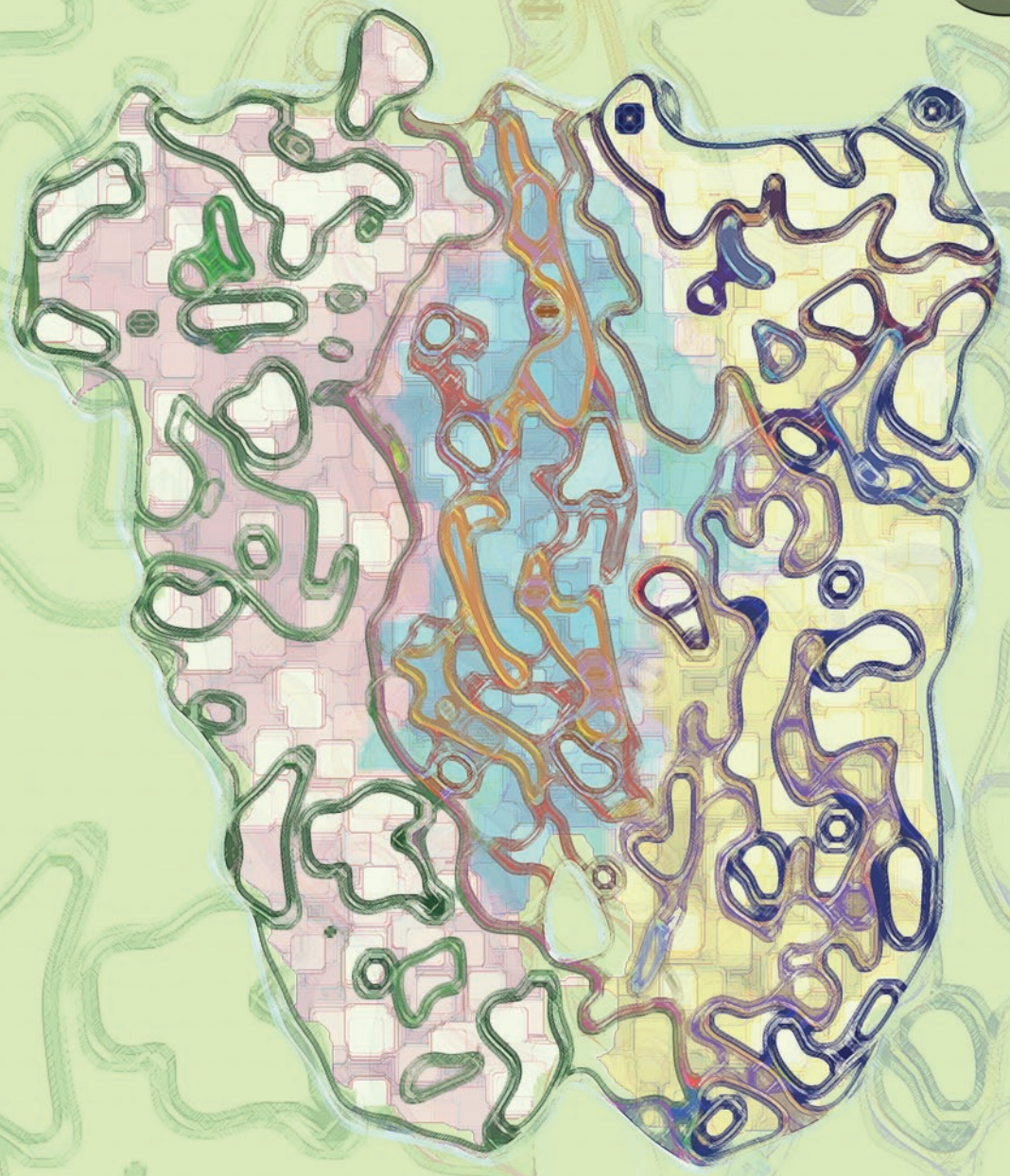
VOLUME 10 NUMBER 12 DECEMBER 2009
www.nature.com/natureimmunology

nature immunology



IL-17RA–IL-17 structure

MicroRNA and MS

Regulating MAVS

nature immunology

Structural basis of receptor sharing by interleukin 17 cytokines

Lauren K Ely, Suzanne Fischer & K Christopher Garcia

Interleukin 17 (IL-17)-producing helper T cells (T_H-17 cells), together with their effector cytokines, including members of the IL-17 family, are emerging as key mediators of chronic inflammatory and autoimmune disorders. Here we present the crystal structure of a complex of IL-17 receptor A (IL-17RA) bound to IL-17F in a 1:2 stoichiometry. The mechanism of complex formation was unique for cytokines and involved the engagement of IL-17 by two fibronectin-type domains of IL-17RA in a groove between the IL-17 homodimer interface. Binding of the first receptor to the IL-17 cytokines modulated the affinity and specificity of the second receptor-binding event, thereby promoting heterodimeric versus homodimeric complex formation. IL-17RA used a common recognition strategy to bind to several members of the IL-17 family, which allows it to potentially act as a shared receptor in multiple different signaling complexes.

Naive T cells are stimulated to differentiate into specialized effector cells mainly through the actions of secreted cytokines. Helper T cells have been typically considered to fall into one of two effector cell lineages; T helper type 1 (T_H1) and T_H2 cells modulate cellular and humoral T cell immunity, respectively, on the basis of their cytokine expression profiles[1]. Subsequent work has described 'T_H-17' cells, a third lineage of effector helper T cells distinct from and in fact antagonized by products of the T_H1 and T_H2 lineages[2,3]. Named for its 'signature' cytokine, interleukin 17 (IL-17), this third subset of helper T cells seems to have evolved as an arm of the adaptive immune system specialized for enhanced host protection against extracellular bacteria and some fungi, as these microbes may not be effectively controlled by T_H1 or T_H2 responses[4,5]. The varied tissue sources of cytokines that induce the differentiation and regulate the homeostasis of T_H-17 cells (IL-23, IL-6 and transforming growth factor-β), together with the presence of IL-17 receptors on both hematopoietic and nonhematopoietic cells, emphasize the complicated relationships that exist between cells of the adaptive and innate immune systems. Although the full scope of T_H-17 cell effector functions is still emerging, the strong inflammatory response promoted by T_H-17 cells has been associated with the pathogenesis of several autoimmune and inflammatory disorders previously attributed to T_H1 or T_H2 cells, including rheumatoid arthritis, multiple sclerosis and psoriasis[4]. Thus, targeting T_H-17 cells for the treatment of autoimmune and inflammatory disorders, either directly through IL-17 blockade or indirectly through inhibition of IL-23, is now being pursued clinically. However, the structural uniqueness of the IL-17 system, combined with a dearth of biochemical and structural information on receptor interactions, remains a barrier to the development of mechanism- or structure-based antagonists.

The IL-17 family is composed of six cytokines and five receptors, and the ligand-receptor pairing is not completely worked out for all members[6]. On the basis of the crystal structure of IL-17F, the six structurally related IL-17 cytokines (IL-17A through IL-17F) are predicted to form a homodimeric fold (or heterodimeric fold in the case of IL-17A–IL-17F) homologous to that of the cysteine-knot growth factors such as nerve growth factor (NGF)[7,8]. T_H-17 cell–derived IL-17A and IL-17F share the greatest homology in the family (50%) and require both IL-17 receptor A (IL-17RA; A001253) and IL-17RC for signaling[9,10]. Although it has been shown that fibroblasts, epithelial and endothelial cells coexpress both IL-17RA and IL-17RC, T cells do not express IL-17RC and express only IL-17RA[11]. It was thought that lymphocytes are not responsive to IL-17; however, it has now been reported that T cells can indeed respond directly to IL-17 (ref. 12).

The five IL-17 receptors (IL-17RA through IL-17RE) are not homologous to any known receptors and show considerable sequence divergence. All seem to contain extracellular domains composed of fibronectin type III (FnIII) domains and cytoplasmic SEF–IL-17R domains that show loose homology to Toll–IL-1R domains[13,14]. The IL-17 receptors mediate signaling events that are distinct from those triggered by the more widely known receptors for type I four-helix cytokines[15,16]. Like stimulation of the Toll-like receptor, stimulation of the IL-17 receptor results in activation of the transcription factor NF-κB and mitogen-activated protein kinases. However, IL-17 receptor signaling does not use the same set of membrane-proximal adaptor molecules as Toll-like receptor signaling does; IL-17R requires the adaptor Act1, which also contains a SEF–IL-17R domain[17–19]. These unique signaling properties of IL-17 receptors enable T_H-17 cells to act as a bridge between cells of the innate and adaptive immune systems. Mechanistically, fluorescence resonance energy transfer studies have

Howard Hughes Medical Institute, Departments of Molecular and Cellular Physiology, and Structural Biology, Stanford University School of Medicine, Stanford, California, USA. Correspondence should be addressed to K.C.G. (kcgarcia@stanford.edu).

Received 20 August; accepted 17 September; published online 18 October 2009; doi:10.1038/ni.1813

Table 1 Data collection and refinement statistics

	Native 1	K_2PtCl_4	SeMet	Native 2
Data collection				
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions				
a, *b*, *c* (Å)	171.6, 171.6, 84.3	171.7, 171.7, 83.6	171.4, 171.4, 82.3	170.7, 170.7, 81.9
Wavelength (Å)	1.00	1.07	0.978	1.03
Resolution (Å)	3.4, 3.4, 3.9	4.5	3.8	3.3
Completeness (%)	100 (100)	99.9 (100)	100 (100)	99.2 (99.9)
Redundancy	7.8 (8.0)	15.0 (15.5)	15.4 (15.6)	6.3 (6.4)
R_{merge}	0.10 (0.47)	0.10 (0.39)	0.12 (0.48)	0.15 (0.45)
I/σI	15.6 (3.8)	19.7 (6.8)	12.7 (7.0)	7.6 (3.2)
Refinement statistics				
Resolution (Å)	40–3.3			
Reflections (total / test)	18,479 / 948			
R_{factor}/R_{free} (%)	22.9 / 25.6			
Atoms				
Protein	3,779			
Carbohydrate	98			
Calcium	1			
r.m.s. deviations				
Bond length (Å)	0.009			
Bond angle (°)	1.33			
Mean *B* value (Å^2)	111			
Ramachandran plot	87.1, 12.6, 0.2, 0.0			

Values in parentheses represent the shell of highest resolution; values for the Ramachandran plot are presented as most favored, allowed, generously allowed, disallowed. K_2PtCl4, heavy metal derivative; SeMet, IL-17RA labeled with selenium-containing methionine (selenomethionine).

suggested that IL-17RA may exist as a preformed dimer on the cell surface that undergoes a conformational change after binding IL-17 to form a heterodimeric signaling complex with IL-17RC. However, the molecular basis for how a homodimeric IL-17 cytokine would pair with two different receptors has remained unknown[14,20].

RESULTS

Structure of IL-17RA bound to IL-17F

We determined the crystal structure of IL-17RA bound to IL-17F at a resolution of 3.3 Å by single isomorphous replacement with anomalous scattering phasing (**Table 1**). We expressed IL-17F from baculovirus and expressed the IL-17RA extracellular domain with human embryonic kidney 293S cells deficient in acetylglucosaminyltransferase I (293S GnTI- cells). To facilitate crystallization, we methylated the complex and 'shaved' the heavily glycosylated receptor extracellular domain with endoglycosidase H before crystallization to improve homogeneity (described in Online Methods) which left one *N*-acetylglucosamine residue at each of the asparagine-linked glycosylation sites (**Fig. 1**). Biochemically, the 'shaved' and 'unshaved' complexes behaved identically (data not shown). By gel filtration, mixtures of IL-17F or IL-17A with extracellular domain of IL-17RA resulted in coelution of complexes with a stoichiometry of 2:2 (two receptors plus one IL-17 dimer) or 1:2 (one receptor plus one IL-17 dimer), with the main species having a stoichiometry of 1:2. We detected the complex with a stoichiometry of 2:2 only at high protein concentrations, whereas at lower concentrations, the complex with a stoichiometry of 1:2 predominated even in the presence of excess IL-17RA. The crystals contained one IL-17RA bound to one IL-17F homodimer (**Fig. 1**). As discussed below, this 'partial' signaling complex may in fact be the biologically relevant form of the IL-17RA–IL-17F and IL-17RA–IL-17A complexes.

The IL-17RA ectodomain is composed of two unusual FnIII domain modules joined by an 18–amino acid linker (**Fig.1** and **Supplementary Fig. 1**). Although it is not apparent from the sequence, the IL-17RA structure is reminiscent of that of hematopoietic cytokine receptors in that it contains tandem β-sandwich domains; however, the domains themselves have some substantial deviations from canonical FnIII folds, and the manner of ligand interaction is entirely distinct from that of other cytokine receptors. We modeled residues 2–272 of the predicted 286 ectodomain residues (where residue 1 is the first amino acid of the mature peptide) into continuous electron density for the receptor chain and clearly visualized five of the potential seven *N*-linked glycans (electron-density map quality, **Supplementary Fig. 2**). The first FnIII domain (D1) has an additional 40–amino acid amino-terminal extension that forms a unique fold (**Supplementary Fig. 1**). The chain makes a hairpin-like turn bridged by a disulfide bond (Cys12-Cys19), and the second strand of the turn forms a β-strand (A′) that extends the FnIII β-sheet and then wraps around the face of D1, forming disulfide bonds with Cys95 of the C′ strand before passing over the domain to start the A strand of the FnIII domain. The interdomain linker region contains a short helix and is stabilized by an internal disulfide bond (Cys154-Cys165). The second FnIII domain (D2) has two atypical disulfide bonds, one linking the C–C′ loop (Cys214) to the D–F loop (Cys245) and a second in the F–G loop (Cys259-Cys263). We predict that a third disulfide bond exists between the F–G loop (Cys246) and the carboxyl terminus of the G strand (Cys272), similar to that observed in class II cytokine receptors[21]; however, this bond is not well defined in the present electron density map.

Although the core structure of the IL-17RA-bound IL-17F molecule was essentially unchanged compared with that of the form of IL-17F not receptor bound[7], peripheral strands and loops underwent structural accommodations to facilitate binding to IL-17RA. The conformation observed in the IL-17F structure that was not receptor bound could not be maintained in the IL-17RA-bound state, as it would generate steric clashes with the amino-terminal coil region of the receptor. Each IL-17F monomer is composed of two pairs of antiparallel β-sheets (strands 1–4), with the second and fourth strands connected by two disulfide bonds in a manner homologous to that of proteins of the cysteine-knot family. There is a 50–amino acid amino-terminal extension of which residues 29–42 are parallel to strands 3 and 4 of the second IL-17F protomer. This coil region is stabilized by many interactions, including several hydrogen bonds with the adjacent strands. In the IL-17RA-bound IL-17F conformation, this region (residues 33–42) moves out to open the binding pocket and interact with the receptor (**Fig. 2a**). The first 24 amino acids of each IL-17F chain and residues 105–109 from the 3–4 loop on one IL-17F protomer could not be modeled. In the IL-17F structure that is not receptor bound, Cys17 forms a disulfide

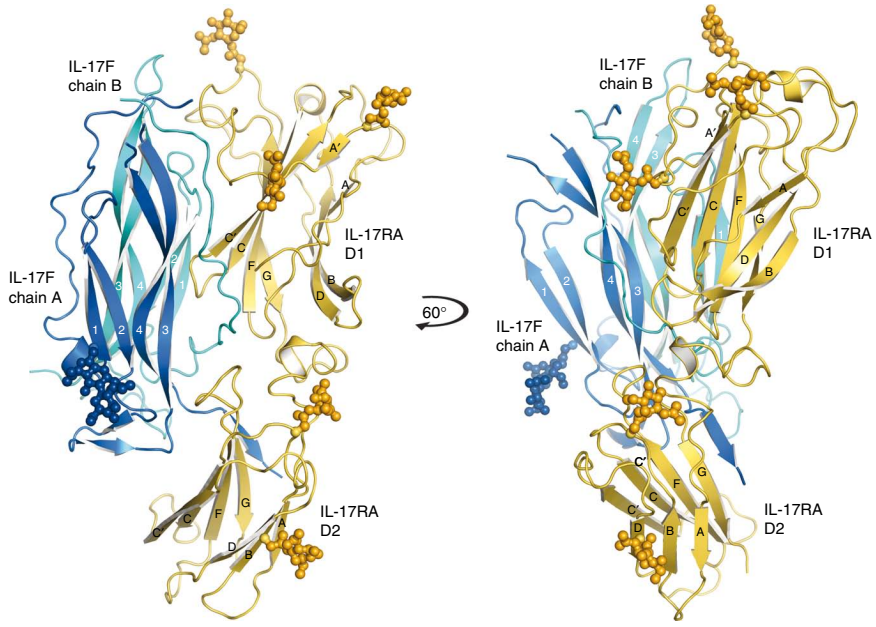


Figure 1 Structure of the IL-17RA–IL-17F complex. IL-17RA (yellow) bound to IL-17F (chain A, blue; chain B, cyan) presented as a ribbon diagram with N-linked glycans presented in 'ball-and-stick' representation. IL-17RA is composed of two FnIII domains (D1 and D2) joined by a short helical linker. Right, complex rotated by 60° around the *y* axis.

bond with Cys107 at the tip of the 3–4 loop on the adjacent IL-17F chain. These interchain disulfide bonds were not modeled but were present, as our protein acted like a disulfide-linked dimer by SDS-PAGE (data not shown).

IL-17RA–IL-17F binding interface

The overall mode for the binding of IL-17F to IL-17RA, in which both receptor FnIII domains bind in a 'side-on' orientation and use edge strands to insert into a crevasse formed at the dimeric interface of the ligand, is unlike that of other cytokine or growth factor receptor complexes. IL-17RA forms an extensive binding interface with IL-17F, burying ~2,200 Å^2 of surface area; ~70% of this buried surface area is mediated by the IL-17RA D1 domain. There are three main interaction sites at the binding interface (**Fig. 2**). Site 1 is formed between the amino-terminal extension of IL-17RA (Thr25–Trp31) and the 1–2 loop (Pro60–Tyr63) plus the carboxy-terminal region of strand 3 (Val100 and Arg102) of IL-17F chain B; this interaction buries ~330 Å^2 (**Fig. 2c**). Trp31 of the receptor is buried in the center of this binding site; the main-chain oxygen atom forms hydrogen bonds with Arg102 and the side chain forms hydrogen bonds with Pro60. Two additional hydrogen bonds are formed between Thr25 and Cys26 of IL-17RA and Tyr63 of IL-17F. Site 2 is the most prominent interface feature of the complex and is composed of the C′–C loop of IL-17RA D1 (Leu86–Arg93), which slots into a deep binding pocket flanked by the amino-terminal extension and strand 2 of IL-17F chain B and strand 3 of IL-17F chain A; this interaction buries almost 550 Å^2 (**Fig. 2a**,**b**). This eight–amino acid IL-17RA loop forms extensive hydrophobic and polar interactions with both chains of IL-17F, including a potential salt bridge between Glu92 of IL-17RA and Arg37 of IL-17F chain B, and a hydrogen bond between the main-chain oxygen atom of Asn89 of IL-17RA and Asn95of IL-17F chain A. Site 3, which encompasses ~410 Å^2 of buried surface area, is formed between the F–G loop of IL-17RA D2 (Cys259–Arg265) and the carboxy-terminal regions of strands 3 and 4 of IL-17F chain A, and the amino-terminal extension of IL-17F chain B (**Fig. 2d**). Site 3 is rich in charged interactions, with nine potential hydrogen bonds and a salt bridge between Asp262 of IL-17RA and Arg47 of IL-17F chain B. Overall the interface is extensive and is composed of many specific contacts. We envisage that an analogous binding mode is used by other IL-17 receptor-cytokine pairs, given the sequence conservation of contact residues (discussed below).

Heterodimeric receptor complex formation

The stoichiometries of the receptor complexes remain to be fully elucidated[6], but the asymmetric IL-17RA–IL-17F complex hints at a 'preference' for the formation of heterodimers with a second,

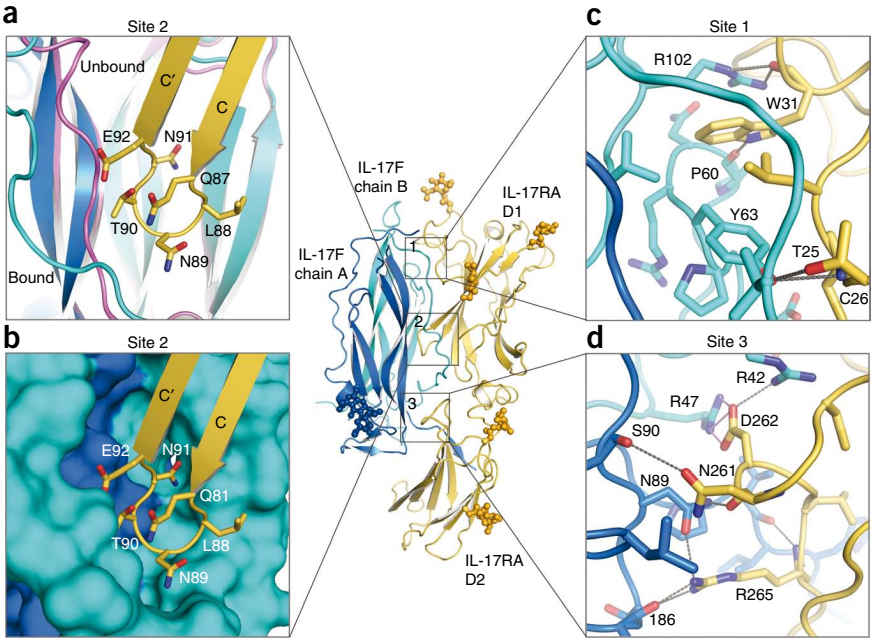


Figure 2 Binding of IL-17F to IL-17RA is mediated by three distinct interfaces. The overall structure of IL-17RA complex with IL-17F is in the center, surrounded by boxed regions presenting regions of the interface in greater detail. (**a**) In site 2, the C–C′ loop of IL-17RA D1 (yellow) inserts between the amino-terminal coil region and strands 1 and 2 of IL-17F chain B (cyan). The amino-terminal coil undergoes a conformational change between the unbound (magenta) and bound (cyan) conformations. (**b**) Surface representation of the 'knob-in-hole' IL-17F binding-pocket complementarity in site 2. (**c**) The amino-terminal binding site of IL-17RA D1 (site 1). (**d**) The binding site for IL-17RA D2 (site 3). Contact residues are presented as 'stick' models; gray dotted lines represent hydrogen bonds; pink dotted lines represent salt bridges.

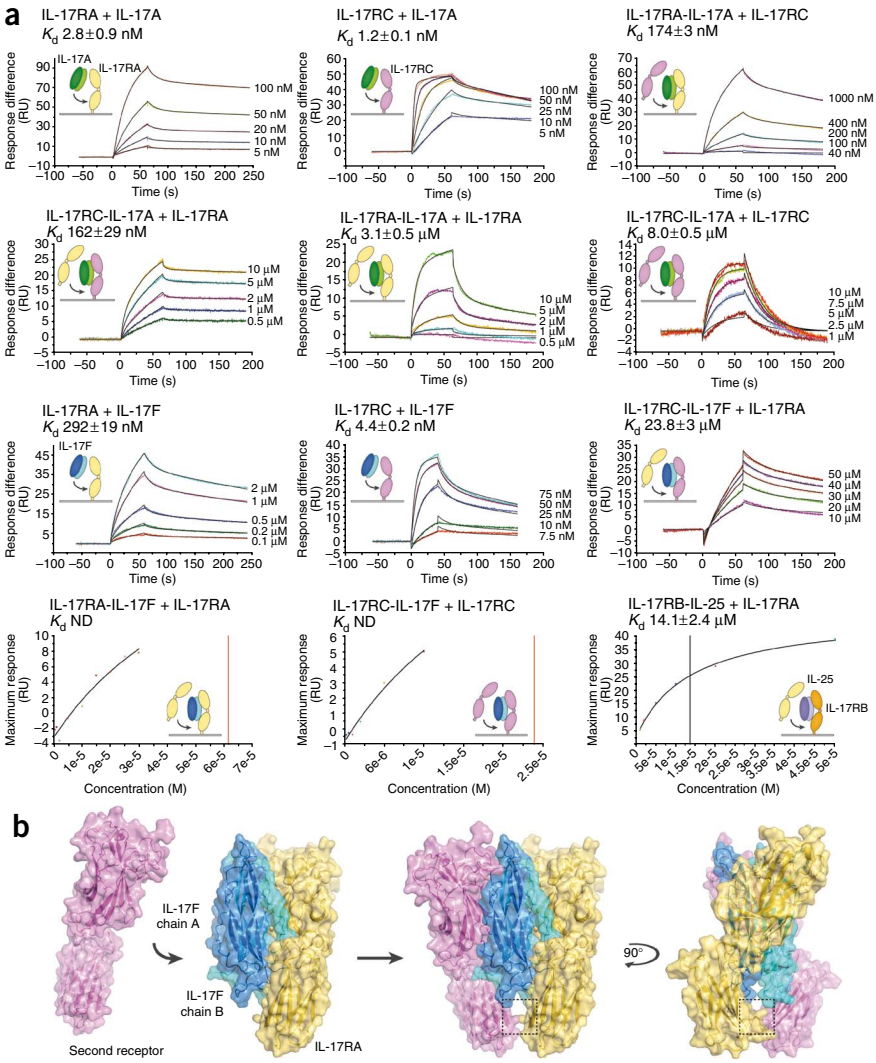


Figure 3 Assembly and model of the heterodimeric IL-17 signaling complex. (**a**) Binding affinity of IL-17A, IL-17F and IL-25 for IL-17RA, IL-17RB and IL-17RC immobilized on SPR chip surfaces. In some experiments, the binding affinity of a second receptor to the preassembled receptor-cytokine complex on the chip was then measured. For kinetic experiments (top three rows), time (s) is plotted against response (in resonance units (RU)); sensorgrams are presented as colored lines and the curve fit is the black line; injected concentrations are along the right margin. For equilibrium experiments (bottom row), injected concentration (M) is plotted against the maximum response; the curve fit is a black line and the dissociation constant (K_d) is a vertical line. Insets show the binding event: yellow, IL-17RA; orange, IL-17RB; magenta, IL-17RC; dark and light green, IL-17A; dark and light blue, IL-17F; dark and light purple, IL-25. Data represent at least two independent experiments (K_d, mean ± s.e.m.). (**b**) Model of heterodimeric signaling complex formation. The second receptor (magenta) was modeled with the assumption that both receptors bind to IL-17F in the same orientation. The carboxy-terminal domains (D2) of the receptors come into close proximity (dashed box; details, **Supplementary Fig. 3**).

different receptor. We therefore investigated the mechanism by which a homodimeric cytokine could possibly coordinate two different receptors. IL-17RA and IL-17RC can each bind independently to IL-17A and IL-17F, but both receptors are necessary for signaling[9,10,22]. To further understand how the signaling complex is formed, we devised a surface plasmon resonance (SPR) strategy using soluble proteins to measure the affinities of both the homodimeric and heteromeric receptor complexes for cytokines *in vitro*. Although the binding affinities of IL-17RA and IL-17RC for IL-17A and IL-17F have been reported[7,22], we considered it pertinent to assess the binding affinity of the second receptor-binding site. Our strategy was to immobilize one receptor on the SPR chip at a low coupling density to minimize possible formation of homodimers (that is, cross-linking) of the receptors on the chip. The dimeric IL-17 cytokine was then captured by this receptor so that each receptor would be bound to one dimeric IL-17 ligand, leaving an exposed and accessible second receptor-binding site. We subsequently passed the second receptor over the preformed receptor-cytokine complexes to measure the affinity of the second receptor-binding event. In this way, we assembled the complex in a stepwise manner and measured each of the binding affinities (**Fig. 3**). IL-17A bound to both IL-17RA and IL-17RC with high affinity (2.8 ± 0.9 nM and 1.2 ± 0.1 nM, respectively). After IL-17A was bound by one IL-17RA molecule, the binding affinity for a second IL-17RA was diminished to 3.1 ± 0.5 μM, whereas the affinity of IL-17RC for this second binding site was 174 ± 3 nM. When IL-17A was originally captured by IL-17RC, a second IL-17RA bound to the existing IL-17RC–IL-17A complex with an affinity of 162 ± 29 nM; the binding affinity of a second IL-17RC to an existing IL-17RC–IL-17A complex was only 8.0 ± 0.5 μM.

We observed a similar pattern for IL-17F, which has a higher affinity for IL-17RC (4.4 ± 0.2 nM) than for IL-17RA (292 ± 19 nM). Given the divergent affinities, it seemed likely that IL-17F would be initially captured by IL-17RC; once bound, the affinity of IL-17RA for the IL-17RC–IL-17F complex was 23.8 ± 3 μM. In contrast, the binding affinity of IL-17RA and IL-17RC for preformed IL-17RA–IL-17F and IL-17RC–IL-17F complexes, respectively, was so weak that it could not be calculated accurately over the concentration range used for these experiments. Thus, these findings show that engagement of IL-17RA or IL-17RC by IL-17A or IL-17F encourages a 'preference' for the second receptor-binding site to engage a different receptor and thereby to form a heterodimeric receptor complex.

IL-17RA has been linked to signaling by IL-17E (also known as IL-25) together with IL-17RB[23]. IL-25 promotes T_H2 inflammatory responses and shares approximately 20% identity with IL-17A and IL-17F. Binding experiments have demonstrated that although IL-25 binds to IL-17RB with high affinity, it has no apparent affinity for IL-17RA[23–25]. We hypothesized that IL-17RA may bind IL-25 only after IL-25 is captured by IL-17RB. To test our hypothesis, we immobilized IL-17RB on an SPR chip, captured IL-25 and measured the affinity of IL-17RA for the IL-17RB–IL-25 complex. In support of our hypothesis, IL-17RA bound to the IL-17RB–IL-25 complex with an affinity of 14.1 ± 2.4 μM (**Fig. 3**). At concentrations up to 50 μM, we found no interaction between IL-17RA and IL-25 or between the IL-17RB–IL-25

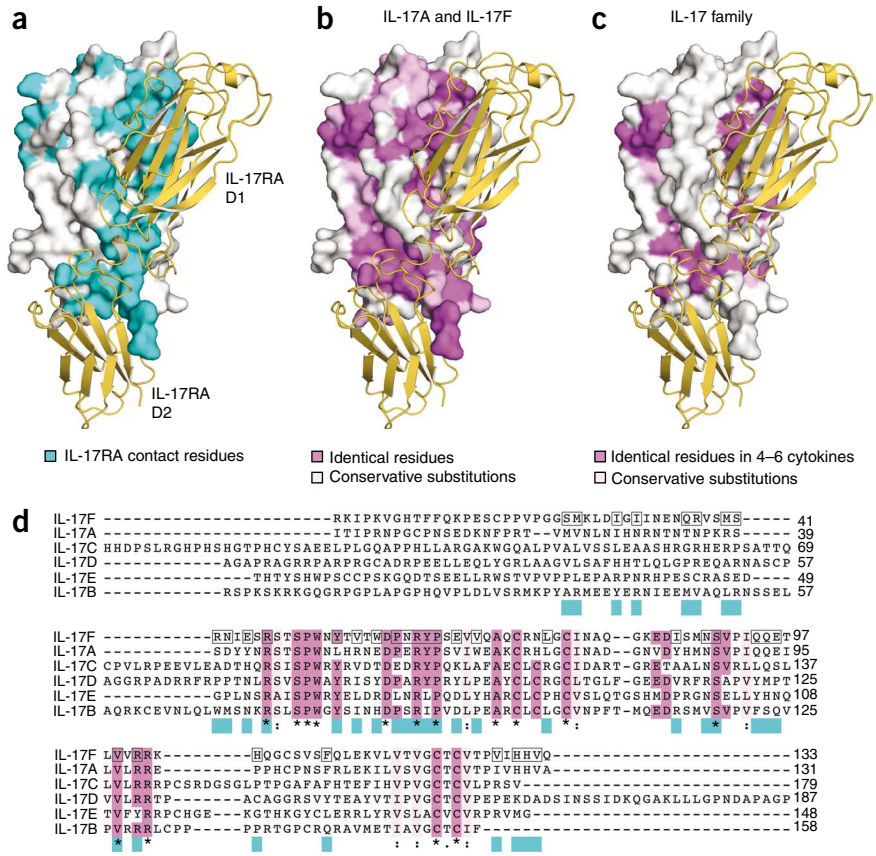


Figure 4 Binding interface and conserved IL-17 residues. (**a**–**c**) Surface representation of IL-17F (white) in complex with IL-17RA (yellow), presented in ribbon format. (**a**) IL-17RA–IL-17F complex, with contact residues in cyan. (**b**) Residues conserved in IL-17A and IL-17F mapped onto the IL-17F structure, showing identical residues (magenta) and conservative substitutions (light pink). (**c**) Residues identical among four, five or six IL-17 cytokine family members (magenta) and substitutions conserved in all six cytokines (light pink). (**d**) Alignment of human IL-17 cytokines: black boxes on the IL-17F sequence and cyan boxes beneath indicate residues that form contacts in the IL-17RA–IL-17F structure; magenta highlighting indicates residues identical in four, five or six cytokines; asterisks indicate residues identical in all six cytokines; conserved groups are light pink with a colon beneath.

complex and a second IL-17RB molecule. Together with the IL-17A and IL-17F binding data, these results indicate that formation of the heteromeric complex may be mediated by allostery and/or an interaction between the receptors.

To further address that idea, we modeled a second IL-17RA molecule to form the hypothetical receptor-cytokine complex with a stoichiometry of 2:2 (**Fig. 3b**). Assuming that the second receptor binds in a way identical to the binding of the first receptor, the base of one IL-17RA D2 would come into very close proximity with D2 of the second IL-17RA (**Fig. 3b**). For two IL-17RA molecules bound to IL-17F, His212 on the C–C′ loop of one IL-17RA would potentially clash with His212 of the second IL-17RA (**Supplementary Fig. 3**). This potential interaction site may allow the receptors to regulate their pairing. Steric clashes may cause lower affinity for a second identical receptor, or favorable receptor-receptor interactions may stabilize heteromeric receptor complexes. We cannot rule out the possibility that homodimeric receptor complexes could form on cells in certain conditions; however, our data indicate that receptor heterodimers are probably the main signaling species (discussed below).

IL-17RA as a common receptor

IL-17RA binds to IL-17A with an affinity ~100-fold higher than its affinity for IL-17F. IL-17A and IL-17F share ~50% identity, and mapping the conserved residues onto the structure of IL-17F showed a horseshoe-shaped ring of variable residues around the receptor-binding pocket (**Fig. 4**). Most C′–C loop interactions of IL-17RA are formed with residues that differ between IL-17A and IL-17F, whereas interactions between the amino-terminal region and F–G loop of IL-17RA D2 involve mainly conserved residues. We have reported here that the extracellular region of IL-17RA can also bind to the IL-17RB–IL-25 complex, and it has been shown that IL-17RD can interact with IL-17RA to mediate IL-17A signaling[26]. Given this association of IL-17RA with diverse IL-17 family members, we speculate that IL-17RA may act as a shared receptor analogous to those used in class I cytokine receptor complexes[27]. To investigate this possibility, we mapped onto the IL-17F surface the residues conserved among all members of the IL-17 family. In our analysis of the location of these residues in the IL-17RA–IL-17F complex, it seemed plausible that IL-17RA contacts these conserved residues with the amino-terminal region of D1 and the F–G loop of D2 (**Fig. 4c**). In contrast, IL-17RA may modulate specificity for each cytokine by contacting nonconserved cytokine residues with the C–C′ loop (**Fig. 4c**). Thus, IL-17RA seems to use a strategy of cross-reactivity based on a subset of conserved contacts, among a background of distinct contacts, with several different IL-17 cytokines. This is similar to the strategy used by the shared p75 receptor for the recognition of different neuro-trophin ligands[28] and is in contrast to the mechanism used for cross-reactivity by, for example, gp130 and the common γ-chain, which form largely disparate molecular interactions with different four-helix cytokines[27].

Receptor-binding modes of cysteine-knot growth factors

Several crystal structures have been reported for complexes of receptor and cysteine-knot growth factor ligand, such as NGF[28–30], vascular endothelial growth factor[31], glial cell–derived neurotrophic factor family members[32] and others; these structures serve as instructive comparisons for the mode of ligand engagement mediated by IL-17RA (**Fig. 5**). In the complex of NGF bound to the p75 neurotrophin receptor (p75NTR; a member of the death receptor family)[28,30], the receptor bears no structural similarity to IL-17RA; however, like IL-17RA, p75NTR engages NGF in a concave groove at the ligand dimer interface (**Fig. 5b**). In the complex of the receptor TrkA with NGF[29,33], an immunoglobulin domain in TrkA, which is structurally related to the FnIII domains of IL-17RA, is used for ligand binding. However, the immunoglobulin domain of TrkA binds 'end-on' to a flat face in the 'saddle' of NGF formed by the NGF β-sheets; thus, the mode of binding is distinct (**Fig. 5c**). Notably,

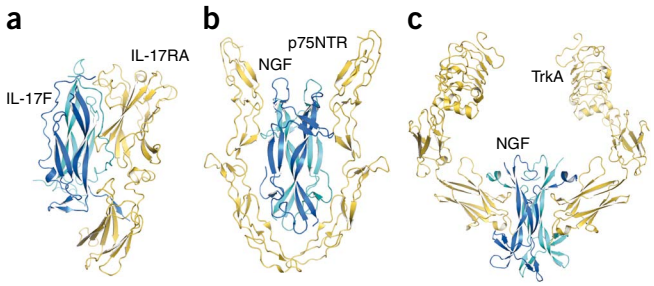


Figure 5 IL-17RA–IL-17F and homodimeric complexes of cysteine-knot growth factor receptors. Complexes of IL-17RA–IL-17F (**a**), p75NTR-NGF (**b**) and TrkA-NGF (**c**) presented as ribbon models with the receptors in yellow and the cytokines and growth factors in blue and cyan.

the NGF-p75NTR complex has been reported as complexes with stoichiometries of 1:2 and 2:2 that may represent partial and complete forms of a homodimeric p75 signaling complex, respectively[28,30]. However, in that case, homodimeric NGF ligand engages two identical p75 molecules and thus does not require a structural mechanism for the symmetric dimeric ligand to form heterodimers with two different receptors.

DISCUSSION

Cytokines of the IL-17 family are central mediators of chronic inflammatory and autoimmune conditions. Here we have reported the crystal structure of IL-17RA bound to IL-17F, which demonstrated both the receptor-binding interface and a potential mechanism by which the IL-17 family of homodimeric cytokines can coordinate two different receptors. Our structure showed one IL-17RA bound to the dimeric IL-17F cytokine, leaving the second potential receptor-binding interface free to engage a second receptor. Biochemical studies demonstrated that the heteromeric complex can be reconstituted *in vitro* and that the binding of the first receptor modulates the affinity of the second.

Although we have shown here that IL-17F forms heterodimeric signaling complexes with IL-17RA as a shared receptor, the possibility remains that, in principle, homodimeric receptor complexes could form in certain conditions[34,35]. The differences in receptor affinities demonstrated by our binding data suggest that in conditions in which one type of receptor has high overexpression relative to the expression of another receptor, homodimeric signaling pairs could be formed. In this manner, cells may be able to 'tune' their responsiveness to IL-17 through heterodimeric versus homodimeric receptor complexes by a mass action–based mechanism based on modulations in relative receptor expression, as has been shown for the IL-4 and IL-13 system[36]. However, the generality of homodimeric complexes *in vivo* is debatable, given that cells from IL-17RA-deficient or IL-17RC-deficient mice are not responsive to IL-17A or IL-17F[9,10]. Experiments based on fluorescence resonance energy transfer have established that IL-17RA chains exist as preformed dimers on the surface of cells[20]. The addition of cytokine causes a decrease in the efficiency of fluorescence resonance energy transfer, which suggests that the IL-17RA chains either undergo a conformational change that separates the intracellular domains or dissociate after binding. Overexpression of both IL-17RA and IL-17RC in the absence of cytokine is not sufficient to induce signaling, consistent with the idea that the cytokine elicits a change necessary for signaling[9]. Given that both IL-17RA and IL-17RC are required for signaling, it seems plausible that cytokine binding imposes the necessary receptor pairing. The wide range of affinities between IL-17 receptors and cytokines further exacerbates this need to modulate receptor pairing. Our data indicate that pairing may be dictated by an interaction between the membrane-proximal (D2) domains of the receptors. For 'like' receptors, this may represent a repulsive force in the bound conformation that decreases the affinity for the second 'like' receptor. It has been reported that the mouse IL-17RA D2 actually mediates homotypic interactions[14]. We have not observed any dimer formation with our extracellular IL-17RA protein (data not shown), despite a high degree of conservation between the extracellular regions of mouse and human IL-17RA. As the SEF–IL-17R domains in the intracellular region of IL-17 receptors and the signaling adaptor Act1 are predicted to form dimers[3], it seems plausible that the intracellular domains may mediate the preassembly of receptor chains, whereas cytokine binding imposes the correct receptor pairing for signaling.

IL-17RA is essential for IL-17A, IL-17F and IL-25 signaling[9,23]. IL-17RA has also been linked to IL-17RD signaling, although the ligand for this interaction remains unknown[26]. Given this emerging trend, along with our observation that IL-17RA contact residues in binding interfaces of site 1 and site 3 are somewhat conserved among members of the IL-17 cytokine family, we propose that IL-17RA may act as a shared receptor for the IL-17 family. Shared receptors such as gp130 and the common γ-chain are the hallmark of many cytokine-receptor signaling complexes[27]. These common receptors bind to an array of multimeric receptor-cytokine complexes, often in a manner mediated by their affinity for the cytokine-bound α-receptor complex rather than a direct binding affinity for the cytokine itself. IL-17RA may represent the newest member of the shared receptor family, serving in effect as a 'common γ-chain' of the IL-17R family and adding a new twist to the paradigm in which the shared receptor-binding site uses conserved 'anchor points' to engage variant ligands.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Accession codes. UCSD-Nature Signaling Gateway (http://www.signaling-gateway.org): A001253; Protein Data Bank: coordinates and structure factors, 3JVF.

Note: Supplementary information is available on the Nature Immunology website.

ACKNOWLEDGMENTS

We thank S. Juo for assistance with data collection and structure determination; E. Özkan for discussions; D. Gorman (DNAX) for cDNA reagents; and the beamline staff of the Stanford Synchrotron Radiation Lightsource, Advanced Light Source and Advanced Photon Source for assistance with data collection. Supported by the National Health and Medical Research Council of Australia (L.K.E.), the US National Institutes of Health (AI51321 to K.C.G.) and the Howard Hughes Medical Institute (K.C.G.).

AUTHOR CONTRIBUTIONS

K.C.G. initiated studies; L.K.E. and K.C.G. designed experiments; L.K.E. and S.F. did experiments; and L.K.E. and K.C.G. analyzed data and wrote manuscript.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/natureimmunology/.

Published online at http://www.nature.com/natureimmunology/.
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/.

1. Mosmann, T.R. & Coffman, R.L. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7**, 145–173 (1989).

2. Harrington, L.E. *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).
3. Park, H. *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**, 1133–1141 (2005).
4. Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V.K. IL-17 and Th17 Cells. *Annu. Rev. Immunol.* **27**, 485–517 (2009).
5. Weaver, C.T., Hatton, R.D., Mangan, P.R. & Harrington, L.E. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.* **25**, 821–852 (2007).
6. Gaffen, S.L. Structure and signalling in the IL-17 receptor family. *Nat. Rev. Immunol.* (2009).
7. Hymowitz, S.G. *et al.* IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *EMBO J.* **20**, 5332–5341 (2001).
8. Wright, J.F. *et al.* Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. *J. Biol. Chem.* **282**, 13447–13455 (2007).
9. Toy, D. *et al.* Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J. Immunol.* **177**, 36–39 (2006).
10. Zheng, Y. *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* **14**, 282–289 (2008).
11. Ishigame, H. *et al.* Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. *Immunity* **30**, 108–119 (2009).
12. O'Connor, Jr W. *et al.* A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat. Immunol.* **10**, 603–609 (2009).
13. Novatchkova, M., Leibbrandt, A., Werzowa, J., Neubuser, A. & Eisenhaber, F. The STIR-domain superfamily in signal transduction, development and immunity. *Trends Biochem. Sci.* **28**, 226–229 (2003).
14. Kramer, J.M. *et al.* Cutting edge: identification of a pre-ligand assembly domain (PLAD) and ligand binding site in the IL-17 receptor. *J. Immunol.* **179**, 6379–6383 (2007).
15. Maitra, A. *et al.* Distinct functional motifs within the IL-17 receptor regulate signal transduction and target gene expression. *Proc. Natl. Acad. Sci. USA* **104**, 7506–7511 (2007).
16. Rochman, Y., Spolski, R. & Leonard, W.J. New insights into the regulation of T cells by γ_c family cytokines. *Nat. Rev. Immunol.* **9**, 480–490 (2009).
17. Claudio, E. *et al.* The adaptor protein CIKS/Act1 is essential for IL-25-mediated allergic airway inflammation. *J. Immunol.* **182**, 1617–1630 (2009).
18. Chang, S.H., Park, H. & Dong, C. Act1 adaptor protein is an immediate and essential signaling component of interleukin-17 receptor. *J. Biol. Chem.* **281**, 35603–35607 (2006).
19. Qian, Y. *et al.* The adaptor Act1 is required for interleukin 17-dependent signaling associated with autoimmune and inflammatory disease. *Nat. Immunol.* **8**, 247–256 (2007).
20. Kramer, J.M. *et al.* Evidence for ligand-independent multimerization of the IL-17 receptor. *J. Immunol.* **176**, 711–715 (2006).
21. Bazan, J.F. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc. Natl. Acad. Sci. USA* **87**, 6934–6938 (1990).
22. Wright, J.F. *et al.* The human IL-17F/IL-17A heterodimeric cytokine signals through the IL-17RA/IL-17RC receptor complex. *J. Immunol.* **181**, 2799–2805 (2008).
23. Rickel, E.A. *et al.* Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J. Immunol.* **181**, 4299–4310 (2008).
24. Shi, Y. *et al.* A novel cytokine receptor-ligand pair. Identification, molecular characterization, and in vivo immunomodulatory activity. *J. Biol. Chem.* **275**, 19167–19176 (2000).
25. Lee, J. *et al.* IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J. Biol. Chem.* **276**, 1660–1664 (2001).
26. Rong, Z. *et al.* IL-17RD (Sef or IL-17RLM) interacts with IL-17 receptor and mediates IL-17 signaling. *Cell Res.* **19**, 208–215 (2009).
27. Wang, X., Lupardus, P., Laporte, S.L. & Garcia, K.C. Structural biology of shared cytokine receptors. *Annu. Rev. Immunol.* **27**, 29–60 (2009).
28. He, X.L. & Garcia, K.C. Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science* **304**, 870–875 (2004).
29. Wehrman, T. *et al.* Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron* **53**, 25–38 (2007).
30. Gong, Y., Cao, P., Yu, H.J. & Jiang, T. Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. *Nature* **454**, 789–793 (2008).
31. Wiesmann, C. *et al.* Crystal structure at 1.7 A resolution of VEGF in complex with domain 2 of the Flt-1 receptor. *Cell* **91**, 695–704 (1997).
32. Wang, X., Baloh, R.H., Milbrandt, J. & Garcia, K.C. Structure of artemin complexed with its receptor GFRα3: convergent recognition of glial cell line-derived neurotrophic factors. *Structure* **14**, 1083–1092 (2006).
33. Wiesmann, C., Ultsch, M.H., Bass, S.H. & de Vos, A.M. Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature* **401**, 184–188 (1999).
34. You, Z. *et al.* Interleukin-17 receptor-like gene is a novel antiapoptotic gene highly expressed in androgen-independent prostate cancer. *Cancer Res.* **66**, 175–183 (2006).
35. Li, T.S., Li, X.N., Chang, Z.J., Fu, X.Y. & Liu, L. Identification and functional characterization of a novel interleukin 17 receptor: a possible mitogenic activation through ras/mitogen-activated protein kinase signaling pathway. *Cell. Signal.* **18**, 1287–1298 (2006).
36. LaPorte, S.L. *et al.* Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259–272 (2008).

ONLINE METHODS

Protein expression and purification. The native signal peptide and extracellular region of human IL-17RA (residues 1–286) was cloned into the BacMam expression vector pVLAD6 (ref. 37). Recombinant protein was transiently expressed at 37 °C in suspended 293 GnTI- cells grown in Pro293 media (Lonza) supplemented with 1% (vol/vol) FCS and 10 mM sodium butyrate. Full length IL-17F with a carboxy-terminal six-histidine tag was cloned into the pAcGP67-A expression vector (BD Biosciences) and protein was secreted by High Five insect cells grown at 27 °C in Insect Xpress medium (Lonza). Supernatants containing IL-17RA and IL-17F protein were mixed and concentrated before nickel-affinity purification. IL-17RA protein was deglycosylated by treatment with endoglycosidase H, and the IL-17RA and IL-17F purification tags were cleaved with 3C protease and carboxypeptidase A (Sigma-Aldrich). Protein complexes were subjected to reductive lysine methylation with dimethylamine-borane complex and formaldehyde as described[38]. The IL-17RA–IL-17F complex was further purified with a Superdex 200 size-exclusion column (GE Healthcare) equilibrated in 10 mM HEPES, pH 7.4, and 150 mM NaCl. Fractions containing the IL-17RA–IL-17F complex were concentrated to ~15 mg/ml for crystallization trials.

Selenomethionine-labeled IL-17RA protein was prepared as described[39] with the following modifications. Untransfected adherent 293 GnTI- cells were cultivated in FBS-supplemented DMEM media (Invitrogen). After a single wash in PBS, the media was replaced with methionine- and cysteine-free DMEM (Invitrogen) supplemented with L-cysteine (40 mg/l), selenium–L-methionine (45 mg/l), 2% (vol/vol) FBS, l-glutamate, sodium pyruvate, IL-17RA BacMam virus and 10 mM sodium butyrate. Expression was allowed to proceed for 72 h. IL-17RA–selenomethionine protein supernatants were mixed with IL-17F and purified as described above.

For binding experiments, proteins were expressed and purified essentially as described above. The extracellular domain of IL-17RA, IL-17RB and IL-17RCs were expressed by 293s GnTI- cells with and without a carboxy-terminal BirA ligase tag. IL-17RC was expressed with an additional carboxy-terminal Fc tag that was cleaved by 3C protease before size-exclusion chromatography. IL-17A, IL-17F and IL-25 were expressed by High Five cells with carboxy-terminal six-histidine tags. Proteins were biotinylated enymatically with BirA ligase and were purified by size-exclusion chromatography.

Crystallization and X-ray data collection. IL-17RA–IL-17F complexes were initially grown by hanging-drop vapor diffusion in 10% (vol/vol) PEG 6000 and 0.1 M bicine, pH 9.0. Optimized native and selenomethionine protein complex crystals were grown in PEG 6000 (4–14%) and 0.1 M 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid buffer, pH 9.1–9.3, with 20 mM $CaCl_2$ or 10 mM $CaCl_2$ and 1.5% (wt/vol) trimethylamine *N*-oxide dihydrate added directly to the protein-precipitant drop. Heavy-metal derivatives were prepared by soaking of the crystals for 6 h in 'well solution' supplemented with 0.5 mM K_2PtCl_4 and 2% (vol/vol) ethylene glycol. Crystals were 'cryoprotected' before data collection in the 'well solution' plus 20–25% (vol/vol) ethylene glycol and were cooled to 100 K. The crystals belonged to the space group $P4_12_12$ and had unit cell dimensions of ~171 Å, 171 Å and 83 Å. The initial native data set was collected at the Stanford Synchrotron Radiation Lightsource beamline 9-2. The platinum-derivative and selenomethionine data sets were collected at the Stanford Synchrotron Radiation Lightsource beamline 11-1. The native data set of higher resolution was collected at the Advanced Photon Source beamline ID-23D. All data were indexed and integrated with the program Mosflm[40] and were scaled with SCALA from the CCP4 suite[41]. The diffraction is anisotropic and the initial native data set was also subjected to ellipsoidal truncation and anisotropic scaling with the diffraction anisotropy server[42], which rendered a data set scaled to 3.4 Å, 3.4 Å and 3.9 Å.

Structure determination and refinement. A molecular-replacement solution for a single IL-17F homodimer was determined with the program Phaser[43] using the published 2.85-Å IL-17F structure as a model (Protein Data Bank accession code, 1JPY)[7]. The initial maps showed additional density on one side of the IL-17F dimer, which indicated the binding site for IL-17RA. Phases were calculated with a K_2PtCl_4 derivative by single isomorphous replacement with anomalous scattering in the program Sharp[44]. Density-modified maps were calculated with the assumption of 71% solvent and inclusion of the partial model from the IL-17F molecular replacement for 10 of 20 rounds. A partial model of the IL-17RA main chain was built manually into this map with the program Coot[45].

The position of the IL-17RA methionine residues was calculated by a fast Fourier transform algorithm to generate an anomalous difference map with the program FFT in the CCP4 suite. As the selenomethionine data set was not isomorphous with the native data set and the signal was too weak to locate the sites by single-anomalous-difference phasing methods, the partially built model was used as a molecular-replacement model for the selenomethionine data set and the calculated phases were used to find the selenium peaks. Three of a potential six selenomethionine residues were located, corresponding to Met159, Met166 and Met218 of IL-17RA. These methionine positions, in addition to the predicted asparagine-linked glycosylation sites and disulfide bonds, were used to register the polypeptide in the density and complete building of the initial IL-17RA model. The program Phenix[46] was used for iterative rounds of coordinate and B-factor refinement, intersected with manual model building in Coot. Initial rounds of model building used *B*-factor-sharpened σ_A-weighted phased-combined maps calculated by the program CNS[47]. The final model was refined to 3.3 Å with R_{factor} and R_{free} values of 22.9% and 25.6%, respectively. There is one IL-17RA–IL-17F complex in the asymmetric unit. The model includes a dimethyl lysine at position 43 of the IL-17RA chain, five single *N*-acetylglucosamine sites on the IL-17RA chain, one site with two *N*-acetylglucosamine residues on IL-17F chain B and a calcium ion. The programs PROCHECK[48] and WHAT_CHECK[49] were used to assess the geometry of the final model. The CCP4 suite programs Contact and Areaimol were used to determined the interface contacts and buried surface area respectively. All structural figures were generated with the program Pymol[50].

Affinity measurements. Binding affinities were calculated by SPR on a Biacore T100 (GE Healthcare). IL-17 receptors with carboxy-terminal biotinylation were coupled to immobilized streptavidin on either an SA or CM4 sensor chip (GE Healthcare). An irrelevant biotinylated protein was captured at immobilization densities equivalent to those of to control flow cells. For measurement of the second receptor-binding interaction, the cytokine was first captured by the immobilized receptor, then the second receptor was injected. Low coupling densities (200–400 RU) and excess cytokine concentrations were used to optimize the number of cytokine homodimers bound to a single receptor. The surface was regenerated with 3 M $MgCl_2$ between each cycle. For kinetic experiments, a flow rate of 50 µl/min was used. Data were analyzed with Biacore T100 evaluation software, version 2.0 (GE Healthcare).

37. Dukkipati, A., Park, H.H., Waghray, D., Fischer, S. & Garcia, K.C. BacMam system for high-level expression of recombinant soluble and membrane glycoproteins for structural studies. *Protein Expr. Purif.* **62**, 160–170 (2008).
38. Walter, T.S. *et al.* Lysine methylation as a routine rescue strategy for protein crystallization. *Structure* **14**, 1617–1622 (2006).
39. Barton, W.A., Tzvetkova-Robev, D., Erdjument-Bromage, H., Tempst, P. & Nikolov, D.B. Highly efficient selenomethionine labeling of recombinant proteins produced in mammalian cells. *Protein Sci.* **15**, 2008–2013 (2006).
40. Leslie, A.G.W. Recent changes to the MOSFLM package for processing film and image plate data. *Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography* **26** (1992).
41. Collaborative Computation Project 4. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **50**, 760–763 (1994).
42. Strong, M. *et al.* Toward the structural genomics of complexes: crystal structure of a PE/PPE protein complex from *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* **103**, 8060–8065 (2006).
43. McCoy, A.J. *et al.* Phaser crystallographic software. *J. Appl. Crystallogr.* **40**, 658–674 (2007).
44. de La Fortelle, E. & Bricogne, G. SHARP: A maximum-likelihood heavy-atom parameter refinement program for the MIR and MAD methods. *Methods Enzymol.* **276**, 472–494 (1997).
45. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 (2004).
46. Afonine, P.V., Grosse-Kunstleve, R.W. & Adams, P.D. The Phenix refinement framework. *CCP4 Newsletter* **42** (2005).
47. Brunger, A.T. Version 1.2 of the Crystallography and NMR system. *Nat. Protoc.* **2**, 2728–2733 (2007).
48. Laskowski, R.A., Moss, D.S. & Thornton, J.M. Main-chain bond lengths and bond angles in protein structures. *J. Mol. Biol.* **231**, 1049–1067 (1993).
49. Hooft, R.W., Vriend, G., Sander, C. & Abola, E.E. Errors in protein structures. *Nature* **381**, 272 (1996).
50. DeLano, W.L. *Pymol Molecular Graphics System* (DeLano Scientific, San Carlos, California, 2002).

doi:10.1038/ni.1813