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Sequence variation of a novel heptahelical leucocyte receptor through alternative transcript formation

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Chemoattractants, including chemokines such as interleukin 8 (IL-8) and related proteins, activate leucocytes via seven-transmembrane-domain G-protein-coupled receptors. A cDNA for a novel receptor of this kind consisting of 327 amino acids was isolated from a human blood monocyte cDNA library. The polypeptide, termed monocyte-derived receptor 15 (MDR15), is an alternative form of the Burkitt's lymphoma receptor 1 (BLR1) encoded by a human Burkitt's lymphoma cDNA [Dobner, Wolf, Emrich and Lipp (1992) Eur. J. Immunol. **22**, 2795–2799]. *MDR15* and *BLR1* cDNAs differ in the 5′ region, where the open reading frame of *MDR15* is shorter by 45 codons. Southern-blot analysis indicates that the two transcripts for *MDR15* and *BLR1* are encoded by the same gene. Northern-blot analysis using a probe that hybridizes with both mRNAs demonstrated high-level expression in chronic B-lymphoid leukaemia and non-Hodgkin's lymphoma cells and, to a lesser extent, peripheral blood monocytes and lymphocytes. Reverse transcription-PCR studies with *MDR15*- and *BLR1*-specific primers showed similar levels of transcripts for both receptors in RNA that was positive in Northern-blot analysis. MDR15 and BLR1 have high structural similarity to receptors for human IL-8 (about 40 % amino acid identity) and other chemokines. However, none of a series of radiolabelled chemokines (IL-8, NAP-2, GROα, PF4, IP10, MCP-1, MCP-2, MCP-3, I-309, RANTES and MIP-1α) and other ligands (C3a and leukotriene B_4) bound to Jurkat transfectants that stably expressed either *MDR15* or *BLR1* mRNA. The fact that *MDR15* and *BLR1* are expressed on leucocytes and show marked sequence similarity to chemokine receptors suggests the existence of as yet unidentified chemokines. Alternative transcript formation affecting the 5′-terminal part of the coding region may be a way to modify ligand-binding selectivity.

INTRODUCTION

Of the numerous factors that attract leucocytes, chemotactic cytokines related to interleukin 8 (IL-8) have gained most attention. Such cytokines, which are now generally termed chemokines, are small (68–79 amino acids) proteins with four conserved cysteine residues [1]. Depending on the position of the first two cysteines, two subfamilies are distinguished: CXC chemokines with the cysteines separated by the one amino acid, and CC chemokines with the cysteines in adjacent positions. IL-8 and related CXC chemokines act mainly on neutrophil leucocytes. CC chemokines, in contrast, are inactive on neutrophils and attract and activate monocytes, eosinophils, basophils and lymphocytes. All CC chemokines described so far [monocyte chemotactic protein (MCP)-1, MCP-2, MCP-3, regulated on activation, normal T-cell expressed and secreted protein (RANTES), macrophage inflammatory protein (MIP)-1α, MIP-1β and I-309] are chemotactic for monocytes, and some have been shown to induce transient changes in cytosolic free Ca^{2+}, the up-regulation of β2-integrins and the respiratory burst [1].

CXC chemokine receptors are well characterized. Two types of IL-8 receptors, IL-8R1 and IL-8R2, are abundantly expressed in neutrophils, and their sequences have been established by cDNA cloning [2,3]. Binding studies have shown that IL-8R1 is selective for IL-8, the only CXC chemokine that is bound with high affinity, whereas IL-8R2 has high affinity for all neutrophil-activating CXC chemokines [4-9]. Using Jurkat cells stably transfected with cDNAs for IL-8R1 or IL-8R2 we recently showed that the two receptors function independently and mediate chemotaxis in response to CXC chemokines [10]. Information about CC chemokine receptors, in contrast, is still fragmentary [1]. Monocytes and THP-1 cells were reported to express several thousands of receptors for MCP-1 (K_d 0.5–2.0 nM) [1–13], and RANTES and MIP-1α also bind to these cells with similar affinity [13,14]. A human leucocyte cDNA was recently isolated and shown to confer high-affinity binding for ^{125}I-MIP-1α when transfected into *Xenopus* oocytes [15] or human embryonic kidney cells [16]. This MIP-1α receptor (MIP-1αR) binds MCP-1, MIP-1β and RANTES with somewhat lower affinity, but is inactive with IL-8 [16]. Also, two isoforms of an MCP-1 receptor (type A and B) with alternatively spliced C-termini were reported that are specific for MCP-1 but not other CC or CXC chemokines [17]. As evidenced by cross-desensitization studies, mononuclear phagocytes express binding sites with different selectivity for CC chemokines [18]. Two chemokine receptors, US28N [16,19] and ECRF3 [20], are encoded by viral genes. Like MIP-1αR, US28N recognizes MCP-1, MIP-1α, MIP-1β and RANTES but not IL-8 [16], whereas ECRF3 is selective for CXC chemokines [20]. All known chemokine receptors belong to the superfamily of seven-transmembrane-domain (7-TM) receptors coupled to G-proteins. Sequence identity of the cloned CC chemokine receptors with IL-8R1 and IL-8R2 is 28–31 %.

In this paper, we described a cDNA, *MDR15*, that was isolated from a human monocyte cDNA library by hybridization with an IL-8 receptor probe. The putative polypeptide, monocyte-derived receptor 15 (MDR15), strongly resembles chemokine

Abbreviations used: IL-8, interleukin 8; IL-8R1 and 2, IL-8 receptor types 1 and 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PHA, phytohaemagglutinin; K_d, binding-affinity constant; RT-PCR, reverse transcription PCR; MDR15, monocyte-derived receptor 15; BLR1, Burkitt's lymphoma receptor 1; 7-TM, seven-transmembrane-domain; B-CLL, chronic B-lymphoid leukaemia; B-ALL, acute B-lymphoid leukaemia; MCP, monocyte chemotactic protein; RANTES, regulated on activation, normal T-cell expressed and secreted protein; MIP, macrophage inflammatory protein; GRO, growth-relating protein.

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receptors and is an isoform of the recently described Burkitt's lymphoma receptor, BLR1 [21]. Northern-blot and PCR studies revealed the expression of transcripts for both receptors in normal and malignant blood leucocytes.

EXPERIMENTAL

Isolation of *MDR15 cDNA*

With total RNA isolated from human monocytes [22], a cDNA library was prepared using the expression vector pcDNA-I (Invitrogen Corp.) and non-self-complementary *Bst*XI adaptors [23,24]. Some 4×10^5 colonies were screened by the colony-lift hybridization method [25], using an 819 bp DNA fragment, *F3R1* [4], corresponding to the rabbit IL-8 receptor [26]. Of three strongly positive clones, two corresponded to IL-8R1 and IL-8R2 and the third cDNA, *MDR15*, was subcloned and sequenced to completion [27].

Synthesis of *BLR1 cDNA*

A cDNA fragment of 556 bp encoding the 5′ region of *BLR1* [21] and including the internal *Bst*XI site at position 675 in *MDR15* was generated by PCR using the cDNA primers SE1-BLR and AS1-MDR (Table 1). Template cDNA was prepared from total RNA from the human lymphoma cell line Daudi, and PCR was carried out for 35 cycles as described below. The amplified *Hin*dIII–*Bst*XI fragment was replaced with the *Bst*XI-proximal segment in *MDR15*, thereby generating the entire *BLR1* coding region, and the construct was sequenced [27].

Northern-blot analysis

Total RNA from normal and malignant human blood leucocytes and lymph node biopsy specimen from patients with non-Hodgkin's lymphoma was extracted by the guanidinium isothiocyanate/LiCl method [22]. RNA samples (10 μg) were electrophoresed on 1% agarose/formaldehyde gels [25], transferred to Nytran membranes (Schleicher and Schuell), immobilized and hybridized at 42 °C for 24 h (50% formamide, 2 × SSC, 5 × Denhardt's, 1% SDS, 10% dextran sulphate, 1 mM EDTA and 200 μg/ml sonicated calf thymus DNA where 1%SSC is 0.15 M NaCl plus 0.015 M sodium citrate and 1 × Denhardt's is 0.02% Ficoll 400/0.02% polyvinylpyrrolidone/0.02% BSA) with the ^{32}P-labelled *Bst*XI–*Xba*I fragment of *MDR15*. After being washed (2 × SSC at room temperature, twice in 0.25 × SSC/0.1% SDS at 55 °C for 10 min) the membranes were exposed to Hyperfilm MP (Amersham International).

Southern-blot analysis

Human genomic DNA was digested with *Bgl*I, *Eco*RI, *Hin*dIII or *Xho*I, electrophoresed in a 0.7% agarose gel, blotted on to a Nytran membrane and hybridized with the ^{32}P-labelled *Bst*XI–*Xba*I fragment of *MDR15* [25]. The membrane was washed under low-stringency [0.25 × SSPE/0.1 × SDS at 50 °C for 30 min where 1 × SSPE is 0.18 M NaCl/10 mM sodium phosphate (pH 7.7)/1 mM EDTA] and high stringency-(0.1 × SSPE/0.1% SDS at 66 °C for 30 min) conditions and examined by autoradiography.

cDNA synthesis and PCR amplification

PCR amplification of RNA was performed as described [4]. Briefly, 2 μg samples of total RNA were reverse-transcribed in 1 × PCR buffer (Perkin–Elmer Cetus), supplemented with $MgCl_2$ to 6 mM, 1 mM dNTPs, 30 units of RNasin and 25 units of AMV-reverse transcriptase (Promega Corp.). PCR amplification mixtures (25 μl) contained 5 μl of cDNA template, 1 × PCR buffer, 0.4 μM primers and 280 μM dNTPs. Amplification was performed for 20–30 cycles (1 min at 64 °C for all PCR primers, 1.5 min at 72 °C and 0.5 min at 96 °C) in a Techne PHC-2 thermal cycler (Brouwer AG). Contamination of RNA preparations with genomic DNA was assessed by mock reverse transcription (no reverse transcriptase added), followed by PCR. The primer sequences are listed in Table 1. Amplified products were analysed by ethidium bromide staining after separation on agarose gels.

Transfection of Jurkat cells and receptor-binding studies

For the generation of stable transfectants, 4×10^6 Jurkat cells were co-transfected by electroporation (Genpulser; Bio-Rad) with 20 μg of pcDNAI-*MDR15* or pcDNAI-*BLR1*, both linearized with *Bcl*I and 10 μg of $pSV2_{neo}$ linearized with *Eco*RI as described [4]. Stable transfected clones were selected by the addition of G418 (Life Technologies) at 800 μg/ml of culture medium and screened for expression of recombinant transcripts by Northern-blot analysis.

Binding studies with transfectant Jurkat cells were performed as described [4,10,24]. Chemokines were prepared by solid-phase synthesis [29]. C3a was kindly provided by Dr. C. Dahinden, Institute of Clinical Immunology, University of Bern, and [^{3}H]leukotriene B4 was purchased from DuPont–NEN.

Table 1 Oligonucleotides for reverse transcription (RT)-PCR and *BLR1* synthesis

The oligonucleotides were synthesized on an Applied Biosystems model 391 automated synthesizer according to standard protocols. Orientation of the primers refers to hybridization to either the sense or anti-sense template DNA. Specificity refers to hybridization specificity of the PCR primers (see also Figures 1 and 4). The oligonucleotide for glyceraldehyde-3-phosphate dehydrogenase cDNA (*GAPDH*) was synthesized based on the sequence of Tokunaga et al. [28].

Primers		Orientation	Specificity
SE1-BLR	5′-CCAAGCTTGGTGACTCACAGCCGGCACAGCC-3′	Sence	*BLR1*, 5′ region
AS1-MDR	5′-GCCCACCAGCCAGATGGTCCC-3′	Anti-sense	*BLR1*/*MDR15*, common sequence
SE2-MDR	5′-GTCCTTAGGTCCTCACCCTCCCG-3′	Sense	*MDR15*, 5′ region
SE2-BLR	5′-CTCTCTAGAGGCACCTGGCGGGG-3′	Sense	*BLR1*, 5′ region
SE-GAP	5′-ACATCAAGAAGGTGGTGAAGCAGG-3′	Sense	
AS-GAP	5′-CTCTTTCCTCTTGTGCTCTTGCTGG-3′	Anti-sense	*GAPDH*

RESULTS

MDR15 cDNA

A cDNA library prepared from human blood monocyte polyadenylated RNA was screened with an 819 bp PCR, *F3R1*, encoding part of the rabbit IL-8 receptor [4,26]. In addition to clones corresponding to the two known human IL-8 receptors, IL-8R1 [2] and IL-8R2 [3], a clone, *MDR15*, was isolated that strongly hybridizes with ^{32}P-*F3R1*. The 2020 bp cDNA insert of *MDR15* was sequenced (EMBL data library accession number X68829). The start codon for the longest open reading frame (981 bp) is at position 289, and is preceded by a sequence, GGGCCCCTCATGG, that is unusual for the pyrimidine base at position -3 (C) but otherwise conforms reasonably well with the consensus sequence for translation-initiation sites in vertebrate mRNAs [30]. Several in-frame translational stop codons are located upstream of this putative start codon. The deduced polypeptide sequence of 327 amino acids comprises seven putative transmembrane segments typical of G-protein-coupled receptors, and shares marked sequence similarity to chemokine receptors (see below).

MDR15 is 93% identical with *BLR1*, the cDNA for an orphan receptor isolated from a Burkitt's lymphoma cDNA library [21]. The *BLR1* gene consists of two exons with an intervening sequence of approx. 9 kb, and the exon 1 contains the first 17 codons for BLR1. *MDR15* differs from *BLR1* in the 5′ position of the exon splice site (Figure 1) resulting in shortening of the putative open reading frame for MDR15 by 135 bp. Additional differences include a G → A transition at position 1183 of *MDR15* causing the mutation of Gly^{299} → Ser, and, in the non-coding 3′ regions of *MDR15*, two insertions of a C at positions 1424 and 1790 as well as C → T transition and a C → G transversion at positions 1580 and 1588 respectively.

Northern-blot analysis of normal and neoplastic cells

To study the expression of *MDR15* and *BLR1*, total RNA from human blood monocytes, neutrophils and lymphocytes as well as myeloid and lymphoid cell lines was probed with the internal *Bst*X–*Xba*I cDNA fragment that is common to *MDR15* and *BLR1* (Figure 1).

As shown in Figure 2(a), monocytes and peripheral blood lymphocytes express *MDR15/BLR1*-specific RNA transcripts. Various non-B-lymphoid leukaemic cell lines lacked detectable levels of *MDR15/BLR1* mRNA. The B-lymphoblastic cell lines Raji and Daudi were strongly positive, which confirms the observations of Dobner et al. [21] for *BLR1*. Analysis of samples from leukaemic patients showed that *MDR15/BLR1* transcripts are abundantly expressed in chronic (B-CLL) but not acute (B-ALL) types of B-lymphoid leukaemia. High-level expression of transcripts was also observed in eight of ten biopsy samples from patients with B-cell non-Hodgkin's lymphomas (Figure 2b). Malignant T-cell lines were negative, suggesting that the expression of *MDR15/BLR1* mRNA is restricted to the B-cell lineage and monocytes.

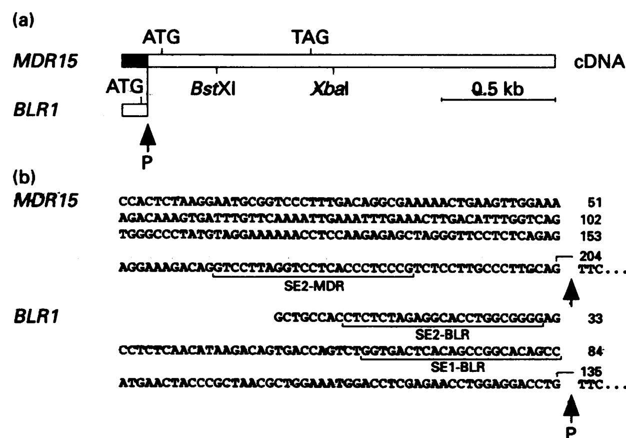

Figure 1 Sequence differences between *MDR15* and *BLR1*

(**a**) Schematic representation of the cDNAs for *MDR15* and *BLR1* [21]. The filled box indicates the 5′ region in *MDR15* which differs from *BRL1*. P denotes the point of divergence. The positions of the start (ATG) and stop (TGA) codons and two critical restriction enzyme sites (*Bst*XI, *Xba*I) are shown. (**b**) Nucleotide sequences of the 5′ regions of *MDR15* and *BLR1*. Underlining indicates the position of the synthetic primers used in the cyclic amplification reactions (see below).

Southern blotting with human genomic DNA

Using the same *Bst*XI–*Xba*I fragment as in Northern blots, analysis of endonuclease-digested human genomic DNA yielded single fragments (Figure 3). Thus the transcripts for *MDR15* and *BLR1* appear to be encoded by the same gene which occurs as a single copy. No additional hybridization bands were revealed by either washing the blots at lower stringency before autoradiography or prolonged autoradiography, suggesting the absence of closely related genes. These findings are in full agreement with the Northern-blot analysis (Figure 2). *LESTR* is abundantly expressed in neutrophils, monocytes and lymphocytes [24], and high levels of transcripts for IL-8R1 and IL-8R2 are found in neutrophils [4], but these RNAs did not cross-hybridize with the Northern-blot probe for *MDR15/BLR1*.

Two oligonucleotide primers that discriminate between *MDR15* and *BLR1*, SE2-MDR and SE2-BLR, and a primer, AS1-MDR, that does not (Table 1, Figure 4a) were then used to analyse human genomic DNA by PCR amplification. The PCR product obtained with the primers for *MDR15* was the same size (521 bp) as the fragments generated with *MDR15* cDNA, the control template (Figure 4b). This indicates that the *MDR15* primers span a genomic sequence that is not spliced during RNA processing. Similarly, *BLR1* fragments of the expected size (608 bp) were amplified using reverse-transcribed *BLR1* mRNA as template and SE2-BLR and AS1-MDR as primers (see below). In contrast, no PCR products were obtained with genomic DNA, which would be expected if a large intervening sequence separates the two primer annealing sites on exons 1 and 2 [21]. These results demonstrate that *MDR15* and *BLR1* correspond to alternative transcript forms derived from the same gene (*MDR15/BLR1*).

Cellular expression of *MDR15* and *BLR1*

To discriminate between the two transcript forms, RT-PCR studies were performed with the same primers as for genomic DNA amplification. B-CLL, Daudi and Raji cells, which were strongly positive on Northern-blot analysis, monocytes and lymphocytes, which showed weaker signals, as well as AML193, HEL, KG1 cells and phytohaemagglutinin (PHA)-treated T-lymphocytes were analysed. All RNA preparations were negative for genomic DNA contamination. Except for AML193 and PHA-treated T-lymphocytes, all samples contained transcripts for both *MDR15* and *BLR1*, and none expressed only one of the two mRNAs (Figure 5a). Transcripts for HEL cells which were not detected by Northern-blot analysis (Figure 2) and KG1 cells appear to be present at low levels. Except for monocytes, the two

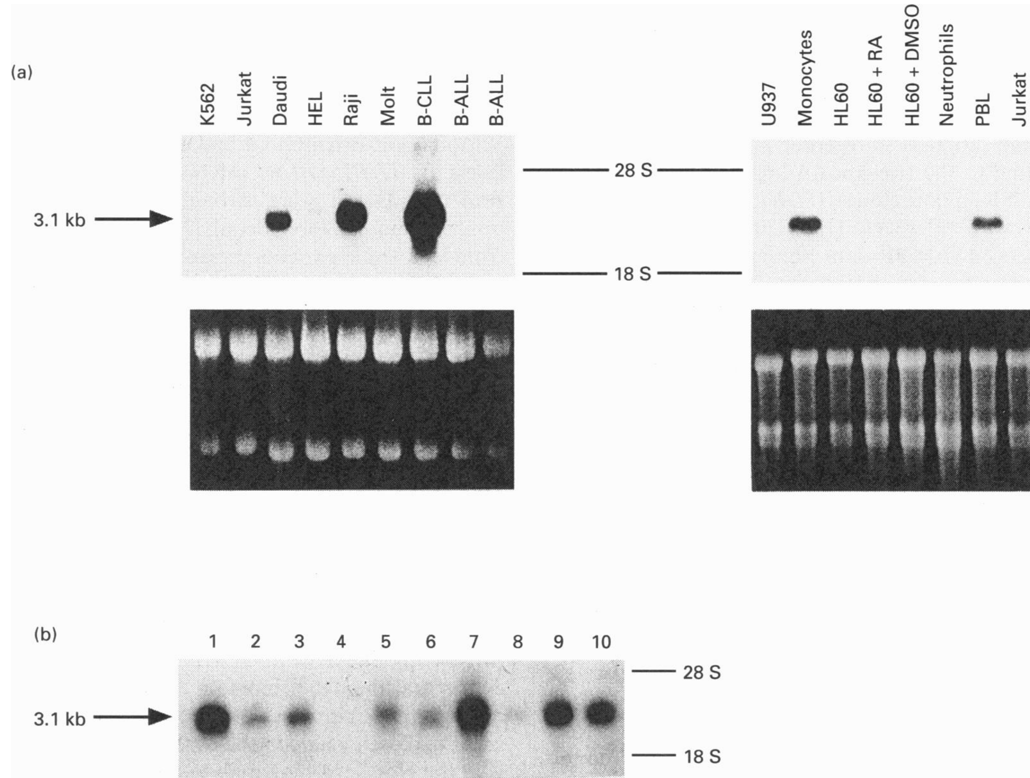

Figure 2 Northern-blot analysis of *MDR15* and *BLR1*

(**a**) Examination of total RNA (10 μg/lane) from human blood leucocytes, related cultured cell lines and primary cultures of malignant B-cells. HL60+DMSO or RA refers to HL60 cells that were treated for 72 h with 1.25% DMSO or 1 μM retinoic acid (RA). PBL, peripheral blood leucocytes; B-CLL, chronic B-lymphoid leukaemia; B-ALL, acute B-lymphoid leukaemia. In the lower gels, RNA was stained with ethidium bromide before transfer. (**b**) Analysis of total RNA (10 μg/lane) from lymph node biopsies of ten patients with chronic B-cell lymphomas. The ^{32}P-labelled internal *Bst*XI–*Xba*I fragment was used as hybridization probe, and the membranes were washed as described in the Experimental section before exposure to Hyperfilm MP.

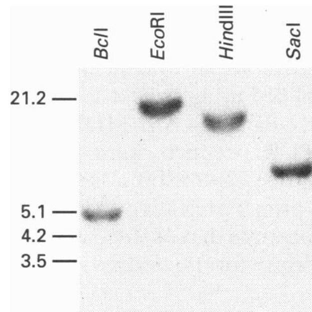

Figure 3 Southern-blot analysis of the *MDR15/BLR1* gene

Human genomic DNA (10 μg/lane) was digested with *Bcl*I, *Eco*RI, *Hin*dIII or *Sac*I, separated on a 0.7% agarose gel, and transferred to a nylon membrane. For hybridization the same ^{32}P-labelled cDNA fragment as in Figure 2 was used. The sizes (in kb) of standard DNA fragments are indicated.

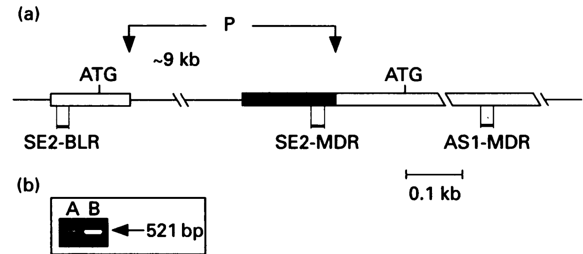

Figure 4 Genomic organization of the *MDR15/BLR1* gene

(**a**) Boxes correspond to the *MDR15* and *BLR1* cDNAs. The filled box designates the unique 5′ region in *MDR15* that is deleted when the intervening sequence of the two-exon *BLR1* gene is spliced during RNA processing. Note that only the 5′ region of the second exon is shown. P denotes the two splice sites, and positions for the start codons (ATG) and PCR primers are indicated. (**b**) PCR analysis of genomic DNA from human blood monocytes (A) and *MDR15* cDNA (B). After 30 amplification cycles with 1 μg of genomic DNA or 1 ng of *MDR15* as template and SE2-MDR and AS1-MDR as primers, the PCR products (521 bp) were analysed by agarose-gel electrophoresis.

forms are expressed in similar amounts in RNA from all positive cells. Using serial dilutions of purified *MDR15*- and *BLR1*-specific PCR products followed by cyclic amplification, the efficiency of the two primer sets was found to be very similar and 100 template molecules were readily detected with 30 PCR cycles (Figure 5b).

Expression of *MDR15* and *BLR1* in Jurkat cells

Sequence alignments of *MDR15* with proteins in the SwissProt database revealed close relationship to chemokine receptors

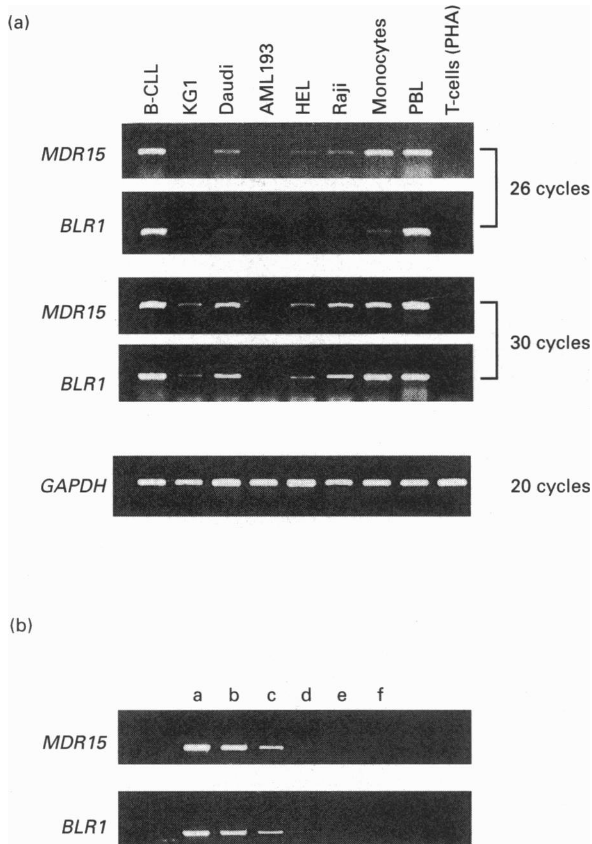

Figure 5 RT-PCR analysis of *MDR15*- and *BLR1*-specific transcripts in normal and malignant leucocytes

Total RNA was reverse-transcribed, amplified by PCR and analysed by agarose-gel electrophoresis and ethidium bromide staining. (**a**) For *MDR15*- and *BRL1*-specific mRNA the primers SE2-MDR/AS1-MDR and SE1-BLR/AS1-MDR were used respectively and for the control amplification (*GAPDH*) the primers SE-GAP/AS-GAP (Table 1). The RT-PCR products of 521 bp for *MDR15*, 608 bp for *BLR1* and 282 bp for *GAPDH* are shown. (**b**) Comparison of the sensitivity of the *MDR15* and *BLR1* amplification reactions. *MDR15* and *BLR1* DNAs were diluted in 10-fold steps in PCR buffer containing 1 μg/ml heat-inactivated salmon sperm DNA. Lanes a–f correspond to 10^5–10^0 molecules of *MDR15* or *BLR1* template DNA.

(Figure 6). MDR15 shares amino acid identity of 40% with the human IL-8 receptors [2,3], 35% with LESTR [23,32], an orphan receptor that is highly expressed in human phagocytes and lymphocytes, 33% with the human MIP-1α receptor [15,16], and 32–34% with the MCP-1 receptors type A and B [17]. MDR15 and BLR1 contain sequence motifs that are highly conserved in chemokine receptors, including Asp-Arg-Tyr-Leu-Ala-Ile-Val-His-Ala in the second intracellular loop which may be critical for G-protein interaction [33,34], and Cys/Phe-Trp-X-Pro-Tyr and His-Cys/Ser-Cys-Leu/Val-Asn-Pro in the transmembrane domains 6 and 7 respectively. As the similarity is more pronounced with chemokine receptors than receptors for other chemoattractants (22–24%) [1], the binding of chemokines to MDR15 and BLR1 was tested in transfectant Jurkat cell lines that stably express either *MDR15* or *BLR1* mRNA (Figure 7). Untreated Jurkat cells do not contain detectable levels of *MDR15* or *BLR1* transcripts, but respond to CXC chemokines when transfected with IL-8 receptor cDNAs [10]. Of the 16 known chemokines, 11 [IL-8, growth-relating protein (GRO)α(3Y), neutrophil activating protein (NAP)-2, platelet factor (PF)-4, interferon-inducible protein of 10 kDa (IP10), MCP-1, MCP-2, MCP-3, MIP-1α, I-309 and RANTES] were labelled with ^{125}I and used in binding studies. Three chemokines that lack tyrosine residues (GROβ, GROγ and ENA-78) were excluded, and GCP-2 and MIP-1β were not available [1]. Under experimental conditions that are optimal for binding of ^{125}I-labelled IL-8, GROα and NAP-2 to native and recombinant IL-8 receptors [4–6], no specific binding of the selection of chemokines to the Jurkat transfectants could be detected (results not shown). Two additional agonists, leukotriene B_4 and C3a, were also negative.

DISCUSSION

MDR15 was isolated from a human blood monocyte cDNA library, and its homologue, *BLR1*, was derived from human Burkitt's lymphoma RNA [21]. The open reading frames of the two receptor cDNAs are identical, except for minor variations, from the region encoding the first transmembrane domain to the 5′ end. The few single-base differences between the two cDNAs could be due to point mutations or sequencing artifacts. A major difference is observed, however, in the regions coding for the extracellular N-terminal domains which are believed to be involved in ligand recognition. The *BLR1* gene consists of two exons that are separated by a 9 kb intervening sequence [21]. The first exon contains the 5′ untranslated region, the putative translation-initiation codon and an open-reading-frame sequence encoding the 17 N-terminal amino acids. Southern-blot analysis revealed that *MDR15* and *BLR1* are encoded by the same gene. In contrast with *BLR1* [21], PCR studies with primers to the unique 5′ region of *MDR15* and genomic DNA as template show that this region is not interrupted by intervening sequences, indicating that the open reading frame of *MDR15* is contained on a contiguous stretch of genomic DNA. This led us to conclude that *MDR15* and *BLR1* are derived from alternative forms of RNA that are encoded by the same gene.

MDR15- and *BLR1*-specific transcripts were detected in total RNA from human monocytes and lymphocytes, and to a greater extent in B-lymphoblastic cell lines and primary isolates of B-CLL. Owing to the increased sensitivity of RT-PCR over Northern-blot analysis, low levels of transcripts were also detected in KG1 and HEL cells but not U937 and activated T-lymphocytes. A similar situation was reported for both human IL-8 receptors, IL-8R1 and IL-8R2, the mRNAs of which are demonstrated in different types of leucocytes and non-lymphoid cells by RT-PCR [4], whereas only in neutrophils, the primary target cells for IL-8, were transcripts readily observed by Northern blotting [1,4]. Except for monocytes, most cells analysed in this study expressed similar levels of *MDR15* and *BLR1* mRNA, and expression was particularly high in B-cell non-Hodgkin's lymphoma and B-CLL, but not in B-ALL. The lack of transcripts in the acute forms of lymphoid disorders and the apparent absence of expression in malignant T-cells suggest that the expression may be primarily restricted to B-cell lineage and related to the stage of maturation.

Formation of alternative RNA species with changes in the 5′-coding region may represent a mechanism for regulation of the ligand specificity of 7-TM receptors. Studies with N-terminal-substitution variants of the two types of IL-8 receptor have shown that the N-terminal domain is critical for discriminating between IL-8 and other CXC chemokines [8,35]. Mutagenesis studies revealed two regions in IL-8R1 (the receptor that is selective for IL-8), one in the third extracellular loop and one in the N-terminus, that are essential for high-affinity binding of IL-8 [36]. Functionally different receptor forms resulting from alternative RNA splicing were reported recently for two G-

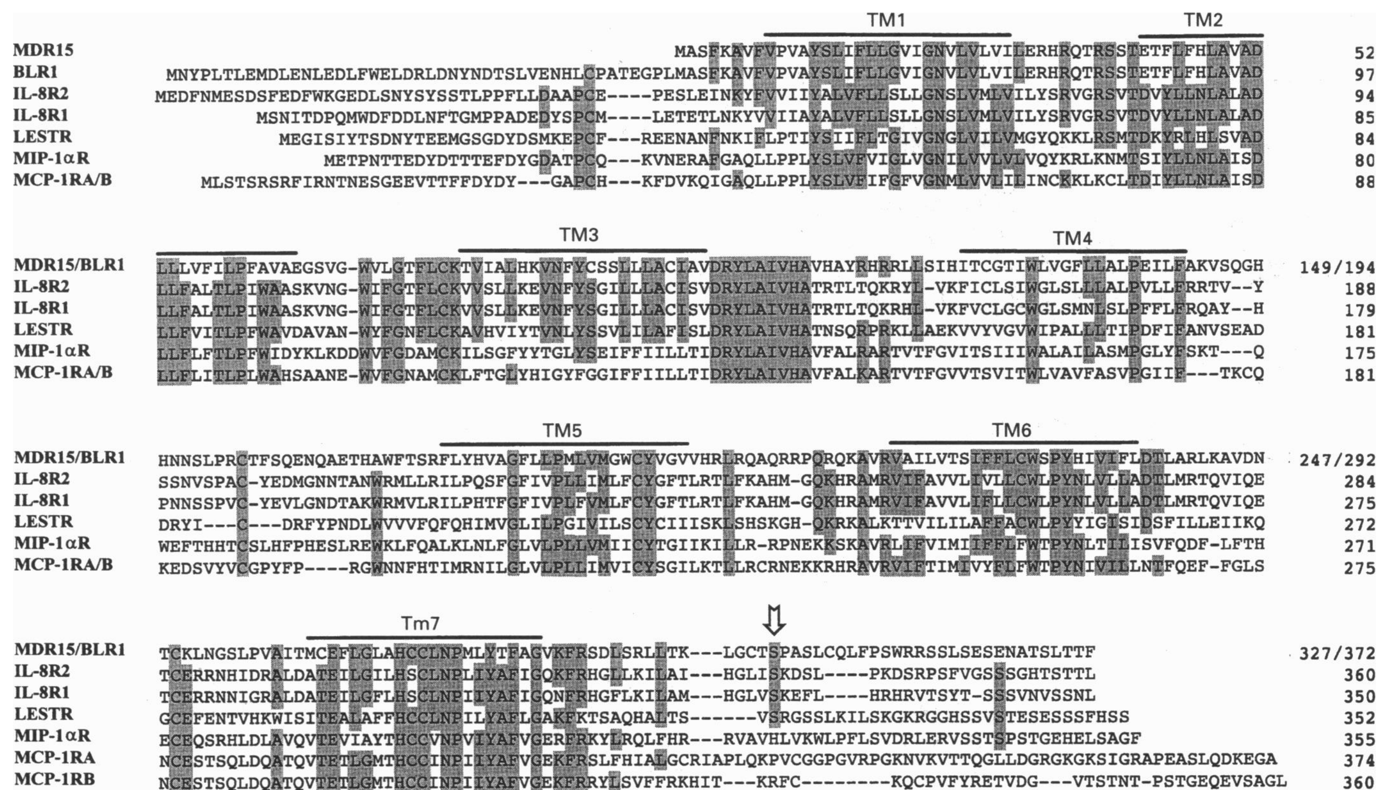

Figure 6 Sequence alignment of MDR15 with related G-protein-coupled receptors

The multiple protein alignment according to Higgins and Sharp [31] includes the human IL-8 receptors, IL-8R1 [2] and IL-8R2 [3], the orphan human blood monocyte receptor, LESTR [23,32], the MIP-1α receptor, MIP-1αR [15,16], and the human type A and B MCP-1 receptors, MCP-1RA and MCP-1RB [17]. Shaded areas indicate amino acid residues that are identical in four or more sequences, and overbars denote the seven putative transmembrane regions (TM1–TM7). Except for a serine residue (arrow) that is replaced by a glycine in BLR1, MDR15 is identical with the large C-terminal portion of BLR1.

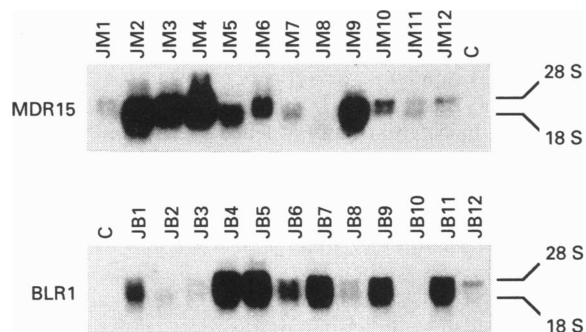

Figure 7 Expression of *MDR15*- and *BLR-1*-specific transcripts in Jurkat transfectant cell lines

Total RNA (10 μg/lane) from Jurkat cell lines transfected with *MDR15* or *BLR1* cDNA was analysed by Northern blot exactly as described in Figure 2. JM2 and JM9 for *MDR15*-expressing cell lines, and JB4 and JB5 for *BLR1*-expressing cell lines were selected for further studies. As a control (C), RNA from untreated Jurkat cells was used.

protein-coupled receptors, the prostaglandin receptor subtype EP3 (EP3R) [37] and the type-I receptor for the pituitary adenylate cyclase-activating polypeptides PACAP-27 and PACAP-38 (PACAPR) [38]. The differences in the transcripts affected the amino acid sequence in the third intracellular loop of PACAPR [38] and the intracellular C-terminal domain of EP3R [37]. In both systems the receptor isoforms showed unaltered ligand specificities but differed in their coupling to G-proteins and consequently in their mode of signalling. The same situation may exist for the two types of MCP-1 receptors, as they differ only in their intracellular C-terminal regions [17]. Finally, variation in the 5′ region was reported for mRNAs that encode the human platelet-activating factor receptor which also belongs to the superfamily of 7-TM receptors, but in this case the coding region was not affected [39].

MDR15 and BLR1 are members of an exclusive family of G-protein-coupled receptors primarily of lymphocyte origin (or prominent expression therein). Transcripts for EBI 1 and EBI 2, two orphan 7-TM receptors, are highly enriched in the Epstein–Barr virus-immortalized primary B-lymphoblastoid cell line, IB4, and Epstein–Barr virus-treated Burkitt's lymphoma cell line, BL41, but not in myeloid cells [40]. Also, as for MDR15 and BLR1, transcripts for EBI 1 are weakly expressed and transcripts for EBI 2 are absent from PHA-stimulated peripheral T-lymphocytes. In contrast, 6H1, an orphan 7-TM receptor of 308 amino acids, is primarily expressed in activated chicken T-lymphocytes [41]. Finally, LESTR, encoded by a cDNA that was isolated from the same blood monocyte cDNA library as *MDR15*, is highly expressed not only in blood lymphocytes and PHA-treated T-cells but also in monocytes, neutrophils and many

related blood cell lines [23,32]. The natural ligand has not been identified for any of these receptors.

Within the superfamily of G-protein-coupled 7-TM receptors, MDR15 and BRL1 show the highest degree of amino acid sequence identity with chemokine receptors. It is therefore reasonable to assume that their natural ligands may be as yet unidentified chemokines with selectivity for normal leucocytes as well as malignant B-cells, in which *MDR15/BLR1* transcripts are highly expressed. In transformed B-cells, chemokines may regulate growth and/or differentiation rather than chemotaxis and the release of inflammatory mediators.

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