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Identification of the IL-17 Receptor Related Molecule IL-17RC as the Receptor for IL-17F

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Abstract

The proinflammatory cytokines IL-17A and IL-17F have a high degree of sequence similarity and share many biological properties. Both have been implicated as factors contributing to the progression of inflammatory and autoimmune diseases. Moreover, reagents that neutralize IL-17A significantly ameliorate disease severity in several mouse models of human disease. IL-17A mediates its effects through interaction with its cognate receptor, the IL-17 receptor (IL-17RA). We report here that the IL-17RA-related molecule, IL-17RC is the receptor for IL-17F. Notably, both IL-17A and IL-17F bind to IL-17RC with high affinity, leading us to suggest that a soluble form of this molecule may serve as an effective therapeutic antagonist of IL-17A and IL-17F. We generated a soluble form of IL-17RC and demonstrate that it effectively blocks binding of both IL-17A and IL-17F, and that it inhibits signaling in response to these cytokines. Collectively, our work indicates that IL-17RC functions as a receptor for both IL-17A and IL-17F and that a soluble version of this protein should be an effective antagonist of IL-17A and IL-17F mediated inflammatory diseases.

Six members of the IL-17 family have been identified based on their similarity to its founding member, originally identified as IL-17, now designated IL-17A (1,2). The other members of the family are IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F (3–5). Among this group, IL-17A and IL-17F are by far the most similar to one another sharing 55% identity (4). In addition to their sequence similarity, both of these cytokines are produced by activated, memory $CD4^+$ T cells (6–10). Both have been similarly implicated as contributing agents to progression and pathology of a variety of inflammatory and autoimmune diseases in humans and in mouse models of human diseases (11–14). In fact, IL-17A, and to a lesser extent IL-17F, have been implicated as effector cytokines that trigger inflammatory responses and thereby

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contribute to a number of autoinflammatory diseases including multiple sclerosis (7,15,16), rheumatoid arthritis (3,8,12,14,17–21), and inflammatory bowel diseases (22,23). The $CD4^+$ T cells that produce IL-17A and IL-17F have recently been shown to be induced by the combined effects of TGFβ and IL-6 (24,25), and to be further influenced in ways that are incompletely understood by IL-23 (26). These cells have been postulated to represent a unique developmental pathway in helper T cell development akin to the well-established Th1-Th2 development paradigm. This lineage has been termed Th_{17} and the number of these cells clearly correlates with disease progression and severity in mouse models of human autoimmune diseases (6–8,15). Although the involvement of IL-17A and IL-17F in inflammatory diseases seems clear, the target cells for these cytokines have not been identified due in part to the fact that a receptor for IL-17F has not been identified.

At the time IL-17A was identified, the cognate receptor was also described and designated the IL-17 receptor (2,10), now referred to as IL-17RA. Bioinformatic analysis led to identification of four additional IL-17 receptor-related molecules and these are now designated IL-17RB, IL-17RC, IL-17RD, and IL-17RE (3). Despite the relatedness of the IL-17 and IL-17R family, the receptor for only two of the members of the cytokine family has been unambiguously identified: IL-17A binds to IL-17RA and IL-17E binds to IL-17RB (27). However, it has been recently reported that IL-17RA and IL-17RC function as a heterodimer in IL-17RA deficient fibroblasts (28).

We and others (29) identified IL-17RC using bioinformatic analysis, and we report here that human IL-17RC (hIL-17RC)[2] is the cognate receptor for human IL-17F (hIL-17F), but that it also binds human IL-17A (hIL-17A) with high affinity. This has led us to propose that soluble hIL-17RC may be a useful therapeutic for blocking effects of both hIL-17A and hIL-17F. In contrast, human IL-17RA (hIL-17RA) binds hIL-17A effectively, but binds hIL-17F with ~1000-fold lower affinity. However, although the above information pertains to the human cytokines and receptors, the binding pattern of mouse IL-17A and IL-17F is dramatically different. This significantly impacts how the effectiveness of such a therapeutic can be assessed in mouse models of human diseases.

Materials and Methods

Production and biotinylation of mouse and human IL-17A and IL-17F

Mouse and hIL-17A and hIL-17F were either purchased from R&D Systems or were produced as recombinant proteins in transiently transfected 293F cells. A cDNA encoding each cytokine was engineered to include a C-terminal His-tag and this DNA was transiently transfected into 293F cells (Invitrogen Life Technologies) using Lipofectamine 2000 (Invitrogen Life Technologies) under conditions recommended by the supplier. Protein was purified from conditioned medium by Ni^{2+}-agarose affinity chromatography. The binding and biological activity of the His-tagged proteins was found to be indistinguishable from the untagged versions produced in *E. coli* using assays discussed below.

Cytokines described above were biotinylated using either the EZ-Linkr Sulfo-NHS-LC-Biotin (human and mouse IL-17A and mouse IL-17F) or the EZ-Linkr Sulfo-NHS-PEO-Biotin (hIL-17F) kits from Pierce under conditions recommended by the supplier except as noted below. These steps were essential to prevent precipitation of the cytokines during the biotinylation reaction:

[2]Abbreviations used in this paper: hIL-17RC, human IL-17RC; hIL-17F, human IL-17F; hIL-17A, human IL-17A; BB, binding buffer; SAEC, small airway epithelium cell; BAL, bronchial alveolar lavage; HBE, human bronchial epithelial cell; BHK, baby hamster kidney cell.

mIL-17A. Used a 2:1 molar ratio of biotin instead of the suggested 20:1 ratio. Added 0.1 ml NaOAc to protein before adding biotin. Borate was not added before biotinylation and the reaction was not quenched with Tris.

mIL-17F. Borate was not added before biotinylation.

hL-17F. Used a 5:1 molar ratio of biotin to cytokine. The reaction was not quenched with Tris.

Production of soluble hIL-17RC-Fc

An expression construct for hIL-17RC-Fc protein was generated by fusing DNA sequences encoding the prepro signal sequence from human tissue plasminogen activator, the extracellular domain of hIL17RC (splice variant Δ7), and the Fc region from the human FcG1 H chain. The fusion protein was purified from supernatants derived from CHO DXB-11 cells by Protein A and size-exclusion chromatography.

Production of polyclonal antisera against mouse and hIL-17RC

Fusion proteins including mouse or hIL-17RC sequences fused in-frame to maltose binding protein (MBP) were generated and IL-17RC-MBP fusion proteins were purified on Amylose resin under conditions recommended by the supplier (New England BioLabs). Female New Zealand white rabbits were immunized with the purified MBP-fusion proteins, and mouse or hIL-17RC-specific polyclonal antisera was affinity purified using the fusion protein coupled to a SEPHAROSE 4B protein column (Pharmacia LKB). IL-17RC-specific Abs were characterized using ELISA, immunoblotting, and FACS. All assays revealed that each antisera was specific for IL-17RC from the appropriate species, but that it recognized all splice variants examined.

Cell transfections and FACS binding analysis

BHK and 293F cells were transfected with expression vectors using Lipofectamine 2000 (Invitrogen Life Technologies) following manufacturer's protocol. Transient 293F cell lines were incubated for 48 h before FACS binding analysis. Stable BHK cell lines were grown out under selection in 1 μM methotrexate.

Transfected BHK or 293F cells were harvested with Versene (Invitrogen Life Technologies) and resuspended in staining medium (HBSS/1%BSA/0.1% NaAzide/10 mM HEPES). Cells were stained at 2×10^6 cells/ml with 1 μg/ml biotinylated ligand for 30 min at 4°C. For competition assays, unlabeled competitor was titrated and preincubated with labeled ligand for 15 min. Stained cells were washed 1× in staining medium, and stained with Streptavidin-PE and anti-human CD8-APC (BD Biosciences). Samples were analyzed on a FACSCalibur Flow Cytometer using CellQuest Software (BD Biosciences).

Cytokine labeling and saturation binding

hIL-17A and -F were iodinated using Iodotubes (Pierce) under conditions recommended by the supplier. Iodinated cytokines were compared with unlabeled forms and found to have similar activities using the activity assays described below. BHK cells expressing hIL-17RA, hIL-17RC, or both receptors were plated at densities of 10^5 cells/well for two days in 24-well dishes. Cells were washed with binding buffer (BB) at 4°C (RPMI 1640, 20 mM HEPES, pH 7.4, 3 mg/ml BSA) and binding reactions were performed in 250 μl BB containing ^{125}hIL-17A or ^{125}hIL-17F in a concentration range of 0.045–10 nM for one hour on ice. Nonspecific binding was determined by including a 100-fold molar excess of unlabeled protein. Cells were washed three times with cold BB; bound counts were extracted into 1 ml of 1 N NaOH, and

quantitated on a gamma counter. Analysis of the binding was done using Prizm4 (GraphPad Software).

Biacore analysis

Goat-anti-human-Fcγ specific Abs (Jackson ImmunoResearch Laboratories) were immobilized onto Biacore CM5 sensor chips at 20 μg/ml in 10 mM acetate buffer pH 5.0 via amine coupling. Soluble hIL-17RC-Fc was then bound to the chip and a concentration series of ligands (0.39–100 nM) was injected across the receptor to observe association and dissociation. After each run, the surface was regenerated with 2–30 s injections of 10 mM Glycine, pH 1.75. Biacore Evaluation Software (v3.2) was used to define kinetic values.

Sequences and splice variants

Sequences for each of the splice variants discussed can be found with the following accession numbers: hIL-17RC (EF676033), hIL-17RCΔ7 (BD292072), hIL-17RCΔ7,12 (EF676032), and hIL-17RCΔ12 (EF676034); mIL-17RC (AX360310), mIL-17RCΔ7 (CS251263), mIL-17RCΔ7,8 (AX360307), and mIL-17RCΔ8 (CS251267).

Asthma model

BALB/c mice between 6 and 8 wk of age, were sensitized i.p. with 10 μg chicken egg albumin (OVA) (Calbiochem) in 50% Imject Alum adjuvant (Pierce) on day 0 and day 6. On days 13 and 14, mice were challenged under anesthesia intranasally with either PBS alone or 20 μg OVA in 50 μl PBS. Forty-eight hours after the last challenge, bronchioalveolar lavage was collected. Lung tissue was also removed and mRNA was isolated using Qiagen RNeasy isolation kits (Qiagen) as per the manufacturer's instructions. All RNA samples were subsequently treated with DNase using Ambion DNA-free kits (Ambion) before quantitative real-time PCR analysis. All animal procedures and care were conducted in accordance with approved ethical guidelines under the auspices of the ZymoGenetics Institutional Animal Care and Use Committee.

Quantitative real-time PCR

Expression of *mil-17ra* and *mil-17rc* mRNA were measured using multiplex real-time quantitative RT-PCR (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). mRNA levels were normalized to the expression of HPRT mRNA and determined by the comparative threshold cycle method (PE Applied Biosystems). Each data point represents expression results from a pooled mRNA sample containing five mice. The primers and probe for each was as follows: *mil-17ra:* forward primer, 5′ CCCAGTAATCTCAAATACCACAGTTC; reverse primer 5′ CGATGAGTGTGATGAGGCCATA; probe, 5′ CAGCCAGTTGCAGA CTACATTCCCCTGT; *mil-17rc:* forward primer, 5′ GAGTCCCTGC CAGCCACTT; reverse primer, 5′ ACTGGAAATCTTGTGGCTCATTC; probe, 5′ ACCAGTGCCCCAGAAGAACGCCAC.

Analysis of IL-17RC splice variants

RNA was isolated from small airway epithelium cells (SAEC) and expression of hIL-17RC splice variant mRNAs were measured with multiplex real-time quantitative RT-PCR method (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). RT-PCR primers and probe were designed to differentiate either the inclusion or exclusion of exon 7 or exon 12. The expression levels of the exon specific regions of *IL-17RC* were normalized to the expression levels of an amplified region of *IL-17RC* common to all possible splice variants. In addition, the expression levels of the exon specific regions of *IL-17RC* were normalized to expression levels of the house keeping gene HPRT using the comparative

threshold cycle method (User Bulletin 2; PE Applied Biosystems). Individual DNA templates containing each of the splice variants were used as controls for verification of the specificity of the primers and probes. The primers and probe for hIL-17RC include.

Primers that amplify splice variants that include exon 7: primer, 5′-GAGCTGCTGGGCCCTGC; primer, 5′-ACATTCAGAACCAGATGCACGTT; probe: 5′-GGCTCAACGTGTCAGCAGATGGTG. Primers that amplify splice variants that exclude exon 7: primer, 5′-AGCTGCCTGCCCTGCC; primer, 5′-ACATTCAGAACCAGATGCACGTT; probe, 5′-GGCTCAACGTGTCAGCAGATGGTG. Primers that amplify splice variants that include exon 12: primer, 5′-CGCTTTCCTGGGAGAACGT; primer, 5′ - GAGGTTAGGGTGGCCTTTCAG; probe, 5′-ACTGTGGACAAGGTTCTCGAGTTCCCATT. Primers that amplify splice variants that exclude exon 12: primer, 5′-ACCGCTTTCCTGGGAGA; primer, 5′-CACAAGCACTCCTGCAGCTG; probe, 5′-GTCACTGTGGACGTGAACAGCTCG. Primers that amplify a region of IL-17RC common in all splice variants: primer, 5′-CCTTCCAGGCCTACCCTACTG; primer, 5′-TATACCACAGAGCCCACAGACTG; probe, 5′-TGCGTCCTGCTGGAGGTGCAA.

Alternatively, first-strand cDNA was synthesized using 5 μg of human SAEC or human adrenal gland total RNA using the superscript first strand synthesis system (Invitrogen Life Technologies). PCRs were prepared using first strand cDNA as the template. Hotstar polymerase (Qiagen) was used according to the manufacturer's recommendations. Reactions were run using a 51°C annealing temperature with 40 cycles of amplification and primers as follows: sense primer, 5′-TCTCCAGGCCCAAGTCGTGCTCT′; antisense primer, 5′-TGTCCTGGGGGCCTCGTGTCTCC-′.

PCR products were subcloned using the TOPO TA kit for sequencing (Invitrogen Life Technologies) and the resultant clones were analyzed by DNA sequence determination.

Luminex-based phospho-IκB assay

National Institutes of Health-3T3 cells transfected with expression vectors encoding hIL-17RA, hIL-17RC, or both receptors were plated at 7500 cells/well in growth medium (DMEM with L-Glutamine plus 5% FBS, 1% Sodium Pyruvate, and 1 μM MTX) in 96-well plates. Cells were switched to assay medium (DMEM with L-Glutamine plus 0.1% BSA and 10 mM HEPES) on day two and were stimulated with cytokine at 37°C for 10 min on day 3. Lysates were prepared by addition of 50 μl/well lysis buffer (Bio-Plex cell lysis kit, Bio-Rad Laboratories) and agitation for 20 min at 4°C. Plates were centrifuged at 4500 rpm at 4°C for 20 min. Supernatants were analyzed for phosphorylated I-κBα using a Bio-Plex Phospho-IκBα Assay (Bio-Rad Laboratories) under conditions recommended by the supplier. Data was analyzed using analytical software (Bio-Plex Manager 3.0, Bio-Rad Laboratories) and EC_{50} values were calculated using GraphPad Prism 4 software (GraphPad Software).

Northern blot analysis

Northern blot analysis was performed using Clontech multiple tissue Northern blots according to manufacturer's recommendations.

A 436 bp probe was generated by PCR, using the following primers: sense, 5′-CGGCGTGGTGGTCTTGCTCTT; antisense, 5′-CCCGTCCCCCGCCCCAGGTC.

This fragment was labeled by random priming with a Rediprime kit from Amersham according to manufacturer's recommendations and blots were washed in 0.1 × SSC/0.1% SDS at 65°C.

Human bronchial epithelial cell (HBE) culture and stimulations

HBEs (Cambrex) were cultured based upon the method of Gray and colleagues (30) and grown as previously described (11). Under these conditions, HBEs formed a well-differentiated mucociliary phenotype, as previously described (11). A total of three donors were used to complete these studies.

Mature, ciliated HBE cells (14–21 days post seeding) were placed in fresh antibiotic-free medium. The next day cells were stimulated with hIL-17A (10 ng/ml) or hIL-17F (100 ng/ml) plus TNF-α (1 ng/ml). Cytokines were applied to the serosal side of the cell monolayer in the presence or absence of the soluble IL-17RC-Fc construct at 10 μg/ml. Basolateral medium was collected at 24 h and assayed for G-CSF and GRO-α using bead array technology (BioSource International).

Results

IL-17RC binds IL-17A and -F

We used a bioinformatics approach to search for proteins related to IL-17RA and identified a cDNA encoding the IL-17 receptor-related protein IL-17RC (S.R. Presnell, S.K. Burkhead, S.L. Pownder; unpublished observations). Despite its obvious similarity to the IL-17 receptor (IL-17RA), which binds to the prototypical member of the IL-17 family IL-17A, and the identification of five other members of the IL-17 cytokine family, a specific ligand for IL-17RC had not been previously reported. To assess whether one or more members of the IL-17 family could specifically interact with cells transfected with IL-17RC, baby hamster kidney cells (BHK) were stably transfected with constructs encoding either hIL-17RA or hIL-17RC. Expression of receptors on the surface was confirmed by FACS analysis using either a mAb to hIL-17RA or a polyclonal antiserum to hIL-17RC (data not shown). To assess cytokine binding, we used biotinylated forms of human IL-17A, -C, -D, -E, and -F and fluorochrome-conjugated streptavidin to detect cytokine binding to transfected cells by flow cytometry. Fig. 1a shows that stably transfected BHK cells expressing hIL-17RA clearly bound hIL-17A as expected (Fig. 1a), whereas those transfected with empty expression vector failed to bind any members of the IL-17 family tested (data not shown). Less robust binding of hIL-17F to hIL-17RA-transfected cells was also observed (Fig. 1a), but there was no significant binding of other members of the IL-17 family tested (data not shown). We also examined binding of IL-17 family members to hIL-17RC-transfected cells and noted that these cells showed significant binding to hIL-17F (Fig. 1a). In addition, we saw significant binding of hIL-17A to these cells (Fig. 1a), but no binding of hIL-17C, -D, or -E (data not shown). This data suggested that hIL-17RC might be the receptor for hIL-17F, but that it also bound to hIL-17A.

An examination of the level of fluorescence over a range of cytokine concentrations revealed information on the relative affinities of hIL-17A and -F for hIL-17RA and hIL-17RC. By comparing mean fluorescence intensities of the individual cytokines on each transfectant, we noted that hIL-17A bound much better to hIL-17RA than hIL-17F did, but that both cytokines seemed to bind equally well to hIL-17RC-transfected cells (Fig. 1b). It has recently been reported that both hIL-17RA and hIL-17RC were essential for regenerating a fully functional receptor for hIL-17A in mouse IL-17RA-deficient fibroblasts (28). However, we noted in this assay that cytokine binding to cells that expressed both receptors seemed to be additive with no evidence of cooperativity (Fig. 1b).

We next investigated the specificity of these interactions by attempting to compete for binding with unlabeled cytokine. Transfected BHK cells were incubated with a fixed concentration of biotinylated cytokine and increasing concentrations of unlabeled cytokine and the amount of bound biotinylated material was quantitated by FACS. Fig. 1c illustrates that the binding of

both hIL-17A and -F to hIL-17RC was specific because increasing concentrations of unlabeled cytokine interfered with binding of the biotinylated material. In fact, unlabeled hIL-17A and -F effectively cross-competed for binding of biotinylated forms of both cytokines to hIL-17RC-transfected cells (Fig. 1c), suggesting that the two cytokines were binding hIL-17RC with similar affinities, and that they were binding to overlapping, if not identical sites. These experiments also confirmed that the biotinylation reactions did not significantly impact the binding activity of the IL-17-family molecules, because the nonbiotinylated forms seemed to compete for binding of the biotinylated forms at close to a 1:1 ratio (Fig. 1c). In contrast, unlabeled hIL-17A effectively competed for binding of both biotinylated hIL-17A and -F to hIL-17RA-transfected cells, but unlabeled hIL-17F showed essentially no ability to compete for hIL-17A binding to hIL-17RA (Fig. 1c). This indicated that although hIL-17F showed specific binding to hIL-17RA, the avidity of this interaction appeared to be significantly lower than the interaction of hIL-17A and hIL-17RA. This is consistent with a previous report that hIL-17F bound hIL-17RA with low affinity (31).

Affinities of hIL-17A and hIL-17F for hIL-17RC and hIL-17RA

Saturation binding studies were done to measure the affinity of hIL-17A and -F binding to hIL-17RC and hIL-17RA. BHK lines stably expressing hIL-17RA or hIL-17RC were incubated with ^{125}I-labeled hIL-17A or -F under saturation binding conditions to determine the affinity constants of each cytokine for each receptor. We found that hIL-17A bound both hIL-17RA and hIL-17RC with comparable affinities (Table I). In addition, the affinity of hIL-17F for hIL-17RC was very similar to the affinity of hIL-17A for this receptor (Table I). However, consistent with results obtained using biotinylated cytokines, the affinity of hIL-17F for hIL-17RA was considerably lower relative to other affinities measured (Table I). This indicated that hIL-17A and -F bound hIL-17RC with similar affinities, but that their affinities for hIL-17RA differed dramatically.

In addition, as discussed above, evidence has emerged suggesting that IL-17RA and IL-17RC form a heterodimeric receptor for IL-17A (28), and therefore we examined the affinities of hIL-17A and hIL-17F for cells expressing both hIL-17RA and hIL-17RC. Binding studies of IL-17A and -F on BHK cells expressing both receptors revealed two binding sites with affinities similar to those of the individual receptors (Table I), suggesting that each receptor influenced binding independently with no evidence of any dramatic cooperative binding effect.

The receptor for mIL-17F is a splice variant of mIL-17RC

Because of the proposed role for IL-17A and -F in the progression of a number of inflammatory diseases, we proposed to test soluble IL-17RC as therapeutic agent in mouse models of human diseases. Before attempting this, the binding properties of mIL-17A and -F to mIL-17RA and mIL-17RC were examined. The mouse cytokines were biotinylated and used to assess binding to cells transfected with the corresponding mouse receptors. Human 293F cells were transiently transfected with empty expression vectors, or those encoding mIL-17RC or mIL-17RA. The vector used included an internal ribosome entry site downstream of the cytokine receptor, followed by a cDNA encoding the extracellular and transmembrane region of the human CD8 protein. This allowed us to restrict binding analysis to cells that had taken up DNA, because only transfected cells would express CD8 and hence the cytokine receptor of interest. Transfected cells were simultaneously tested for binding to anti-CD8 Abs and biotinylated forms of mouse or hIL-17A and -F by FACS analysis. Surprisingly, mIL-17RC-transfected cells did not bind either mIL-17A or mIL-17F (Fig. 2). However, the receptor was clearly expressed on the surface of these cells, because hIL-17A and -F did bind to CD8 positive cells from this transfection (Fig. 2), and a polyclonal Ab specific for mIL-17RC clearly stained transfected cells (data not shown). Moreover, we were able to detect binding of both of the biotinylated mouse cytokines to cells transfected with mIL-17RA (Fig. 2) indicating that the

biotinylation had not altered the ability of the mouse cytokines to bind cells expressing an appropriate receptor. This data indicated that the binding patterns of these IL-17-family members differed between humans and mice.

These results suggested that mIL-17RC was not the receptor for mIL-17F, but that mIL-17RA was behaving qualitatively similar to hIL-17RC with respect to mIL-17A and -F binding. We and others (3) have previously recognized the existence of a large number of splice variants in transcripts encoding both the human and mouse IL-17RC protein. The particular splice variant of the mouse receptor used in the experiments described above (Fig. 2) was identical in terms of exon composition to the human isoform used to establish binding of hIL-17A and -F. This variant excludes the seventh exon of the IL-17RC gene and was designated IL-17RCΔ7 (see Table II, Fig. 3). In fact, all of the splice variants we have identified from mouse sequences differ in their inclusion or exclusion of exons 7 and 8. Exon 7 is either completely included or excluded, while the 5′end of exon 8 is either included or excluded due to alternative use of splice acceptors at the 5′ end of exon 8, or within the middle of exon 8 (see Table II and Fig. 3). Four mouse variants have been identified that have been designated mIL-17RC (including all exons); mIL-17RCΔ7; mIL-17RCΔ7,8; and mIL-17RCΔ8 and their precise exon compositions are illustrated in Table II and shown schematically in Fig. 3. We tested each of these splice variants in our transient transfection system for binding of biotinylated mouse and human IL-17A and -F. Consistent with our previous results, we saw binding of mIL-17RCΔ7 to hIL-17A and -F, but not to either mouse cytokine (Fig. 2, Table II). However, mIL-17RC, which includes all exons of the IL-17RC gene, bound to mIL-17F as well as hIL-17A and -F, but not to mIL-17A (Fig. 2, Table II). mIL-17RCΔ7,8 and mIL-17RCΔ8 did not show binding to any of the cytokines tested (Table II, data not shown). These nonbinding receptors were clearly expressed on the surface however, because a polyclonal antiserum against mIL-17RC clearly stained the $CD8^+$ cells (data not shown).

In addition to testing these mouse splice variants in transient transfection assays, we also tested them in BHK cells that were stably expressing each receptor. The binding results obtained matched perfectly with what was seen in the transiently transfected 293 cells (data not shown), providing additional confirmation that the transient transfection assays were appropriate for this analysis.

Binding patterns of splice variants of hIL-17RC

Given the surprising results obtained with the different splice variants of mIL-17RC, a similar analysis of hIL-17RC splice variants was also performed. The number of splice variants in humans is much greater and therefore we performed our initial experiments on only a subset of these molecules. Those chosen for this analysis also differed in their inclusion or exclusion of exon 7, but unlike the mouse, all splice variants incorporated all of exon 8. The cryptic splice acceptor found in the middle of the mouse exon 8 sequence is not present in human exon 8. However, the other splice variants tested either included or excluded hIL-17RC exon 12. These variants were designated hIL-17RC (identical in exon composition to the full-length mIL-17RC above); hIL-17RCΔ7 (identical in exon composition to mouse Δ7 above); hIL-17RCΔ7,12; and hIL-17RCΔ12 (Table II and Fig. 3). Again, these splice variants were transiently expressed in 293F cells and were tested for their ability to bind biotinylated mouse and human IL-17A and -F and the results are summarized in Table II. Consistent with the experiments presented earlier, hIL-17RCΔ7 bound to both hIL-17A and -F, but did not bind to either mouse cytokine. hIL-17RC also bound to both human cytokines, and like its mouse counterpart, it bound to mIL-17F, but not mIL-17A. hIL-17RCΔ7,12 and Δ12 failed to bind any of the four cytokines tested (Table II), although they were clearly expressed on the surface of transfected cells because a polyclonal antiserum against hIL-17RC stained $CD8^+$ cells (data not shown). These binding results were faithfully recapitulated in stably transfected BHK cells as well (data not

shown). Collectively, these data allow us to draw conclusions regarding essential portions of the IL-17RC protein required for binding to the mouse and human cytokines (see *Discussion*).

Soluble hIL-17RCΔ7 inhibits binding of both hIL-17A and -F

The high affinity binding of hIL-17A and -F to hIL-17RCΔ7-transfected cells suggested that a soluble form of hIL-17RC might be an effective inhibitor of these two cytokines. To test this directly, we produced a soluble form of hIL-17RCΔ7 as an Fc-fusion protein (hIL-17RC-Fc) and tested its ability to inhibit the binding of both hIL-17A and -F. These effects were then compared with results obtained using a soluble form of hIL-17RA. Increasing concentrations of hIL-17RC-Fc or hIL-17RA-Fc were included in binding reactions and FACS analysis was used to assess effects of the soluble receptors on binding of biotinylated cytokines to stably transfected BHK cells. We noted that hIL-17RC-Fc inhibited the binding of both hIL-17A and -F to a similar extent, whereas an Fc-fusion protein of another member of the IL-17R family (hIL-17RD) had no effect (Fig. 4a). In contrast, soluble hIL-17RA-Fc effectively blocked binding of hIL-17A, but had essentially no effect on the binding of hIL-17F (Fig. 4a). The results shown in Fig. 4a are from BHK cells transfected with both hIL-17RA and hIL-17RCΔ7, but similar results were obtained using cells transfected with only one of these receptors as well. These data are consistent with results obtained from affinity measurements and indicate that the soluble forms of IL-17RA and IL-17RC are exhibiting the same general binding properties as their membrane-anchored forms.

To more precisely determine the effectiveness of each of these soluble receptors in neutralizing binding of hIL-17A and hIL-17F, we performed detailed inhibition of binding studies to obtain IC_{50} values for each receptor against each cytokine. Results of this analysis are shown in Fig. 4b. As expected, hIL-17RA-Fc was unable to inhibit binding of biotinylated hIL-17F to transfected BHK cells, but effectively blocked binding of hIL-17A with an IC_{50} of ~100 nM (Fig. 4b). In contrast, hIL-17RC-Fc effectively inhibited binding of both hIL-17A and -F, with IC_{50} values of 285 and 234 nM respectively (Fig. 4b). Collectively, this data supports the idea that hIL-17RC-Fc can effectively prevent binding of both hIL-17A and -F to cells expressing appropriate receptors.

As an additional assessment of the capacity of hIL-17RC-Fc to bind to hIL-17A and -F, the affinity of the soluble receptor for these cytokines was assessed using Biacore analysis. Soluble hIL-17RC bound to both hIL-17A and -F with high affinity (Table III), providing additional support for the idea of using this reagent as an antagonist for the effects of both hIL-17A and -F in vivo. However, no detectable binding of mIL-17A or -F was observed (Table III), indicating that this reagent could not be used in mouse models of human IL-17A/F-mediated disease.

hIL-17RC-Fc blocks signaling in response to hIL-17A and -F

The ability of hIL-17RC-Fc to block cytokine binding to cells suggested this reagent would also be capable of blocking subsequent intracellular signaling events. IL-17A binding to its cognate receptor has been shown to activate the NF-κB signaling pathway through the adapter proteins Traf6 and Act1 (32–35). We had previously shown that mouse National Institutes of Health-3T3 fibroblasts respond to both hIL-17A and hIL-17F by activating NF-κB presumably by virtue of these cytokines binding to and activating the endogenous mouse receptors. However, we have also shown that transfecting these cells with hIL-17RC dramatically enhances their responsiveness to both hIL-17A and -F. (R. E. Kuestner, D. W. Taft, T. Brender, and S. D. Levin, unpublished observations). To assess whether hIL-17RC-Fc could block signaling responses to hIL-17A and -F, we stimulated National Institutes of Health-3T3 fibroblasts that had been stably transfected with hIL-17RC and examined NF-κB activation

using Abs that specifically detected phosphorylated IκB (P-IκB) in a Luminex-based assay. The hIL-17RC-transfected National Institutes of Health-3T3 cells exhibited dose-dependent increases in P-IκB 10 min after stimulation (Fig. 5a). As expected, hIL-17RA-Fc inhibited IκB phosphorylation in response to hIL-17A in a dose dependent manner, but it failed to affect responses to hIL-17F (Fig. 5, b and c). However, consistent with the inhibition of binding data observed, hIL-17RC-Fc inhibited responses to both hIL-17A and -F (Fig. 5, b and c). Collectively, this data supports the notion that hIL-17RC serves as a functional receptor for both hIL-17A and -F, and that a soluble form of this receptor blocks both binding and signaling in response to either cytokine.

To confirm that the same held true for primary cells that respond to both IL-17A and -F, and that express physiological levels of IL-17 receptors on their surface, we examined effects mediated by these cytokines on human bronchial epithelium (HBE). These cells produce proinflammatory cytokines and chemokines in response to either hIL-17A or -F (11), including G-CSF, IL-6, CXCL1, and IL-8. Moreover, it had been previously established that hIL-17RA-Fc was able to block the induction of proinflammatory cytokines and chemokines from HBE in response to hIL-17A, but not in response to hIL-17F (11). Therefore, the capacity of hIL-17RC-Fc to block these effects in vitro was examined. HBE respond to hIL-17A alone to some extent, but responses are generally not as robust to hIL-17F alone. However, these cells respond synergistically when stimulated with both TNF-α and hIL-17A or -F, producing elevated levels of G-CSF and CXCL-1. Such synergy has been clearly established in multiple cell types including HBE (11) and synoviocytes from RA patients (19,21). Therefore, we tested the ability of hIL-17RC-Fc to neutralize this synergistic induction of G-CSF and CXCL1. Soluble hIL-17RC blocked induction of G-CSF (Fig. 6a) and CXCL1 (data not shown) induced by hIL-17A plus TNF-α in HBE from three separate donors. Moreover, hIL-17RC-Fc also inhibited hIL-17F-mediated effects similarly (Fig. 6b and data not shown). Collectively, our data supports the use of hIL-17RC-Fc as a therapeutic agent to neutralize the proinflammatory effects of hIL-17A and -F.

Coexpression of hIL-17RA and hIL-17RC does not enhance the sensitivity of cells to hIL-17A or -F

Because of the recent report that hIL-17RA and hIL-17RC form a heterodimeric receptor complex for hIL-17A (28), we assessed differences in signaling capacity in cells that overexpress hIL-17RA, hIL-17RC, or both receptors. We chose the variant including all of the extracellular domain exons of IL-17RC in this case because it corresponds to the splice variant used by Toy et al. in their study (28), although we have also shown that the Δ7 splice variant behaves similarly (data not shown). National Institutes of Health-3T3 fibroblasts that stably expressed either hIL-17RA or hIL-17RC alone or that expressed both of these receptors were assessed for activation of NF-κB in response to either cytokine using the P-IκB assay described above. As discussed above, National Institutes of Health-3T3 cells respond to both hIL-17A and -F without transfection of the human receptors, indicating that the mouse receptors expressed in these cells are sufficient to trigger signaling. However, these cells clearly become more sensitive to cytokine when hIL-17RC is provided, and they become more sensitive to hIL-17A when hIL-17RA is provided. As shown in Fig. 7, cells transfected with hIL-17RA or hIL-17RC responded to hIL-17A in a dose-dependent manner with EC_{50} values of 1.5 and 0.38 nM respectively. Similarly, cells expressing either receptor singly also respond to hIL-17F, although in this case the EC_{50} values differ significantly (5.9 nM for hIL-17RA transfected cells vs 0.38 nM for hIL-17RC transfected cells). In fact the response of hIL-17RA transfected cells to hIL-17F is virtually identical with untransfected National Institutes of Health-3T3 cells (data not shown) indicating that expression of hIL-17RA does not appreciably affect the sensitivity of these cells to hIL-17F, which is consistent with the binding data discussed above. To evaluate the idea that hIL-17RA and hIL-17RC in fact function as a heterodimeric complex,

responses of cells over-expressing both receptors were examined. The magnitude of the response to both hIL-17A and -F was higher in cells expressing both receptors, but the EC_{50} values for the double transfectants mirrored the EC_{50} value associated with IL-17RC overexpressing cells (Fig. 7). Therefore, although overexpression of both receptors increased the magnitude of the response, this is most likely due to the increased number of signaling receptors at the surface. However, expression of both receptors did not alter the sensitivity of the cells to either cytokine, which suggests that coexpression of the two receptors does not lead to a synergistic signaling effect.

Differential expression of IL-17RA and IL-17RC

The observation that hIL-17RC bound both hIL-17A and -F with high affinity suggests that cells expressing hIL-17RC should be equally capable of responding to hIL-17A and -F. In contrast, because hIL-17RA bound hIL-17A with high affinity, but hIL-17F ~1000-fold less well, the implication is that cells expressing hIL-17RA would, under physiologic conditions, only respond to hIL-17A. Previous reports indicate that hIL-17RA is expressed ubiquitously, although levels appear to be higher in hemopoietic cells relative to other cell types (2). Therefore, we examined the expression of hIL-17RC to determine the extent of overlap in the expression patterns. Northern blot analysis showed that hIL-17RC was expressed at high levels in glandular tissues such as adrenal gland, prostate, liver, and thyroid with no detectable expression in hemopoietic tissues (Fig. 8a).

To further investigate expression of these receptors in hemopoietic cells, we examined binding of biotinylated hIL-17A and -F to PBMC by multiparameter FACS analysis. We found that hIL-17A bound to virtually all PBMC subsets examined, whereas hIL-17F failed to show detectable binding to any of these populations (Fig. 8b and data not shown). This is consistent with the capacity of hIL-17RA to bind hIL-17A with high affinity, but not hIL-17F (Table I), and with our failure to detect hIL-17RC mRNA in PBMC (Fig. 8a and data not shown). Collectively, these data indicate that IL-17RC is preferentially expressed in nonhemopoietic tissues, whereas IL-17RA is preferentially expressed in hemopoietic cells.

Finally, we examined expression in lung tissue and infiltrating hemopoietic cells from mice that had been sensitized and exposed to an inhaled Ag (OVA) in a mouse model of human asthma. Mice were sensitized with OVA or PBS as a control, and then exposed to inhaled OVA or PBS as described in *Materials and Methods*. Following challenge, animals were sacrificed and hemopoietic cells were collected by bronchial alveolar lavage (BAL) and these cells and remaining lung tissue were processed to generate mRNA. Expression of mIL-17RA and mIL-17RC mRNA in each tissue was then assessed using quantitative RT-PCR. Consistent with Northern blot and FACS data discussed above, infiltrating hemopoietic cells (BAL) expressed higher levels of mIL-17RA whereas lung tissue showed detectable but reduced levels mIL-17RA mRNA (Fig. 8c). Also consistent with data discussed previously, BAL cells showed essentially no expression of mIL-17RC, whereas lung tissue showed significant transcript levels (Fig. 8c). Importantly, expression of both receptors was unaffected by sensitization and/or challenge (Fig. 8c). Collectively, this data supports the notion that the IL-17RC is preferentially expressed in nonhemopoietic tissue, and that nonhemopoietic tissue appears to express both IL-17RA and IL-17RC. It also indicates that the general expression patterns for IL-17RA and IL-17RC are conserved between mice and humans despite the apparent differences in responsiveness conveyed by these receptors between the two species.

To evaluate the expression of different splice variants of hIL-17RC within a hIL-17A/F responding population we chose to examine SAEC, which respond to both hIL-17A and -F (data not shown). Because the splice variants of hIL-17RC we examined differed only with respect to their inclusion or exclusion of exons 7 and 12, we initially designed a quantitative RT-PCR strategy to determine the fraction of transcripts that included these exons. Results

from this analysis are shown in Table IV. The distribution of transcripts that included or excluded exon 7 was 44 vs 56% respectively, suggesting a roughly equal distribution of exon 7 inclusion or exclusion. However, our analysis of exon 12 usage indicated a heavy bias toward including exon 12 (89 inclusion vs 11% exclusion). This suggests that most of the IL-17RC transcripts in SAECs are either full length hIL-17RC or the Δ7 splice variants (Table II; Fig. 3). To further analyze the distribution of IL-17RC transcripts, we designed a PCR strategy to amplify full-length IL-17RC cDNA's from mRNA. This amplified cDNA was then subcloned and sequenced and the nature and number of each splice variant evaluated. Consistent with the analysis discussed above, 15/40 clones sequenced represented the Δ7 splice variant whereas 8/40 represented the full-length variant (Table V). We found no Δ7,12 transcripts among the 40 clones sequenced and only one Δ12 transcript. The remaining transcripts sequenced represented largely mis-spliced transcripts that included frame-shifts generating sequences that no longer encoded IL-17RC protein sequences. In contrast, a similar analysis of another tissue that expresses IL-17RC (adrenal gland) had 4/15 Δ7 clones; 4/15 full-length clones; 2/15 Δ7,12 clones; and no Δ12 clones (Table V). Collectively our data suggests that there is a bias toward expression of binding-competent hIL-17RC splice variants in tissues that respond to IL-17A and -F.

Discussion

We have identified the cognate receptor for IL-17F as the IL-17R-related protein IL-17RC. Notably, hIL-17RC binds to hIL-17A and -F with similar affinities (Table I). Consistent with a previous report, hIL-17RC and -F also bound to hIL-17RA (31). Because these two members of the IL-17 family share 55% sequence identity, it is perhaps not surprising that they share receptors. However, hIL-17RA binds hIL-17A with high affinity, but binds hIL-17F with an affinity that is nearly 1000-fold lower, suggesting that under physiologic conditions, hIL-17RA would not bind hIL-17F. The implication is that cells that express hIL-17RC should respond to both hIL-17A and -F, whereas cells that express only hIL-17RA will only respond to hIL-17A. This difference has the potential to impact how these cytokines affect different tissues. Our own expression analysis revealed that, although hIL-17RA is expressed ubiquitously, it is more highly expressed in hemopoietic cells, whereas hIL-17RC tends to be expressed in nonhemopoietic tissues with no expression in the hemopoietic compartment (Fig. 8 and data not shown). Consistent with this, all subsets of human PBMC bind hIL-17A, but do not bind hIL-17F (Fig. 8 and data not shown). Collectively, this suggests that nonhemopoietic tissues should respond to both hIL-17A and -F, whereas hemopoietic cells should only respond to hIL-17A.

It has also been suggested that hIL-17RA and hIL-17RC function as a heterodimeric receptor complex for at least hIL-17A and perhaps hIL-17F (28). Although we could not definitively rule out this possibility, our data did not provide information that might explain this mechanistically. The coexpression of both human receptor subunits in mouse BHK cells did not significantly affect the ability of the cells to bind either hIL-17A or -F, and in fact our evidence suggests that coexpression appeared to convey only the individual receptor binding characteristics (Fig. 1,Table I). Moreover, expression of both hIL-17RA and hIL-17RC in National Institutes of Health-3T3 cells did not alter the sensitivity of the cells to IL-17A or -F beyond their additive individual contributions (Fig. 7). However, in contrast to the original report (28), our experiments were not performed in the complete absence of IL-17RA. The National Institutes of Health-3T3 cells express both mIL-17RA and mIL-17RC and in fact this mouse cell line responds to both hIL-17A and -F without provision of human receptors. Although providing these human receptors individually renders cells more sensitive to the cytokines in a way that's understandable based on what we know of their binding properties (e.g., expression of either the Δ7 or full-length variant of hIL-17RC renders National Institutes of Health-3T3 cells more sensitive to both hIL-17A and -F, whereas expression of hIL-17RA

only renders them more sensitive to hIL-17A), it's formally possible that endogenous mIL-17RA or RC levels may be sufficient to allow responses through a heterodimeric receptor complex. It's also possible that the high level of overexpression achieved in the transfectants allows the cells to overcome an obligate requirement for formation of a heterodimeric signaling complex. In fact, some evidence suggests that we still do not fully understand the biology underlying the ability of these receptors to convey signaling potential. For example, it is curious that the parental National Institutes of Health-3T3 cells respond to mIL-17A and to both hIL-17A and -F without expressing a human IL-17 receptor, but they respond very poorly to mIL-17F. However, these cells can be converted to mIL-17F responders by transfecting them with hIL-17RC (R.K., D.W.T., T.B., and S.D.L., unpublished). Additionally, we have previously shown that soluble hIL-17RA-Fc inhibits signaling in response to hIL-17A, but not -F in HBE (11), and we show here that hIL-17RC-Fc inhibits responses to both cytokines in the same cells. Both of these results are supported by the binding characteristics demonstrated here, but we have also shown that a blocking anti-IL-17RA Ab inhibits responses to both cytokines (11), perhaps reinforcing the idea that IL-17RA is an essential signaling component in responses to both IL-17A and -F.

In contrast with the human receptors and cytokines, mIL-17RA binds both mIL-17A and -F, whereas mIL-17RC (the full-length variant) binds only mIL-17F (Fig. 2, Table II). This implies that mouse cells expressing mIL-17RA should respond to both mIL-17A and -F, whereas those expressing the correct splice variant of mIL-17RC will respond only to mIL-17F. Curiously, we note the same general expression pattern for the receptors in mice with IL-17RA expressed more abundantly in hemopoietic cells, while IL-17RC is expressed at higher levels in nonhemopoietic cells (Fig. 8 and data not shown). This suggests that the relative contributions of IL-17A and -F in inflammation could be different in mice and humans and this should be considered when testing IL-17 family antagonists in mouse models of human diseases.

Examination of cytokine binding to the different IL-17RC splice variants has revealed two portions of the receptor that are essential for cytokine binding, and there are subtle differences in the binding characteristics of the mouse and human cytokines. Moreover, these characteristics are consistent for the cytokines regardless of the species of the receptor examined. From the data presented in Table II, we conclude that exon 12 and all of exon 8 are required for hIL-17A and -F to bind to IL-17RC, because these cytokines only bind to the human and mouse IL-17RCΔ7 and IL-17RC full-length variants. Each of these isoforms includes all of exons 8 and 12, although they differ with respect to whether exon 7 is included or not. This implies that exon 7 is dispensable for binding of the human cytokines, although further study is required to see whether there might be subtle differences in the binding and signaling capacity of hIL-17RCΔ7 and the full-length form. In contrast, mIL-17A does not bind to any of the IL-17RC isoforms appreciably, and exon 7 is clearly required for binding of mIL-17F (as are all of exons 8 and 12). The nature of the requirement for these exons is unknown and the amino acids encoded could represent actual points of contact with the cytokine, or could simply provide necessary structural framework.

Finally, we suggest that soluble hIL-17RC should serve as an antagonist for the biological effects of both hL-17A and -F. To test this, we generated soluble hIL-17RCΔ7 as an Fc-fusion protein and tested its ability to antagonize binding and signaling in response to hIL-17A and -F in vitro. This reagent showed high affinity binding to hIL-17A and -F (Table III) and readily inhibited binding of both hIL-17A and -F to cells expressing an appropriate receptor (Fig. 4). Moreover, it blocked immediate intracellular signaling events, as judged by NF-κB activation (Fig. 5), as well as more distal events such as the release of proinflammatory cytokines and chemokines (Fig. 6). It has also been recently noted that hIL-17A and -F can be secreted as a heterodimeric complex from Th17 cells (36). This is an interesting observation and although it has not yet been tested directly, we would anticipate that hIL-17RC-Fc would bind to and

neutralize effects of this heterodimeric molecule as well, because it does this with the individual homodimeric cytokines. However, the soluble form of the human receptor we prepared (Δ7) does not bind either mIL-17A or mIL-17F and therefore, this reagent is not useful in mouse models of human disease. Based on our binding data, soluble mIL-17RA in mice should behave as we predict soluble hIL-17RC would behave in humans – that is both receptors should bind to and neutralize IL-17A and -F. This suggests that mIL-17RA-Fc should serve as a useful surrogate in understanding the potential benefit of hIL-17RC-Fc in human inflammatory diseases.

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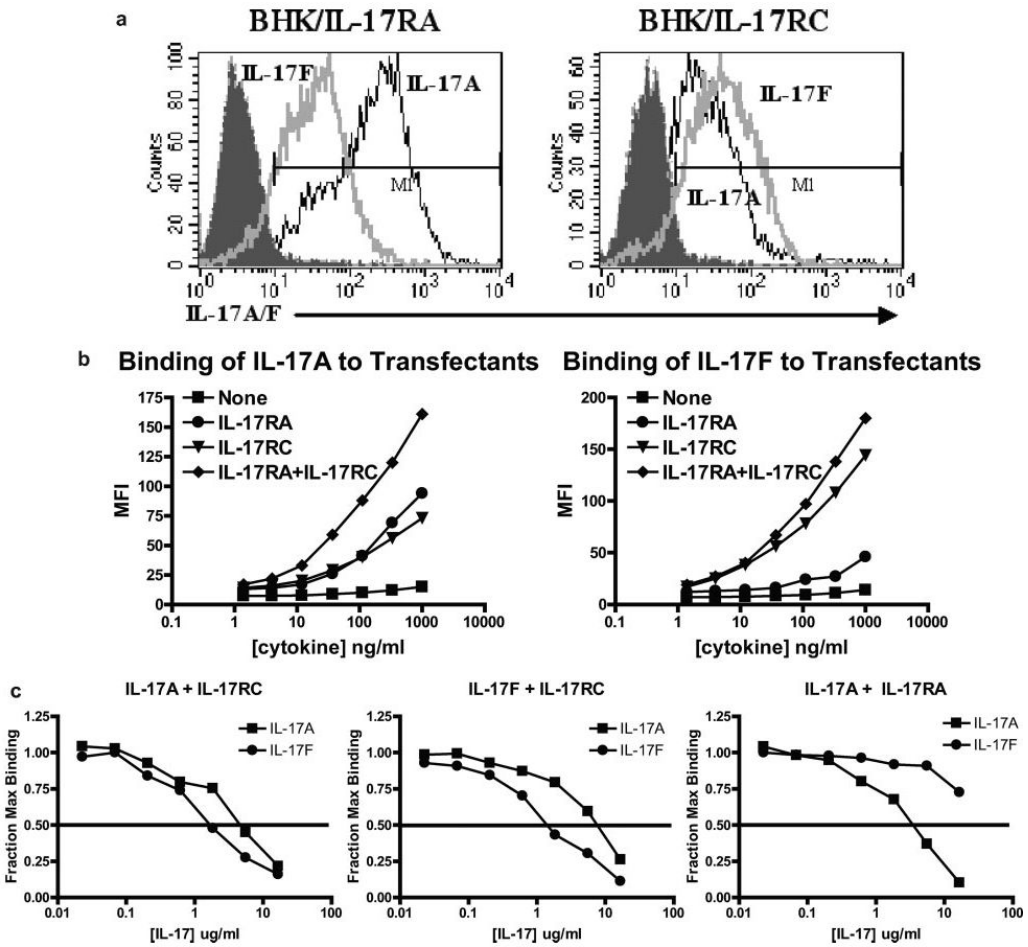

FIGURE 1.

hIL-17A and F both bind to hIL-17RC. Binding of biotinylated forms of each cytokine was tested using FACS analysis as described in *Materials and Methods. a*, Binding of hIL-17A (thin line) and hIL-17F (thick line) on cells transfected with the indicated receptor is shown on single parameter FACS plots. Untransfected cells with hIL-17A (shaded histogram) or hIL-17F (dotted line superimposed on shaded histogram) are also shown. *b*, A quantitative analysis of binding was done by plotting the MFI vs cytokine concentration on cells transfected with the indicated receptor. *c*, hIL-17A and -F cross-compete for binding. Unlabeled hIL-17A or hIL-17F was titrated over a dose-range with cells transfected with the indicated receptor and biotinylated cytokine shown at the top of each graph. Biotinylated cytokines were used at 1 μg/ml and the cytokines titrated are indicated with squares (hIL-17A) or circles (hIL-17F).

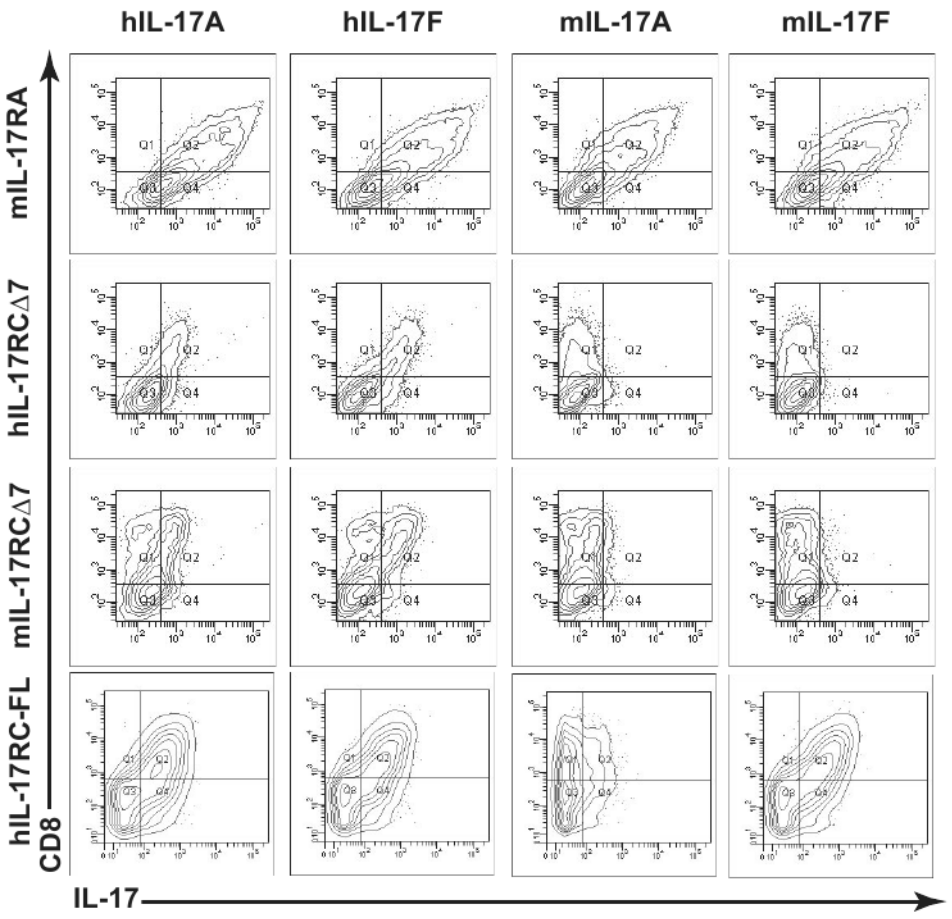

FIGURE 2.

Binding of mouse and hIL-17A and -F to mIL-17RA and mIL-17RC. 293F cells were transiently transfected with bicistronic expression vectors encoding the indicated cytokine receptor upstream of a human CD8 cDNA as described in *Materials and Methods*. Cells were stained with anti-human CD8 mAb to identify transfected cells and costained with the indicated biotinylated cytokine. CD8 expression is shown on the *y*-axis and IL-17 binding on the *x*-axis. See Fig. 3, Table II, and *Materials and Methods* for specific information on composition of the splice variants indicated.

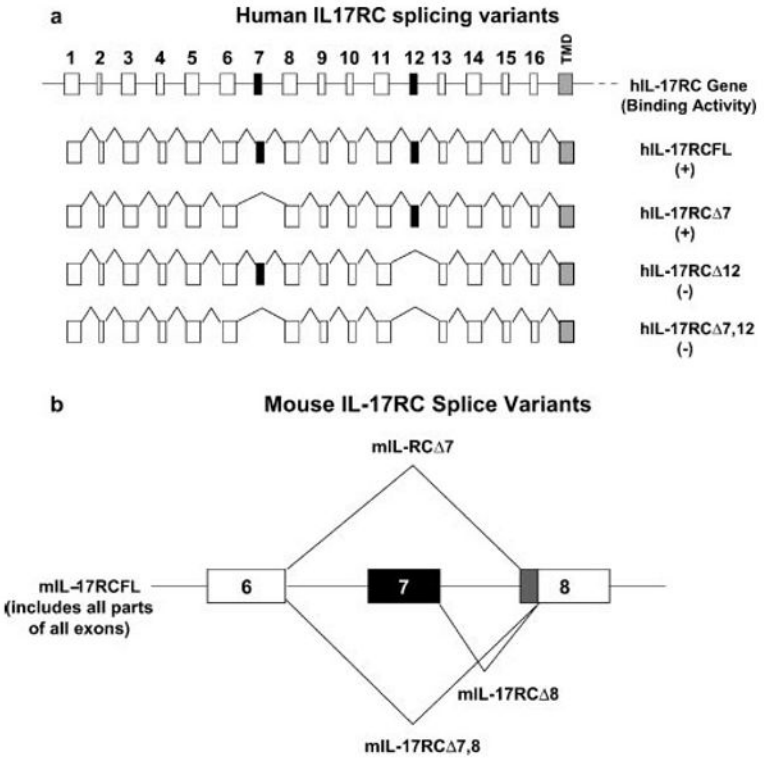

FIGURE 3.

Diagrammatic representation of human (*a*) and mouse (*b*) IL-17RC splice variants. See also Table II and *Materials and Methods* for additional information.

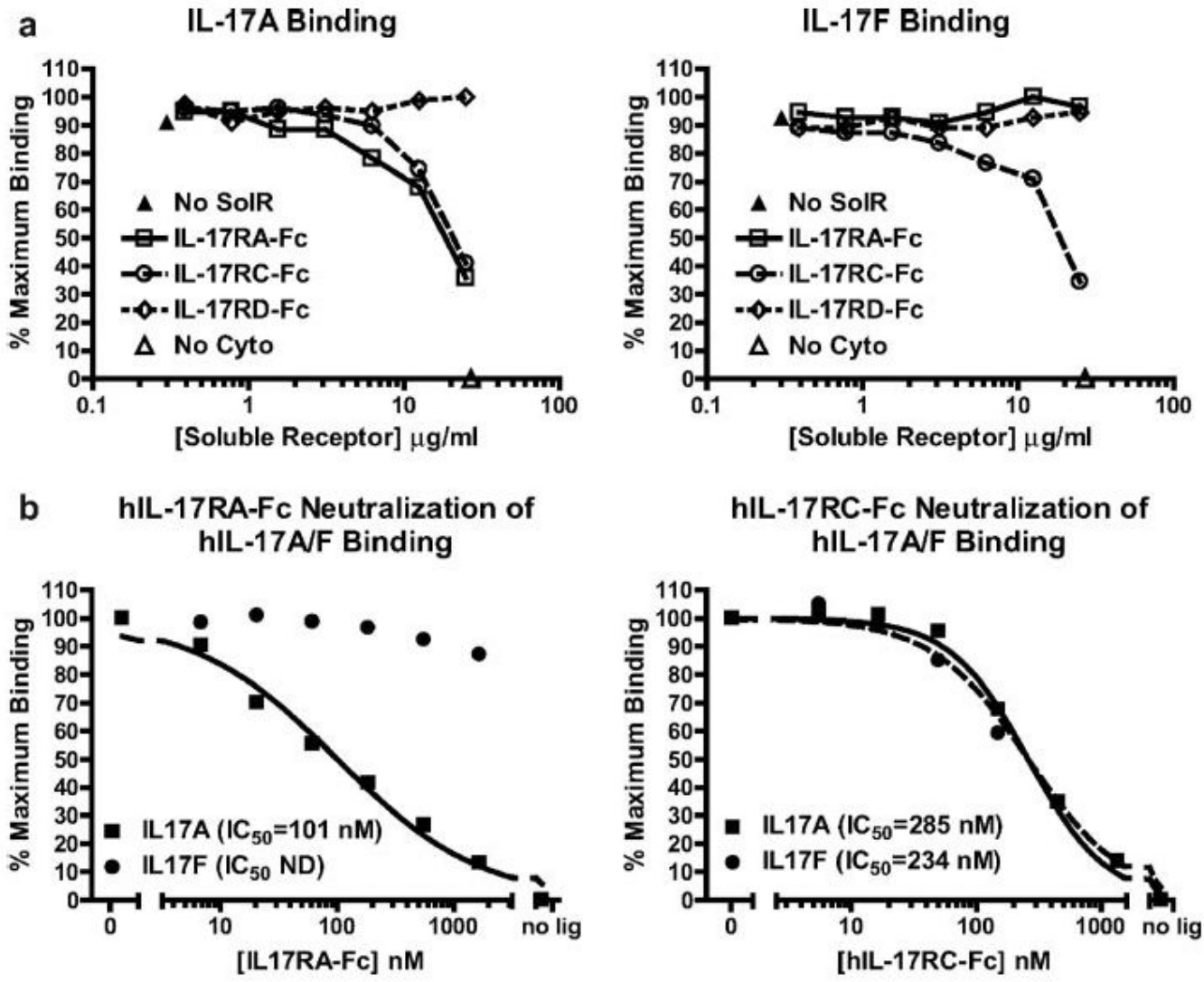

FIGURE 4.

Soluble hIL-17RC competes for binding of both hIL-17A and -F. *a*, hIL-17RA-Fc, hIL-17RC-Fc, or hIL-17RD-Fc were incubated with BHK cells stably transfected with hIL-17RA plus hIL-17RCΔ7 at the indicated concentrations and binding of the indicated biotinylated cytokine was assessed by FACS. *b*, The same soluble receptors were titrated over a range of concentrations and effects on cytokine binding were monitored by FACS. IC_{50} values were determined from the concentration of soluble receptor required to inhibit 50% of the binding as described in *Materials and Methods*. The hIL-17RC-Fc protein used is derived from the Δ7 splice variant. Results are representative of at least three experiments.

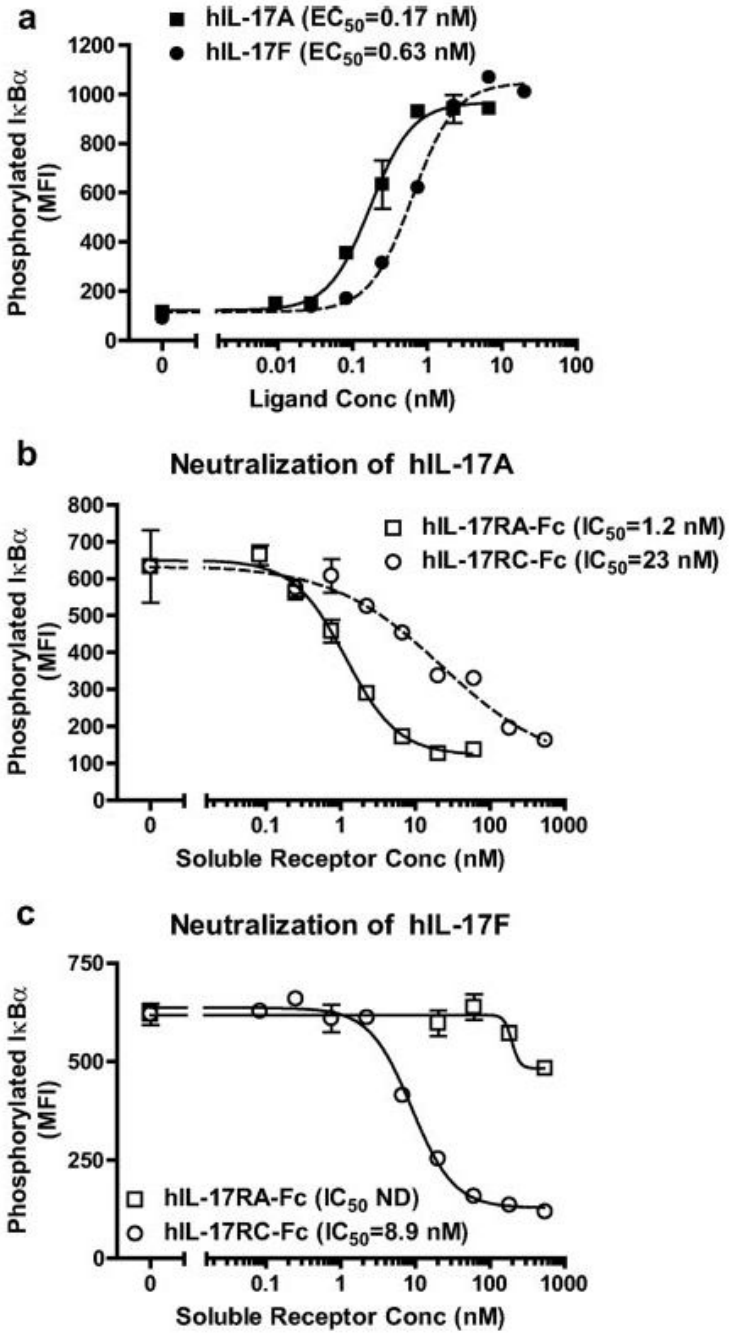

FIGURE 5.

Soluble hIL-17RC inhibits signaling in response to hIL-17A and -F. *a*, National Institutes of Health-3T3 cells transfected with hIL-17RC respond to hIL-17A and -F in a dose-dependent manner. Stably transfected cells were exposed to the indicated cytokine over a range of concentrations and responses were assessed by measuring the amount of P-I*κ*B*α* as described in *Materials and Methods. b*, hIL-17RA-Fc and hIL-17RC-Fc both inhibit responses of these cells to hIL-17A with IC_{50} values of 1.2 nM and 23 nM respectively. *c*, hIL-17RC-Fc inhibits responses to hIL-17F with an IC_{50} of 8.9 nM, but hIL-17RA-Fc does not (ND, not determined due to insufficient inhibition).

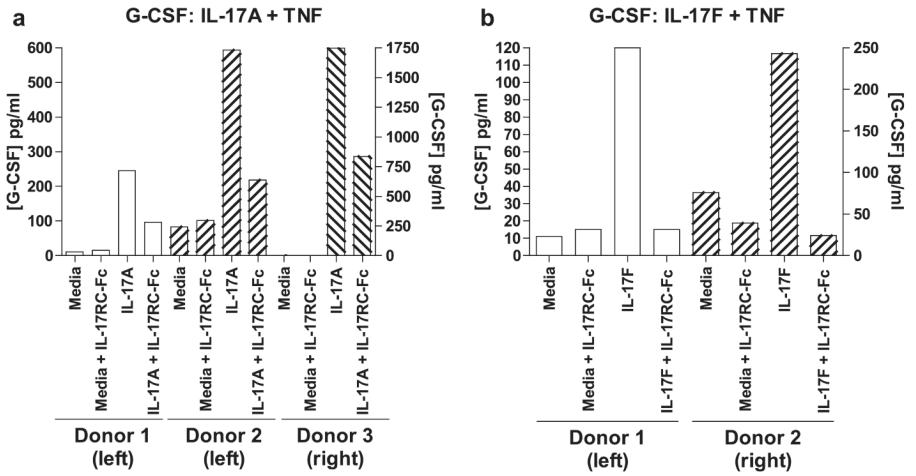

FIGURE 6.

hIL-17RC-Fc inhibits IL-17A and -F mediated production of G-CSF by HBE. *a*, HBE cells were stimulated with TNF-α (1 ng/ml) in the presence of medium or hIL-17A (10 ng/ml) with or without soluble hIL-17RC-Fc (10 μg/ml) as indicated. Culture supernatants were collected 24 h later and analyzed for G-CSF content by Luminex analysis. Results shown are from three separate donors. Because of donor to donor variation in the amount of cytokine produced, donors 1 and 2 are plotted on the left *y*-axis and donor 3 is plotted on the right *y*-axis. *b*, HBE from two donors were stimulated as above except with hIL-17F (100 ng/ml) instead of hIL-17A. G-CSF levels for donor 1 are plotted on the left *y*-axis and for donor 2 on the right *y*-axis. Data in this figure is all from cultures treated with TNF-α because this treatment alone did not trigger a significant response.

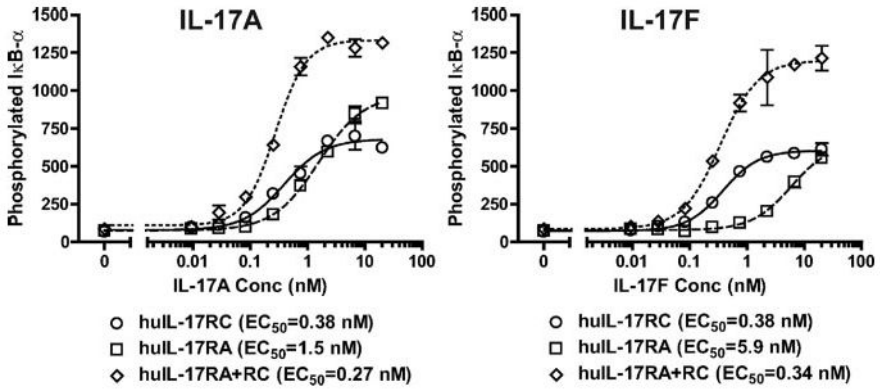

FIGURE 7.

Expression of both hIL-17RA and hIL-17RC does not enhance the sensitivity of transfected cells to hIL-17A and -F. Responses of National Institutes of Health-3T3 cells transfected with the indicated receptor(s) to hIL-17A and -F were assessed over a range of cytokine concentrations using P-IκBα detection and EC_{50} values were determined as described in *Materials and Methods*.

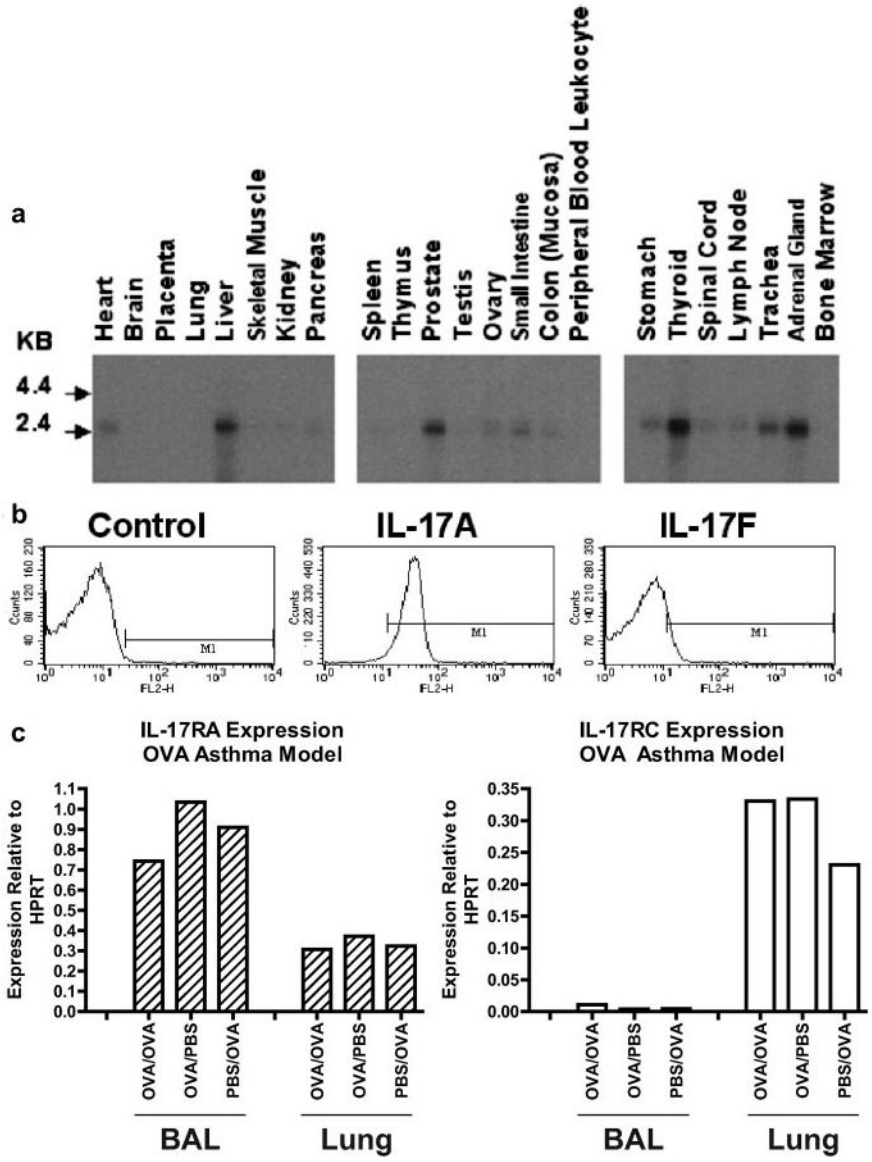

FIGURE 8.

Expression of IL-17RA and IL-17RC. *a*, Multitissue northern blots were probed with a hIL-17RC-specific probe as described in *Materials and Methods. b*, hIL-17A binds to hemopoietic cells, but hIL-17F does not. Biotinylated hIL-17A and hIL-17F were used to examine binding of these cytokines to various hemopoietic lineages by FACS analysis. Data shown is for $CD8^+$ T cells, but results were the same for all lineage markers examined including $CD4^+$ (helper T cells), $CD14^+$ (monocytes), $CD19^+$ (B cells) and $CD56^+$ (NK cells). *c*, Expression of mIL-17RA and mIL-17RC in lung tissue during asthma challenge. Mice were sensitized with PBS or OVA as described in *Materials and Methods*. Sensitized animals were then challenged with either PBS or OVA. After challenge, animals were sacrificed and infiltrating cells were harvested by BAL. Cells obtained from BAL or remaining lung tissue were processed for RNA and levels of mIL-17RA and mIL-17RC were assessed by RT-PCR analysis. Expression levels are presented relative to HG-PRT levels. OVA/OVA, Mice sensitized and challenged with OVA; OVA/PBS, mice sensitized with OVA and challenged with PBS; PBS/OVA, mice sensitized with PBS and challenged with OVA.

Table I

Affinity of hIL-17A and F for hIL-17RA and hIL-17RC via saturation binding on transfected cells[a]

	hIL-17A	hIL-17F
hIL-17RC (Δ7)[b]	0.6 nM	1.0 nM
hIL-17RA	1.9 nM	1.5 μM
hIL-17RA + IL-17RC(Δ7)	0.2 nM/3.0 nM	0.5 nM/>1 μM

[a] BHK cells transfected with the indicated receptors were used to establish K_d values for hIL-17A and hIL-17F as described in *Materials and Methods*. Results shown are mean K_d values derived from triplicate determinations. Results presented are typical of at least two experiments.

[b] Δ7 splice variant of hIL-17RC (see *Materials and Methods*, Table II, and Fig. 3).

Table II

Cytokine binding to IL-17RC splice variants[a]

		Exons[b]			Cytokine Binding[c]			
Species	**Variant**	**7**	**8**	**12**	**hIL-17A**	**hIL-17F**	**mIL-17A**	**mIL-17F**
Mouse	IL-17RC	+	+	+	+	+	–	+
	IL-17RCΔ7	–	+	+	+	+	–	–
	IL-17RCΔ7,8	–	–	+	–	–	–	–
	IL-17RCΔ8	+	–	+	–	–	–	–
Human	IL-17RC	+	+	+	+	+	–	+
	IL-17RCΔ7	–	+	+	+	+	–	–
	IL-17RCΔ7,12	–	+	–	–	–	–	–
	IL-17RCΔ12	+	+	–	–	–	–	–

[a] 293F cells transiently expressing the indicated splice variants were assessed for binding using biotinylated cytokines and FACS analysis as described in *Materials and Methods*. The inclusion of exons 7, 8, and 12 in each splice variant is indicated and the binding capacity of each variant for each of the four cytokines is indicated (see also Fig. 3). Identical results were obtained using stably transfected BHK cells as well.

[b] Exons completely included in transcript. For the mouse variants, the 3′ end of exon 8 is always included, but the – symbol indicates the 5′ end of it is excluded.

[c] +, Detectable cytokine binding as assessed by a significant increase in fluorescence by FACS. −, No significant change in fluorescence.

Table III

Affinity of IL-17A and F for hIL-17RC-Fc using Biacore analysis[a]

hIL-17RC-Ig	k_a (on-rate)	k_d (off-rate)	K_D
mIL-17A		No detectable binding	
mIL-17F		No detectable binding	
hIL-17A	1.05E+06	4.90E-04	0.469 nM
	1.24E+06	4.38E-04	0.352 nM
hIL-17F	9.91E+05	4.31E-04	0.435 nM
	1.11E+06	3.84E-04	0.346 nM

[a] Soluble receptor was captured onto chips and binding experiments were performed as described in *Materials and Methods*. Results shown represent data obtained from two independent experiments.

Table IV

Percentage of hIL-17RC transcripts from SAEC that include or exclude exons 7 and 12[a]

Exon 7		Exon 12	
+	−	+	−
44%	56%	89%	11%
Full-length, Δ12	Δ7; Δ7,12	Full-length, Δ7	Δ12; Δ7,12

[a]The fraction of hIL-17RC transcripts that include or exclude exons 7 and 12 was determined as described in *Materials and Methods*. The specific splice variants that fall into each category are indicated below the percentage (see also Table II).

Table V

The fraction of sequenced hIL-17RC transcripts identified from SAEC or Adrenal gland mRNA[a]

	Δ7	Δ7,12	FL	Δ12
SAEC	15/40	0/40	8/40	1/40
Adrenal	4/15	2/15	4/15	0/15

[a]Full-length hIL-17RC cDNA was generated from either SAEC or adrenal gland mRNA by RT-PCR and the products were cloned and sequenced. Sequence was obtained from 40 SAEC clones and 15 adrenal clones and the number that fall into each category here are indicated. Most of the remaining clones sequenced represented mis-spliced transcripts that included frame-shifts and had lost the capacity to encode hIL-17RC protein. FL, Full length.