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A Receptor for the Heterodimeric Cytokine IL-23 Is Composed of IL-12Rβ1 and a Novel Cytokine Receptor Subunit, IL-23R1

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IL-23 is a heterodimeric cytokine composed of the IL-12p40 “soluble receptor” subunit and a novel cytokine-like subunit related to IL-12p35, termed p19. Human and mouse IL-23 exhibit some activities similar to IL-12, but differ in their capacities to stimulate particular populations of memory T cells. Like IL-12, IL-23 binds to the IL-12R subunit IL-12Rβ1. However, it does not use IL-12Rβ2. In this study, we identify a novel member of the hemopoietin receptor family as a subunit of the receptor for IL-23, “IL-23R.” IL-23R pairs with IL-12Rβ1 to confer IL-23 responsiveness on cells expressing both subunits. Human IL-23, but not IL-12, exhibits detectable affinity for human IL-23R. Anti-IL-12Rβ1 and anti-IL-23R Abs block IL-23 responses of an NK cell line and Ba/F3 cells expressing the two receptor chains. IL-23 activates the same Jak-stat signaling molecules as IL-12: Jak2, Tyk2, and stat1, -3, -4, and -5, but stat4 activation is substantially weaker and different DNA-binding stat complexes form in response to IL-23 compared with IL-12. IL-23R associates constitutively with Jak2 and in a ligand-dependent manner with stats. The ability of cells to respond to IL-23 or IL-12 correlates with expression of IL-23R or IL-12Rβ2, respectively. The human IL-23R gene is on human chromosome 1 within 150 kb of IL-12Rβ2. The Journal of Immunology, 2002, 168: 5699–5708.

The growth, differentiation, and function of cells in the immune system are regulated by an array of soluble proteins termed cytokines. Cytokines can be classified into several protein families whose members share structural homology, e.g., the TNF family, IL-1 and related proteins, and hemopoietic cytokines. The latter family is a large group of cytokines that have a four α-helical bundle structure and collectively play important and varied roles in immune regulation.

IL-23 is a covalently linked heterodimeric hemopoietic cytokine that shares the p40 (soluble cytokine receptor-like) subunit of IL-12 but is distinguished from the latter by its cytokine subunit, p19 (1). IL-23-like IL-12 induces proliferation and IFN-γ production by human T cells. In contrast to IL-12, IL-23 preferentially stimulates memory as opposed to naive T cell populations in both human and mouse (1). Transgenic mice ubiquitously expressing the p19 subunit of IL-23 develop a severe multiorgan inflammatory syndrome with elevated expression levels of TNF and IL-1 (2), suggesting direct or indirect effects on myeloid cells as well as NK and T cells. This pathology can be transferred with transgenic bone marrow, suggesting that the principal source of the proinflammatory mediator (IL-23) is hemopoietic cells (2).

Consistent with the structural and biological similarities of IL-12 and IL-23, the IL-23R complex shares a subunit with that of IL-12 (IL-12Rβ1); however, it does not use or detectably bind to IL-12Rβ2 (1). To facilitate a more detailed understanding of IL-23’s function, we have identified and characterized an additional subunit of the IL-23R complex, termed “IL-23R.” In this study, we show that IL-23R is a novel member of the hemopoietin receptor superfamily encoded by a gene that maps within 150 kb of the gene for IL-12Rβ2 on human chromosome 1. IL-23 uses the same Jak-stat signaling molecules as IL-12, and IL-23R associates with Jak2 and stat3. The ability of cells to respond to either IL-12 or IL-23 is determined by expression of IL-12Rβ2 or IL-23R, respectively.

Materials and Methods

Cytokines

Human (h) IL-12 and mouse (m) IL-12 were purchased from R&D Systems (Minneapolis, MN). rhIL-23 and rmIL-23 were covalently linked fusion proteins (3) containing FLAG epitope tags as described (1).

Antibodies

Goat anti-hIL-12Rβ1 was from R&D Systems. Anti-FLAG epitope Abs were from Sigma-Aldrich (St. Louis, MO). Rabbit anti-hIL-23 anti-serum was prepared by immunizing rabbits (Josman LLC, Napa, CA) with rIL-23 extracellular domain containing C-terminal V5 (Invitrogen, Carlsbad, CA) and His6 epitope tags (soluble s)hIL-23R-V5-His6). Rabbit IgG fraction was prepared from serum by caprylic acid precipitation (4). hIL-23R-specific Ab was purified by absorption and elution from an affinity column containing rhIL-23R extracellular domain fused to the Fc region of IgG1 (hIL-23R-Ig).

Cells and cell lines

The hIL-2-2-dependent T cell line Kit225 (5) which binds and responds to IL-12 (6, 7) was kindly provided by Dr. J. Johnston (DNAX, Palo Alto).

6 Abbreviations used in this paper: h, human; m, mouse; BAC, bacterial artificial chromosome; s, soluble; HTG, high-throughput genomic.
CA). mIL-3-dependent Ba/F3 cells were as described (1, 8), Human PHA blast preparation was as described (1).

Human T cell clone 37 was derived from CD4+CD45RA+ peripheral blood T cells following two rounds of activation by anti-CD3 cross-linked on L cells expressing CD32, CD80, and CD55 in the presence of IL-2 (20 U/ml; R&D Systems) and IL-12 (1 ng/ml; R&D Systems). This population of cells was subsequently cloned by limiting dilution using PHA and feeder cells (9). T cell clone 37 has a Th1 phenotype. The human NK leukemia cell line NKL (10) was cultured in Yssel's medium supplemented with 1% human serum and 100 U/ml IL-2.

**NKl assay**

NKL cells (2 × 10^5 cells/well) were incubated in 200 μl Yssel’s medium in the absence or presence of IL-2 (200 U/ml), IL-12 (1 ng/ml), IL-18 (100 ng/ml), and IL-23 (100 ng/ml) either alone or in specified combinations for 72 h. Supernatants were harvested and the amount of IFN-γ determined by specific ELISA (R&D Systems).

**BALB/c bone marrow-derived macrophages**

BALB/c bone marrow-derived macrophages were cultured for 10 days in GM-CSF (40 ng/ml) + anti-mIL-10R (1 μg/ml). On day 10, cultures were stimulated for 24 h under each of the following conditions: anti-IL-10R or IFN-γ (5 ng/ml) + LPS (10 μg/ml), anti-IL-10R + IFN-γ, anti-IL-10R + LPS, and anti-IL-10R only.

cDNA libraries and expression cloning of hIL-23R

Total cellular RNA was isolated by lysis of cells in guanidinium isothiocyanate and centrifugation through a CsCl pad. Poly(A)+ mRNA was selected using an Oligotex mRNA kit (Qiagen, Valencia, CA). Retroviral expression cDNA libraries were prepared in the pMX expression vector (11) using the Superscript RT cDNA library kit (Life Technologies, Rockville, MD). Retrovirus was generated from cDNA libraries via transient transfection of BOSC23 cells as described (12) and used to infect 10^7 Ba/F3 cells expressing hIL-12Rβ1 (1) for 24–48 h on petri dishes coated with 30 μg/ml recombinant fibronectin fragments (Retronectin; Takara Bio, Shiga, Japan). Infection efficiencies of 80–98% were regularly obtained. Infected cells were cultured in M-3 for 2–3 days, then washed three times to remove M-3, plated at 1.2–1.5 × 10^5 cells/well in 96-well plates in medium containing 50 ng/ml hIL-23, and allowed to grow for 2 wk. Cultures were supplemented with fresh hIL-23-containing medium every 4–5 days. Cells growing in the presence of hIL-23 were tested for hIL-23-dependent proliferation in an assay using Alamar Blue (Tre Diagnostische Systems, Westlake, OH) as described (1, 8). Genomic DNA was isolated (DNasey Tissue kit; Qiagen) from hIL-23-dependent cell lines and subjected to PCR amplification using pMX vector-specific primers (11) (5′-CCCGGAGGGCTGAGGACCTCTCT-3′ and 5′-CTCAAGGTGGTGTCCTTCATTCATC-3′) followed by another round of amplification using a second, “nested” set of pMX vector primers (5′-TTGGATACACGCCGGCCCAACGTGAAGGCTGCCGA-3′ and 5′-CTTTATTATTTATGGTACCTGCAGACTGTTGTCGG-3′). High m.w. (>2 kb) PCR products were cloned in pCR2.1-TOPO (Invitrogen) and sequenced.

A candidate hIL-23R cDNA identified by this method (see Results) was used to screen K1225 and human NK cell cDNA libraries by hybridization and several of the resulting full-length hIL-23R cDNAs were subcloned in pMX containing a puromycin resistance gene (pMX-puro; Ref. 11). Retrovirus was generated and used to infect Ba/F3-hIL-12Rβ1 cells. Cells resistant to 2 μg/ml puromycin were tested for hIL-23-dependent growth.

**hIL-23R and mIL-23R genomic genes and mIL-23R cDNA**

The hIL-23R gene was determined to be closely linked to the gene encoding hIL-12Rβ2 on chromosome 1p13.2-3.12 (see Results). Accordingly, we obtained mIL-12Rβ2 bacterial artificial chromosome (BAC) genomic DNA clones from C57BL/6 and 129 ES cell genomic DNA libraries (Incyte Genomics, St. Louis, MO) by hybridization to an mIL-12Rβ2 cDNA probe (1, 13). BAC DNA maps were restricted and analyzed by pulsed-field electrophoresis (CHEF MapperXA system; Bio-Rad, Richmond, CA) and DNA blot hybridization using either mIL-12Rβ2 or hIL-23R cDNAs as probes. Restriction fragments hybridizing to the hIL-23R probe were subcloned and sequenced, allowing identification of several exons of the mIL-23R gene. PCR-amplified exons were used to probe a cDNA library derived from BALB/c bone marrow-derived macrophages. Primers were sequenced and full-length cDNAs transferred to pMX-puro for expression in Ba/F3 cells expressing mIL-12Rβ1 (1). Cells were assessed for responsiveness to hIL-23 and hIL-23 as described above.

**Binding of IL-23 to cells and rIL-23R**

Binding of IL-23 to cells expressing hIL-12Rβ1, hIL-23R, or both hIL-12Rβ1 and hIL-23R was detected by FACS staining using anti-FLAG or anti-p40 Abs as described (1). To detect this interaction by ELISA, plates were coated with 1 μg/ml hIL-23R-Ig, then incubated with various concentrations of hIL-23 or an irrelevant ligand (FLAG-hIL-10) in PBS + 10% FCS for 2 h at room temperature, followed by successive incubations with biotin-anti-FLAG (Sigma-Aldrich) and streptavidin-HRP (BD Pharmingen, San Diego, CA).

Interaction of hIL-23 and the soluble extracellular domain of hIL-23 was also demonstrated by immunoprecipitation. shIL-23R-V5-His was expressed in 293T cells; similarly, FLAG-hp40 and FLAG-hp19 were coexpressed in 293T cells. The respective supernatants were combined for immunoprecipitation experiments and incubated overnight at 4°C. Immunoprecipitations were performed in the presence of 0.1% Brij96 using anti-FLAG-M2 agarse (Sigma-Aldrich) or Ni-NTA agarose (Qiagen). Precipitated proteins were separated by nonreducing SDS-PAGE and detected by Western blot using anti-FLAG-HRP (Sigma-Aldrich) or anti-V5-HRP (Invitrogen) Abs according to the manufacturer’s recommendations.

**Quantitative PCR**

A total of 50 ng of DNA from various cDNA libraries was analyzed for expression of hIL-23R or mIL-23R by the fluorogenic 5′-nuclease PCR amplification by the ABI Prism 7700 Sequence Detection System (PerkinElmer, Foster City, CA) and as described (1). The following primer and probes were used: hIL-23R, forward: GCACAAACTCTGATTTGCA, reverse: ATGGGTCTTCCCTGGACAGA, probe: 6-carboxyfluorescein-TTCTTGTCTCAACACTGTTGATAATTACACCAAAAACC-6-carboxytetramethylrhodamine; hIL-12Rβ1, forward: GACATGGAAGCTGAGGTGTC, reverse: CACGGAGAGGATCTGAGGAA, probe: 6FAM-TGGGCTCATTCTCTCCAGTTGGCCTTCCCTG-TAMRA; hIL-12Rβ2, forward: AAGACACAGCTGGCCCACTGGA, reverse: TTGACAGCCTGATTTGCTACGCTACGACG, probe: 6FAM-CTCCG TATGACTATGGCCAGGCCTGTGTA, TAMRA; and mIL-23R, forward: TGAAAGAGCCACTTACCTCCTTTGA, reverse: CAGGAAATTGGGAGATTGGGATATGTGT, probe: 6FAM-ACCAGAGCATGGACTGGTTGTGGC TTCCACGTCCACGTCGTC, TAMRA. Cells grown to log phase were serum-starved for 4–6 h in RPMI 1640 + 0.5% BSA. Cells were centrifuged and resuspended in 2–4 × 10^6 cells/ml and stimulated with cytokine for 10 min at 37°C. After incubation, cells were centrifuged and washed in PBS + 1.5 mM sodium vanadate, then lysed in Brij buffer (10 mM Tris (pH 7.5), 2 mM EDTA, 0.15 M NaCl, 0.875% Brij 97 (Sigma-Aldrich)), 0.125% NP-40, complete protease inhibitors (Roche, Indianapolis, IN), and 3 mM Pefabloc protease inhibitor (Roche). Lysates were centrifuged and supernatants were either immunoprecipitated or prepared for SDS-PAGE by addition of reducing NuPAGE LDS loading buffer (Invitrogen). For immunoprecipitation, 2 μg Ab and 50 μl of protein A agarose beads (Sigma-Aldrich) were added to each reaction. Tubes were agitated at 4°C for 2–24 h. Following washes with Brij buffer and PBS, beads were resuspended in 10 μl of protein G/M-28, washed with reducing NuPAGE LDS loading buffer. Lysates or immunoprecipitates were subjected to 4–12% NuPAGE SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) per manufacturer’s recommendations. Membranes were blocked in 3% BSA/TBST (for anti-phosphotyrosine blots) or 5% milk blocking buffer. Membranes were first probed with anti-phosphotyrosine (4G10, Upstate Biotechnology), and then stripped and reblotted with anti-Stat1, Stat2, Stat3, Stat4 (BD Transduction Laboratories, Covington, KY), STAT 5, 6 (Santa Cruz Biotechnology), or Jak1 (BD Transduction Laboratories), Jak2 (Upstate Biotechnology or Santa Cruz Biotechnology), or Jak3 or Tyk2 (Santa Cruz Biotechnology) as appropriate.

Receptor communoprecipitation experiments were done as described above, using Kit225, or Ba/F3 transfected with hIL-12Rβ1 and c-myc epitope-tagged hIL-23R. Lysates were immunoprecipitated with rabbit anti-shh-23R-V5-His. Western blots were probed with anti-c-myc, rat anti-hIL-23R polyclonal Ab (provided by T. Churakov, DNAX), or anti-Jak/ Stat Abs as described above.
Results

Kit225 cells respond to both IL-12 and IL-23

Kit225 is a human T cell line that proliferates in response to IL-2 and IL-12 (5, 14–16). Although Kit225 cells readily lost growth factor dependence in culture and were thus not suitable for routine cytokine bioassays, we nonetheless demonstrated a proliferative response to IL-23 (Fig. 1).

Expression cloning of hIL-23R

We prepared cDNA libraries from Kit225, human PHA blasts (1), and human T cell clone 37 in the retroviral expression vector pMX. Ba/F3 cells expressing hIL-12Rβ1 were infected with cDNA library retrovirus and then allowed to grow in mIL-3 for 48–72 h. The resulting cells were washed, distributed at 1–1.5 × 10^5/well in 96-well plates, and cultured in hIL-23. Individual cell cultures growing in the presence of hIL-23 were analyzed for hIL-23-dependent growth. Ba/F3 cells expressing both hIL-12Rβ1 and human T cell clone 37 in the retroviral expression vector pMX. We prepared cDNA libraries from Kit225, human PHA blasts (1), and IL-12 and IL-23 (5, 14–16). Kit225 is a human T cell line that proliferates in response to IL-2 and IL-12 (5, 14–16). Among these subclones we identified a 2.9-kb cDNA encoding a 629-aa type I transmembrane protein with homology to cytokine receptors; its closest relatives appeared to be IL-12Rβ2 (Fig. 2a) and gp130 (data not shown). Retrospective PCR analysis of IL-23-dependent cell lines isolated from screens of all three cDNA libraries revealed that 51/54 such cell lines harbored integrated retrovirus containing this “hIL-23R” cDNA insert.

To confirm function as a receptor for hIL-23, candidate hIL-23R cDNAs were expressed in Ba/F3-hIL-12Rβ1 cells, and the resulting cells tested for growth in hIL-23. Ba/F3 cells expressing both hIL-12Rβ1 and IL-23R (“8c”), but not the individual subunits, responded to hIL-23 (Fig. 3a) and mIL-23 (data not shown). This response could be inhibited by anti-hIL-12Rβ1 (Fig. 3b) and affinity-purified rabbit anti-hIL-23R Abs (Fig. 3c). Moreover, the response of human NKL cells to hIL-23 was also inhibited by anti-hIL-12Rβ1 and anti-hIL-23R Abs (Fig. 3d).

Human and mouse genomic IL-23R genes

A basic local alignment search tool search of the high-throughput genomic (HTG) database located the hIL-23R gene on human chromosome 1 (1p31.2-32.1) on the same 151-kb segment as hIL-12Rβ2. Using sequence derived from several HTG entries, we assembled what appears to be the complete hIL-23R gene structure (Table 1). The hIL-23R ORF is encoded by 10 exons spanning ~92 kb of human genomic DNA.

To obtain the mIL-23R genomic gene and cDNA, we isolated eight BAC genomic clones from two different libraries by hybridization to a mIL-12Rβ2 cDNA. Two of eight BAC clones also hybridized to the hIL-23R probe. We subcloned and sequenced two HindIII fragments that hybridized to hIL-23R. These fragments contained mIL-23R exons corresponding to exons 8 and 10 of the hIL-23R gene.

PCR-amplified mIL-23R exons were then used to screen a cDNA library of BALB/c mouse 7-day Th2-polarized T cells; two full-length cDNA clones were obtained encoding identical 644-aa type I transmembrane proteins with 66% identity and 77% similarity to hIL-23R (Fig. 2a). DNA sequence homology was ~84% in the protein-coding regions (data not shown).

mIL-23R cDNA was expressed in Ba/F3 cells together with mIL-12Rβ1 (1). Ba/F3 cells expressing both mIL12Rβ1 and mIL-23R responded to IL-23 (Fig. 3e). Both mIL-23 and hIL-23 were active on mouse and human cells as reported earlier (1) and on cells expressing the respective rIL-12Rβ1/hIL-23R complexes (Fig. 3e).

Structure of IL-23R

The extracellular domain of IL-23R contains a signal sequence, an N-terminal Ig-like domain and two cytokine receptor domains (Fig. 2, a and b). The extracellular domains are all related to corresponding domains of IL-12Rβ2 (Fig. 2a). In contrast to IL-12Rβ2, IL-23R does not contain three transmembrane-proximal fibronectin type III domains. There are eight and seven potential N-linked glycosylation sites in mIL-23R and hIL-23R, respectively.

The transmembrane-proximal cytokine receptor domain of both mIL-23R and hIL-23R contains a sequence (WQPWS) similar to the cytokine receptor signature WSXWS motif. Interestingly, the mIL-23R cDNA sequence contains a 20-aa duplication including this motif. We also found this duplication in BAC genomic DNA clones (129 strain). Moreover, among eight mouse cDNA libraries from BALB/c and C57BL/6 that contained mIL-23R cDNAs, we

Table 1. Exon and intron distribution of the hIL-23R gene on human chromosome 1. The complete hIL-23R genomic sequence was assembled from the following HTG entries: AC026054, AL109843, AL389925, AC068536, AC025693, and AL358512

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found only mIL-23R cDNAs containing this duplication (data not shown). Thus, we conclude that the mIL-23R gene and protein contain this 20-aa duplication.

The 252-aa hIL-23R cytoplasmic domain contains seven tyrosine residues, six of which are conserved in mIL-23R (hIL-23R Y463 is not conserved). Three of these tyrosines define potential Src homology 2 domain-binding sites, Y399, Y484, and Y611 (Fig. 2a). The Y399EDI sequence is a potential SHP2 binding site (17) and Y611FPQ is a potential stat1 and stat3 binding site (18). The GY484KPQIS sequence resembles to a degree the motif in stat4 and IL-12Rβ2 known to bind to stat4 (GYL/VPS; Refs. 19 and 20). Although it is difficult to discern a proline-rich region that

FIGURE 2. Structure of a receptor complex for IL-23. a, Alignment of amino acid sequences of hIL-23R, mIL-23R, and hIL-12Rβ2. Only the Ig-like and cytokine receptor domains of hIL-12Rβ2 are shown. Predicted signal sequences, the WSXWS boxes, and transmembrane domains are indicated by black boxes. Potential N-linked glycosylation sites are indicated by dark red boxes and potential Src homology 2 domain binding sites by blue boxes. b, Schematic of receptors for IL-12 and IL-23. Ig-like domains are shown in light gray, cytokine receptor domains in white, and fibronectin type III domains in dark gray. The WSXWS box motif is indicated by the thick black line and conserved cysteine residues by thin black lines. IL-23R cDNA sequences have been deposited in the GenBank database with accession numbers AF461422 (hIL-23R) and AF461423 (mIL-23R).
would be predicted to bind a Jak kinase, IL-23R does associate with Jak2 (see Fig. 7).

**Interaction of IL-23 with IL-23R**

Binding of hIL-23 to hIL-23R was readily detected by FACS analysis (Fig. 4a); hIL-12 did not bind to hIL-23R (data not shown) and hIL-23R did not associate with hIL-12Rβ1 in the presence of hIL-12 (see Fig. 7). hIL-23 bound to shIL-23R-V5-His6 coated on ELISA plates (Fig. 4b). In addition, FLAG-hp19 and FLAG-hp40 expressed together in 293T cells could associate with shIL-23R-V5-His6 in solution (Fig. 4c).

We were unable to demonstrate ability of hIL-23R-Ig or shIL-23R-V5-His6 to act as effective antagonists of hIL-23 stimulation of 8c cells (data not shown), nor could we see evidence of these proteins’ ability to augment cytokine activity.

**IL-23R expression**

hIL-23R mRNA was expressed at levels too low to detect by RNA blot except in Kit225 cells which expressed a 2.8–3-kb hIL-23R mRNA (Fig. 5a), consistent with the size of the hIL-23R cDNA clone. Therefore, we analyzed expression of both hIL-23R and mIL-23R by quantitative real-time PCR (Taqman). hIL-23R mRNA is expressed by a human Th1 and Th0 clone as well as several NK cell lines and clones, including NKL (Fig. 5b). cDNA libraries prepared from several cultured monocyte and dendritic cell populations (Fig. 5b) expressed very low levels of hIL-23R. Relatively low but detectable hIL-23R mRNA levels were observed in EBV-transformed B cells and anti-CD3/anti-CD28/LPS-activated PBMC (Fig. 5b). This expression pattern was similar to that of hIL-12Rβ2 (Fig. 5c). PBMC used in these experiments were activated for short time periods up to 24 h; higher receptor expression levels were seen at 48–72 h (data not shown). In addition, nine hIL-23R expressed sequence tags were found by basic local alignment search tool search; all were derived from a cDNA library of bone marrow from chronic myelogenous leukemia patient(s).

mIL-23R expression (Fig. 5, d and e) was detected in BALB/c 7-day polarized Th1 and Th2 cells and 3-wk polarized, activated Th2 cells and bone marrow dendritic cells. Neither the J774 macrophage cell line nor several mast cell lines expressed significant mIL-23R mRNA levels. These results suggest either a degree of

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**FIGURE 3.** IL-23R is required for cellular responses to IL-23. a, Ba/F3-hIL-12Rβ1/hIL-23R (8c) cells respond to hIL-23. Inhibition of 8c response to IL-23 by anti-hIL-12Rβ1 (b) and anti-hIL-23R (c). d, Anti-hIL-23R and anti-hIL-12Rβ1 inhibit the response of human NKL cells to IL-23. e, Ba/F3-mIL-12Rβ1/mIL-23R cells respond to m,hIL-23. Error bars represent the range of duplicate measurements.
difference in the expression patterns of hIL-23R and mIL-23R, or more likely that the respective human and mouse cell populations examined are not functionally equivalent.

Bone marrow macrophages activated by LPS in the presence of either IL-10 or neutralizing anti-IL-10R mAb (21) expressed mIL-23R, in contrast to peritoneal macrophages (Fig. 5d). However, these same cells expressed little or no detectable IL-12R. Therefore, we examined IL-12Rβ1 and IL-23R expression in these cells under different conditions and found that activation in the presence of anti-IL-10R mAb and IFN-γ induced expression of both receptor subunits while in the presence of LPS IL-12Rβ1 mRNA expression was not detected (Fig. 5, d–f). Our observation of IL-12Rβ1 expression by monocyte/macrophage cells is consistent with a number of reports in the literature (22–29).

mCD4⁺CD45RBlow memory T cells respond to IL-23 but relatively poorly or not at all to IL-12; in contrast, CD4⁺CD45RBhigh cells respond well to IL-12, but poorly to IL-23 (1). Therefore, we analyzed mIL-23R and mIL-12Rβ2 expression in these cells. Consistent with these cells’ cytokine responses, CD4⁺CD45RBlow cells expressed IL-23R mRNA and low levels of IL-12Rβ2, while CD4⁺CD45RBhigh cells expressed IL-12Rβ2 and little or no detectable IL-23R (Fig. 5g).

Signal transduction in response to IL-23

In view of the structural and biological activity similarities exhibited by IL-23 and IL-12, we examined the Jak kinase and Stat transcription factor molecules activated by IL-23. Previous work showed that IL-23, like IL-12, activates Stat4, although to a lesser extent (1). Results shown in Fig. 6, a–d, demonstrate that IL-23 activates the same spectrum of Jak/Stat molecules as IL-12 (30–37): Jak2, Tyk2, and Stat1, -3, -4, and -5. However, Stat4 activation is notably weaker in response to IL-23 (Fig. 6b), and differences were also evident in EMSA experiments (Fig. 7, a and b). In contrast to IL-12, which induces a strong Stat4-containing EMSA band (33, 35, 38), IL-23 induces a heterogeneous set of at least three bands. In Kit225 cells, the top and bottom bands contain
FIGURE 5. Expression of IL-23R mRNA. a, RNA blot demonstrating 2.8 kb hIL-23R mRNA in Kit225 cells. b and c, hIL-23R, hIL-12Rβ1, and hIL-12Rβ2 Taqman expression profiles; d and e, mIL-23R, mIL-12Rβ1, and mIL-12Rβ2 Taqman expression profiles. f, Mouse bone marrow macrophages activated in the presence of anti-mIL-10R and IFN-γ express both IL-12Rβ1 and IL-23R mRNA. g, mIL-23R expression in murine CD4⁺CD45RB⁺⁺ and CD4⁺CD45RB⁺⁺⁺ T cells. Units are fg/100 ng cDNA in a–d and g; f, Expression levels are given relative to ubiquitin mRNA.
Stat3 and Stat1, respectively, as shown by “supershift” experiments. Stat5 was not detected with the M67 SIE oligonucleotide probe, as noted by others (39, 40). Ab supershift experiments suggested that the middle band, faintest of the three, may contain both Stat3 and Stat4. In PHA blast T cells (Fig. 7b) and NKL cells (data not shown), the composition of the three bands was somewhat different: as for Kit225 the prominent upper band contained Stat3, but the lower band contained Stat4, and the middle band clearly contained both Stat3 and Stat4. We could not reliably detect IL-23- or IL-12-induced Stat1 activation in PHA blasts and NKL cells.

In the presence of IL-23, IL-23R can be immunoprecipitated in association with IL-12Rβ1 (Fig. 7c). In addition, Jak2 and Stat3 physically associate with IL-23R, the latter in a ligand-dependent manner, and IL-23R itself is tyrosine-phosphorylated in response to IL-23 (Fig. 7d). Thus, like IL-12Rβ2, IL-23R associates with Jak2. However, we were unable to consistently demonstrate association of Stat4 with IL-23R such as that reported for IL-12Rβ2 (Refs. 19 and 20; data not shown).

**Discussion**

We have cloned and characterized a novel hemopoietic cytokine receptor that is a subunit of the receptor for IL-23, IL-23R. IL-23R and IL-12Rβ1 together comprise the IL-23 receptor complex on IL-23-responsive cells, as evidenced by: 1) the proliferative response to IL-23 of cells expressing rIL-23R and rIL-12Rβ1, and 2) the ability of anti-IL-23R Abs to block responses of cells to this cytokine (Fig. 3). Although we cannot rule out that the IL-23R complex contains yet an additional subunit, our findings so far do not support this possibility. IL-23R is a member of the hemopoietic cytokine receptor family related to IL-12Rβ2 and gp130. However, unlike its close relatives, the IL-23R extracellular region does not contain three membrane-proximal fibronectin III-like domains; rather it contains one Ig-like domain and two cytokine receptor domains, similar to IL-6Rα, IL-11Rα, and CNTFRα (18, 41). The latter “short” cytokine receptor subunits generally provide little in the way of signal-transducing functions in the cytokine-receptor complex; IL-23R thus appears to be an exception in this regard. Mouse IL-23R contains a 20-aa duplication including the signature cytokine receptor WSXWS box (WQPWS, Fig. 2a). This duplication was present in mIL-23R mRNA or genomic DNA from three different mouse strains and thus appears to be encoded in the mIL-23R gene. Despite this insertion, mIL-23R is functional (Fig. 3e). Whether this structural difference between hIL-23R and mIL-23R has any consequences for the biological roles of IL-23 in the human and mouse systems respectively is presently unknown.

IL-23 signal transduction engages the same Jak-Stat signaling molecules as IL-12: Jak2, Tyk2, Stat1, Stat3, Stat4, and Stat5 (Fig. 6). These results suggest that, consistent with the reported biology...
of IL-23 (1, 2), some signal transduction mechanisms engaged by IL-23 are similar to those of IL-12 (30–37).

However, in contrast to IL-12, the most prominent Stat induced by IL-23 is Stat3 rather than Stat4. Moreover, the compositions of DNA-binding Stat complexes induced by IL-12 and IL-23 exhibit potentially important differences. As shown in Fig. 7, IL-12 induces a DNA-binding complex containing only Stat4, while IL-23 induces several complexes containing Stat3, Stat1, Stat4, and possibly Stat3/Stat4. This pattern of EMSA bands induced by IL-23 is similar to that reported to be induced by IL-12 at longer times of stimulation (35). Thus, while the same Jak kinases and Stat proteins are activated by IL-12 and IL-23, the resulting DNA-binding Stat transcription factor complexes can be different. These observations suggest that significant differences should be expected in the biological responses induced by IL-23 and IL-12. This area is the subject of ongoing investigation.

Human IL-23R is expressed by both T cells and NK cells, including NKL cells (Fig. 5, a and b), consistent with the ability of these cells to respond to IL-23. We demonstrate here that IL-23 enhanced the production of IFN-γ by NKL cells. The combination of IL-2, IL-18, and IL-23 induced levels of IFN-γ that were substantially greater than those induced by either cytokine alone or when combined solely with IL-2. Polyclonal anti-IL-23R and anti-IL-12Rβ1 Abs inhibited the enhanced production of IFN-γ induced by IL-23 (Fig. 3d).

The hIL-23R expression pattern overlaps with that of hIL-12Rβ2, in agreement with the observation that most human cells that respond to IL-23 also respond to IL-12 (Ref. 1 and R. de Waal Malefyt, unpublished observations). The proximity of the IL-23R and IL-12Rβ2 genes is also consistent with possible coordinate regulation of expression.

Mouse bone marrow macrophages express IL-23R. Activation in the presence of IFN-γ induced coordinate expression of IL-23R and IL-12Rβ1, while in the presence of LPS induction of IL-12Rβ1 mRNA was markedly reduced (Fig. 5, d–f; D. Rennick, J. Cheung, and T. McClanahan, unpublished observations). These observations suggest the intriguing possibility that IFN-γ (perhaps induced by accessory cell IL-12) induces macrophages to become responsive to IL-23. Moreover, the pathology of the p19 transgenic mouse (2) also suggests the possibility of as yet uncharacterized biologic responses of myeloid cells to IL-23.

Mouse CD45RBlow memory cells respond to IL-23 but poorly to IL-12, while naive CD45RBhigh cells respond well to IL-12 but poorly to IL-23 (1). We have demonstrated in this study (Fig. 5g) that IL-23R is expressed by CD45RBlow cells and at comparatively much lower levels by CD45RBhigh cells. Moreover, for IL-12Rβ2, the reciprocal expression pattern is observed. Thus, the responses of these mouse cell types to IL-12 or IL-23 correlate with the relative expression levels of IL-12Rβ2 or IL-23R, respectively.

The identification of both IL-23 and its specific receptor component IL-23R defines a new cytokine produced by accessory cells that stimulates T cells, NK cells, and possibly certain macrophage/myeloid cells. In contrast to anti-IL-12p70 mAbs that target both

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**FIGURE 7.** IL-23R signal transduction and association with signaling molecules. a and b, EMSA pattern of DNA-binding Stat transcription factor complexes induced by IL-12 and IL-23 in Kit225 and PHA blast cells. 32P-labeled double-stranded M67 SIE probe (5’-CATTTCCCGTAAATC-3’) was used; cells were stimulated for 10 min with IL-12 or IL-23. c, IL-23R associates with IL-12Rβ1 in the presence of IL-23, but not IL-12 in Kit225 cells; d, IL-23R associates constitutively with Jak2, inducibly with stat3, and is tyrosine-phosphorylated in response to IL-23 in Ba/F3 cells expressing hIL-12Rβ1 and c-myc-epitope-tagged hIL-23R.
IL-12 and IL-23, specific reagents such as anti-IL-23R neutralizing mAbs will facilitate distinguishing the functions of IL-12 and IL-23 in the induction and development of immune responses. Of particular interest will be the respective roles played by these two cytokines in regulation of memory T cells, as the ability to therapeutically alter memory T cell responses has important implications for treatment of autoimmune diseases. In addition, genetic deficiencies in the IL-12p40, IL-12β1, IL-12β2, IFN-γR1, and IFN-γR2 genes have been associated with diminished ability to combat mycobacterial infections and enhanced atopic disease (42). It is possible that mutations in either the IL-23p19 or IL-23 genes may explain similar deficiencies in individuals who lack genetic defects in the IL-12/IFN-γR ligand systems.

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