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Inflammatory Signals Direct Expression of Human IL12RB1 into Multiple Distinct Isoforms

Nicole R. Ford,* Halli E. Miller,* Allison E. Reeme,* Jill Waukau,† Christine Bengtson,† John M. Routes,‡ and Richard T. Robinson* 

IL12RB1 is essential for human resistance to multiple intracellular pathogens, including Mycobacterium tuberculosis. In its absence, the proinflammatory effects of the extracellular cytokines IL-12 and IL-23 fail to occur, and intracellular bacterial growth goes unchecked. Given the recent observation that mouse leukocytes express more than one isoform from IL12rb1, we examined whether primary human leukocytes similarly express more than one isoform from IL12RB1. We observed that human leukocytes express as many as 13 distinct isoforms, the relative levels of each being driven by inflammatory stimuli both in vitro and in vivo. Surprisingly, the most abundant isoform present before stimulation is a heretofore uncharacterized intracellular form of the IL-12R (termed “isoform 2”) that presumably has limited contact with extracellular cytokine. After stimulation, primary PBMCs, including the CD4+, CD8+, and CD56+ lineages contained therein, alter the splicing of IL12RB1 RNA to increase the relative abundance of isoform 1, which confers IL-12/IL-23 responsiveness. These data demonstrate both a posttranscriptional mechanism by which cells regulate their IL-12/IL-23 responsiveness, and that leukocytes primarily express IL12RB1 in an intracellular form located away from extracellular cytokine. The Journal of Immunology, 2012, 189: 000–000.

The cytokines IL-12 and IL-23 influence numerous aspects of adaptive and innate human immunity, including the differentiation of helper T and NK cell subsets (1–6), enhancing cytolytic T cell responses (7), promoting B cell production of select Ig isotypes (8), and regulating dendritic cell activity (9). The biological activities of IL-12 and IL-23 are dependent on IL-12Rβ1, a type I integral membrane protein that serves as a low-affinity receptor for the p40 subunit of both cytokines (10–12). Reflecting the multifaceted influence of IL-12Rβ1 on human immunity, deleterious mutations in the gene IL12RB1 associate with susceptibility to numerous maladies (e.g., atopic dermatitis, Crohn’s disease, neurofibromatosis type I, and sarcoidosis) (13–17) and intracellular bacterial infections (e.g., salmonellosis, leishmaniasis, coccidioidomycosis, and mycobacterial disease) (18–23). In the case of Mycobacterium tuberculosis infection, mouse models demonstrate that IL-12 is important for T and NK cell secretion of IFN-γ; in its absence, activation of infected macrophages is reduced and intracellular M. tuberculosis growth goes unchecked (24–26). As further evidence of its immunological significance, interruption of IL-12/IL-23’s interaction with IL-12Rβ1 is the target of psoriasis treatments ustekinumab and ABT-874 (27–29). Targeting IL-12Rβ1 activity via IL-12 is also being investigated as a cancer therapeutic (30).

To relay the immunological effects of IL-12 or IL-23, IL-12Rβ1 must both physically associate with each cytokine and signal in complex with either IL-12Rβ2 or IL-23R, respectively (10–12). Although the extracellular portion of IL-12Rβ1 contains the cytokine-binding region essential for physical association with IL-12 or IL-23, the cytoplasmic portion acts in concert with IL-12Rβ2/IL-23R to transmit intracellular signals via preassociated JAKs TYK2 and JAK2 (31). TYK2 associates with the cytoplasmic tail of IL-12Rβ1, whereas JAK2 associates with either IL-12Rβ2 or IL-23R (31, 32). Reflecting the sensitivity of IL-12Rβ1 activity to changes in either its extracellular or intracellular regions, amino acid substitutions in either of these regions can enhance or interrupt IL-12Rβ1 function (33). Posttranscriptional modifications to these same amino acids (e.g., spliceosome-mediated deletions) are also likely to influence IL-12Rβ1 function and, possibly, the efficacy of any current or experimental therapy targeting them.

Alternative RNA splicing has been demonstrated to produce cytokine receptors with distinct, isoform-specific functions (34). For example, the IL-1 type II receptor and soluble IL-6Rα are generated through alternative RNA splicing and serve, respectively, as an antagonist and agonist of their cognate cytokines (35, 36). Given the recent observation that mouse leukocytes express two distinct mRNA isoforms from il12rb1 (37) (the mouse homolog of human IL12RB1), we sought to determine whether human leukocytes and tissues similarly express more than one IL12RB1 mRNA isoform. We observed that under both homeostatic and inflammatory conditions, leukocytes and lung tissues transcribe IL12RB1 into as many as 13 unique mRNA isoforms: 2 major isoforms (1 located on the cell surface and another in an intracellular compartment), as well as 11 minor isoforms. The predicted amino acid sequences of 12 of these isoforms deviate from the IL-12Rβ1 amino acid sequence originally reported (10). These isoforms are generated through a combination of alternate

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Abbreviations used in this article: Ct, threshold cycle; hnRNP, heterogeneous nuclear ribonucleoprotein; MCW, Medical College of Wisconsin; PDI, protein disulfide isomerase; STX, syntaxin; UTR, untranslated region.

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The Journal of Immunology
3’ splice- and polyadenylation-site activation within the IL12RB1 genomic sequence, alternate 3’ exon inclusion, exon skipping, partial exon inclusion, as well as alternate 5’ exon site usage. Furthermore, the relative representation of these isoforms varies with activation or disease status. The variable expression of multiple IL12RB1 isoforms in different tissues and lymphocyte lineages may contribute to tissue- and lineage-specific responses to IL-12 and IL-23.

Materials and Methods

Isolation of PBMCs

Units of blood from healthy adult donors were collected into sodium citrate-treated bags at the Blood Center of Wisconsin (Milwaukee, WI); donors were excluded if they were taking (or had taken within 2 wk before collection) any of the following categories of immunosuppressant medications: antineoplastic agent, antiviral agent, corticosteroid (either dermatological or nondermatological), disease-modifying antirheumatic drug, or immunosuppressive mAb drugs. After deidentification, blood units were delivered to our laboratory via courier the same day for PBMC isolation. For this, blood was diluted 1:2 in sterile PBS (Ca2+ and Mg2+ free) and immediately spun over a room-temperature Ficoll-Histopaque 1107 gradient (GE Healthcare, Piscataway, NJ) according to established protocols (38). Buffy coats were washed twice in PBS, counted, and then used for either total RNA purification or leukocyte subset purification as detailed earlier.

Isolation of PBMC subsets

For purifying leukocyte subsets of interest, PBMCs were suspended in a slurry of magnetic bead-Ab conjugates recognizing either human CD4 (clone L200), CD8 (clone SK1), or CD56 (clone NCAM16.2) according to the manufacturer’s protocols (BD Biosciences, San Diego, CA). After positive selection and two rounds of washing in PBS, subsets were counted and immediately lysed for RNA extraction. Leukocyte subsets were purified away from PBMCs both before and after PBMC culture in PHA to remove activated lymphocytes. After PBMCs were purified, they were cultured in complete RPMI 1640 containing CD2-activator (39) PHA-P (1 μg/ml final concentration; Sigma-Aldrich, St. Louis, MO). After 3 d, cells were washed and either lysed for RNA use or used for leukocyte subset purification as detailed earlier.

In vitro PHA stimulation

After PBMCs were purified, they were cultured in complete RPMI 1640 containing CD2-activator (39) PHA-P (1 μg/ml final concentration; Sigma-Aldrich, St. Louis, MO). After 3 d, cells were washed and either lysed for RNA use or used for leukocyte subset purification as detailed earlier.

Lung tissue specimens

All tissues were supplied by the National Resource Center of the National Disease Research Interchange. Relevant donor information is presented in Supplemental Table II. Between 1 and 5 h postmortem, affected areas of sarcoid lungs (or from control lungs) were resected and snap frozen for the National Disease Research Interchange repository. Upon transfer to our laboratory, these tissues were divided into smaller pieces for RNA extraction, ELISA analysis of select cytokine levels, or histological analysis.

RNA extraction

Total RNA was extracted using the Qiagen RNeasy method (Valencia, CA). For total mononuclear cells and select lymphocyte populations, cells were counted and pelleted for RNA extraction. For frozen lung tissue, portions of sarcoid or control lung were placed in a mass-adjusted volume of Qiagen Buffer RLT (with 2-ME) and immediately homogenized at high speed using a Millenyi gentleMACS dissociator (specifically, using the “RNA 02.01” program).

cDNA synthesis

First-strand cDNA synthesis was performed using Roche Transcriptor High Fidelity cDNA Synthesis Kit (Indianapolis, IN). Anchored poly-dT primers (supplied with the kit) were used to initiate reverse transcription.

IL12RB1 isoform sequencing and amplification

cDNA samples were amplified using the Roche FastStart High Fidelity PCR System (Indianapolis, IN) with any of the following primer pairs: 5’-IL12RB1 and 3’-isoform 1 (for amplification of IL-12RB1 isoform 1), 5’-IL12RB1 and 3’-isoform 2 (for amplification of IL-12RB1 isoform 2), or rGAPDH_F and rGAPDH_R (for amplification of GAPDH). Because we were able to amplify the entire 5’-untranslated region (5’-UTR) with the same primer for both isoforms, the transcription start site is presumably the same for both isoforms. The sequences of all primers used can be found in Supplemental Table I. All PCR amplicons were either cloned into bacterial vector pUC19 using standard PCR cloning methods or TA-cloned using the Promega pGEM-T system (Madison, WI). Individual colonies (as donor) were sequenced by Sanger sequencing using an ABI 3730xl DNA Sequencer (Functional Biosciences, Madison, WI). Chromatograms were individually analyzed to ensure the integrity of each sequence (Sequencer 5.0; Gene Codes Corporation, Ann Arbor, MI).

IL12RB1 isoform copy number assay

The mRNA copy number of both IL12RB1 isoforms 1 and 2 was determined by real-time quantitative PCR. A 25-μl reaction was used with 2x iQ SYBR Green (Bio-Rad), 5% DMSO, cDNA derived from indicated cells/tissue, and the following 0.4 mM primers: 12 F and 12/13_R for amplification of isoform 1, 9/9F2 and 9b_R for amplification of isoform 2 (sequences are listed in Supplemental Table I). The parameters for these reactions were the following: incubation at 95°C for 2 min (1 cycle); denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s (40 cycles) on qiQ RT-PCR machine (Bio-Rad). The threshold cycles (Ct values) for individual amplifications were determined using iQ5 software. IL12RB1 isoform 1 and 2 cDNAs cloned into pUC19 served as standards to calculate mRNA copy number from the Ct values given. Melting curve analysis was used to determine amplification specificity and was performed immediately after the amplification reaction. The relative abundance of isoform 1 to isoform 2 was determined by dividing the number of isoform 1 mRNA copies in a cDNA sample by the number of isoform 2 mRNA copies in the same sample.

Determining IL12RB1 mRNA isoform half-life

To determine the relative half-lives of IL12RB1 isoforms in the presence or absence of PHA, PBMCs were purified as detailed earlier and placed in complete media in the presence or absence of PHA. Four hours later, either actinomycin D (1 μg/ml final concentration) or vehicle control (DMSO) was added to each culture. For a given PBMC preparation, multiple wells were set up in this manner (5 × 105 well in six-well plates) to collect data from three experimental replicates per condition per time point. Cells were collected at designated times after actinomycin D or DMSO treatment; total RNA was isolated using the Qiang RNeasy method and frozen for future analysis (allowing us to perform, in one setting, the IL12RB1 isoform copy number assay described earlier with samples from all time points). For cDNA synthesis, the concentration of total RNA of each sample was normalized (as determined via NanoDrop) and cDNA prepared for all samples using the same lot of reverse transcription reagents. The mRNA copy number of both IL12RB1 isoforms 1 and 2 was determined as detailed earlier and normalized to the total concentration of RNA collected. Data were analyzed using GraphPad Prism and its bundled software via nonlinear regression analysis; both goodness of fit (R2) and calculated half-life values are indicated.

Cell lines and transfection

HEK293T cells were cultured in DMEM supplemented with 5% newborn calf serum and 5% FBS in the presence of puromycin (Sigma-Aldrich) as needed. HEK293T cells stably expressing IL12RB1 isoform 1 or 2 cDNAs were generated by Lipofectamine-mediated transfection (Invitrogen, Carlsbad, CA) with the vector pPyCAGIP (40) containing either cDNA (together with their 3’-UTRs) under the CAG promoter. Isoforms were subcloned into a multiple cloning site upstream of an internal ribosomal entry site and puromycin N-acetyl transferase gene (paa) to allow for puromycin selection. HEK293T cells stably transfected with empty pPyCAGIP were used as negative controls for some experiments. BSC40 cells (used for localization studies) were cultured in DMEM supplemented with 10% FBS and transfected with pPyCAGIP isoform 1, pPyCAGIP isoform
2, pYcAGIP-empty, or pAcGFP-N1 (Clontech, Mountain View, CA) using the Lipofectamine method (Invitrogen). Transient transfectants were used for immunofluorescent analyses. The leukemic Jurkat T cell line was used for our initial assessment of IL12RB1 isoform expression (see Fig. 1A) and was cultured with or without indicated concentrations of Con A (Sigma-Aldrich) in complete RPMI 1640.

**Western analysis**

Protein lysates were prepared from indicated cell populations in SDS reducing sample buffer, run on 7% SDS-PAGE gels, and transferred onto nitrocellulose membrane via a Semi-Dry Transfer system (Bio-Rad). Membranes were blocked in a milk and TBS solution, and probed with a rabbit polyclonal anti-human IL-12Rβ1 Ab (ProteinTech Group, catalog no. 13287-1-AP) generated against an amino acid sequence shared between both IL12RB1 isoforms 1 and 2 (aa 44–381; for the relative location of these amino acids in isoforms 1 and 2, see Fig. 2B, 2C). After incubation with an HRP-conjugated secondary Ab and multiple washes in TBS, blots were exposed to film for indicated lengths of time.

**Immunoprecipitation**

IL12RB1 isoforms 1 and 2 were immunoprecipitated using the Protein A Dynabeads System (Invitrogen). Specifically, cells transfected with both isoform 1 and 2 cDNAs were puromycin selected (as detailed earlier) and lysed in NP-40 lysis buffer for immunoprecipitation with polyclonal anti–IL-12Rβ1/Protein A beads (per the manufacturer’s instructions). For negative controls, immunoprecipitates were similarly prepared from both empty-vector transfectants (i.p. using anti–IL-12Rβ1/Protein A) and from isoform-transfected cells (i.p. using nonspecific IgG/Protein A). After immunoprecipitation, samples were linearized and treated with recombinant PNGase F (New England Biolabs), per the manufacturer’s instructions. These treated samples, as well as untreated controls, were then subjected to Western analysis as described earlier.

**Immunofluorescence**

BSC40 transfectants grown on glass coverslips were washed with PBS, fixed with 4% paraformaldehyde in PBS, permeabilized with 0.5% saponin in PBS and 3% BSA, incubated with primary Abs (polyclonal anti–IL-12Rβ1 [mentioned earlier], anti-MHC class I clone W6/32), or anti-protein disulfide isomerase [anti-PDI, clone 34/PDI], washed, and incubated with Alexa 488- or 594-conjugated secondary Abs (Invitrogen). Cells were imaged using a Nikon Eclipse TE2000-U inverted microscope, with a 60× oil immersion objective, and analyzed using Nikon Elements software (Nikon Instruments, Melville, NY).

**Histological analysis**

Frozen tissues were sectioned and stained with H&E by the MCW Children’s Research Institute Histology Core. Visual analysis of serial sections (100 μm apart) was used to confirm the relative abundance of inflammatory cells in the sarcoid lung compared with control specimens. Images were taken with a Nikon Eclipse TE2000-U inverted microscope, with a 60× oil immersion objective, and analyzed using Nikon Elements software (Nikon Instruments, Melville, NY).

**ELISA analysis**

Lung specimens of similar size were homogenized at high speed in ice-cold PBS using a Millenyi gentleMACS dissociator (specifically, using the “Protein” program). Homogenized lungs were then centrifuged to separate any debris; the supernatants were then aliquoted for ELISA analysis of human IFN-γ, TNF-α, and IL-12p40 levels (BD Biosciences).

**Graphing and statistics**

Graphs were prepared using Graph Pad Prism version 5.0. Statistical analyses used the bundled software of Prism; comparisons involving mRNA levels of IL12RB1 isoform 1, IL12RB1 isoform 2, or the ratio of these two isoforms to one another used a two-tailed paired t test. Differences between groups were considered significant if p < 0.05 and are graphically indicated by an asterisk.

**Results**

**Primary human leukocytes express two major IL12RB1 isoforms**

Because mouse leukocytes express two distinct il12r1b isoforms (37), we predicted that human leukocytes would also express more than one IL12RB1 isoform. Consistent with this, amplification of cDNA from PBMCs of healthy donors, syngeneic PBMCs stimulated with PHA-P, or the Jurkat T cell leukemia line with primers spanning IL12RB1 produced two major isoforms (Fig. 1A); a previously characterized larger isoform (10) (hereafter referred to as “isoform 1”) and an uncharacterized shorter isoform (“isoform 2”; Figs. 1B, 2C, 2D) originally cloned from human testes (National Center for Biotechnology Information accession no. BC029121; http://www.ncbi.nlm.nih.gov/nuccore/bc029121). Whereas isoform 1 results from transcriptional inclusion of all 17 exons of the IL12RB1 gene (Fig. 2A, 2B), isoform 2 results from the inclusion of exons 1–9, an alternate 3′-exon and alternate 3′-UTR that are otherwise excluded from the sequence of isoform 1 (Figs. 1B, 2). This alternate 3′-exon (hereafter referred to as “exon 9b”) is 125 nt in length, and contains a coding sequence terminated by a stop codon and an alternate polyadenylation site (Fig. 2D). Although the 3′-UTR of isoform 1 is 47 nt long, the 3′-UTR adjacent of isoform 2 is 647 nt long and contains several pyrimidine-rich elements (Fig. 2D). The translated product of the isoform 2 mRNA, as deduced from its sequence, retains the cytokine binding region but loses the transmembrane and intracellular sequences of isoform 1 (Fig. 2A–C); these last sequences are replaced with a distinct, shorter C-terminal sequence (Fig. 2C). In this manner, human IL12RB1 isoform 2 differs from its homolog in mice (il12r1bΔtm), which is created by the skipping of mouse il12r1b exon 14 (37). Thus, human leukocytes express two major isoforms from the IL12RB1 gene, the splicing of which is different from mice because it entails transcription of an alternative 3′ exon, alternative polyadenylation site, and a unique 3′-UTR.

**Leukocyte activation alters the relative representation of IL12RB1 isoforms 1 and 2**

Visual analysis of IL12RB1 isoform 1 and 2 expression by syngeneic PBMCs before and after PHA stimulation suggested that the ratio of these two isoforms changes with stimulation status (Fig. 1A, compare lanes 3 and 4). This result would indicate that the pathways activated by PHA initiate the alternative RNA splicing of IL12RB1 isoforms 1 and 2. To quantify these changes and determine whether they could be generalized to PBMCs of multiple individuals, we determined via real-time PCR analysis the mRNA copy numbers of both isoforms before and after PHA stimulation. Real-time PCR allowed us to reliably detect a minimum of 10^2 to 10^3 mRNA copies for each isoform (Supplemental Fig. 1A–C). For isoform 1, PHA stimulation decreased the C_t value at which this isoform was detected (Fig. 1C), indicating that PHA increased production of this mRNA. Although occasionally PHA stimulation decreased the C_t at which isoform 2 was detected, the difference was greater for isoform 1 than for isoform 2 (compare Fig. 1C with 1D). When expressed as mRNA copy number, the data demonstrate that although PBMCs from seven individuals increased expression of isoform 1 after PHA exposure (Fig. 1E), the relative abundance of isoform 2 remained unchanged (Fig. 1F). As a consequence, the ratio of each isoform to the other changes in the presence of PHA (Fig. 1G). This change in the relative representation of IL12RB1 isoforms 1 and 2 with PHA exposure was observed across multiple unrelated donors (Fig. 1G) and was not associated with changes in the mRNA half-life of either isoform (Supplemental Fig. 1D–L). We conclude from these data that PHA-dependent signaling pathways direct the alternative RNA splicing of IL12RB1 isoforms 1 and 2 in PBMCs.

**Leukocyte activation alters the appearance of multiple minor IL12RB1 isoforms**

In addition to expressing two major isoforms, sequencing of multiple mRNA clones before and after PHA exposure revealed
that PHA also influenced the extent to which *IL12RB1* was further processed into additional minor isoforms. These minor isoforms are listed in Table I together with their associated changes to the amino acid sequence. The frequency with which each minor isoform appears in the presence or absence of PHA (as a percentage of the total number of clones analyzed) is shown in Fig. 3A and 3B. With the exception of minor variant δex16, PHA exposure changed the relative representation of all minor splice variants for isoform 1 (Fig. 3A). PHA exposure also changed the relative representation of half of all minor splice variants observed for isoform 2 (Fig. 3B). In several instances, more than one minor splicing event was observed in a single clone (Fig. 3C). PHA-driven alternative splicing wasgene specific, as multiple *IL12RB1* mRNA clones showed no evidence of alternative splicing of its nine exons (Fig. 3C). Collectively, these data demonstrate that immunogen exposure induces alternative RNA splicing of both *IL12RB1* isoforms 1 and 2, with the relative abundance of both major and minor isoforms being dependent on activation status.

The extent of *IL12RB1* RNA splicing is lineage dependent and can vary between individuals

PBMCs represent an accumulation of multiple distinct leukocyte lineages. As with any population-based analysis, changes in gene expression observed at a total population level may not reflect what occurs in select subpopulations. For this reason, we determined whether changes in *IL12RB1* splicing were observed in CD4⁺, CD8⁺, and CD56⁺ lineages of the same individual before and after PBMC culture with PHA. These lineages were chosen because of the well-described effects of IL-12 on each (30). The results of these experiments are shown in Fig. 4. For two out of three donors, CD4⁺, CD8⁺, and CD56⁺ lines increased their expression of isoform 1 relative to isoform 2 after their culture in total PBMC/PHA (Fig. 4A, 4B). For donor A, CD8⁺ cells expressed the highest ratio of isoform 1 to isoform 2 after PHA exposure (Fig. 4A). For donor B, CD56⁺ cells expressed the highest ratio of isoform 1 to isoform 2 after PHA exposure (Fig. 4B). For a third donor, the relative ratio of isoform 1 to isoform 2 in CD4⁺ and CD8⁺ cells actually decreased after PHA exposure, whereas CD56⁺ cells expressed a small increase (Fig. 4C). From these data, we conclude that although leukocyte activation alters the relative representation of both major *IL12RB1* isoforms, the extent and direction of alternative *IL-12RB1* splicing (i.e., whether isoform 1 or 2 is preferentially expressed after activation) is lineage specific.

*IL12RB1* isoforms 1 and 2 have distinct protein characteristics

Because the 3′-UTR sequence of *IL12RB1* isoform 2 mRNA contains several pyrimidine-rich elements associated with mRNA degradation (41) (see highlighted sequences in Fig. 2D), we wished to determine whether this mRNA was translated into a protein product. For this, the entire *IL12RB1* isoform 2 mRNA (with the 3′-UTR intact) was cloned into mammalian expression vector pPyCAGIP (40) upstream of an internal ribosomal entry site and puromycin-resistance (*pac*) cassette. Puromycin-resistant
Rather than form discrete bands, all products were characterized by smears; this was especially true of the 120-kDa product of isoform 1 and is likely the result of glycosylation. Consistent with this, six asparagine residues of isoform 1 are predicted glycosylation sites (42) (Fig. 2B); two of these residues are shared with isoform 2 (Fig. 2C). Also consistent with glycosylation is that PNGase F treatment of isoforms 1 and 2 (immunoprecipitated from cells transfected with both cDNAs) resulted in a more discrete electrophoretic banding pattern than that of non–PNGase-treated controls (Supplemental Fig. 2A, 2B). Whereas isoform 1-expressing cells produced two products (~250 and ~120 kDa), isoform 2-expressing cells produced three products (~250, ~90, and ~40 kDa; Fig. 5A). Despite loading equal amounts of lysate, isoform 1 consistently reacted more strongly with polyclonal anti–IL-12Rβ1 than did isoform 2; this is demonstrated by different exposure times shown in Fig. 5A (5 min versus 30 s).

To determine whether changes in PBMC IL12RB1 isoform mRNA abundance (Fig. 1E–G) were reflected at the protein level, denatured lysates from untreated and PHA-treated PBMCs were subjected to Western analysis and stained with polyclonal anti–IL-12Rβ1. Lysates prepared from stable HEK293T cells expressing isoform 1 and 2 cDNAs were used as controls. As shown in Fig. 5B, PBMC lysates probed with anti–IL-12Rβ1 had a different electrophoretic pattern than that of either isoform 1- or isoform 2-expressing HEK293T cells. In the absence of PHA, PBMCs expressed a minimum of five different proteins that reacted with polyclonal anti–IL-12Rβ1 (Fig. 5B, bands 1, 2, 3, 5, and 6). In the presence of PHA, a minimum of six different proteins reacted with polyclonal anti–IL-12Rβ1 (Fig. 5B, bands 1–6). We considered the possibility that isoform 2 may be a secreted form of IL-12R. However, attempts to immunoprecipitate isoform 1 or 2 from the media of either transfected HEK293T cells or activated PBMCs were unsuccessful (Fig. 5C). Similar results were obtained using nickel-mediated pull-down of a tagged version of isoform 2 expressing 6x-His downstream of the N-terminal signal peptide (data not shown). We conclude from these analyses that IL12RB1 isoform 1 and 2 cDNAs both express proteins with distinct migration patterns, and that PBMCs express multiple proteins recognized by anti–IL-12Rβ1 whose electrophoretic migration pattern changes after PHA stimulation.

**IL12RB1 isoform 2 localizes to an intracellular compartment**

A BLAST search of the C-terminal amino acid sequence of isoform 2 indicated that this portion of isoform 2 bears homology to proteins known to localize to either the endoplasmic, trans-Golgi network or intracellular endosomes (Supplemental Fig. 2C), including VKORC1 (43), SLC4A1 (44), ADRA1A (45), syntaxin 6 (STX6) (46), and STX8 (47). These similarities, along with the distinct electrophoretic patterns of isoforms 1 and 2, led us to question whether the cellular localizations of isoforms 1 and 2 were also different. BSC40 cells transiently expressing isoforms 1 or 2 were stained with anti–IL-12Rβ1 and used for direct visualization by fluorescent microscopy to test this hypothesis. To discriminate between expression on the cell surface and expression inside the cell, cells were also stained with anti-MHC class I. As we anticipated, cells containing only empty vector displayed minimal reactivity with anti–IL-12Rβ1 (data not shown), whereas GFP-transfected cells displayed a predominantly nuclear fluorescence signal (48) (Fig. 6A). In agreement with the ability of isoform 1 to bind extracellular IL-12 (10), IL12RB1 isoform 1 demonstrated a pattern of fluorescence consistent with cell surface localization (compare Fig. 6B with that of MHC class I staining shown in Fig. 6E). In contrast with isoform 1, isoform 2 localized to an intracellular compartment within transfected cells (Fig. 6C).

**FIGURE 2.** *IL12RB1* isoform 2 is distinct from isoform 1 and is derived from alternate 3′ exon inclusion. (A) The genomic organization of human *IL12RB1*. *IL12RB1* is located on autosomal chromosome 19 at location 19p13.1 (1817037-18197697) and comprises exons 1–9, 9b, and 10–17. The locations of each exon along the *IL12RB1* sequence are shown; exons 1–9 and 10–17 are shown as blue boxes, and exon 9b and its 3′-UTR are shown as red boxes. (B) A schematic of the protein domains of *IL12RB1* isoform 1 protein as reviewed and modeled in van de Vosse et al. (42). On the left of the schematic are indicators of which *IL12RB1* exons encode which portions of the isoform protein; to the right of the schematic are the individual domain names alongside the corresponding amino acid number. Also indicated by green triangles are predicted glycosylation sites (specifically, aa 121, 329, 346, 352, 442, and 456 of isoform 1), the relative domain names alongside the corresponding amino acid number, and the WSxWS motif in the FB2 domain (pink). (C) A schematic of the protein domains of *IL12RB1* major isoform 2 as predicted from its mRNA sequence. This protein retains the cytokine binding region (CBR), two potential glycosylation sites, and the WSxWS motif, but contains a unique N-terminal signal sequence. (D) The mRNA sequence of *IL12RB1* exon 9b comprises 125 bp of protein coding sequence (highlighted yellow with the translated amino acid sequence shown beneath) that is terminated by a stop codon (underlined); this is followed by a 647-bp 3′-UTR that contains several pyrimidine-rich regions (highlighted purple) and a polyadenylation site (highlighted red). Note that the first nucleotide of the sequence shown (a guanine; circled) is derived from exon 9 and not exon 9b.

(*pac*) HEK293T cells were subsequently selected and their expression of isoform 2 protein assessed via Western analysis. Blots were probed with a polyclonal Ab generated against a region shared between isoforms 1 and 2 (see Materials and Methods). *pac* HEK293T cells expressing isoform 1 or empty vector alone were also generated and used as positive and negative controls, respectively. As shown in Fig. 5A, both isoform 1 and 2 cDNAs expressed protein products recognized by polyclonal anti–IL-12Rβ1, whereas empty vector containing HEK293T cells did not.

**Materials and Methods**
Isoform 2 staining exhibited a reticular pattern; nevertheless, this pattern was distinct from that observed for ER-specific PDI (Supplemental Fig. 2D). The absence of isoform 2 on the cell surface was confirmed by examining isoform 2-transfected cells stained with both anti–IL-12Rb1 and anti-MHC class I (compare Fig. 6D with 6E). Therefore, we conclude from our microscopic analysis that IL12RB1 isoforms 1 and 2 have distinct localization patterns, with isoform 1 being expressed on the cell surface and isoform 2 being expressed in an intracellular compartment.

Alternative IL12RB1 splicing is characteristic of inflammatory responses in vivo

Having observed PHA-responsive IL12RB1 splicing in vitro (Figs. 1–4), we wished to determine whether alternative splicing of IL12RB1 cDNA clones were sequenced from the PBMCs (both unstimulated and PHA stimulated) of eight healthy individuals; shown is a dot plot of the relative representation of each of the minor splice variants shown in Table I among both (A) isoform 1 and (B) isoform 2 clones in the presence or absence of PHA. Each minor splice variant has a corresponding color that is indicated in the legend; also indicated above each dot plot is the relative placement of each splicing event along the isoform 1 or 2 mRNA sequence. (C) Several clones of isoforms 1 and 2 were observed to contain more than one splicing event. As a control, multiple GAPDH cDNA clones from the same cells were subjected to an identical analysis. Shown are the number of splicing events observed in all IL12RB1 isoform 1, isoform 2, and GAPDH clones analyzed.

**FIGURE 3.** Activation influences the extent to which both major IL12RB1 isoforms are further processed into additional minor isoforms. Multiple IL12RB1 cDNA clones were sequenced from the PBMCs (both unstimulated and PHA stimulated) of eight healthy individuals; shown is a dot plot of the relative representation of each of the minor splice variants shown in Table I among both (A) isoform 1 and (B) isoform 2 clones in the presence or absence of PHA. Each minor splice variant has a corresponding color that is indicated in the legend; also indicated above each dot plot is the relative placement of each splicing event along the isoform 1 or 2 mRNA sequence. (C) Several clones of isoforms 1 and 2 were observed to contain more than one splicing event. As a control, multiple GAPDH cDNA clones from the same cells were subjected to an identical analysis. Shown are the number of splicing events observed in all IL12RB1 isoform 1, isoform 2, and GAPDH clones analyzed.

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**Table I.** Eleven minor IL12RB1 isoforms are expressed by human leukocytes

<table>
<thead>
<tr>
<th>Minor Isoform</th>
<th>No. of NT Deleted</th>
<th>No. of NT Added</th>
<th>AA Sequence</th>
<th>Domain Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δex3</td>
<td>12</td>
<td>...G----S...</td>
<td>...GSLPGS...</td>
<td>Pre-CBR</td>
</tr>
<tr>
<td>Δex5</td>
<td>140</td>
<td>...SVRGSRFLS...</td>
<td>...SHRLTSTSG*</td>
<td>CBR (FB1)</td>
</tr>
<tr>
<td>Δex5</td>
<td>54</td>
<td>...Q---------G...</td>
<td>...GSLPGS...</td>
<td>CBR (FB1)</td>
</tr>
<tr>
<td>Δex8</td>
<td>21</td>
<td>...P--------E...</td>
<td>...PVGLVLIAE...</td>
<td>CBR (FB2)</td>
</tr>
<tr>
<td>Δex10</td>
<td>168</td>
<td>...TEFVAMLIVQVTTMTYPARAQTGTTYCIEWQPQDGLATCGLTAPDPCGMA...</td>
<td>...M------------A...</td>
<td>FB4</td>
</tr>
<tr>
<td>Δex11</td>
<td>138</td>
<td>...MATYGNRESGAMQEQKCYTTIFASAHPEKLSTLSTYHFGNA...</td>
<td>...M------------A...</td>
<td>FB4</td>
</tr>
<tr>
<td>Δex13</td>
<td>135</td>
<td>...SHPVLQTETQVTLTGLRAGAVTYTVQARDTAHLRGNVEPQPQRFSEIE...</td>
<td>...S------------E...</td>
<td>FB5</td>
</tr>
<tr>
<td>Δex13</td>
<td>72</td>
<td>...S-------------E...</td>
<td>...SREPAMPLKEVPGLRASPAE...</td>
<td>FB5</td>
</tr>
<tr>
<td>Δex16</td>
<td>13</td>
<td>...DRCKARM*</td>
<td>...DRCDR*</td>
<td>INTRA</td>
</tr>
<tr>
<td>Δex17</td>
<td>143</td>
<td>...K*</td>
<td>...KTRATISITTAGY*</td>
<td>INTRA</td>
</tr>
<tr>
<td>Δex16+Δex17</td>
<td>143</td>
<td>...KTRATISITTAGY*</td>
<td>...KTRATISITTAGY*</td>
<td>INTRA</td>
</tr>
</tbody>
</table>
IL12RB1 mRNA characterized inflammatory responses in vivo. For this, we compared the extent of IL12RB1 mRNA splicing in lungs from individuals with sarcoidosis with that observed in healthy controls. Sarcoidosis is a chronic inflammatory disease of undefined cause characterized by the infiltration of activated leukocytes and granulomatous formations in the lung (49). cDNA from affected lung portions was generated and amplified in a manner identical to that done with PBMC cDNA; relevant clinical information on each tissue donor is listed in Supplemental Table II. Consistent with previous reports, sarcoid lung specimens contained granulomas comprising both lymphocytes and epithelioid histiocytes surrounded by extensive fibrosis (Fig. 7B). These features were not observed in control lung specimens (Fig. 7A). Sarcoid lungs were characterized by higher levels of TNF-α compared with control lungs (Fig. 7C); IL-12p40 and IFN-γ levels varied between the tissues we examined (Fig. 7D, 7E). Associated with this inflammatory response in lung sections from two different sarcoidosis patients (from tissue immediately adjacent to that histologically analyzed in Fig. 7B) was an increased ratio of IL12RB1 isoform 1 to isoform 2 (Fig. 7F, 7G) compared with control lungs. Although isoform 1 comprised 15–26% of all IL12RB1 expression in control sections (Fig. 7F), it comprised approximately twice as much (34–60%) in sarcoid sections. We conclude from these data that, similar to in vitro responses to PHA, alternative IL12RB1 splicing is characteristic of inflammatory responses in vivo.

Discussion

IL12RB1 is critical for IL-12- and IL-23-dependent leukocyte responses: CD4+ Th cells of individuals homozygous for non-functioning IL12RB1 (i.e., IL12RB1mut) alleles exhibit defective Th1 and Th17 polarization (19, 20, 50), NK cells from IL12RB1mut individuals are hyporeactive (6), and the frequency of CD56+ T cells in the circulation of IL12RB1mut individuals is low (6). These combined immunological defects leave IL12RB1mut individuals more susceptible to infection by mycobacteria, Salmonella, and Candida species, as reported in a recent extensive international study (20). Smaller and more regional studies have also shown positive associations between IL12RB1 deficiencies (13, 14, 21, 23, 51–53) or IL12RB1 polymorphisms (16, 22, 54–57) and susceptibility to a number of other maladies and infectious diseases. However, notably, beneficial IL12RB1 polymorphisms also have been discovered (58, 59). It is essential that we understand IL12RB1’s basic functions and regulation to fully understand how IL12RB1 modulates immunity and how we can use the expression of this gene or its ligands IL-12 and IL-23 for therapeutic purposes.

In this study, we have demonstrated that 13 distinct IL12RB1 mRNA isoforms are expressed by PBMCs of healthy individuals; 2 major isoforms (isoforms 1 and 2) and 11 minor isoforms (ex6x3, Δex5, 6ex5, Δex8, Δex10, Δex11, Δex13, Δex13, Δex16, Δex13, and Δex16+Δex17). The relative abundance of each isoform changes with leukocytes’ activation state (both in vitro and in vivo), as well as the specific leukocyte lineage or tissue examined. Based on these data, as well as the observation that isoform 2 localizes intracellularly, we propose the following model of IL12RB1 post-transcriptional regulation in leukocytes. Under homeostatic conditions, IL12RB1 is expressed at basal levels predominantly in the form of isoform 2. This isoform lacks any signaling domains and is sequestered intracellularly with limited access to extracellular cytokine (i.e., IL-12 or IL-23). Upon cellular activation, a modification of RNA splicing occurs, resulting in the preferential expression of isoform 1 over that of isoform 2. Isoform 1 localizes to the outer membrane, where it comes into contact with cytokine and confers cytokine responsiveness. Any changes in the relative abundance of each isoform by an individual cell likely “tunes” its response to IL-12/IL-23 in a manner that promotes immunity as a whole.

Because IL12RB1 is a well-known regulator of both adaptive and innate human immune responses, it is surprising that the majority of isoforms observed have not been reported previously. For >20 y, phenotypes associated with IL12RB1mut and polymorphic IL12RB1 alleles have been interpreted on the assumption that only one protein product is expressed from a normal IL12RB1 allele; this protein, IL-12Rb1, corresponds to isoform 1 of our study and was originally cloned by Chua et al. (10). One possible reason isoform 1 has been the only IL12RB1 isoform reported to date was the method of its discovery. This first IL-12R component was cloned by Chua et al. (10) from COS cells transfected with a cDNA library from PHA-activated PBMCs; a COS clone exhibiting both surface reactivity with mAb 2.4E6 and an affinity for extracellular IL-12 was chosen for sequencing (10). Rigorous studies since that time have shown that isoform 1 is integral to the plasma membrane (60, 61), binds the p40 domain of IL-12/IL-23 (11, 62), associates with IL-12Rb2/IL-23R to form high-affinity complexes for IL-12/IL-23 (12, 32), and, because it lacks intrinsic kinase activity, relies on its association with cytoplasmic Tyk2 for relaying IL-12 signals (31, 63). However, the original cloning scheme used by Chua et al. (10) excluded any clones not having reactivity with the Ab 2.4E6 or extracellular IL-12; consequently,
any clones without these reactivities because of either an absence or shielding of the epitope recognized by 2.4E6, or intracellular localization, went unsequenced and uncharacterized. Chua et al. (10) did make mention of a second transcript expressed by PBMCs that can be discriminated by Northern blot via its binding a cDNA probe specific to the exons encoding the extracellular portion of IL-12Rβ1; however, this transcript did not bind a cDNA probe specific to region encoding the cytoplasmic domain of IL-12Rβ1 (10). This transcript was left uncloned and, in hindsight, likely corresponds to either isoform 2 or one of the other isoforms described in this article.

Given both the significance of IL12RB1 expression to human health and our observation that this gene is primarily expressed as heretofore uncharacterized isoform, determining the function of isoform 2 as it pertains to IL-12– and IL-23–dependent immunity is the obvious next step for future investigations. Specifically, it should be determined whether isoform 2 competes with isoform 1 for access to IL-12Rβ2/IL-23R, impacts IL-12/IL-23 binding, or regulates Th1/Th17 lineage commitment. Because isoform 2 localizes to an intracellular compartment, it presumably has limited contact with cytokine at the cell surface. We considered the possibility that isoform 2 was secreted, which would allow it to come into contact with extracellular IL-12/IL-23; however, immunoprecipitation attempts from the supernatants of either activated PBMCs or transfected HEK293T cells failed. The homology of isoform 2’s C terminus to the N terminus of STX6 and STX8 (Supplemental Fig. 2C) may prove informative to future functional studies of isoform 2. Namely, the N terminus of STX tethers SNARE proteins (expressed on vesicles) to the protein MUNC18 (64), which is expressed on target membranes; after SNARE/MUNC18 interaction, vesicle/membrane fusion occurs and vesicle contents are released across the target membrane (65, 66). This information, when combined with the observation of Durali et al. (67) that primary lymphocytes can internalize IL-12, suggests that isoform 2 may play a role in either recycling internalized IL-12 back into the extracellular compartment or promoting a yet to be defined intracellular IL-12R system. Testing this hypothesis will be largely dependent on our generating an Ab specific to the C terminus of isoform 2 and examining the consequences of isoform 2 silencing in primary lymphocytes. Also still to be determined is what posttranslational modifications (in addition to glycosylation)
account for the discrepancy between the isoform 1’s predicted molecular mass (~73 kDa based on amino acid composition alone) and its actual mass (~100–120 kDa) as estimated by both us (Fig. 5) and Chua et al. (10).

Regarding changes in relative isoform abundance, immunogen exposure altered the relative abundance of both major and minor IL12RB1 isoforms in leukocytes. In vitro, this was accomplished by culturing PBMCs in the presence of PHA. In vivo, an altered IL12RB1 isoform 1/2 ratio was characteristic of sarcoid lung granulomas. Although the inflammatory stimulus/stimuli responsible for pulmonary sarcoidosis has yet to be determined definitively, candidates include both viral and bacterial organisms (including mycobacteria) (49). We conclude from both our in vitro and in vivo data that extracellular signals induce alternative splicing of IL12RB1. Although alternative splicing of genes is common in human leukocytes, incidents of signal-induced alternative splicing in this population are relatively rare (68). CD44 and PTPRC (i.e., CD45) are two other human immune-associated genes that undergo signal-induced alternative splicing (69, 70). Regarding the mechanism by which mitogen signals the alternative splicing of CD45, splicing of CD45 is dependent on the spliceosome component PSF (71). In the absence of PMA, the kinase GSK3 phosphorylates PSF, hindering the association of PSF with the CD45 pre-mRNA (71). Upon stimulation with PMA, however, GSK3 activity is reduced, allowing PSF to associate with the CD45 pre-mRNA and promote exon exclusion (71). Whether a similar GSK3/PSF-dependent mechanism governs signal-induced IL12RB1 splicing will be a focus of future experiments. Another possibility is the involvement of heterogeneous nuclear ribonucleoproteins (hnRNPs) (68, 72–74). hnRNPs are known to be phosphorylated, sumoylated, and arginine methylated (75–78); PHA possibly signals a modification of hnRNPH that alters its poly(A)-promoting activity through associated poly(A) factors CstF or poly(A) polymerase (72, 73, 79) and consequently decreases the relative success of poly(A) addition to isoform 2. Finally, we cannot rule out the possibility that IL12RB1 promoter usage varies after cell activation. In mice, the cis elements IRE/ISRE and ETS/PU.1 are located 2508-bp upstream of the il12rb1 transcriptional start site (80); these elements promote il12rb1 isoform 1 transcription via their recruitment of IRF3 and PU.1, respectively. Because isoform 2 shares the first nine exons of isoform 1, its transcription is also likely governed by the IRE/ISRE and ETS/PU.1 elements. However, it is possible that additional, as yet unidentified promoters and trans factors promote the activation of the 3′ splice site and polyadenylation site within intron 9, which terminates the transcript with what we refer to as exon 9b (Fig. 2). Most examples of this form of alternative processing involve cis elements within the affected exon and the flanking introns (79, 81, 82).

Because polymorphisms in IL12RB1 associate with a range of human maladies and infectious diseases (16, 22, 54–57), it will be important to determine, in a manner analogous to studies done with CD45 (83, 84), how these same IL12RB1 polymorphisms influence exon inclusion. Furthermore, because modulators of IL-12/IL-23 signaling are being used as approved and experimental therapies for a variety of clinical applications (27–30, 85, 86), including vaccine adjuvants (87), it will also be important to determine whether differences in IL12RB1 isoform prevalence underlie person-to-person variation in IL-12 and IL-23 responses.

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References


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Disclosures

The authors have no financial conflicts of interest.


### SUPPLEMENTAL TABLE I.  
Primers used in this study. Each primer used was designed for a specific molecular application using bioinformatic software (Amplify v3.1.4, Madison, WI). Listed (from left to right) is the name of each primer, its use in this study, its sequence, and its position in the gene amplified. C, cloning; M, multiplex; S, sequencing; Q, quantitative PCR.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Use</th>
<th>Sequence (5’-3’)</th>
<th>Position in Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’IL12RB1</td>
<td>C</td>
<td>CCCACGTACGAGGTGGCTGAACCTCGCAGGTGGCAG</td>
<td>5’ terminus of <em>IL12RB1</em> transcript; introduces restriction sites for cloning</td>
</tr>
<tr>
<td>3’Isoform1</td>
<td>C</td>
<td>TTTAAGCTTGGGCCTACGTAGCCTCGGCGAGTC</td>
<td><em>IL12RB1</em> Isoform 1 3’ terminus; introduces restriction sites for cloning</td>
</tr>
<tr>
<td>3’Isoform2</td>
<td>C</td>
<td>TTTAAGCTTGGGCCTAGCCTCGGCGAGTC</td>
<td><em>IL12RB1</em> Isoform 2 3’ terminus; introduces restriction sites for cloning</td>
</tr>
<tr>
<td>rGAPDH_F</td>
<td>C</td>
<td>CCCTCGAGAAATTGAGCCCGACAGCTCCCGCTT</td>
<td>5’ terminus of <em>GAPDH</em> transcript</td>
</tr>
<tr>
<td>rGAPDH_R</td>
<td>C</td>
<td>TTGAAGCTTGGGAGCAGGTGGTACTTTATTGT</td>
<td>3’ terminus of <em>GAPDH</em> transcript</td>
</tr>
<tr>
<td>Ex9_F</td>
<td>M,S</td>
<td>CTTCAGAAGGGCTGTCAGGGCTG</td>
<td>Exon 9 of <em>IL12RB1</em> (both isoforms)</td>
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<td>Ex9b_R</td>
<td>M,S</td>
<td>CAAATAGCCGGGTATGGTGAGGGAGAT</td>
<td>Exon 9b of <em>IL12RB1</em> Isoform 2</td>
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<tr>
<td>Ex11_R</td>
<td>M,S</td>
<td>GGCAAGATGGTAATTGTAGTAACACT</td>
<td>Exon 11 of <em>IL12RB1</em> Isoform 1</td>
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<tr>
<td>12_F</td>
<td>Q</td>
<td>ACCTCTGGGTAAGAATTGATACTTTATTGT</td>
<td>Exon 12 of <em>IL12RB1</em> Isoform 1</td>
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<tr>
<td>12/13_R</td>
<td>Q</td>
<td>GATGCTCTGACACCTGGT</td>
<td>Exon 12/13 junction of <em>IL12RB1</em> Isoform 1</td>
</tr>
<tr>
<td>9/9b_F</td>
<td>Q</td>
<td>ACACCCACACAGATGGCATGA</td>
<td>Exon 9/9b junction of <em>IL12RB1</em> Isoform 2</td>
</tr>
<tr>
<td>9b_R</td>
<td>Q</td>
<td>TTAGCCCTGGTATGGTGAGGGAGAT</td>
<td>Exon 9b of <em>IL12RB1</em> Isoform 2</td>
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</table>
## SUPPLEMENTAL TABLE II

Clinical information pertaining to the lung tissue donations used in this study. All tissues used in this study were obtained from the National Disease Research Interchange. Listed here is clinical information (that accompanied each frozen specimen) relevant to our analysis of inflammation, cytokine protein expression, and *IL12RB1* isoform mRNA expression in each tissue.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age</th>
<th>Race</th>
<th>Sex</th>
<th>Cause of Death</th>
<th>Medications/Drugs</th>
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</thead>
<tbody>
<tr>
<td>CTRL 1</td>
<td>28</td>
<td>C</td>
<td>M</td>
<td>Cardiac Arrest</td>
<td>Phenytoin, Lamotrigine</td>
</tr>
<tr>
<td>CTRL 2</td>
<td>16</td>
<td>B</td>
<td>M</td>
<td>Anoxic Injury</td>
<td>None</td>
</tr>
<tr>
<td>SARC 1</td>
<td>50</td>
<td>B</td>
<td>M</td>
<td>Cerebral Edema</td>
<td>Prednisone, Cortisone, Benzoylmethylecgonine</td>
</tr>
<tr>
<td>SARC 2</td>
<td>68</td>
<td>C</td>
<td>M</td>
<td>Cardiac Arrest</td>
<td>Prednisone, Atorvastatin, Carvedilol, Tiotropium</td>
</tr>
</tbody>
</table>
A

<table>
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<tr>
<th>I.P. Input (Lysate)</th>
<th>I.P.</th>
</tr>
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<tbody>
<tr>
<td>1 Empty vector</td>
<td>αL12Rβ1</td>
</tr>
<tr>
<td>2 Isoform 1 &amp; 2</td>
<td>IgG</td>
</tr>
<tr>
<td>3 Isoform 1 &amp; 2</td>
<td>αL12Rβ1</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>I.P.</th>
<th>-PNGase</th>
<th>+PNGase</th>
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<tbody>
<tr>
<td>①</td>
<td>◯</td>
<td>◯</td>
</tr>
<tr>
<td>②</td>
<td>◯</td>
<td>◯</td>
</tr>
<tr>
<td>③</td>
<td>◯</td>
<td>◯</td>
</tr>
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C

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
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<tr>
<td>VKORC1-2</td>
<td>SGTISAHCNLRPLGSSNSPSAASRAVAGITGCMCHRARLIFVF</td>
</tr>
<tr>
<td>SLC48A1</td>
<td>--I-ASAHCNLRLPGSSNSPSAASQVAGITGICHXRRQIFVF</td>
</tr>
<tr>
<td>IL12RB1-2</td>
<td>DGMISAHCNRLPDGRDPSAASRVAGITGIChHLRILYF</td>
</tr>
<tr>
<td>ADRA1A</td>
<td>SGMLASHCNRLPGSRDPSAASAOAAGTGMCHQ--</td>
</tr>
<tr>
<td>STX6</td>
<td>NGAIASAHCNLHLPSSNSPSAASQVAGILGMRHQALQILYF</td>
</tr>
<tr>
<td>STX8</td>
<td>NGVISAHCNLRLGGSSPSAASOVAIGTGTRHAWLIF--</td>
</tr>
</tbody>
</table>

D

α-Protein Disulfide Isomerase (PDI)
SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. Quantitation of IL12RB1 Isoform copy number and mRNA half-life measurements. IL12RB1 Isoforms 1 and 2 were cloned into pUC19 and used as standards of defined copy number. For each real-time assay performed, serial dilutions of these standards were made to generate amplification curves for $10^7$, $10^5$, $10^3$, $10^2$ cDNA copies as well as a no template control. (A) Representative real-time fluorescence curves of IL12RB1 Isoform 1 standards amplified with primers 12_F and 12/13_R in the presence of SYBR Green. (B) Representative real-time fluorescence curves of IL12RB1 Isoform 2 standards amplified with primers 9/9b_F and 9b_R in the presence of SYBR Green. (C) Post-amplification melting curve analysis of PBMC cDNA (from either +/- PHA conditions) amplified with either primers 12_F and 12/13_R (for Isoform 1) or 9/9b_F and 9b_R (for Isoform 2). Data in (A-C) are representative of the real time data generated for each assay performed. (D-L) PBMCs collected from healthy individuals were cultured in the presence or absence of PHA. Four hours after establishing each culture, either actinomycin D (+ActD) or vehicle control (-ActD) were added; after select periods of time, cells from each culture were collected and the mRNA copy number of both Isoform 1 and Isoform 2 determined by real time PCR. Shown in (D-K) are the data from two separate trials representing PBMCs from two different individuals (Donors 12 and 13). Shown in (D-E, H-I) are Donor 12’s expression of (D-E) Isoform 1 or (H-I) Isoform 2 in either the (E,I) presence or (D,H) absence of PHA; similarly, (F-G, J-K) shows Donor 13’s expression of either (F-G) Isoform 1 or (J-K) Isoform 2 in either the (G,K) presence or (F,J) absence of PHA. For all data collected from ActD-treated cells, non-linear regression was used to determine the half-life of each Isoform; shown within each graph are the calculated $\frac{1}{2}$-life and R$^2$ values for
each isoform under each condition. The cumulative results of both individuals are shown in (L), with each bar representing the mean (±SD) half-life for both isoforms under each condition (three separate cultures per condition per time point per donor).

SUPPLEMENTAL FIGURE 2. Analysis of IL12RB1 Isoform glycosylation and homology. (A-B) Lysates were prepared from cells (1) transfected with empty vector or (2-3) co-transfected with Isoform 1 and Isoform 2 expressing vectors. Cell numbers were normalized prior to lysis so that an equivalent amount of lysate was subjected to downstream i.p. analysis. Shown in (A) is a GAPDH protein blot of input lysates prior to i.p. (B) Immunoprecipitates were prepared from either (1) empty transfectant lysates using αIL12Rβ1, (2) isoform transfectants with nonspecific IgG, or (3) isoform transfectants using αIL12Rβ1. Precipitated proteins were then linearized and either left untreated (-PNGase) or treated with PNGase (+PNGase); untreated and treated samples were subsequently analyzed by Western analysis. Molecular weights of all products were estimated using simultaneously run size standards. Results in (A-B) are representative of two separate experiments. (C) The results of a standard protein BLAST search of the NCBI human protein database using the C-terminal amino acid sequence of IL12RB1 Isoform 2. The blasted sequence of Isoform 2 is indicated in the third row from the top and is shown (from top to bottom) in alignment with proteins VKORC1, SLC48A1, ADRA1A, STX6 and STX8. (D) BSC40 cells were stained for ER-specific protein disulfide isomerase (PDI). Shown is a representative micrograph of the punctate ER-staining as observed in all BSC40 cells regardless of transfection with either Isoform 1 or Isoform 2.