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The Human IL-17F/IL-17A Heterodimetric Cytokine Signals through the IL-17RA/IL-17RC Receptor Complex

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IL-17A and IL-17F, produced by the Th17 CD4+ T cell lineage, have been linked to a variety of inflammatory and autoimmune conditions. We recently reported that activated human CD4+ T cells produce not only IL-17A and IL-17F homodimers but also an IL-17F/IL-17A heterodimeric cytokine. All three cytokines can induce chemokine secretion from bronchial epithelial cells, albeit with different potencies. In this study, we used small interfering RNA and Abs to IL-17RA and IL-17RC to demonstrate that heterodimeric IL-17F/IL-17A cytokine activity is dependent on the IL-17RA/IL-17RC receptor complex. Interestingly, surface plasmon resonance studies indicate that the three cytokines bind to IL-17RC with comparable affinities, whereas they bind to IL-17RA with different affinities. Thus, we evaluated the effect of the soluble receptors on cytokine activity and we find that soluble receptors exhibit preferential cytokine blockade. IL-17A activity is inhibited by IL-17RA, IL-17F is inhibited by IL-17RC, and a combination of soluble IL-17RA/IL-17RC receptors is required for inhibition of the IL-17F/IL-17A activity. Altogether, these results indicate that human IL-17F/IL-17A cytokine can bind and signal through the same receptor complex as human IL-17F and IL-17A. However, the distinct affinities of the receptor components for IL-17A, IL-17F, and IL-17F/IL-17A heterodimer can be exploited to differentially affect the activity of these cytokines. The Journal of Immunology, 2008, 181: 2799–2805.
biological activities among homodimers and heterodimer of IL-17A and IL-17F cytokines may be attributed to affinity differences among these cytokines for IL-17RA and IL-17RC receptors.

Materials and Methods

Reagents

Human IL-17F, IL-17A, and IL-17F/IL-17A were purified as previously described (20) and used in all experiments except small interfering RNA (siRNA) experiments, where IL-17A from R&D Systems was used. IL-17F, IL-17A, and IL-17F/IL-17A were biotinylated according to the manufacturer’s protocol using FluorReporter Minibiotin-XX protein labeling kit (Molecular Probes). Anti-IL-17RA and anti-IL-17RC polyclonal Abs used in functional assays were purchased from R&D systems. Human IL-17RA.Fc and IL-17RC.Fc were purified as described below.

Cloning of the human IL-17 receptors

Full-length human IL-17RA and IL-17RC were PCR amplified from unstimulated MG63 CDNA and sequence confirmed. The extracellular portions of human IL-17RA (residues 1–317) and human IL-17RC (residues 1–452) were fused in frame with a GSGSGSG linker and the human IgG1 Fc portion (28). PCR-derived fusion receptors were subcloned into a CMV promoter-driven mammalian expression construct and sequence verified.

Expression and purification of the human IL-17RA.Fc and IL-17RC.Fc receptors

HEK 293 cells were transiently transfected with IL-17RA.Fc or IL-17RC.Fc expressing vectors using TransIT-LT1 (Mirus Bio). Twenty-four hours post transfection, media was removed and replaced with serum-free media. Conditioned media was harvested at 48 h and protein production evaluated by Western blot analysis. Media containing IL-17RA.Fc or IL-17RC.Fc was flowed over a protein A column (Amersham Biosciences), washed with PBS, and protein eluted with 20 mM citric acid, 200 mM NaCl (pH 3). Protein aggregates were removed by size exclusion chromatography (PBS (pH 7.2)) and the resulting preparations dialyzed (PBS (pH 7.2)) and characterized by SDS-PAGE, Western blot analysis, and analytical size-exclusion chromatography.

Cell-based assay

BJ human foreskin fibroblast cells (American Type Culture Collection) were maintained in DME + 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, and 100 U/ml penicillin + 100 µg/ml streptomycin. BJ cells were seeded at 5 × 10^4 cells/well in 96-well flat-bottom microtiter plates in which cytokines had been prediluted in culture medium with or without soluble receptors. In treatments where Abs to cell-surface receptors were used, Abs to the receptors were added (20 µg/ml) to the cells before the addition of cytokine at the indicated concentration. Cells were incubated at 37°C for 16–24 h, and supernatants were collected and analyzed for GRO-related oncogene (GRO)-α production by ELISA (R&D Systems).

ELISA

Binding of human IL-17A, IL-17F, and IL-17F/IL-17A to human IL-17RA.Fc and IL-17RC.Fc was determined by indirect sandwich ELISA. ELISA plates (Costar) were coated with goat anti-human IgG-Fc (10 µg/ml, Bethyl Laboratories). Human IL-17RA.Fc (6 ng/ml) or IL-17RC.Fc (30 ng/ml) was added to plates and incubated for 3 h, followed by serial dilutions of biotinylated IL-17A, IL-17F, and IL-17F/IL-17A for 2 h at room temperature. ELISA was developed with poly-HRP streptavidin (Pierce Biotechnology) and tetramethylbenzidine substrate (Kirkegaard & Perry Laboratories).

siRNA

Twenty-four hours before transfection, BJ cells were seeded at 10^3 cells/well in 96-well plates. Cells were transfected with siRNA (20 nM) using DharmaFECT 1 transfection reagent (Dharmacon) according to the manufacturer’s instructions. Each transfection contained one of eight siRNAs listed in Table I. siRNAs were obtained from Qiagen. At 24 h, transfection medium was removed and replaced with fresh medium or culture medium containing IL-17A, IL-17F, or IL-17F/IL-17A at the indicated concentrations. After 16 h, cells were harvested for GRO-α production by ELISA (R&D Systems).

TaqMan

The TaqMan gene expression assay probe-primer sets for IL-17R (Hs00234888_m1, 20×) and IL-17RC (Hs00262062_m1, 20×) were obtained from Applied Biosystems. BJ cell mRNA was isolated using TurboCapture mRNA kit (Qiagen) according to the manufacturer’s instructions; mRNA was eluted in 50 µl of elution buffer. Ten microliters of mRNA per sample and 1× of probe-primer mixture per samples were used in 25 µl TaqMan PCR reactions (30 min at 48°C, 10 min at 95°C, then 40 cycles each of 15 s at 95°C and 1 min at 60°C on MicroAmp optical 96-well plates/caps) performed on an ABI Prism 7700 DNA sequence detector (Applied Biosystems). Each plate contained triplicates of the test mRNA templates and no-template controls for each reaction mix. The expression for each gene was normalized to human β2-microglobulin gene expression.

Western blot analysis

HEK 293 cells (1.2 × 10^5 cells/ml) stably transfected with human IL-17RA or IL-17RC plasmid were seeded in 96-well plates. After 16–24 h the cells were transfected with 20 nM of siRNA and DharmaFECT transfection reagent (Dharmacon) according to the manufacturer’s protocol. At 48 h, the cells were washed once with PBS and lysed on ice using M-PER, mamalian protein extraction reagent (Pierce Biotechnology). Extracted protein was then loaded on an SDS-PAGE gel, transferred to a nylon membrane, and the membrane blocked with 5% nonfat dried milk in PBS containing 0.1% Tween 20. IL-17RA or IL-17RC Ab was added at 1/4000 and incubated overnight at 4°C. Membranes were washed three times (PBS with 0.1% Tween 20) and incubated with donkey anti-goat IgG-HRP (1/2000 dilution, Santa Cruz Biotechnology). Proteins were visualized using Western Lightning Western Blot Chemiluminescence Reagent Plus (PerkinElmer).

Binding kinetics of the IL-17 cytokines with receptors

Kineti analysis was performed using a Biacore 2000 (Biacore). All experiments were performed at 22°C. IL-17RA.Fc or IL-17RC.Fc was immobilized between 1000 and 2000 resonance units (RU) using amine coupling as directed by the manufacturer. Human IL-17F, IL-17A, and IL-17F/IL-17A heterodimer were each diluted into HBST buffer (10 mM HEPES with 0.15 M NaCl, 3.4 mM EDTA, and 0.05% surfactant F20) at concentrations of 400, 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 nM. Each sample was injected at 50 µl/min for 3 min followed by 10-min dissociation. The surface was regenerated by a 30-s injection of a solution of 0.549 M MgCl2, 0.138 M potassium thiocyanate, 0.276 M urea, and 0.549

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* Abbreviations used in this paper: siRNA, small interfering RNA; GRO-α, growth-related oncogene-alpha.

**Table I. siRNAs used for human IL-17RA and IL-17A knockdown**

<table>
<thead>
<tr>
<th>siRNA</th>
<th>Cat. No.</th>
<th>Sequence</th>
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<tr>
<td>Hs_IL17R_1_HP</td>
<td>S100104979</td>
<td>CAG CGG TCT GAT CTT GAT CTA</td>
</tr>
<tr>
<td>Hs_IL17R_2_HP</td>
<td>S100104986</td>
<td>CAG CAC CTA GGT ACT CTA</td>
</tr>
<tr>
<td>Hs_IL17R_3_HP</td>
<td>S100104993</td>
<td>CAG GAA GGT CTA CTA CTA</td>
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<tr>
<td>Hs_IL17RC_1_HP</td>
<td>S100144165</td>
<td>ACC GCA GAT CAT TAC CTT GAA</td>
</tr>
<tr>
<td>Hs_IL17RC_2_HP</td>
<td>S100144172</td>
<td>CAG GTC TGA GTC TCT GCT CA</td>
</tr>
<tr>
<td>Hs_IL17RC_3_HP</td>
<td>S100144179</td>
<td>CAG GAC TTA AAT AAA GAC AGA</td>
</tr>
<tr>
<td>Hs_IL17RC_4_HP</td>
<td>S100144186</td>
<td>CCG GCC GTC TCT GCT CCT CTA</td>
</tr>
</tbody>
</table>
M guanidine-HCl followed by two consecutive 60-s HBST buffer injections. Data were double referenced (29) using Scrubber2 software (Bio-Logic Software), then fit to a 1:1 binding model using Biacore evaluation software version 3.2.

Results

Functional activity of IL-17A, IL-17F, and IL-17F/IL-17A on BJ cells

We have previously reported that IL-17F, IL-17A, and IL-17F/IL-17A induce the secretion of GRO-α from the BEAS-2B human bronchial epithelial cell line (20). We have extended this observation to BJ, a primary human foreskin fibroblast cell. ELISA analysis of conditioned medium obtained from BJ cells cultured with purified IL-17F, IL-17A, or IL-17F and incubated at 37°C for 16–24 h. Supernatants were collected and analyzed in triplicate for GRO-α, IL-6, and IL-8 production by ELISA. Stimulation index represents the data normalized to those obtained with media control. Data represent three experiments. B. Human primary foreskin fibroblast (BJ) cells were seeded at 5 × 10⁵ cells/well into 96-well plates containing IL-17A (1 ng/ml), IL-17F/IL-17A heterodimer (5 ng/ml), or IL-17F (50 ng/ml) and incubated at 37°C for 16–24 h. Supernatants were collected and analyzed in triplicate for GRO-α production by ELISA. Data are representative of seven experiments.

FIGURE 1. Functional activity of IL-17 cytokines. A, Human primary foreskin fibroblast (BJ) cells were seeded at 5 × 10⁵ cells/well into 96-well plates containing IL-17A (1 ng/ml), IL-17F/IL-17A heterodimer (5 ng/ml), or IL-17F (50 ng/ml) and incubated at 37°C for 16–24 h. Supernatants were collected and analyzed in triplicate for GRO-α, IL-6, and IL-8 production by ELISA. Stimulation index represents the data normalized to those obtained with media control. Data represent three experiments. B. Human primary foreskin fibroblast (BJ) cells were seeded at 5 × 10⁵ cells/well into 96-well plates containing serial dilutions of purified IL-17A, IL-17F/IL-17A, or IL-17F and incubated at 37°C for 16–24 h. Supernatants were collected and analyzed in triplicate for GRO-α production by ELISA. Data are representative of seven experiments.

FIGURE 2. IL-17F/IL-17A heterodimer can bind independently to IL-17RA and IL-17RC. Goat anti-human IgG (10 ng/ml) was used to capture IL-17RA.Fc (6 ng/ml) or IL-17RC.Fc (30 ng/ml), and biotinylated IL-17A, IL-17F, or IL-17F/IL-17A was added at the indicated concentrations. ELISA was developed as described in Materials and Methods. A, IL-17A, IL-17F, and IL-17F/IL-17A binding to IL-17RC.Fc. B, IL-17A, IL-17F, and IL-17F/IL-17A binding to IL-17RA.Fc. Data are representative of two experiments.

IL-17F, IL-17A, and IL-17F/IL-17A bind to the IL-17RA/IL-17RC receptor complex with different kinetics

Indirect sandwich ELISAs were done to evaluate the binding of IL-17F, IL-17A, and IL-17F/IL-17A to IL-17RC.Fc and IL-17RA.Fc (Fig. 2). We confirmed, as previously reported (30), that IL-17A and IL-17F homodimers can bind independently to IL-17RA or IL-17RC. IL-17F/IL-17A heterodimer can also bind independently to either IL-17RC or IL-17RA (Fig. 2). All three of the IL-17 cytokines bind to IL-17RC.Fc with approximately the same EC₅₀ of 12–17 ng/ml (Fig. 2A). However, the EC₅₀ for the IL-17 cytokines is different for binding to IL-17RA.Fc. The lowest EC₅₀ occurs between IL-17A and IL-17RA.Fc with a value of 19 ng/ml, whereas IL-17F binds weakly to IL-17RA.Fc with an EC₅₀ > 2000 ng/ml. The IL-17F/IL-17A heterodimer is an EC₅₀ of 329 ng/ml, which is 10-fold higher than the binding of IL-17A but ~10-fold lower than the IL-17F, as shown in Fig. 2B.
To better characterize the binding kinetics of human IL-17F, IL-17A, and IL-17F/IL-17A to each receptor component, the association and dissociation rate constants were measured by surface plasmon resonance. The calculated dissociation constants are shown in Table II. Each IL-17 cytokine tested binds IL-17RA.Fc with a different $K_d$, which is reflected by differences in the on and off rates. IL-17A binds with the fastest on rate ($1.4 \times 10^5$ M/s) and slowest off rate ($2.9 \times 10^4$ s$^{-1}$), which result in the lowest $K_d$ of 2.2 nM. IL-17F has about a 10-fold slower on rate ($9.4 \times 10^3$ M/s) and 10-fold slower off rate ($1.6 \times 10^3$ s$^{-1}$), which results in about a 100-fold higher $K_d$ for IL-17RA.Fc (170 nM) compared with IL-17A. Interestingly, the IL-17F/IL-17A heterodimer has an on rate ($4.3 \times 10^4$ M/s) that is intermediate between IL-17A and IL-17F whereas the off rate ($1.0 \times 10^2$ s$^{-1}$) is the same as IL-17F. Overall, the $K_d$ of the IL-17F/IL-17A heterodimer (26 nM) is intermediate between IL-17A and IL-17F. In contrast to their different binding kinetics for IL-17RA.Fc, IL-17F, IL-17A, and IL-17F/IL-17A, each bind IL-17RC.Fc with similar on and off rates and hence also have similar $K_d$ values of 10–20 nM. Altogether, these results indicate that IL-17A binds better to IL-17RA than IL-17RC, IL-17F binds to IL-17RC with $\sim 10$-fold higher affinity than to IL-17RA, while IL-17F/IL-17A binds with similar affinity to both receptors.

**Table II.** Kinetic rate constants of IL-17 cytokines binding to IL-17RA.Fc and IL-17RC.Fc as determined by Biacore

<table>
<thead>
<tr>
<th>Injected Analyte</th>
<th>Immobilized Ligand</th>
<th>$k_{on}$ (M/s)</th>
<th>$k_{off}$ (s$^{-1}$)</th>
<th>$K_d$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17A</td>
<td>IL-17RA-Fc</td>
<td>1.39 $\pm$ 0.15 $\times 10^5$</td>
<td>2.94 $\pm$ 0.70 $\times 10^{-4}$</td>
<td>2.15 $\pm$ 0.73 $\times 10^{-9}$</td>
</tr>
<tr>
<td>IL-17F</td>
<td>IL-17RA-Fc</td>
<td>9.43 $\pm$ 0.38 $\times 10^3$</td>
<td>1.64 $\pm$ 0.10 $\times 10^{-3}$</td>
<td>1.74 $\pm$ 0.07 $\times 10^{-7}$</td>
</tr>
<tr>
<td>IL-17F/IL-17A</td>
<td>IL-17RA-Fc</td>
<td>4.28 $\pm$ 1.46 $\times 10^4$</td>
<td>1.03 $\pm$ 0.01 $\times 10^{-3}$</td>
<td>2.55 $\pm$ 0.83 $\times 10^{-8}$</td>
</tr>
<tr>
<td>IL-17A</td>
<td>IL-17RC-Fc</td>
<td>8.92 $\pm$ 0.39 $\times 10^4$</td>
<td>1.79 $\pm$ 0.08 $\times 10^{-3}$</td>
<td>2.01 $\pm$ 0.18 $\times 10^{-8}$</td>
</tr>
<tr>
<td>IL-17F</td>
<td>IL-17RC-Fc</td>
<td>1.28 $\pm$ 0.07 $\times 10^5$</td>
<td>2.12 $\pm$ 0.20 $\times 10^{-3}$</td>
<td>1.66 $\pm$ 0.06 $\times 10^{-8}$</td>
</tr>
<tr>
<td>IL-17F/IL-17A</td>
<td>IL-17RC-Fc</td>
<td>1.44 $\pm$ 0.15 $\times 10^5$</td>
<td>1.51 $\pm$ 0.16 $\times 10^{-3}$</td>
<td>1.06 $\pm$ 0.22 $\times 10^{-8}$</td>
</tr>
</tbody>
</table>

*Shown are the means and SDs from three independent experiments.

FIGURE 3. Effect of siRNA on the expression of IL-17RA and IL-17RC. BJ cells were transfected with either IL-17RA or IL-17RC siRNA as described in the Materials and Methods. A. The amount of knockdown for IL-17RA and IL-17RC in the transfected BJ cells was measured by quantitative PCR and plotted as percentage response to a siRNA control (NTC1). B. HEK 293 cells expressing IL-17RA or IL-17RC were transfected with either IL-17RA or IL-17RC siRNA. At 48 h, cells were collected, protein was extracted, and receptor expression was analyzed by Western blot.

FIGURE 4. Effect of IL-17RA and IL-17RC siRNA on IL-17 cytokine response. BJ cells were transfected with IL-17RA or IL-17RC siRNAs identified in Fig. 3. The transfection medium was removed and replaced with culture medium containing IL-17A (A), IL-17F (B), or IL-17F/IL-17A (C) at indicated concentrations. After 16 h, supernatants were collected and analyzed in triplicate for GRO-$\alpha$ secretion by ELISA. Data are representative of three experiments.
IL-17A. BJ cells were transfected with various IL-17RA (RA-1 to RA-4) or IL-17RC (RC-1 to RC-4) siRNAs, and the level of IL-17RA and IL-17RC transcript expression was determined by quantitative PCR (Fig. 3A). Additionally, the effect of siRNA on IL-17RA and IL-17RC protein expression in HEK 293 cells overexpressing IL-17RA or IL-17RC was determined by Western blot analysis (Fig. 3B). Based on the specific inhibition of both mRNA transcripts and protein, two siRNAs per receptor were selected to evaluate IL-17RA (RA-3 and RA-4) and IL-17RC (RC-2 and RC-4) activity in functional assays. IL-17F, IL-17A, and IL-17F/IL-17A were added at three different concentrations to BJ cells transfected with IL-17RA, IL-17RC, or control (NTC1) siRNAs (Fig. 4). IL-17RA and IL-17RC siRNAs decreased GRO-α production in response to IL-17A, IL-17F, or IL-17F/IL-17A at all three concentrations tested relative to the siRNA control. These results suggest that the IL-17F/IL-17A heterodimer activity depends on both IL-17RA and IL-17RC and thus, this cytokine shares a common receptor complex with IL-17A and IL-17F.

**Anti-IL-17RA and anti-IL-17RC decrease the functional activity of IL-17F, IL-17A, and IL-17F/IL-17A**

To further delineate the role of IL-17RA and IL-17RC, we evaluated whether Abs to IL-17RA and IL-17RC could block IL-17F/IL-17A functional activity. It has been previously shown that Abs to IL-17RA and IL-17RC can block the activity of IL-17F and IL-17A (30). BJ cells were stimulated with suboptimal concentrations of cytokines in the presence or absence of Abs to IL-17RA and IL-17RC. As shown in Fig. 5A, IL-17A-, IL-17F/IL-17A-, and IL-17F-dependent induction of GRO-α was significantly decreased when the cells were treated with anti-human IL-17RA or anti-human IL-17RC Abs. However, anti-IL-17RC Ab had a more profound effect on the activity of IL-17F than on the activities of IL-17A and IL-17F/IL-17A heterodimer. These results, in conjunction with the siRNA knockdown experiments, strongly support the notion of a common receptor complex for IL-17A, IL-17F, and IL-17F/IL-17A.

**Preferential inhibition of homodimers and heterodimer of IL-17A and IL-17F cytokines by soluble forms of IL-17RA and IL-17RC**

Our surface plasmon resonance results demonstrated different binding affinities between IL-17 cytokines and IL-17RA and IL-17RC receptor components (Table II), so we investigated whether the soluble form of the receptors would affect differentially the functional activity of IL-17A, IL-17F, and IL-17F/IL-17A. Soluble receptor forms were generated by in-frame fusing of the extracellular portions of IL-17RA or IL-17RC to human IgG1 Fc portions, and protein production was done in HEK 293 cells. The ability of the soluble receptors to inhibit cytokine activity was determined at a 50-fold molar excess of receptor to cytokine. As shown in Fig. 5B, IL-17RA.Fc and IL-17RC.Fc significantly neutralize the activity of IL-17A or IL-17F, respectively, at >50% inhibition. Neither IL-17RA.Fc nor IL-17RC.Fc alone could significantly inhibit the activity of the IL-17F/IL-17A heterodimer. Interestingly, a combination of soluble IL-17RA and IL-17RC neutralized ~50% of the activity of the IL-17F/IL-17A heterodimer. These findings suggest that a combination of both receptors is required to neutralize the activity of the IL-17F/IL-17A heterodimer whereas only the higher binding affinity receptor, either IL-17RA or IL-17RC, is required for neutralizing the activity of IL-17A or IL-17F homodimers, respectively.

**Discussion**

In this study, we show that human IL-17F/IL-17A heterodimer, the recently identified member of the IL-17 cytokine family, utilizes the same receptor complex as the IL-17F and IL-17A cytokines. Using various experimental approaches, including surface plasmon resonance and siRNA gene knockdown, we characterized the physical and functional interactions of IL-17F/IL-17A, IL-17F, and IL-17A with the IL-17RA and IL-17RC receptors and propose that all three cytokines require both receptors for their biological activity.

Toy et al. demonstrated that human IL-17A or IL-17F could not induce CXCL1 expression in IL-17RA–/– fibroblast cells and that transfection of human IL-17A did not rescue the expression of CXCL1 as was seen in wild-type cells (26). When the cells were cotransfected with both human IL-17A and IL-17RC and treated with either human IL-17A or IL-17F, production of CXCL1 was restored, suggesting that a heterodimeric IL-17RA/IL-17RC receptor was required for signaling (26). Recently, it has been reported that IL-17A is also required for murine IL-17F/IL-17A signaling (21). We confirm and extend these findings by assessing the function of human IL-17F/IL-17A heterodimer in more physiological primary human foreskin fibroblast cells. We find that Ab blockade or siRNA knockdown of either receptor component leads to loss of both homodimeric and heterodimeric cytokine activity. These data are consistent with a model in which both receptor chains are required to form a functional receptor complex. Although IL-17A
and IL-17F homodimers can bind independently to IL-17RA and IL-17RC (Table II), both chains are required for IL-17A and IL-17F biological function.

In our study, direct binding measurements with purified IL-17 cytokines and receptors indicate that while all three IL-17 cytokines bind with similar affinities to IL-17RC, they bind very differently to IL-17RA. The order of binding to the receptors and the binding affinities for receptor chains may determine the molecular mechanisms that differentiate the biological functions of these cytokines. Based on the binding kinetics, we propose that IL-17F is likely to associate with IL-17RC and not IL-17RA, unless IL-17RA is in great abundance. On the other hand, IL-17A would be predicted to preferentially bind IL-17RA complexes. The IL-17F/IL-17A heterodimer is likely to interact equally with either receptor chain complex. The pattern of binding affinities that we demonstrated for IL-17A and IL-17F is the same as in previous reports (17, 27), but the dissociation constants differ. The difference in relation to IL-17RA may be attributed to the monomeric form of the IL-17RA protein used in previous experiments (17) vs the dimeric IL-17RA receptor Ig Fc fusion construct used in the present study. Consistent with the higher affinity of IL-17A compared with IL-17F for IL-17RA, we show that the IL-17RA.Fc fusion protein inhibits cellular responses mediated by IL-17A better than those mediated by either IL-17F or IL-17F(IL-17A heterodimer (Fig. 5B and Ref. 30). Both soluble receptors, IL-17RA.Fc and IL-17RC.Fc, which have approximately the same binding affinity for the IL-17F/IL-17A heterodimer, are required to inhibit the activity of IL-17F/IL-17A. The affinities we report for IL-17A and IL-17F binding to IL-17RC are weaker than a previous report even though both studies used the same spliced form of IL-17RC and both used a receptor Ig Fc reagent (27). However, these studies differed in how the receptor was immobilized on the sensor chip surface, which, in turn, may affect binding affinity of the ligand (31).

Biological activity of the IL-17 family may differ among cell types as a result of IL-17 receptor composition (homodimeric and heterodimeric forms). For instance, we observed that soluble IL-17RC.Fc, when used at 50-fold molar excess, can neutralize the biological activity of IL-17F but not IL-17A on primary human foreskin fibroblast cells even though they have a similar binding affinity for the IL-17RC (Fig. 5B). On human bronchial epithelial cells, Kuestner et al. (27) have reported neutralization of IL-17A activity (in combination with TNF-α) by IL-17RC used at a 217-fold molar excess. These disparate results may be partially attributed to differences in receptor composition among cell lines tested (primary human foreskin fibroblasts vs bronchial epithelial cells), cytokine activity examined (IL-17A vs IL-17A plus TNF-α), and/or molar excess of receptor to cytokine employed (50 vs 217 molar excess). Thus, further characterization of receptors in these cell lines is warranted.

IL-17A and IL-17F share 50% identity at the amino acid level. The crystal structure of IL-17F contains a cysteine knot fold characterized by two sets of paired β strands with disulfide bonds connecting the second and fourth strand (17). Conservation of the disulfide linkage in both the IL-17A homodimer and the IL-17F/IL-17A heterodimer suggests that these cytokines have similar structures (20). Although reports have characterized the TGF-β superfamilies as containing a cysteine knot motif, the lack of the third disulfide bond is unique to IL-17F. The crystal structure also showed that IL-17F contains a large cavity (one per monomer) at the cytokine dimer interface (17), as was seen with nerve growth factor (19). These cavities have been proposed as likely sites of cytokine/receptor interaction, based on the binding of nerve growth factor to its high affinity receptor, TrkA (17). Recently, it has been shown that IL-17RA is a preformed multimeric complex and that receptor association is reduced in the presence of IL-17A or IL-17F, suggesting a conformational change of the receptor due to ligand binding (25). Furthermore, it has been proposed that ligand binding alters the conformation of IL-17RA to facilitate a functional, heterotypic interaction with IL-17RC (26). It is tempting to speculate that one of the receptors binds to the large cavity present in the homodimer or heterodimer interface, leading to a conformational change and recruitment of the second receptor.

The recent emergence of the effector Th17 T cell lineage has reshaped the Th1/Th2 landscape and presents a new arm of adaptive immunity that provides explanations to some aspects of immune regulation and immune pathogenesis. While considerable data related to the expression of IL-17A and IL-17F cytokines have been generated recently, detailed functional studies of the IL-17 receptor family have not yet been reported. Currently, it is not known if IL-17F, IL-17A, and IL-17F(IL-17A have redundant roles in certain disease settings. Along these lines, studies have shown that IL-17A-deficient mice are resistant to the development of collagen-induced arthritis, suggesting that IL-17F cannot compensate for IL-17A in the pathogenesis of collagen-induced arthritis (15). By contrast, intranasal administration of either IL-17A or IL-17F adenovirus leads to pulmonary neutrophilia and inflammatory gene expression in the lungs (32).

Based on our finding of a shared use of receptors by IL-17A, IL-17F, and IL-17F(IL-17A, an interesting question arises from our studies as to whether IL-17F(IL-17A contributes partially to the activity observed for IL-17A in vitro and in vivo. Tissue expression comparing IL-17F(IL-17A heterodimer, IL-17F, and IL-17A might define the role of the heterodimer. In conclusion, we have shown that IL-17F(IL-17A, IL-17F, and IL-17A share a common receptor complex. However, due to binding affinity differences between the IL-17 cytokines and soluble receptors, each receptor component may be exploited to target either IL-17A, IL-17F, or IL-17F(IL-17A cytokine. Elucidating the complexity related to regulation of the expression of various IL-17A–F family members and the biologic significance of their interactions with their cognate receptor(s) will enhance their potential as therapeutic targets in inflammation and autoimmunity.

Disclosures
The authors have no financial conflicts of interest.

References


