and Lo-1071) were designed to selectively amplify from the unique 5′-end of CXCR4-Lo transcripts from HL-60 cells. Hybridization of amplified cDNA with the CXCR4-Lo cDNA-specific 346-bp DNA probe revealed the expected specificity of the 1071-bp PCR product (Fig. 1C). In

FIGURE 2. Tissue distribution of human CXCR4 and CXCR4-Lo by Northern blots using the common 515-bp cDNA probe. The 1.7-kb and 4.0-kb bands specific for CXCR4 and CXCR4-Lo mRNAs are indicated by arrows. A, Take note of the relatively high expression of both CXCR4 and CXCR4-Lo isoforms in PBL and various leukemia cell lines with the striking exception of K-562, which is devoid of CXCR4 expression. B, Northern blot analysis of CXCR4-Lo and CXCR4 expression in regions of the human brain.
addition, Northern analysis with the 346-bp probe also confirmed expression of the ~4.0-kb CXCR4-Lo mRNA in PBLs and other tissues (Fig. 1D).

The qualitative comparison of CXCR4 and CXCR4-Lo mRNA expression in tissues was done using the common 515-bp cDNA probe (7). As noted previously (4, 5), CXCR4 is highly expressed in most human tissues upon Northern blot analysis (Fig. 2). While overall CXCR4-Lo expression is either proportionally lower or absent in all tissues (Fig. 2A), there is relatively more expression of its mRNA in spleen, lung, PBLs, and cancer cell lines like HL-60 and MOLT-4. Such selective differences in tissue-specific expression of the unspliced CXCR4-Lo isoform implies that its expression is regulated, rather than being caused by a random or passive absence of normal splicing. However, additional studies including the role of the “coding intron” in modulating transcript-

tion are desirable to assess the functional significance of CXCR4-Lo expression. Furthermore, although CXCR4 expression is low in whole brain (Fig. 2, A and B), especially high mRNA expression was observed in spinal cord, medulla, and frontal lobe, with moderate to low expression in putamen, temporal lobe, cerebellum, and cerebellar cortex. Significant, though lower, concomitant expression of the ~4.0-kb CXCR4-Lo mRNA was also observed in the spinal cord, medulla, substantia nigra, and subthalamic nucleus (Fig. 2B). The functional consequence of such an expression pattern of both CXCR4 and CXCR4-Lo in brain is not known, although it was recently shown that fetal cerebellar development is impaired in the CXCR4-knockout mice (10).

### Heterologous functional expression of CXCR4-Lo and comparison with CXCR4

Stably transfected RBL cell lines were generated from full-length ORFs of both CXCR4 and CXCR4-Lo, and their functional response to SDF-1α-mediated [Ca^{2+}]_{i} flux and chemotaxis was compared. Expression of CXCR4-Lo-specific mRNA in transfected RBL cell lines was confirmed by isoform-specific RT-PCR (with Lo-346 primers) and Northern blot analysis of total RNA (data not shown). Furthermore, as shown in Fig. 3A, FACS analysis revealed comparable levels of chemokine receptor surface expression on both CXCR4- and CXCR4-Lo-transfected RBL cell lines. Upon treatment with SDF-1α, there was a rapid [Ca^{2+}]_{i} flux in CXCR4-Lo-transfected RBL cells in a concentration-dependent manner (Fig. 3B). Similar results were obtained with SDF-1β also (data not shown). Altogether, CXCR4-Lo was found to be less potent and efficacious (EC_{50} of ~20 nM) in its response to SDF-1, in comparison with CXCR4-transfected RBL cells (EC_{50} of ~6 nM) in the FLIPR [Ca^{2+}]_{i} assay (Fig. 3B). Similarly, the chemotactic response of CXCR4-Lo-transfected RBL cells, although significant, was also comparatively attenuated in the transwell migration assay (Fig. 3C).

Our results clearly demonstrate that the human CXCR4 gene is expressed in two alternate functional forms: the highly expressed known CXCR4, which is the primary receptor for SDF-1; and a longer, low abundance but functional CXCR4-Lo unspliced variant. In contrast to other chemokines (25), both CXCR4 and SDF-1 genes are remarkably conserved with >90% identity across diverse species (19). Such identity suggests a fundamental role for them during development, and it is noteworthy that the exceptional lethal phenotype caused by their genetic knockouts (8–10) is not similarly observed for other chemokines and chemokine receptors (26–28). Therefore, it is not surprising that in the unlikely event of failure in the splicing mechanism during embryogenesis, the critical requirement to conserve SDF-1 functions may be fulfilled by a backup functional receptor in the form of an unspliced CXCR4-Lo. Our data with CXCR4-Lo also shows that the NH2-terminal EC domain of CXCR4 is critical to elicit an efficacious biological response to SDF-1. This is in accordance with a multisite model for chemokine-chemokine receptor interaction (15) in which one or more subsites determine chemokine activation. Furthermore, CXCR4-Lo may also have significant independent role as an additional coreceptor for strains of HIV-1. If this is indeed true, further exploration on involvement of the CXCR4-Lo receptor in HIV pathogenesis becomes imperative.

### Acknowledgments

We thank Mary Brawner and James Fornwald for help with transfection in RBL cells and James Foley for FLIPR analysis. We also thank John White and Paul Lysko for critical discussion throughout this work.
References


16. Pease, J. E., J. Wang, P. D. Ponath, and P. M. Murphy. 1998. The N-terminal extracellular segments of the chemokine receptors CCR1 and CCR3 are determinants for MIP-1α and ectein binding, respectively, but a second domain is essential for efficient receptor activation. *J. Biol. Chem.* 273:19972.


