A Soluble Form of IL-27Rα Is a Natural IL-27 Antagonist

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A Soluble Form of IL-27Rα Is a Natural IL-27 Antagonist

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IL-27 is a cytokine of the IL-12 family that plays a key role in the regulation of inflammatory and T cell responses. Its receptor is composed of IL-27Rα and gp130 and activates the STAT pathway. We show in this study, using an ELISA that we developed, that a naturally occurring soluble form of IL-27Rα (sIL-27Rα) is produced by human activated CD4+ and CD8+ T cells, B cells, myeloid cells, and various cell lines. sIL-27Rα is present at a mean concentration of 10,344 ± 1,274 pg/ml in the sera from healthy individuals. Biochemical studies showed that sIL-27Rα is released as two N-glycosylated variants of ~90 and ~70 kDa. In IL-27Rα–transfected COS7 cells, primary cells, and cell lines, production of sIL-27Rα is inhibited by the metalloprotease inhibitors GM6001 and TAPI-0. Importantly, natural sIL-27Rα binds rIL-27, inhibits IL-27 binding to its cell surface receptor, and is a potent inhibitor of IL-27 signaling, as shown by its ability to specifically block IL-27–mediated STAT activation, at low molar excess over IL-27. Also, we found that serum levels of sIL-27Rα were elevated in patients with Crohn’s disease, a Th1-mediated disease. These findings suggest that sIL-27Rα may play important immunoregulatory functions under normal and pathological conditions.


Interleukin-27 is a heterodimeric cytokine of the IL-12 family composed of two subunits, EBV-induced gene 3 (EBI3) and p28 (1, 2). In humans, it is expressed at high levels by monocytes/macrophages and dendritic cells upon activation and by placental trophoblasts (2–4). IL-27 displays broad immunological functions and plays a key role in the regulation of inflammatory and T cell responses. Although it was initially described as a factor promoting the initiation of Th1 responses, it was later found to play a major T cell–suppressive function by limiting Th1 responses, inhibiting Th2 and Th17 cell differentiation, and regulating the development of Th1 and other T regulatory cell populations. In addition to its role as an immunoregulator, IL-27 also regulates angiogenesis, hematopoiesis, and osteoclastogenesis (reviewed in Refs. 5, 6).

IL-27 signals through a heterodimeric receptor composed of two chains, IL-27Rα (formerly called TCCR or WSX-1) and gp130, and activates the STAT pathway, predominantly STAT1 and STAT3 (7). Cytokine signaling and activity can be modulated by cytokine receptors can exist in both membrane and soluble forms (11). These latter can bind to cytokines and modulate their activity in different ways. They can either play an antagonistic role by preventing cytokine association with the membrane form of the receptor, or alternatively play a positive role by extending the half-life of the cytokine and potentiating its activity as described for IL-15Rα or IL-7Rα (12, 13). Gp130 naturally exists as a soluble form that inhibits IL-6/IL-6R complex signaling (14) but does not inhibit IL-27 signaling (15). Mouse neuronal cells express an alternatively spliced IL-27Rα isoform of 33 kDa, lacking exons 7–14, that encodes part of the extracellular domain (16). This truncated IL-27Rα isoform was reported, not to act as a dominant negative, but to associate with CNTF-R and gp130 similarly to full-length membrane IL-27Rα, to form a tripartite functional receptor for humanin, a neurotrophic peptide (16, 17). In this study, we investigated whether human IL-27Rα can be detected as a soluble form that could modulate IL-27 activity. We found that soluble forms of IL-27Rα (sIL-27Rα) are spontaneously released from cells as N-glycosylated proteins of 70/90 kDa, through proteolytic cleavage by metalloproteases. sIL-27Rα can bind rIL-27 in vitro, is complexed with IL-27 in vivo, and inhibits IL-27 signaling. It was present at detectable levels in the sera from healthy individuals and was upregulated in the sera of patients with Crohn’s disease (CD).

Materials and Methods

Human cells and sera

Cos7, Hodgkin (KMH2), and Burkitt (BL2) B lymphoma cell lines were grown in DMEM (COST) or RPMI 1640 medium (KMH2 and BL2) supplemented with 10–15% FBS, 1% glutamine, and 1% antibiotics (Life Technologies) (complete medium). COS7 cells were transfected by electroporation (Bio-Rad) with pcDNA3 vector, empty or encoding full-length C-terminal V5-6His–tagged IL-27Rα (gift of H. Gascan, Angers, France), and GFP expression vector (Clontech) and incubated for various times in complete DMEM or Optimem medium.

Human CD4+ T cells (purity >97%) or CD8+ T cells (purity >95%) were purified by negative magnetic cell isolation (Miltenyi Biotec) from PBMC of adult healthy donors (Etablissement François du Sang, Paris, France), as previously described (18). Purified T cells were cultured (1 × 10^6/ml) for 3–7 d in complete RPMI 1640 medium in the presence of IL-2 (20 U/ml; Roche), and either PHA (4 μg/ml; Sigma-Aldrich) or beads

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Abbreviations used in this article: ADAM, a disintegrin and metalloproteinase; CD, Crohn’s disease; EBI3, EBV-induced gene 3; MMP, matrix metalloproteinase; sIL-27Rα, soluble form of IL-27Rα.

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(ratio 1 bead per 2 cells) coated with CD2/CD3/CD28 Abs (T cell activation/expansion kit; Miltenyi Biotec). Human tonsillar B cells (purity >99%) were purified by magnetic depletion using CD4 microbeads (Miltenyi Biotec) and cultured (2 × 105/ml) for 2–4 d in complete RPMI 1640 medium in the presence of CD40 Ab (0.5 µg/ml; R&D Systems), as described (19). Monocytes (purity >97%) were purified from PBMC by positive selection using CD14 microbeads (Miltenyi Biotec) and cultured (3 × 105/ml) for 3 d in the presence of LPS (100 ng/ml; Invivogen). Monocyte-derived mature dendritic cells were generated from human monocytes cultured for 6 d in the presence of GM-CSF, followed by a 2-d maturation with TNF-α (20). Two cell-permeable inhibitors of metalloproteases, GM6001 or TAPI-0 (Merck Chemicals), were used at concentrations that were not toxic to cells. Because inhibitors were reconstituted in DMSO, DMSO was added at a similar dilution in the control cultures.

Normal human sera were obtained from healthy, nonpregnant volunteers (n = 28). Sera (n = 43) from six women with normal pregnancies, collected at various times during pregnancy (six to eight sera per individual) for serological diagnosis (Béclère Hospital), were used in this study. Sera from pediatric patients (n = 52) with active CD were collected for diagnosis purpose (Necker Hospital) before initiation of treatment with infliximab. Studies were conducted in accordance with the Declaration of Helsinki and were approved by the hospital ethics committee.

ELISA

To detect sIL-27Rα, a sandwich ELISA was developed by using mouse anti-human IL-27Rα mAb (191106; R&D Systems) as coating Ab and goat biotinylated polyclonal anti-human IL-27Rα Ab (R&D Systems) as detection Ab. Both Abs were raised against the extracellular domain of IL-27Rα. Binding of the detection Ab was detected using streptavidin HRP conjugate (GE Healthcare) and tetramethylbenzidine (R&D Systems) as a substrate. Human rIL-27-Fc fusion protein (R&D Systems) was used as a control. Because the m.w. of IL-27-Fc is higher than that of sIL-27Rα, values for sIL-27Rα, expressed in pg/ml, were corrected based on the molarity of each protein. The limit of sensitivity was 30 pg/ml sIL-27Rα. This ELISA detects sIL-27Rα, bound or not to IL-27. In some cases, culture supernatants were concentrated using Amicon Ultra centrifugal filter device (Millipore) before ELISA. To investigate whether sIL-27Rα could form complexes with IL-27 in the sera, we developed an ELISA by using mouse anti-human IL-27Rα mAb or a control isotype mAb as coating Abs, and goat biotinylated polyclonal anti-human IL-27 Ab (R&D Systems) as detection Ab. Binding of the detection Ab was detected as indicated above. Serum levels of IL-27 were detected using a commercial kit (R&D Systems) with some modifications of the procedure.

Immunoprecipitation, Western blotting, and N-glycanase treatment

sIL-27Rα was immunoprecipitated from either the lysate of transfected COS7 cells (lysate buffer: 1% Nonidet P-40, 50 mM Tris [pH 7.4], 150 mM NaCl, 3% glycerol, 1.5 mM EDTA), 10- to 30-fold concentrated KMH2 culture supernatant, or normal human sera. All samples were supplemented with protease inhibitors (1 mM PMSF, 1 µg/ml pepstatin, and 1 µg/ml leupeptin) and precleared with protein G-Sepharose beads (GE Healthcare) before incubation with Abs. sIL-27Rα was immunoprecipitated using either mouse mAb or goat polyclonal anti-IL-27Rα Ab (R&D Systems), in parallel to control Abs (mouse MOPC141 mAb or purified goat IgG; Sigma Aldrich), followed by incubation with protein G-Sepharose beads, or mouse anti-human IL-27Rα mAb covalently linked to agarose beads using AminoLink Plus immobilization kit (Thermo Scientific). After washes in lysis buffer, immunoprecipitates were eluted, separated by SDS-PAGE, and transferred to nitrocellulose membranes. Primary Abs for immunoblotting included goat biotinylated polyclonal anti-human IL-27Rα Ab, mouse V5 mAb (Invitrogen), and 2G4H6 anti-EBI3 mAb (3). Binding of primary Abs was detected using HRP-conjugated secondary reagents and chemiluminescence reagents (Pierce). When indicated, immunoprecipitates were submitted to N-glycanase treatment with PNGase F set (Sigma-Aldrich) before analysis by SDS-PAGE and immunoblotting.

Purification of natural sIL-27Rα

Natural sIL-27Rα was purified from the culture supernatant (450 ml) of KMH2 cells (10³ cells/ml) incubated for 48 h in X-Vivo 15 media (Lonza). The culture supernatant was concentrated by 45-fold using Amicon Ultra centrifugal filter units and supplemented with protease inhibitors before overnight incubation with anti-IL-27Rα beads. Beads were washed extensively, and bound proteins were recovered by acidic elution. After neutralization and addition of BSA as a carrier, the eluate was dialyzed against PBS and concentrated (Microcon centrifugal filter device; Millipore). Concentration of purified sIL-27Rα was determined by ELISA.

STAT activation assay

After overnight starvation in serum-free media, BL2 cells were incubated in 48-well plate (10⁶ cells per 100 µl) in RPMI 1640 medium supplemented with 0.5% BSA (RPMI/BSA media). Prior to addition to cells, IL-27 (6His-tagged covalently linked EBI3-p28 fusion protein; R&D Systems) was incubated for 15 min at 37°C in 100 µl RPMI/BSA media containing various concentrations of rIL-27-Fc chimera, recombinant gp130-Fc chimera (R&D Systems), or purified natural sIL-27Rα. The mixture was then added to BL2 cells for 15 min at 37°C and 5% CO2. The reaction was stopped, and cells were lysed in ice-cold 1% Nonidet P-40 lysis buffer supplemented with protease and phosphatase inhibitors. Phosphorylated and total STAT1 were immunoprecipitated using anti-EBI3-p28 fusion protein; R&D Systems) was incubated with 0.5% BSA (RPMI/BSA media). Prior to addition to cells, IL-27 (6His-tagged covalently linked EBI3-p28 fusion protein; R&D Systems) was incubated for 15 min at 37°C in 100 µl RPMI/BSA media containing various concentrations of rIL-27-Fc chimera, recombinant gp130-Fc chimera (R&D Systems), or purified natural sIL-27Rα. The mixture was then added to BL2 cells for 15 min at 37°C and 5% CO2. The reaction was stopped, and cells were lysed in ice-cold 1% Nonidet P-40 lysis buffer supplemented with protease and phosphatase inhibitors. Phosphorylated and total STAT1 were detected by immunoblot using rabbit polyclonal Abs from Cell Signaling Technology. Quantification of STAT1 inhibition was determined by densitometric analysis of autoradiography bands using ImageJ software.

FACS analysis

Cells were first saturated in PBS containing 20% normal human serum. Cell surface IL-27Rα was detected using PE-conjugated anti-IL-27Rα mAb

FIGURE 1. sIL-27Rα is released from different primary human cell types. (A) Purified CD4+ or CD8+ T cells were cultured in the presence of IL-2 and either PHA or CD2/CD3/CD28-coated beads. After the indicated time, cell culture supernatants were collected and tested by sIL-27Rα ELISA. Cell culture supernatants from CD40-stimulated purified B cells (B) or LPS-stimulated purified monocytes (C) were tested by sIL-27Rα ELISA before concentration (1×) or after a 6- or 8-fold concentration (6× and 8×). Data shown in (A)–(C) represent the mean value ± SEM of three to four experiments performed with different donors. (D) sIL-27Rα levels were measured by ELISA in the culture supernatant from TNF-α-matured dendritic cells (DC) derived from nine different donors. ND, not detected.

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(R&D Systems), in parallel to an IgG2b-PE control mAb. Cells were analyzed on FACSCalibur, and data were analyzed using FlowJo software. For binding experiments, BL2 cells were saturated in PBS containing 5% veinglobulins and incubated for 40 min on ice with 6His-tagged rIL-27 that had been preincubated or not with natural purified sIL-27Ra for 15 min at 37˚C. Binding of IL-27 was detected with an anti-hexahistidine mAb (ThermoScientific), followed by PE-conjugated F(ab‘)2 anti-mouse IgG (eBioscience).

Statistical analysis
Statistical significance was determined using Student’s paired t test (*p < 0.05, **p < 0.01, ***p < 0.001) or unpaired t test (p values indicated on the graphs). Pearson’s test was used for correlation. Pearson correlation coefficients (r² values) and p values are indicated on the graphs.

Results
A soluble form of IL-27Ra is detected in the cell culture supernatant from various primary human cell types
To investigate whether IL-27Ra exists as a soluble form, we first developed a sandwich ELISA, as described in Materials and Methods, and screened supernatants from various human primary cell types. Because activated human CD4+ T cells express high levels of IL-27Ra mRNA and protein (2, 18), we first tested these cells for sIL-27Ra production. Purified CD4+ T cells were stimulated for 3–7 d with PHA or beads coated with CD2/CD3/CD28 Abs in the presence of IL-2. In both conditions of stimulation, detectable levels of sIL-27Ra were measured in the culture supernatant after 3 d of stimulation, and these levels increased over the activation period (up to 339 pg/ml on average at day 7) (Fig. 1A, left). Comparable levels of sIL-27Ra were detected in the culture supernatant from CD8+ T cells activated with CD2/CD3/CD28 beads (mean 401 ± 13 pg/ml at day 7; Fig. 1A, right). Human tonsillar B cells stimulated with CD40 Ab for 2–4 d or CD14+ monocytes stimulated with LPS for 3 d also released sIL-27Ra, albeit at lower levels (Fig. 1B, 1C). Mature monocyte-derived dendritic cells that constitutionally express high amounts of IL-27Ra mRNA (7) produced variable amounts of sIL-27Ra, constitutively (Fig. 1D).

Screening of supernatants from human cell lines revealed that various cell lines, including B and T cell lines and melanoma cell lines, released sIL-27Ra. Of these, the KMH2 lymphoma cell line contained among the highest levels of sIL-27Ra (>250 pg/ml).

Detection of sIL-27Ra in sera from healthy human subjects
Next, we tested sera from healthy individuals (n = 28) for the presence of sIL-27Ra. As shown in Fig. 2A, sIL-27Ra was detected in all samples, at concentrations ranging from 3,108 to 31,757 pg/ml (mean 10,344 ± 1,274 pg/ml). Serum levels of sIL-27Ra were compared with those of IL-27, as measured by IL-27 ELISA. No significant correlation was observed between serum levels of IL-27 and sIL-27Ra (Fig. 2B).

In previous studies (3, 4), we had shown that levels of EB13, released by placental trophoblast cells, are strongly upregulated in the sera from pregnant women and gradually increase with gestational age. Determination of sIL-27Ra levels in sera from six pregnant women collected at various times during pregnancy (43 sera, from 9 to 40 wk of pregnancy) showed that serum levels of sIL-27Ra did not increase during pregnancy (Fig. 2C), in contrast to the situation previously observed for EB13.

Soluble forms of cytokine receptors can be complexes with the cytokine in the serum (21). To investigate whether sIL-27Ra forms complexes with IL-27 in the serum, we developed an ELISA by using an anti–IL-27Ra mAb as a capture Ab and a biotinylated anti–IL-27 Ab as a detection Ab. As positive and negative controls, an anti–IL-27 Ab or a control isotype mAb was used, respectively, as capture Ab. Sera from eight healthy individuals (four nonpregnant and four pregnant individuals) were tested in this ELISA. As shown in Fig. 2D, increased IL-27 binding over the control was observed when anti–IL-27Ra mAb was used as a capture Ab, indicating that a fraction of sIL-27Ra is associated with IL-27 in the serum.

Biochemical analysis of sIL-27Ra
We further characterized the soluble form of IL-27Ra by using immunoprecipitation and Western blot. Mature human full-length IL-27Ra contains a 482-aa extracellular domain comprising seven potential N-glycosylation sites, a 26-aa transmembrane domain, and a 96-aa intracellular domain, resulting in a transmembrane protein with a predicted molecular mass of 66 kDa (22).

Western blot analysis of the cell lysate of COS7 cells transfected with a plasmid encoding a C-terminal V5-6His-tagged full-length IL-27Ra, showed that cell-associated IL-27Ra is detected as a doublet of ∼115 and ∼95 kDa (Fig. 3A). These isoforms corresponded to two different N-glycosylated variants, as immunoprecipitation of IL-27RaV5His and removal of N-linked sugars by treatment of the immunoprecipitate with N-glycanase resulted in a single band of ∼80 kDa (Fig. 3B). This apparent molecular mass is higher than the predicted one (68 kDa) and suggests that the specific amino acid composition of human IL-27Ra results in aberrant migration on SDS-PAGE.
Immunoprecipitation of sIL-27Rα with goat polyclonal or mouse anti–IL-27Rα mAbs from the concentrated supernatant of KMH2 cells (Fig. 3C, left) or from normal human serum (Fig. 3C, right) resulted in each case in detection of two diffuse bands of ~90 and ~70 kDa, as shown by anti–IL-27Rα immunoblot. Treatment of anti–IL-27Rα immunoprecipitate with N-glycanase reduced the apparent molecular mass of sIL-27Rα to a single band of ~60 kDa (Fig. 3D), slightly above the predicted size of the extracellular domain of IL-27Rα (52.2 kDa).

Role of metalloproteases in sIL-27Rα production

Soluble forms of cytokine receptors can be generated by alternative splicing or by proteolytic cleavage and shedding of the cell surface cytokine receptor. Because former Northern blot analyses of human IL-27Rα (22, 23) did not evidence alternative transcripts that could code for the entire extracellular domain of IL-27Rα and no alternative splicing was predicted in ASPIc database, we investigated a potential role of metalloproteases in the generation of sIL-27Rα.

Transient transfection of COS7 cells with full-length IL-27RαV5His resulted in cell surface expression of the protein, as expected (Fig. 4A), but also in the production of sIL-27Rα that accumulated over time in the culture medium, as detected by Western blot (Fig. 4B) or ELISA (Fig. 4C). Production of this soluble form was strongly reduced in the presence of two chemical inhibitors of metalloproteases, GM6001 or TAPI-0 (respectively >80 and 90% inhibition on average, at 48 h) (Fig. 4C). This decrease was specific to the soluble form given that no significant decrease of full-length IL-27RαV5His expression was detected by immunoblot analysis of the cell lysate (Fig. 4D).

Similarly, incubation of KMH2 cells (Fig. 4E) or CD2/CD3/CD28-activated CD4+ T cells (Fig. 4F) with GM6001 or TAPI-0 significantly decreased sIL-27Rα production, in a dose-dependent manner (~90% inhibition in KMH2 cells and >60% inhibition in CD4+ T cells, at the highest concentrations tested), without affecting cell proliferation. In transfected COS7 cells or in KMH2 cells, inhibition of IL-27Rα cleavage did not substantially increase its cell surface level (data not shown), indicating that only a small proportion of IL-27Rα was spontaneously shed from the cell surface, as commonly observed for cytokine receptors (24). However, in activated CD4+ T cells, a small but significant increase of surface IL-27Rα was observed in cells treated with GM6001 or TAPI-0 (Fig. 4G, 4H).

sIL-27Rα binds rIL-27 and antagonizes IL-27 signaling

Former coimmunoprecipitation studies performed with a recombinant soluble extracellular form of IL-27Rα have shown that it could bind EB13/p28 heterodimer, but not individual subunits (2). In addition, a recombinant soluble dimeric IL-27R-Fc fusion protein was reported to act as an inhibitor of IL-27 activity, whereas recombinant soluble gp130-Fc fusion protein had no effect (15, 25). Therefore, we verified that natural and presumably monomeric sIL-27Rα behaves similarly to the recombinant proteins and could bind IL-27 and inhibit its biological activity.

Immunoprecipitation of natural sIL-27Rα from concentrated supernatant of KMH2 cells in the presence of rIL-27 (100 ng per immunoprecipitate) resulted in coprecipitation of rIL-27, but not of free EB13 naturally present at high levels in KMH2 supernatant (500 ng per immunoprecipitate) (Fig. 5A), consistent with the findings described above.

To investigate a potential inhibiting role of natural sIL-27Rα, we took advantage of the BL2 cell line that responds to low concentrations of IL-27. Titration experiments revealed that 2 ng/ml (33 pM) rIL-27 induced significant activation of STAT1, but not other STATs, in these cells. In this assay, preincubation of rIL-27 with IL-27R-Fc prior to cell stimulation resulted in a marked reduction of STAT1 activation in a dose-dependent manner, whereas preincubation with gp130-Fc had no effect, even at high doses, as expected (Fig. 5B). When used at higher concentrations (5 and 10 ng/ml), rIL-27 also activated STAT3 in BL2 cells. Interestingly, not only STAT1, but also STAT3 activation was inhibited when IL-27 was preincubated with either low (10 ng/ml) or high (200 ng/ml) doses of sIL-27Rα-Fc (Supplemental Fig. 1). This latter finding indicates that IL-27 complexed with its soluble receptor does not activate the gp130/STAT3 axis by trans-signaling, as has been described for IL-6/IL-6Rα complex (14).

Importantly, preincubation of rIL-27 with almost equimolar doses of purified natural sIL-27Rα (2 ng/ml, 22–29 pM) induced a 50% inhibition of STAT1 activation, whereas a complete inhibition was observed at 10 ng/ml (3- to 4-fold molar excess).
This inhibition was specific to IL-27, because preincubation of another STAT1-inducing cytokine, IFN-γ, with recombinant or natural sIL-27Ra did not inhibit STAT activation (Fig. 5D). Inhibition of IL-27 signaling by sIL-27Ra was most likely due to a competition effect and inhibition of IL-27 binding to its cell surface receptor. Flow cytometry experiments confirmed that preincubation of rIL-27 with natural sIL-27Ra inhibited IL-27 binding to BL2 cells (Fig. 5E). Taken together, these data indicate that natural sIL-27Ra is a potent and specific antagonist of IL-27.

Serum levels of sIL-27Ra are elevated in CD patients

Next, we investigated whether altered serum levels of sIL-27Ra could be observed under pathological conditions. We focused on CD, a Th1-associated granulomatous inflammatory bowel disease characterized by the accumulation of activated myeloid and CD4⁺ T cells at the sites of disease. We previously showed that IL-27 is produced in situ by epithelioid granulomatous cells of CD patients (26). In addition, genetic studies identified IL-27 p28 as a candidate gene for CD susceptibility and suggested that dysregulated IL-27 expression could contribute to the pathology (27–29).

Analysis of sera from CD patients with active disease (n = 52) showed that serum levels of sIL-27Ra were significantly higher than those of healthy controls (Fig. 6A). In certain patients, values were up to 23-fold higher than the average value observed in controls. Similarly, serum IL-27 levels were higher (by 10-fold...
on average) in CD patients than in controls (Fig. 6B). Overall, a positive correlation was observed between serum levels of sIL-27Rα and IL-27 in CD patients (Fig. 6C). However, not all patients had a concomitant increase of IL-27 and sIL-27Rα levels, and the molar ratio between serum levels of IL-27 and sIL-27Rα was highly variable among patients, ranging from 0.01 to 20.6 (Fig. 6D).

Discussion

IL-27 is a potent immunoregulator that has drawn interest as a tool or a target for immunotherapy. Therefore, understanding the mechanisms by which IL-27 activity can be modulated is important. In this study, to our knowledge, we report the first evidence that the IL-27Rα component of the IL-27R complex exists naturally as a soluble form that antagonizes IL-27 activity.

We show that sIL-27Rα is spontaneously released from different human lymphoid and myeloid primary cell types and from cell lines and is present in the serum of healthy individuals. Immunoblot analysis of sIL-27Rα immunoprecipitates from culture supernatants or from human sera indicated that sIL-27Rα is produced as glycosylated proteins of ~90 and ~70 kDa that result from differential N-glycosylation. We demonstrated in in vitro experiments that natural sIL-27Rα can bind IL-27 and inhibits its signaling. Because preparations of purified sIL-27Rα contained both isoforms of sIL-27Rα, we do not know whether both isoforms are equally efficient at inhibiting IL-27 signaling. Of note, only a few fold molar excess of sIL-27Rα over IL-27 was sufficient to block IL-27 signaling. It should be noted that all in vitro experiments were performed using recombinant covalently linked EBI3-p28 heterodimer. Although we cannot exclude that the affinity of rIL-27 for sIL-27Rα might be different from that of natural IL-27, our results suggest that sIL-27Rα, at concentrations found in the sera of healthy individuals, could inhibit IL-27 activity.

IL-27 and its receptor are produced by different cell types. Whereas in normal conditions IL-27 is predominantly expressed by activated cells of the myeloid lineage, IL-27Rα is constitutively expressed by numerous immune on nonimmune cells. This non-overlapping spectrum of production may account for the non-correlated levels of IL-27Rα and IL-27 detected in sera from healthy individuals. In pregnant women, release of EBI3 and IL-27 by placental cells into maternal blood was not associated with increased levels of sIL-27Rα in the serum, suggesting that pla-
central cells are not a major source of sIL-27Rα production. Also, we showed by ELISA that part of IL-27 is complexed with sIL-27Rα in human sera. Of note, the Abs used to detect IL-27 in this ELISA are polyclonal Abs recognizing both EBI3 and p28, and therefore able to detect not only IL-27 heterodimer, but also free subunits. However, because IL-27Rα does not bind individual subunits, the association we detected in serum between IL-27 and sIL-27Rα is unlikely to be due to direct interaction between sIL-27Rα and free EBI3 or p28. Nevertheless, it remains unknown whether this association results from direct interaction between IL-27 and sIL-27Rα or from an indirect association of IL-27 and sIL-27Rα with other serum proteins.

Two different mechanisms have been described for the generation of soluble cytokine receptors, as follows: synthesis of an alternatively spliced mRNA or proteolytic cleavage of the membrane-anchored receptor. Several lines of evidence support the involvement of the second mechanism in the generation of sIL-27Rα. First, there is to date no evidence for a spliced isoform of human IL-27Rα coding the entire extracellular domain. Second, all cell lines and primary cell types (T, B, and myeloid cells) that produce sIL-27Rα in the culture supernatant also exhibit membrane expression of IL-27Rα. Third, transfection of COS7, which does not express endogenous IL-27Rα, with full-length IL-27Rα cDNA resulted in sIL-27Rα production in the culture supernatant. Last, in transfected COS7, KMH2 cells, and activated CD4+ T cells, production of sIL-27Rα was blocked by the addition of two hydroxamate-based metalloprotease inhibitors, GM6001 and TAPI-0. The fact that metalloproteases are involved in sIL-27Rα production does not exclude, however, that sIL-27Rα could be generated by alternative splicing in other cell types and/or under different conditions.

The specific metalloproteases involved in the cleavage of surface IL-27Rα remain to be identified. Previous studies have shown that a given receptor is often cleaved by more than one metalloprotease (11). GM6001 is a broad spectrum inhibitor of matrix metalloproteases (MMP) that inhibits numerous MMPs and members of the ADAM (a disintegrin and metalloproteinase) family. Similarly, TAPI-0, a potent inhibitor of ADAM-17, inhibits other ADAM family members and several MMPs. Therefore, further investigation will be required to uncover the specific sheddase(s), ADAM-17 or others, responsible for IL-27Rα cleavage.

IL-27Rα shedding leads to release of an IL-27 antagonist and may also, by decreasing levels of cell surface IL-27Rα, lower subsequent IL-27 response in producing cells. Production of sIL-27Rα by activated cells can therefore constitute a negative feedback mechanism to dampen IL-27 effects in vivo. sIL-27Rα may play a beneficial role in pathological settings characterized by excessive IL-27 production and response linked to the severity of the pathology such as psoriasis (30, 31). Conversely, in pathologies associated with defective IL-27 production/response such as asthma (32, 33), production of sIL-27Rα by activated T cells may worsen the pathology.

Increased production of IL-27 has been observed in sarcoidosis, tuberculosis, CD, visceral leishmaniasis, psoriasis, rheumatoid arthritis, and cancer (26, 34–37). The exact role of IL-27 in CD remains to be elucidated. IL-27 can both promote and/or limit excessive Th1 response and inhibit Th17 cell differentiation, and, in murine studies of colitis, IL-27 has been associated with either promotion or attenuation of the disease, depending on the model (38–41). Interestingly, human genetics studies identified polymorphisms and epigenetic modifications in p28 locus, suggesting that dysregulated p28/IL-27 production may contribute to CD pathology (27–29). The elevated levels of IL-27 that we observed in the sera of CD patients most likely result from high levels of IL-27 production by epithelioid cells of granuloma. With respect to sIL-27Rα, both activated T cells and epithelioid cells could contribute to its production. Further studies would be necessary to determine whether variable ratios between serum IL-27 and sIL-27Rα levels may be indicative of different clinical features and outcome of disease.

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Disclosures
The authors have no financial conflicts of interest.

References

FIGURE 6. Analysis of sIL-27Rα and IL-27 levels in the sera of CD patients. Serum levels of sIL-27Rα (A) and IL-27 (B) were determined by ELISA in 52 CD patients and 28 healthy individuals. The correlation between sIL-27 and IL-27 serum levels (C) and the molar ratio between IL-27 and sIL-27Rα serum values (D) are shown. Although a logarithmic scale is used in (C), linear regression was calculated using the actual values.
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