IL-17A Antagonists

Field of the Invention

The present invention relates to interleukin-17A (IL-17A) antibody antagonists, polynucleotides encoding IL-17A antibody antagonists or fragments thereof, and methods of making and using the foregoing.

Background of the Invention

Interleukin-17A (IL-17A, CTLA-8, IL-17) is a cytokine secreted by activated Th17 cells, $CD8^+$ T cells, γδ T cells and NK cells in response to cytokines such as IL-23 and TGF-β, and regulates production of mediators such as antimicrobial peptides (defensins), proinflamatory cytokines and chemokines from multiple cell types such as fibroblasts and synoviocytes that are involved in neutrophil biology, inflammation, organ destruction and host defense (reviewed in Weaver *et al.*, Annu. Rev. Immunol. 25:821-52, 2007; Aggarwal *et al.*, J. Biol. Chem. 278:1910-4, 2003; Mangan *et al.*, Nature 441:231-4, 2006). IL-17A synergizes with other cytokines, such as TNF-α and IL-1β to potentiate the pro-inflammatory environment.

The IL-17A cytokine family consists of six homologs designated IL-17A, B, C, D, E and F, each with divergent and distinct biological roles (Kawaguchi *et al.*, J. Allergy Clin. Immunol. 114:1265-73, 2004; Kolls and Linden, Immunity 21:467-76, 2004; Moseley *et al.*, Cytokine Growth Factor Rev. 14:155-74, 2003). Of the family members, IL-17F is most homologous to IL-17A and shares many similar functional properties such as induction of neutrophilia in the lung and induction of pro-inflammatory cytokines; however, in man, IL-17F is about 10-fold less potent than IL-17A (Moseley *et al.*, Cytokine Growth Factor Rev. 14:155-74, 2003; Kolls *et al.*, Immunity, 21: 467-76, 2004; McAllister *et al.*, J. Immunol. 175:404-12, 2005). IL-17A and IL-17F can also form heterodimers, which have intermediate

bioactivity *in vitro* (Wright *et al.*, J. Biol. Chem. 282:13447-55, 2007).

IL-17A mediates it effects by interacting with the Interleukin-17 receptor A (IL-17RA) and receptor C (IL-17RC) (Moseley *et al.*, Cytokine Growth Factor Rev. 14:155-74, 2003; Toy *et al.*, J. Immunol. 177:36-9, 2006). IL-17F signals through the same receptors, although IL-17F affinity to the receptors is significantly lower (Kuestner *et al.*, J. Immunol. 179:5462-73, 2007). Crystal structures of human IL-17F and human IL-17F/IL-17RA complex identified a putative receptor-binding cavity in the IL-17F homodimer (Hymowitz *et al.*, EMBO J. 20:5332-41, 2001; Ely *et al.*, Nat. Immunology 10:1245-51, 2009). A similar cavity was identified in the crystal structure of human IL-17A in complex with a neutralizing Fab, although the cavity was partially occupied (Gerhardt *et al.*, J. Mol. Biol. 394:905-21, 2009).

Inappropriate or excessive production of IL-17A is associated with the pathology of various diseases and disorders, including rheumatoid arthritis (Lubberts, Cytokine 41:84-91, 2008), airway hypersensitivity including allergic airway disease such as asthma (reviewed in Linden, Curr. Opin. Investig. Drugs. 4:1304-12, 2003; Ivanov, Trends Pharmacol. Sci. 30:95-103, 2009), psoriasis (Johansen *et al.*, Br. J. Dermatol. 160:319-24, 2009), dermal hypersensitivity including atopic dermatitis (Toda *et al.*, J. Allergy Clin. Immunol. 111:875-81, 2003), systemic sclerosis (Fujimoto *et al.*, J. Dermatolog. Sci. 50:240-42, 2008), inflammatory bowel diseases including ulcerative colitis and Crohn's disease (Holtta *et al.*, Inflamm. Bowel Dis. 14:1175-84, 2008; Zhang *et al.*, Inflamm. Bowel Dis. 12:382-88, 2006), and pulmonary diseases including chronic obstructive pulmonary disease (Curtis *et al.*, Proc. Am. Thorac. Soc. 4:512-21, 2007).

Antibodies to IL-17A have been proposed for use in the treatment of IL-17A mediated diseases and disorders (PCT Publ. Nos: WO08/021156, WO07/070750, WO07/149032, WO06/054059, WO06/013107, WO08/001063, WO10/034443; US Pat. Appl. Nos. US2008/095775, US2009/0175881;). As the pharmacokinetic,

efficacy and safety profiles of antibody therapeutics will be dependent on specific compositions, there is a need for improved antibodies to human IL-17A that are suitable for use in the treatment of IL-17A mediated diseases and disorders.

Brief Description of the Drawings

Fig. 1. A-H. CDR sequences of Family 2, 6a, 6b, 19a, and 19b IL-17A antibody antagonists.

Fig. 2. Exemplary A) IGLV3 and IGLJ; and B) IGHV3 and IGHJ germline genes as scaffolds for grafting paratope residues. mAb6785 sequence is shown above. CDR regions are underlined and core contact sites in mAb6785 light chain (Y31, D49, Y90, F92, F93) and heavy chain (S52, T54, F57, Y59, Q99, L100 and T101) are denoted by an asterix "*". Framework 4 regions in B) are double underlined. Sequence shown are *01 alleles unless specifically indicated otherwise.

Fig. 3. Kabat and Chotia numbering for select antibody A) light and B) heavy chains. Locations of Kabat CDRs and Chothia HVs are highlighted in gray.

Fig. 4. Competitive binding assays of labeled A) and B) mAb1926; C) mAb317; D) mAb3171; E) and F) mAb7357 with IL-17A in an ELISA format.

Fig. 5. A) H/D exchange maps of the IL-17A complexed with different anti-IL-17A mAbs. Numbering above the protective blocks corresponds to mature IL-17A (SEQ ID NO: 105) sequence numbering.

Fig. 6. A) The overall molecular structure of IL-17A/ Fab6468 complex. The dimer of IL-17A is shown dark gray and light gray. The two Fab molecules are shown in dark gray and light gray, repectively; B) Comparison of monomer of IL-17A (light gray) and IL-17F (dark gray); C) Dimer of IL-17A (light and dark gray); D) Dimer of IL-17F (light and dark gray).

Fig. 7. The two binding sites and the core epitope on IL-17A for Fab6468. Protomers 1 and 2 are dark and light gray, respectively. The core epitope is indicated by the black oval. The broken line represents disordered residues.

Fig. 8. Comparison of IL-17A and IL-17F putative receptor binding pockets. A) Front and Back views of the the P1 and P2 pockets of IL-17A. The FF motif of mAb6468 light chain CDR3 is shown in the P2 pocket. B) IL-17F with N-terminal FF motif in the P2 pocket. C) Sequence alignment of IL-17A and IL-17F and the conservation of P1 and P2 pockets.

Fig. 9. Binding specificity of mAb1926 to different species of IL-17A proteins in an ELISA format.

Summary of the invention

One aspect of the invention is an isolated antibody or fragment thereof, wherein the antibody binds specifically to human IL-17A having the sequence shown in SEQ ID NO: 105 at amino acid residues 56-68 (SEQ ID NO: 157) and 100-116 (SEQ ID NO: 158); or at residues L26, R55, E57, P59, E60, R61, Y62, S64, V65, W67, R101, E102, P103 and F110.

Another aspect of the invention is an isolated antibody or fragment thereof, wherein the antibody binds specifically to a P2 pocket cavity on human IL-17A, the P2 pocket cavity comprising amino acid residues V22, V24, L26, I28, Y62, L99, R101, F110, and L112 of SEQ ID NO: 105.

Another aspect of the invention is an isolated antibody or fragment that binds specifically to human IL-17A that competes for human IL-17A binding with a monoclonal antibody comprising the amino acid sequences of certain heavy chain complementarity determining regions (CDR) 1, 2 and 3 (HCDR1, HCDR2, HCDR3), the amino acid sequences of certain light chain complementarity determining reigons (CDR) 1, 2 and 3 (LCDR1, LCDR2, LCDR3), the amino acid sequences of certain heavy chain variable regions (VH)

or the amino acid sequences of certain light chain variable regions (VL).

Another aspect of the invention is an isolated antibody or fragment that binds specifically to human IL-17A, comprising certain heavy chain variable region paratope amino acid residues and certain light chain variable region paratope amino acid residues that interact with certain residues of human IL-17A having the amino acid sequence shown in SEQ ID NO: 105.

Another aspect of the invention is an isolated antibody or fragment that binds specifically to human IL-17A, comprising a heavy chain variable region and a light chain variable region, wherein the antibody comprises a heavy chain variable region paratope selected from Chothia residues F56 and Y58; and a light chain variable region paratope selected from Chothia residues Y91, F93 and F94.

Another aspect of the invention is an isolated antibody or fragment that binds specifically human IL-17A, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the antibody comprises the amino acid sequences of certain heavy chain complementarity determining regions (CDR) 1, 2 and 3 (HCDR1, HCDR2, HCDR3), the amino acid sequences of certain light chain complementarity determining reigons (CDR) 1, 2 and 3 (LCDR1, LCDR2, LCDR3), the amino acid sequences of certain heavy chain variable regions (VH) or the amino acid sequences of certain light chain variable regions (VL).

Another aspect of the invention is an isolated antibody or fragment that specifically binds human IL-17A, wherein the antibody comprises the amino acid sequences of certain heavy chains and the amino acid sequences of certain light chains.

Another aspect of the invention is a pharmaceutical composition comprising the isolated antibody or fragment of the invention and a pharmaceutically acceptable carrier.

Another aspect of the invention is an isolated antibody heavy chain comprising the amino acid sequence shown in SEQ ID NOs: 67,

68, 69, 81, 82, 83, 84, 85, 86, 92, 93, 94, 95, 96, 97, 98, 99, or 100.

Another aspect of the invention is an isolated antibody light chain comprising the amino acid sequence shown in SEQ ID NOs: 76, 77, 78, 79, 80, 87, 88, 89, 90, or 91.

Another aspect of the invention is an isolated polynucleotide encoding an antibody heavy chain comprising the amino acid sequence shown in SEQ ID NO: 67, 68, 69, 81, 82, 83, 84, 85, 86, 92, 93, 94, 95, 96, 97, 98, 99, or 100.

Another aspect of the invention is an isolated polynucleotide encoding an antibody light chain comprising the amino acid sequence shown in SEQ ID NO: 76, 77, 78, 79, 80, 87, 88, 89, 90, or 91.

Another aspect of the invention is a vector comprising at least one polynucleotide of the invention.

Another aspect of the invention is a host cell comprising the vector of the invention.

Another aspect of the invention os a method of inhibiting interaction of human IL-17A with IL-17RA comprising: providing a human IL-17A and IL-17RA; and
contacting the human IL-17A with an antagonist that binds the human IL-17A at at least one amino acid residue selected from the group consisting of V22, V24, L26, I28, Y62, L99, R101, F110, and L112.

Another aspecf of the invention si a method of inhibiting IL-17A biological activity, comprising: providing a human IL17-A and IL-17RA; and contacting the human IL-17A with an antagonist that binds the human IL-17A at at least one amino acid residue selected from the group consisting of V22, V24, L26, I28, Y62, L99, R101, F110, and L112.

Another aspect of the invention is a method of treating an inflammatory condition comprising administering a therapeutically effective amount of the isolated antibody of claim 3 or 7 to a patient in need thereof for a time sufficient to treat the inflammatory condition.

Detailed Description of the Invention

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, exemplary materials and methods are described herein. In describing and claming the present invention, the following terminology will be used.

The term "antagonist" as used herein means a molecule that partially or completely inhibits, by any mechanism, IL-17A activity. Exemplary antagonists are antibodies, fusion proteins, peptides, peptidomimetics, nucleic acids, oligonucleotides and small molecules. The agent can be identified using well known assays for IL-17A activity described below.

The term "IL-17A antibody antagonist" or an "antibody reactive with IL-17A" as used herein refers to an antibody that is capable of, directly or indirectly, reducing or inhibiting IL-17A biological activity, blocking binding of IL-17A to its receptor, or inhibiting IL-17A receptor activation. For example, an antibody reactive with IL-17A can bind directly to IL-17A and neutralize IL-17A activity, *i.e*, block IL-17A signaling to reduce cytokine and chemokine release.

The term "IL-17A" (CTLA-8, IL-17, interleukin-17A) refers to a human IL-17A polypeptide having an amino acid sequence shown in GenBank Acc. No. NP_002181. SEQ ID NO: 105 shows the amino acid sequence of the mature human IL-17A. IL-17A *in vivo* forms homodimers of two monomers, which are designated monomer A and

monomer B, or protomer A and protomer B, or protomer 1 and protomer 2, or chain A and chain B. IL-17A can also form a heterodimer with IL-17F. The term "IL-17A" comprises the monomer, the homodimer, and the heterodimer forms. The term "IL-17Amut6" refers to a variant of IL-17A having A70Q and A132Q substitutions. The amino acid sequence of the mature IL-17Amut6 is shown in SEQ ID NO: 106, and the cDNA sequence in SEQ ID NO: 112. IL-17A and IL-17Amut6 have comparable activities (PCT. Pat. Appl. No. WO09/003096).

The term "IL-17A receptor" as used herein comprises both receptor polypeptides, IL-17RA (GenBank Acc no: NP_055154, SEQ ID NO: 107) and IL-17RC (GenBank Acc No NP_703191, SEQ ID NO: 113), and homodimers or heterodimers of the two polypeptides.

The term "antibodies" as used herein is meant in a broad sense and includes immunoglobulin molecules including polyclonal antibodies, monoclonal antibodies including murine, human, human-adapted, humanized and chimeric monoclonal antibodies, antibody fragments, multispecific antibodies formed from at least two intact antibodies, dimeric, tetrameric or multimeric antibodies.

The term "monoclonal antibody" (mAb) as used herein means an antibody (or antibody fragment) obtained from a population of substantially homogeneous antibodies. Monoclonal antibodies are highly specific, typically being directed against a single epitope. The modifier "monoclonal" indicates the substantially homogeneous character of the antibody and does not require production of the antibody by any particular method.

Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA_1, IgA_2, IgG_1, IgG_2, IgG_3 and IgG_4. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

The term "antibody fragments" comprise at least a portion of an immunoglobulin molecule, such as a heavy chain complementarity determining region (HCDR), a light chain complementarity determining region (LCDR), a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain constant region (CH), a light chain constant region (CL), or a framework region (FR) from either antibody heavy or light chain. An antibody may be a Fab, F(ab'), $F(ab')_2$, scFv, dsFv, or diabody. Structures of the above mentioned antibody fragments, and techniques for the preparation and use of the antibodies and fragments thereof are well known in the art.

An antibody variable region consists of a "framework" region interrupted by three "antigen-binding sites". The antigen-binding sites are defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3), and three in the VL (LCDR1, LCDR2, LCDR3), are based on sequence variability (Wu and Kabat, J. Exp. Med. 132:211-250, 1970; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) "Hypervariable regions", "HVR", or "HV", three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3), refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk, Mol. Biol. 196:901-917, 1987). Other terms include "IMGT-CDRs" (Lefranc *et al.*, Dev. Comparat. Immunol. 27:55-77, 2003) and "Specificity Determining Residue Usage" (SDRU) (Almagro, Mol. Recognit. 17:132-143, 2004). The International ImMunoGeneTics (IMGT) database (http://www_imgt_org) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.*, Dev. Comparat. Immunol. 27:55-77, 2003.

"Chothia residues" as used herein are the antibody VL and VH residues numbered according to Al-Lazikani (Al-Lazikani *et*

al., J. Mol. Biol. 273:927-48, 1997). Correspondence between the two most used numbering systems, Kabat (Kabat *et al.*, Sequences of Immunological Interest, 5th Ed. Public Health Service, NIH, Bethesda, MD, 1991) and Chothia (Chothia and Lesk, Mol. Biol. 196:901-17, 1987) in relation to sequential polypeptide numbering is shown in Figure 3 for exemplary antibodies of the invention.

"Framework" or "framework sequences" are the remaining sequences of a variable region other than those defined to be antigen-binding site. Because the antigen-binding site can be defined by various terms as described above, the exact amino acid sequence of a framework depends on how the antigen-binding site was defined.

The term "substantially identical" as used herein means that the two antibody or antibody fragment amino acid sequences being compared are identical or have "insubstantial differences." Insubstantial differences are substitutions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in an antibody or antibody fragment amino acid sequence that do not adversely affect antibody properties. Amino acid sequences substantially identical to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. Percent identity can be determined for example by pairwise alignment using the default settings of the AlignX module of Vector NTI v.9.0.0 (Invitrogen, Carslbad, CA). The protein sequences of the present invention can be used as a query sequence to perform a search against public or patent databases to, for example, identify related sequences. Exemplary programs used to perform such searches are the XBLAST or BLASTP programs (http_//www_ncbi_nlm/nih_gov), or the GenomeQuest™ (GenomeQuest, Westborough, MA) suite using the default settings.

The term "in combination with" as used herein means that the described agents can be administered to an animal together in

a mixture, concurrently as single agents or sequentially as single agents in any order.

The term "inflammatory condition" as used herein refers to acute or chronic localized or systemic responses to harmful stimuli, such as pathogens, damaged cells, physical injury or irritants, that are mediated in part by the activity of cytokines, chemokines, or inflammatory cells (*e.g.*, neutrophils, monocytes, lymphocytes, macrophages) and is characterized in most instances by pain, redness, swelling, and impairment of tissue function.

The term "IL-17A-mediated inflammatory condition" as used herein refers to an inflammatory condition resulting at least partially from IL-17A biological activity, or caused by IL-17A activity. Exemplary IL-17A-mediated inflammatory conditions are psoriasis and rheumatoid arthritis.

The term "IL-17A-mediated condition" as used herein encompasses all diseases and medical conditions in which IL-17A plays a role, whether directly or indirectly, in the disease or medical condition, including the causation, development, progress, persistence or pathology of the disease or condition.

The term "epitope" as used herein means a portion of an antigen to which an antibody specifically binds. Epitopes usually consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope can be composed of either or both contiguous or discontiguous amino acids that form a conformational spatial unit. For a discontiguous epitope, amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule.

The term "paratope" as used herein means a portion of an antibody to which an antigen specifically binds. A paratope can

be linear in nature or can be discontinuous, formed by a spatial relationship between non-contiguous amino acids of an antibody rather than a linear series of amino acids. A "light chain paratope" and a "heavy chain paratope" or "light chain paratope amino acid residues" and "heavy chain paratope amino acid residues" refer to antibody light chain and heavy chain residues in contact with an antigen, respectively.

The term "specific binding" as used herein refers to antibody binding to a predetermined antigen with greater affinity than for other antigens or proteins. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less, and binds to the predetermined antigen with a K_D that is at least ten fold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein, or any other specified polypeptide) other than the predetermined antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody specifically binding to an antigen" or "an antigen specific antibody" e.g. an IL-17A specific antibody. The dissociation constant can be measured using standard procedures.

The term "IL-17A biological activity" or "IL-17A activation" as used herein refers to any activity occurring as a result of IL-17A binding to the IL-17A receptor. Exemplary IL-17A biological activities result in increased secretion of IL-6 or IL-8, NF-κB activation, or regulation of downstream kinases sucn as ERK1, ERK2 and p38 upon binding to the IL-17A receptor. The release of cytokines and chemokines from cells, tissues or in circulation, NF-κB activation, or kinase phosporylation events can be measured using well known methods, for example immunoassays, immunoblotting, or reporter gene systems (Yao *et al.*, Immunity 3:811-21, 1995; Awane *et al.*, J. Immunol. 162:5337-44, 1999).

The term "vector" means a polynucleotide capable of being duplicated within a biological system or that can be moved

between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers, that function to facilitate the duplication or maintenance of these polynucleotides in a biological system. Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

The term "expression vector" means a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

The term "polynucleotide" means a molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. Double and single-stranded DNAs and RNAs are typical examples of polynucleotides.

The term "polypeptide" or "protein" means a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than 50 amino acids may be referred to as "peptides".

Conventional one and three-letter amino acid codes are used herein as follows:

Amino acid	Three-letter code	One-letter code
Alanine	ala	A
Arginine	arg	R
Asparagine	asn	N
Aspartate	asp	D
Cysteine	cys	C
Glutamate	glu	E
Glutamine	gln	Q
Glycine	gly	G
Histidine	his	H

Isoleucine	ile	I
Leucine	leu	L
Lysine	lys	K
Methionine	met	M
Phenylalanine	phe	F
Proline	pro	P
Serine	ser	S
Threonine	thr	T
Tryptophan	trp	W
Tyrosine	tyr	Y
Valine	val	V

Compositions of matter

The present invention provides IL-17A antibody antagonists capable of inhibiting IL-17A biological activity and uses of such antibodies. Exemplary mechanisms by which IL-17A activation may be inhibited by such antibodies include *in vitro*, *in vivo* or *in situ* inhibition of IL-17A homo-or heterodimerization, and blocking binding of IL-17A to the IL-17A receptor, inhibition of receptor dimerization, inhibition of kinase activity of downstream signaling pathways, or inhibition of IL-17A mRNA transcription. Other antibody antagonists capable of inhibiting IL-17A activation by other mechanisms are also within the scope of the various aspects and embodiments of the invention. These antagonists are useful as research reagents, diagnostic reagents and therapeutic agents.

The invention provides novel antigen-binding sites derived from human immunoglobulin gene libraries. The structure for carrying an antigen-binding site is generally an antibody heavy or light chain or portion thereof.

The invention provides an isolated antibody or fragment thereof that binds specifically to human IL-17A, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the antibody comprises the heavy chain complementarity determining region (CDR) 1, 2 and 3 (HCDR1, HCDR2

and HCDR3) amino acid sequences and the light chain complementarity determining region (CDR) 1, 2 and 3 (LCDR1, LCDR2 and LCDR3) amino acid sequences as shown in Table 1a.

Table 1a.

Family	MORmAb#	mAb#	SEQ ID NO:					
			LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
2	7702		1	4	7	23	26	52
	7701		1	4	7	23	27	52
	7708	624	1	4	7	23	28	52
	8297		1	4	7	23	29	52
	8298		1	4	7	23	30	52
	7785	3077	1	4	7	23	31	52
	8104	7024	1	4	7	23	32	52
	8105		1	4	7	23	33	52
	7786		1	4	7	23	34	52
	Consensus sequence		1	4	7	23	35	52
6a	Clone 10		2	5	8	24	36	53
	Clone 11		2	5	9	24	36	53
	Clone 12		2	5	10	24	36	53
	Consensus sequence		2	5	11	24	36	53
6b	Clone 13		2	5	12	24	36	53
	7706	4538	2	5	13	24	36	53
	8299	3584	2	5	13	24	36	54
	8300		2	5	13	24	36	55
	8301		2	5	13	24	36	56
	Clone 15		2	5	14	24	36	53
	Clone 16		2	5	15	24	36	53
	7775	732	2	5	16	24	36	53
	8101		2	5	16	24	36	54
	8102		2	5	16	24	36	55
	8103	4168	2	5	16	24	36	56
	Consensus sequence		2	5	17	24	36	57

Family	MORmAb#	mAb#	SEQ ID NO:					
			LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
19a	Clone 179		3	6	18	25	37	58
	Clone 180		3	6	18	25	38	58
	7709		3	6	18	25	39	58
	Clone 182		3	6	18	25	40	58
	7700	1926	3	6	18	25	41	58
	8095		3	6	18	25	42	58
	8096		3	6	18	25	43	58
	8097		3	6	18	25	41	59
	8098		3	6	18	25	41	60
	8141		3	6	19	25	41	58
	8142		3	6	20	25	41	58
	8143		3	6	21	25	41	58
	8160	7146	3	6	19	25	41	60
	8161		3	6	20	25	41	60
	8162		3	6	21	25	41	60
	8302	6785	3	6	18	25	43	60
	8303		3	6	20	25	43	60
		5548	3	6	19	25	43	60
	7768		3	6	18	25	44	58
	Clone 185		3	6	18	25	45	58
	Consensus sequence		3	6	22	25	46	61
19b	Clone 186		3	6	18	25	47	58
	Clone 187		3	6	18	25	48	58
	Clone 188		3	6	18	25	49	58
	Clone 189		3	6	18	25	50	58
	Consensus sequence		3	6	18	25	51	58

In certain embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 23, 35 and 52, wherein the HCDR2 of SEQ ID NO: 35 is further defined as shown in Formula (I):

Xaa_1-I-I-P-W-F-G-Xaa_2-T-Xaa_3-Y-A-Q-K-F-Q-G,

(I)

wherein

Xaa_1 may be His, Met, Arg, Ser or Tyr;

Xaa_2 may be Trp, Thr or Tyr; and

Xaa_3 may be Tyr, Phe, Ser or Asp.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the LCDR1, LCDR2 and LCDR3 amino acid sequences as shown in SEQ ID NOs: 2, 5 and 11, wherein the LCDR3 of SEQ ID NO: 11 is further defined as shown in Formula (II):

Xaa_4-Q-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10},

(II)

wherein

Xaa_4 may be His or Gln;

Xaa_5 may be Phe or Gly;

Xaa_6 may be Thr, Val or Asn;

Xaa_7 may be Ile, Thr or Tyr;

Xaa_8 may be Pro or Arg;

Xaa_9 may be Ser or Pro; and

Xaa_{10} may be His, Phe or Leu.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to binding human IL-17A, comprising a VH and a VL, wherein the antibody comprises the LCDR1, LCDR2, and LCDR3 amino acid sequences as shown in SEQ ID NOs: 2, 5 and 17, wherein the LCDR3 of SEQ ID NO: 17 is further defined as shown in Formula (III):

Xaa_{11}-Q-Xaa_{12}-Xaa_{13}-Xaa_{14}-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-T,

(III)

wherein

Xaa_{11} may be Gln or Thr;

Xaa_{12} may be Ser or Tyr;

Xaa_{13} may be Asn, Arg, Val or Tyr;

Xaa_{14} may be His or Ser;

Xaa_{15} may be Ile, Thr, Leu, Ala or Ser;

Xaa_{16} may be Pro, Leu or Ser;

Xaa_{17} may be Pro, Ser, Phe or Leu; and

Xaa_{18} may be Ala, Leu or Asp.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A,

comprising a VH and a VL, wherein the antibody comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 24, 36 and 57, wherein the HCDR3 of SEQ ID NO: 57 is further defined as shown in Formula (IV):

E-V-D-S-Xaa_{19}-Y-Y-S-Y-F-D-I,

(IV)

wherein

Xaa_{19} is Met, Ile, Leu or Thr.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the LCDR1, LCDR2 and LCDR3 amino acid sequences as shown in SEQ ID NOs: 3, 6 and 22, wherein the LCDR3 of SEQ ID NO: 22 is further defined as shown in Formula (V):

G-S-Y-D-F-F-L-G-Xaa_{20}-I-V,

(V)

wherein

Xaa_{20} is Met, Leu, Thr or Tyr.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 25, 46 and 61, wherein the HCDR2 of SEQ ID NO: 46 is further defined as shown in Formula (VI):

Xaa_{21}-I-Xaa_{22}-Xaa_{23}-Xaa_{24}-Xaa_{25}-Xaa_{26}-Xaa_{27}-Xaa_{28}-Xaa_{29}-Y-A-D-S-V-K-G,

(VI)

wherein

Xaa_{21} may be Ala, Gly, Thr or Val;

Xaa_{22} may be Asn or Ser;

Xaa_{23} may be Gly, Met, Lys, Ile, Leu or His;

Xaa_{24} may be Leu, Asp, Ala, His, Thr, Gly or Ser;

Xaa_{25} may be Gly or Ser;

Xaa_{26} may be Thr, Gly, Tyr or Asp;

Xaa_{27} may be His, Trp, Tyr or Phe;

Xaa_{28} may be Lys, Thr or Ile; and

Xaa_{29} may be Tyr, Phe or Asn, and

the HCDR3 of SEQ ID NO: 61 is defined as shown in Formula (VII):

$Q\text{-}L\text{-}Xaa_{30}\text{-}L\text{-}D\text{-}V$,

(VII)

wherein

Xaa_{30} may be Met, Leu or Thr.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 25, 51 and 58, wherein the HCDR2 of SEQ ID NO: 51 is further defined as shown in Formula (VIII):

$V\text{-}T\text{-}S\text{-}Xaa_{31}\text{-}Xaa_{32}\text{-}Xaa_{33}\text{-}Xaa_{34}\text{-}T\text{-}Y\text{-}Y\text{-}A\text{-}Xaa_{35}\text{-}S\text{-}V\text{-}K\text{-}G$,

(VIII)

wherein

Xaa_{31} may be Ala, Lys, Met or His;

Xaa_{32} may be Asn, Met, Thr or Arg;

Xaa_{33} may be Gly or Asp;

Xaa_{34} may be Arg, His or Asn; and

Xaa_{35} may be Asp or Gly.

Antibodies whose antigen-binding site amino acid sequences are substantially identical to those shown in Table 1a (SEQ ID NOs: 1-61) are encompassed within the scope of the invention. Typically, this involves one or more amino acid substitutions with an amino acid having similar charge or hydrophobic or stereochemical characteristics, and are made to improve antibody properties, for example stability or affinity. For example, a conservative substitution may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan

et al., Acta Physiol. Scand. Suppl. 643:55-67, 1998; Sasaki *et al.*, Adv. Biophys. 35:1-24, 1998). Conservative substitutions will produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. Non-conservative substitutions in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (1) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (2) the charge or hydrophobicity of the molecule at the target site, or (3) the size of the molecule. Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify residues important for the function of the antibodies, such as residues affecting affinity, or residues that impart undesireable properties such as aggregation. Exemplary amino acid substitutions are shown in Table 1b, and Figure 1.

Substitutions in the framework regions, in contrast to antigen- binding sites may also be made as long as they do not adversely affect the properties of the antibody. Framework substitutions can be made for example at the Vernier Zone residues (US. Pat. No. 6,649,055) to improve antibody affinity or stability. Substitutions can also be made at those framework positions in the antibody that differ in sequence when compared to the homologous human germline gene sequences to reduce possible immunogeneicity. These modifications can be done for example to antibodies derived from *de novo* antibody libraries, such as pIX libraries.

Table 1b.

Original residue	Examplary substitutions	More conservative substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser, Ala	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. Amino acid substitutions can be done for example by PCR mutagenesis (US Pat. No. 4,683,195). Libraries of variants can be generated using well known methods, for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (ACDEGKNRSYW), and screening the libraries or variants with desired properties, as shown in Example 1. Figure 1 shows substitutions made to five parent IL-17A antibody antagonists within the LCDR3, HCDR2 and HCDR3 regions to improve antibody properties. Improved properties, such as affinity or stability can be measured by well known methods.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises certain VH and VL sequences, and also provides for each isolated VH and VL as shown in Table 2.

Table 2.

MOR#	SEQ ID NO:		mAb#	SEQ ID NO:			
	VL	VH		VL	VH	Light chain	Heavy chain
Family 2							
7708	62	67	624	76	67	87	92
7785	62	68	3077	76	68	87	93
8104	62	69	7024	76	69	87	94
Family 6b							
7706	63	70	4538	77	81	88	95
8299	63	71	3584	77	82	88	96
7775	64	70	732	78	81	89	95
8103	64	72	4168	78	83	89	97
Family 19a							
7700	65	73	1926	79	84	90	98
8160	66	74	7146	80	85	91	99
8302	65	75	6785	79	86	90	100
			5548	80	86	91	100

Although the embodiments illustrated in the Examples comprise pairs of variable regions, pairs of full length antibody chains, or pairs of CDR1, CDR2 and CDR3 regions, one from a heavy chain and one from a light chain, a skilled artisan will recognize that alternative embodiments may comprise single heavy chain variable regions or single light chain variable regions, single full length antibody chains, or CDR1, CDR2 and CDR3 regions from one antibody chain, either heavy or light. The single variable region, full length antibody chain or CDR1, CDR2 and CDR3 region of one chain can be used to screen for corresponding domains in another chain, the two chains capable of forming an antibody that specifically binds IL-17A. The

screening may be accomplished by phage display screening methods using, e.g., a hierarchical dual combinatorial approach disclosed in PCT Publ. No. WO92/01047. In this approach, an individual colony containing either a H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H), and the resulting two-chain specific antigen-binding domain is selected in accordance with phage display techniques as described.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL having amino acid sequences at least 90% identical to the VH and VL amino acid sequences shown in Table 2.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL having amino acid sequences at least 95% identical to the VH and VL amino acid sequences shown in Table 2.

In another aspect, the invention provides an isolated antibody or fragment having certain heavy chain and light chain amino acid sequences as shown in Table 2. In addition to numbering antibody residues sequentially, polypeptides encoding antibody chains can be numbered based on Kabat's or Chothia's numbering (Kabat *et al.*, *s*equences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991; Chothia and Lesk, Mol. Biol. 196:901-917, 1987). Examples of correspondence between sequential, Kabat and Chotia numbering for a select antibody chains are shown in Figure 3. The positions highlighted in gray indicate antibody CDR regions.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises a heavy chain variable region paratope selected from Chothia residues S51, T53, F56, Y58, Q95, L96 and T97 and a light chain variable region paratope selected from Chothia residues Y32, D50, Y91, F93

and F94. The heavy chain paratope and the light chain paratope Chothia residues correspond to heavy chain residues S52, T54, F57, Y59, Q99, L100 and T101 of SEQ ID NO: 86 and light chain residues Y31, D49, Y90, F92 and F93 of SEQ ID NO: 79.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising heavy chain variable region paratope amino acid residues that interact with residues of human IL-17A having the amino acid sequence shown in SEQ ID NO: 105, comprising:

a first threonine residue that interacts with R55 or E57 of human IL-17A;

a glutamine residue that interacts with R55 or E57 of human IL-17A;

a lysine residue that interacts with E57 of human IL-17A;

a tyrosine residue that interacts with P59, E60 or R101 of human IL-17A;

a phenylalanene residue that interacts with E60, R101, E102 or P103 of human IL-17A;

a serine residue that interacts with E60 of human IL-17A; and

a second threonine residue that interacts with E60 of human IL-17A.

In other embodiments, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising light chain variable region paratope amino acid residues that interact with residues of human IL-17A having the amino acid sequence shown in SEQ ID NO: 105, comprising:

a first phenylalanine residue that interacts with L26 of human IL-17A;

an aspartic acid residue that interacts with R55 or W67 of human IL-17A;

a first tyrosine residue that interacts with P59, S64 or R101 of human IL-17A;

a second phenylalanine residue that interacts with P59, E60, R61, Y62, R101 or F110 of human IL-17A; and

a second tyrosine residue that interacts with V65 of human IL-17A.

In another embodiment, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising heavy chain variable region paratope amino acid residues and light chain variable region paratope amino acid residues that interact with residues of human IL-17A having the amino acid sequence shown in SEQ ID NO: 105, comprising:

a tyrosine residue in the heavy chain variable region that interacts with R101 of human IL-17A;

a phenylalanine residue in the heavy chain variable region that interacts with R101, of human IL-17A;

a first phenylalanine residue in the light chain variable region that interacts with Y62 and R101 of human IL-17A;

a second phenylalanine residue in the light chain variable region that interacts with L26 and F110 of human IL-17A; and

a tyrosine residue in the light chain variable region that interacts with R101 of human IL-17A.

In another embodiment, the invention provides an isolated antibody or fragment that binds specifically to human IL-17A, comprising a heavy chain variable region and a light chain variable region, wherein the antibody comprises:

a heavy chain variable region paratope selected from Chothia residues F56 and Y58; and

a light chain variable region paratope selected from Chothia residues Y91, F93 and F94.

The heavy chain paratope Chothia residues F56 and Y58 and the light chain paratope Chothia residues Y91, F92 and F94 are residues in direct contact with IL-17A residues L26, Y62, R101 and F110. These IL-17A residues are part of both the Fab6468 epitope and the P2 pocket cavity (see below). While not wishing

to be bound to any particular theory, it is believed that interaction between the Fab6468 and IL-17A at these select residues may be sufficient for the antibody to block IL-17A activity.

Fully human mAbs lacking any non-human sequences can be prepared and optimized from phage display libraries by techniques referenced in, *e.g.*, Knappik *et al.*, J. Mol. Biol. 296:57-86, 2000; and Krebs *et al.*, J. Immunol. Meth. 254:67-84 2001. In an exemplary method, the antibodies of the invention are isolated from libraries expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein. The antibody libraries are screened for bining to human IL-17mut6 (SEQ ID NO: 105), and the obtained positive clones are further characterized, the Fabs isolated from the clone lysates, and expressed as full length IgGs. Exemplary antibody libraries and screening methods are described in Shi *et al.*, J. Mol. Biol. 397:385-96, 2010; PCT Pat. Appl. No. WO09/085462, and U.S. Ser. No. 12/546850; U.S. Pat. Nos. 5,223,409, 5,969,108, and 5,885,793).

The resulting mAbs can further be modified in their framework regions to change certain framework residues to those present in a matching human germline, as exemplified within.

Anibodies of the invention binding specific IL-17A epitopes can be made by immunizing humanized mice expressing human immunoglobulin loci (Lonberg *et al.*, Nature 368:856-9, 1994; Fishwild *et al.*, Nature Biotechnology 14:845-51, 1996; Mendez *et al.*, Nature Genetics 15:146-56, 1997, US. Pat. Nos. 5,770,429, 7,041,870, and 5,939,598) or Balb/c mice with the peptides encoding the epitopes, for example peptide $_{56}$NEDPERYPSVIWE$_{68}$ (SEQ ID NO: 157) or $_{100}$RREPPHCPNSFRLEKIL$_{116}$ (SEQ ID NO: 158) and using the hybrodima method of Kohler *et al.*, Nature 256:495-97. The resulting antibodies are tested for their binding to the epitope using standard methods. The identified mAbs can further be modified by incorporating altered framework support residues to preserve binding affinity by techniques such as those disclosed

in Queen *et al.*, Proc. Natl. Acad. Sci. (USA), 86:10029-32, 1989 and Hodgson *et al.*, Bio/Technology, 9:421, 1991.

Isolated antibodies having certain paratope residues (eg., the core paratope residues defined in Table 10) that bind specifically to human IL-17A can be made, for example, by grafting the paratope residues into a suitable scaffold, assembling the engineered scaffolds into full antibodies, expressing the resulting antibodies, and testing the antibodies for binding to IL-17A or for an effect on IL-17A biological activity. Exemplary scaffolds are amino acid sequences of human antibody variable regions encoded by human germline genes. The scaffolds can be selected based on for example overall sequence homology, % identity between the paratope residues, or canonical structure class identity between the scaffold and an exemplary antibody, such as mAb6785. Human antibody germline genes are disclosed in, for example, Tomlinson *et al.*, J. Mol. Biol 227:776-798, and at the International ImMunoGeneTics (IMGT) database (http_://_www_imgt_org). Consensus human framework regions can also be used, e.g., as described in U.S. Pat. No. 6,054,297. Selection of suitable scaffold can be done for example according to methods described in PCT Publ. No. WO10/045340.

Exemplary human germline genes that can be used as scaffolds onto which the paratope residues are grafted are the genes encoded by the Vλ3, Vh3, Jλ, and the Jh frameworks. Exemplary Vκ3 genes are IGLV3-1, IGLV3-9, IGLV3-10, IGLV3-12, IGLV3-16, IGLV3-19, IGLV3-21, IGLV3-22, IGLV3-25, IGLV3-27, and IGLV3-32 (IMGT nomenclature, *01 alleles), (SEQ ID NOs: 117-127, respectively). Exemplary Jλ genes are IGLJ1, IGLJ2, IGLJ3, IGLJ4, IGLJ5, IGLJ6, and IGLJ7 (SEQ ID NOs: 128-134, respectively). Exemplary Vh3 genes are IGHV3-7, IGHV3-9, IGHV3-11, IGHV3-16, IGHV3-19, IGHV3-20, IGHV3-21, IGHV3-23, IGHV3-30, IGHV3-30*03, IGHV3-33, IGHV3-45, IGHV3-48, IGHV3-64, and IGHV3-74 (IMGT nomenclature, *01 alleles except when different allele is

specificed) (SEQ ID NO:s 135-150, respectively). Exemplary Jh genes are IGHJ1, IGHJ2, IGHJ3, IGHJ4, IGHJ5, and IGHJ6 (SEQ ID NO:s 151-156, respectively). The germline J-regions are used in their entirety or in part to select FR4 sequences. For example, the mAb6785 light chain paratope residues can be grafted into a Vλ3 protein framework encoded by IGLV3-1 (SEQ ID NO: 117) that is joined to the J region sequence encoded by IGLJ2 (SEQ ID NO: 129) with insertion of a single amino acid residue between the IGLV3-1 and IGLJ2 sequences, for example methionine. The Vλ3 protein framework encoded by IGLV3-1 may contain additional substitutions, for example a substitution of cysteine residue at position 33 of SEQ ID NO: 117 ("ACW") with for example asparagine; and substitution of residues 1-3 of SEQ ID NO: 117 ("SYE") with an amino-terminal sequence common to other lambda chain families, such as "QSV" of IGLV1 family. Sequences from other exemplary functional Vλ3 and Jλ genes can be used for grafting mAb6785 light chain paratope residues with the insertion of zero, one, or two amino acid residues between the carboxy-terminus encoded by the Vλ3 genes and the amino terminus encoded by the Jλ genes, such that the length of the CDR3 region is 11 amino acids. For example, methionine and isoleucine can be inserted between IGLV3-22 (SEQ ID NO: 124) and IGLJ2 (SEq ID NO: 129). Figure 2A shows alignment of exemplary light chain scaffolds that can be used for grafting. The mAb6785 heavy chain paratope residues can be grafted onto for example a Vh3 framework encoded by IGHV3-23 (SEQ ID NO: 142), that is joined to the J region FR4 sequence (11 C-terminal amino acids e.g. "WGQGTLVTVSS") of IGHJ1 (SEQ ID NO: 151), with the insertion of about about 5-7 residues, for example 6 residues, constituting HCDR3, between the V and the J regions. The inserted HCDR3 about 5-7 residues include insertion of glutamine, leucine and threonine, e.g. 3 of the paratope residues from mAb6785 Vh (Table 10). Sequences from other exemplary functional Vh3 and Jh genes can be used for grafting mAb6785 heavy chain paratope residues.

In some cases, one C-terminal amino acid from the Vh3 gene may be deleted before insertion of the about 5-7 residues constituting the HCDR3 so that only FR3 sequences are included in the scaffold. Sequences from other Vh3 genes that encode a CDR2 of 17 residues (residues 50-66 of IGHV3-23 (SEQ Id NO: 142) can also be used, and the FR4 sequences of other Jh genes can be substituted in place of IGJH1.

The specific binding to human IL-17A and biological activity of the resulting antibody can be evaluated using standard methods. Alignments of the mAb6785 light chain variable regions and heavy chain variable regions with the exemplary Vh3, Vλ3, Jλ or Jh genes are shown in Figures 2A and 2B. Alternatively, the extended paratope residues of mAb6785, as defined in Table 10, can be used in place of the core paratope residues. The paratope-grafted engineered antibodies can further be modified by substitutions of the Vernier Zone residues (U.S. Pat. No. 6,639,055) or the Affinity Determining Residues (U.S. Pat. Appl. No. 2010/0261620; Cobaugh *et al.*, J Mol Biol. 378:622-33, 2008) to improve antibody properties for example affinity. As long as the paratope-grafted antibody retains binding to IL-17A, the framework amino acid sequence in the paratope-grafted antibody may be 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the mAb6785 framework sequences. Allelic variants of the exemplary germline gene frameworks can be used in place of the V and J region protein sequences. Th sequences of the allelic variants are well known and can be obtained at the International ImMunoGeneTics (IMGT) database (http_://_www_imgt_org).

Sequences from the antigen-binding sites can be grafted in addition to the paratope residues using standard methods. For example, a complete HCDR3 or LCDR3 may be grafted.

Another embodiment of the invention is an isolated antibody or fragment that binds specifically to human IL-17A that competes for human IL-17A binding with a monoclonal antibody comprising

certain HCDR1, HCDR2 and HCDR3, and LCDR1, LCDR2 and LCDR3 amino acid sequences. Examplary monoclonal antibodies of the invention are an isolated antibody comprising HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 25, 43 and 60 and the LCDR1, LCDR2 and LCDR3 amino acid sequences as shown in SEQ ID NOs: 3, 6 and 18.

Competition between specific binding to IL-17A can be assayed *in vitro* using well known methods. For example, binding of MSD Sulfo-Tag™ NHS-ester –labeled antibody to IL-17A in the presence of an unlableled antibody can be assessed by ELISA.

Another embodiment of the invention is an isolated antibody or antibody or fragment thereof, wherein the antibody binds specifically to human IL-17A having the sequence shown in SEQ ID NO: 105 at amino acid residues 56-68 (SEQ ID NO: 157) and 100-116 (SEQ ID NO: 158); or at residues L26, R55, E57, P59, E60, R61, Y62, S64, V65, W67, R101, E102, P103 and F110.

Several well known methodologies can be employed to determine the binding epitope of the antibodies of the invention. For example, when the structures of both individual components are known, *in silico* protein-protein docking can be carried out to identify compatible sites of interaction. Hydrogen-deuterium (H/D) exchange can be carried out with the antigen and antibody complex to map regions on the antigen that may be bound by the antibody. Segment and point mutagenesis of the antigen can be used to locate amino acids important for antibody binding. Co-crystal structure of antibody-antigen complex is used to identify residues contributing to the epitope and paratope.

Previously described anti-IL-17A antibodies bind to epitopes on IL-17A distinct from the epitope for Fab6468 described in the present invention. Antibodies binding human IL-17A (SEQ ID NO: 105) residues 74-85, 46-53, 71-87, 80-86, 11-18, 29-41 or 54-62 have been described (PCT Publ. Nos. WO08/021156, WO07/106769, WO07/149032, WO07/070750; US Appl. No. US2008/095775, respectively). Conformational epitopes have been

described in PCT Publ. No. WO09/130459 and Gerhardt *et al.*, J. Mol. Biol: 394:901-21, 2009.

Another embodiment of the invention is an isolated antibody or fragment thereof, wherein the antibody binds specifically to a P2 pocket cavity on IL-17A, the P2 pocket cavity comprising of amino acid residues V22, V24, L26, I28, Y62, L99, R101, F110, and L112 of SEQ ID NO: 105.

Co-crystal structure of IL-17A homodimer with the anti-IL-17A Fab6468 identified a hydrophobic P2 pocket cavity on the surface of the IL-17A homodimer, which is likely to be involved in IL-17RA binding (see Examples). The "P2 pocket cavity" as used herein refers to a tertiary hydrophobic structural cavity on IL-17A homodimer, where the surface exposed residues in the P2 pocket are V24, L26, I28, Y62, L99, R101, F110 and L112 on monomer A and V22, V24 and L112 on monomer B, and vice versa. Select antibodies of the invention reactive with IL-17A, for example Fab6468, have direct contacts with the P2 pocket cavity residues L26, Y62, R101 and F110, which residues are also part of the Fab6468 epitope. While not wishing to be bound by any particular theory, it is assumed that the antibodies of the invention binding the select IL-17A P2 pocket cavity residues block interaction between IL-17A and IL-17RA. Based on co-crystal structure, the phenlylalanine motif (FF) at residues 93 and 94 in a light chain (SEQ ID NO: 79) of Fab6468 blocks the IL-17A/ IL-17RA interaction, and thus is a P2 pocket cavity blocker. Other P2 pocket cavity blocker antagonists are also within the scope of this invention, such as novel peptides or small molecules. These can be modeled based on the IL-17A/Fab6468 co-structure, and screened for their ability to replace Fab6468 binding to IL-17A. For example, peptide inhibitors can be screened from random peptide libraries that have incorporated the FF motif (for example libraries of XXXXFFXX; X indicated any amino acid; F = phenylalanine) and displayed on bacteriophage as a fusion with for example pIII, pVII or pIX coat protein (US5,223,409; Gao *et al.*, Proc. Natl. Acad. Sci. USA, 96:6025-30,

1999, Tornetta *et al.*, J. Immunol. Methods. 360:39-46, 2010; Shi *et al.*, J. Mol. Biol. 397:385-96, 2010) and subsequently tested for their inhibition of Fab6468 binding to IL-17A, and inhibition of IL-17A activity.

Small molecules may be screened using libraries of synthetic or natural compounds, or any combination thereof, and the resulting primary positive hits can be readily modified to produce structural analogs of the agents. Methods of making peptide libraries and pIX fusions, and screening the resulting libraries are well known.

Another embodiment of the invention of a method of inhibiting interaction of human IL-17A with IL-17RA comprising:

providing human IL-17A and IL-17RA; and

contacting human IL-17A with an antagonist that specifically binds human IL-17A at at least one amino acid residue selected from the group consisting of: V22, V24, L26, I28, Y62, L99, R101, F110, and L112.

Another embodiment of the invention is a method of inhibiting human IL-17A biological activity, comprising:

providing human IL17-A and IL-17RA; and

contacting human IL-17A with an antagonist that specifically binds human IL-17A at at least one amino acid residue selected from the group consisting of: V22, V24, L26, I28, Y62, L99, R101, F110, and L112.

Human IL-17A and IL-17RA can be provided as isolated proteins or fusion proteins. Human IL-17A homodimer can be purified from media of activated Th17 cells prepared by *in vitro* stimulation of naïve CD4 T cells by two anti-CD3/anti-CD28 stimulation in the presence of IL-2, IL-23 and IL-1β. The IL-17RA can be associated with cells or cell membranes, can be native or overexpressed, or can be a fragment of IL-17RA, for example the extracellular domain of the receptor. The IL-17RA can be a human IL-17RA, or IL-17RA from other species such as from mouse, rat or monkey. Antagonists binding to human IL-17A residues V22, V24,

L26, I28, Y62, L99, R101, F110, and L112 can be identified by the ability of the antagonist to replace Fab6468 binding to IL-17A, by mutagenesis studies or by co-crystal structures. Fusion proteins of human IL-17A and IL-17RA can be made by well known methods. Exemplary fusion protein is a soluble IL-17RA fused to an immunoglobulin Fc domain.

Another aspect of the invention is an isolated polynucleotide encoding any of the antibody heavy chains or the antibody light chains or fragments thereof of the invention or their complement. Certain exemplary polynucleotides are disclosed herein, however, other polynucleotides which, given the degeneracy of the genetic code or codon preferences in a given expression system, encode the antibody antagonists of the invention are also within the scope of the invention. Exemplary polynuceotides are shown in SEQ ID NOs: 101, 102, 103 and 104.

Exemplary antibody antagonists may be antibodies of the IgG, IgD, IgE, IgA or IgM isotypes. Additionally, such antibody antagonists can be post-translationally modified by processes such as glycosylation, isomerization, deglycosylation or non-naturally occurring covalent modification such as the addition of polyethylene glycol (PEG) moieties (pegylation) and lipidation. Such modifications may occur *in vivo* or *in vitro*. For example, the antibodies of the invention can be conjugated to polyethylene glycol (PEGylated) to improve their pharmacokinetic profiles. Conjugation can be carried out by techniques known to those skilled in the art. Conjugation of therapeutic antibodies with PEG has been shown to enhance pharmacodynamics while not interfering with function. See Deckert *et al.*, nt. J. Cancer 87:382-90, 2000; Knight *et al.*, Platelets 15:409-18, 2004; Leong *et al.*, Cytokine 16:106-19, 2001; and Yang *et al.*, Protein Eng. 16:761-70, 2003.

Pharmacokinetic properties of the antibodies of the invention can be enhanced through Fc modifications by techniques known to those skilled in the art. The “Fc” of an antibody is

not involved directly in binding of an antibody to an antigen, but exhibits various effector functions. An antibody "Fc" is a term well known and is defined on the basis of papain cleavage of antibodies. The Fc of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. Such complement and Fc receptor binding sites are well known and include for example L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to EU index of Kabat) (Brekke *et al.*, Eur. J. Immunol. 24:2542-7, 1995; US. Pat. Nos. 5,624,821, 7,597,889, Canfield and Morrison, J. Exp. Med. 173:1483-91, 1991). For example, mutation of Leu234/Leu235 in the hinge region of IgG1 to L234A/L235A or Phe235/Leu236 in the hinge region of IgG4 to P235A/L236A minimizes FcR binding and reduces the ability of the immunoglobulin to mediate complement dependent cytotoxicity and ADCC. A Ser to Pro substitution in the Cys-Pro-Ser-Cys (CPSC) motif in the hinge region of IgG4 heavy chains capable of forming either inter- or intra-heavy chain disulfide bonds *in vivo* via action of isomerases (Aalberse and Schuurman, Immunology 105:9-19, 2002), results in "IgG1-like behavior", *i.e.*, the Pro-substituted molecules are unable to form intra-heavy chain disulfide bonds. The location of the CPSC motif is typically found at residue 228 of a mature heavy chain but can change depending on CDR lengths. An exemplary IgG1 Fc region having the Leu234/Leu235 residues has an amino acid sequence shown in SEQ ID NO: 114, wherein the residues L117 and L118 correspond to the Leu234/Leu235 residues in the mature heavy chain. An exemplary IgG4 Fc region having the Cys-Pro-Ser-Cys (CPSC) motif and the Leu234/Leu235 residues has an amino acid sequence shown in SEQ ID NO: 115, where the CPSC motif is located at residues 106-109 and the Leu234/Leu235 residues at positions 122 and 123.

Antibodies or fragments thereof of the invention modified to improve stability, selectivity, cross-reactivity, affinity, immunogenicity or other desirable biological or biophysical

property are within the scope of the invention. Stability of an antibody is influenced by a number of factors, including (1) core packing of individual domains that affects their intrinsic stability, (2) protein/protein interface interactions that have impact upon the HC and LC pairing, (3) burial of polar and charged residues, (4) H-bonding network for polar and charged residues; and (5) surface charge and polar residue distribution among other intra- and inter-molecular forces (Worn *et al.*, J. Mol. Biol. 305:989-1010, 2001). Potential structure destabilizing residues may be identified based upon the crystal structure of the antibody or by molecular modeling in certain caases, and the effect of the residues on antibody stability can be tested by generating and evaluating variants harboring mutations in the identified residues. One of the ways to increase antibody stability is to raise the thermal transition midpoint (Tm) as measured by differential scanning calorimetry (DSC). In general, the protein Tm is correlated with its stability and inversely correlated with its susceptibility to unfolding and denaturation in solution and the degradation processes that depend on the tendency of the protein to unfold (Remmele *et al.*, Biopharm. 13:36-46, 2000). A number of studies have found correlation between the ranking of the physical stability of formulations measured as thermal stability by DSC and physical stability measured by other methods (Gupta *et al.*, AAPS PharmSci. 5E8, 2003; Zhang *et al.*, J. Pharm. Sci. 93:3076-89, 2004; Maa *et al.*, Int. J. Pharm., 140:155-68, 1996; Bedu-Addo *et al.*, Pharm. Res., 21:1353-61, 2004; Remmele *et al.*, Pharm. Res., 15:200-8, 1997). Formulation studies suggest that a Fab Tm has implication for long-term physical stability of a corresponding mAb. Differences in amino acids in either framework or within the CDRs could have significant effects on the thermal stability of the Fab domain (Yasui, *et al.*, FEBS Lett. 353:143-6, 1994).

The antibody antagonists of the invention may bind IL-17A with a K_d less than or equal to about 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}

or 10^{-12} M. The affinity of a given molecule for IL-17A, such as an antibody can be determined experimentally using any suitable method. Such methods may utilize Biacore or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art.

Antibody antagonists binding human IL-17A with a desired affinity can be selected from libraries of variants or fragments by techniques including antibody affinity maturation. Antibody antagonists can be identified based on their inhibition of IL-17A biological activity using any suitable method. Such methods may utilize reporter-gene assays or assays measuring cytokine production using well known methods and as described in the application.

Another embodiment of the invention is a vector comprising at least one polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotides of the invention into a given organism or genetic background by any means.

Another embodiment of the invention is a host cell comprising any of the polynucleotides of the invention such as a polynucleotide encoding a polypeptide comprising an immunoglobulin heavy chain variable region having the amino acid sequence shown in SEQ ID NOs: 67-75 and 81-86 or an immunoglobulin light chain variable region having the amino acid sequence shown in SEQ ID NOs: 62-66 and 76-80 or an an immunoglobulin heavy chain having the amino acid sequence shown in SEQ ID NOs: 92-100 or an immunoglobulin light chain having the amino acid sequence shown in SEQ ID NOs: 87-91. Such host cells may be eukaryotic cells, bacterial cells, plant cells or archeal cells. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell

Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATTC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

Another embodiment of the invention is a method of making an antibody reactive with IL-17A comprising culturing a host cell of the invention and recovering the antibody produced by the host cell. Methods of making antibodies and purifying them are well known in the art. For expression, the engineered family 2, 6a, 6b, 19a and 19b heavy chain sequences can include an N-terminal leader sequence such as MAWVWTLLFLMAAAQSIQA (SEQ ID NO:109). Exemplary nucleotide sequences encoding the heavy chain of candidate mAb6785 (family 19) with a leader sequence and the mature form (without a leader sequence) are shown in SEQ ID NOs: 101 and 102, respectively. Likewise, for expression, the light chain sequences of the family 2, 6a, 6b antibodies of the invention can include an N-terminal leader sequence such as MGVPTQVLGLLLLWLTDARC (SEQ ID NO: 110)and the light chain sequences of the family 19a and 19b antibodies of the invention can include an N-terminal leader sequence such as MAWSPLLLTLLAHCTGSWA (SEQ ID NO: 116). Exemplary nucleotide sequences encoding the light chain of codon optimized mAb6785 with a leader sequence and the mature form (without a leader sequence) are shown in SEQ ID NOs: 103 and 104, respectively.

Another embodiment of the invention is a hybridoma cell line that produces an antibody of the invention.

Methods of Treatment

IL-17A antagonists of the invention, for example IL-17A antibody antagonists, may be utilized in any therapy where it is desired to reduce the effects of IL-17A in the animal patient. IL- 17A may be circulating in the body or may be present in an undesirably high level localised at a particular site in the

body, for example a site of inflammation. While not wishing to be bound by any particular theory, the antagonists of the invention provide beneficial therapy by preventing or reducing IL-17A binding to its receptor, or homo- or heterodimerization of IL-17A. The methods of the invention may be used to treat an animal patient belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals.

Antibodies of the invention may be useful for the prophylaxis and treatment of IL-17A mediated conditions, such as inflammatory conditions, allergies and allergic conditions, hypersensitivity reactions, autoimmune diseases, severe infections, and organ or tissue transplant rejection. The antibodies of the invention are also useful in the preparation of a medicament for such treatment, wherein the medicament is prepared for administration in dosages defined herein. Exemplary IL-17A mediated conditions are inflammatory conditions, immune and proliferative disorders, including rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, osteoarthritis, osteoporosis, uveitis, inflammatory fibrosis (e.g., scleroderma, lung fibrosis, and cirrhosis), inflammatory bowel disorders (e.g., Crohn's disease, ulcerative colitis and inflammatory bowel disease), asthma (including allergic asthma), allergies, COPD, multiple sclerosis, psoriasis, systemic lupus erythematosus, diabetes and cancer. Positive outcomes in patients treated with anti-human IL-17A therapies have been described in rheumatoid arthritis, psoriasis and non-infectious uveitis (Genovese *et al.*, Arthritis Rheum. 62:929-39, 2010; Hueber *et al.*, Sci. Transl. Med. 2: 52ra72., 2010).

Inflammatory pulmonary condition is an example of an inflammatory condition. Exemplary inflammatory pulmonary conditions include infection-induced pulmonary conditions including those associated with viral, bacterial, fungal, parasite or prion infections; allergen-induced pulmonary conditions; pollutant-induced pulmonary conditions such as

asbestosis, silicosis, or berylliosis; gastric aspiration-induced pulmonary conditions, immune dysregulation, inflammatory conditions with genetic predisposition such as as cystic fibrosis, and physical trauma-induced pulmonary conditions, such as ventilator injury. These inflammatory conditions also include asthma, emphysema, bronchitis, chronic obstructive pulmonary disease (COPD), sarcoidosis, histiocytosis, lymphangiomyomatosis, acute lung injury, acute respiratory distress syndrome, chronic lung disease, bronchopulmonary dysplasia, community-acquired pneumonia, nosocomial pneumonia, ventilator -associated pneumonia, sepsis, viral pneumonia, influenza infection, parainfluenza infection, rotavirus infection, human metapneumovirus infection, respiratory syncitial virus infection and aspergillus or other fungal infections. Exemplary infection-associated inflammatory diseases may include viral or bacterial pneumonia, including severe pneumonia, cystic fibrosis, bronchitis, airway exacerbations and acute respiratory distress syndrome (ARDS). Such infection-associated conditions may involve multiple infections such as a primary viral infection and a secondary bacterial infection. Dysregulated IL-17A production may play a role in the pathology of pulmonary diseases such as asthma and Chronic Obstructive Pulmonary Disease (COPD) (reviewed in Alcorn *et al.*, Annu. Rev. Physiol. 72:495-516, 2010). IL-17A has been shown to regulate neutrophilic inflammation in the lungs - a hallmark of severe asthma as well as COPD - owing to the capacity of IL-17A to induce factors important in neutrophil recruitment, survival and activation from lung resident epithelial cells (e.g, IL-6, IL-8, GM-CSF, G-CSF). The antibodies aof the present invention suppress IL-6, IL-8, and GM-CSF secretion from lung epithelial cells, and thus may be beneficial in the therapeutic or prophylactic treatment of subjects with pulmonary inflammatory conditions, such as asthma and COPD. Commonly used animal models for asthma and airway inflammation include the ovalbumin challenge model and methacholine sensitization models (Hessel *et al.*, Eur. J.

Pharmacol. 293:401-12, 1995). Inhibition of cytokine and chemokine production from cultured human bronchial epithelial cells, bronchial fibroblasts or airway smooth muscle cells can also be used as *in vitro* models. The administration of antagonists of the present invention to any of these models can be used to evaluate the use of those antagonists to ameliorate symptoms and alter the course of asthma, airway inflammation, COPD and the like.

Psoriasis is another example of an inflammatory condition. Psoriasis is characterized by T cell mediated hyperproliferation of keratinocytes coupled with an inflammatory infiltrate. The inflammation and hyperproliferation of psoriatic tissue is associated with a different histological, antigenic, and cytokine profile than normal skin. Among the cytokines associated with psoriasis are: TNFα, IL-19, IL-18, IL-15, IL-12, IL-7, IFNγ, IL-17A and IL-23 (Gudjonsson *et al.*, Clin. Exp. Immunol. 135:1-8, 2004). IL-17A has been found overexpressed in psoriatic lesions (US Pat. No. 7,776,540) and positive outcomes in patients treated with anti-human IL-17A therapies have been described (Hueber *et al.*, Sci. Transl. Med. 2: 52ra72., 2010).

Arthritis, including osteoarthritis, rheumatoid arthritis, arthritic joints as a result of injury, and the like, are common inflammatory conditions, which would benefit from the therapeutic use of anti-inflammatory proteins, such as the antagonists of the present invention. Activation of IL-17A signaling may perpetuate inflammation and further tissue damage in the inflamed joint. Several animal models for rheumatoid arthritis are known. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human rheumatoid arthritis. Administration of the IL-17A antibodies of the present invention to the CIA model mice can be used to evaluate the use of these antagonists to ameliorate symptoms and alter the course of diseases.

Exemplary gastrointestinal inflammatory conditions are inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD), colitis induced by environmental insults (*e.g.*, gastrointestinal inflammation (*e.g.*, colitis) caused by or associated with (*e.g.*, as a side effect) a therapeutic regimen, such as administration of chemotherapy, radiation therapy, and the like), infections colitis, ischemic colitis, collagenous or lymphocytic colitis, necrotizing enterocolitis, colitis in conditions such as chronic granulomatous disease or celiac disease, food allergies, gastritis, infectious gastritis or enterocolitis (*e.g.*, Helicobacter pylori-infected chronic active gastritis) and other forms of gastrointestinal inflammation caused by an infectious agent. Several animal models for gastrointestinal inflammatory conditions exist. Some of the most widely used models are the 2,4,6-trinitrobenesulfonic acid/ethanol (TNBS)-induced colitis model or the oxazalone model, which induce chronic inflammation and ulceration in the colon (Neurath *et al.*, Intern. Rev. Immunol 19:51-62, 2000). Another model uses dextran sulfate sodium (DSS), which induces an acute colitis manifested by bloody diarrhea, weight loss, shortening of the colon and mucosal ulceration with neutrophil infiltration. Another model involves the adoptive transfer of naïve $CD45RB^{high}$ CD4 T cells to RAG or SCID mice. In this model, donor naïve T cells attack the recipient gut causing chronic bowel inflammation and symptoms similar to human inflammatory bowel diseases (Read and Powrie, Curr. Protoc. Immunol. Chapter 15 unit 15.13, 2001). The administration of antagonists of the present invention in any of these models can be used to evaluate the potential efficacy of those antagonists to ameliorate symptoms and alter the course of diseases associated with inflammation in the gut, such as inflammatory bowel disease.

Renal fibrosis can develop from either an acute insult (ex. graft ischemia/reperfusion) (Freese *et al.*, Nephrol. Dial. Transplant. 16:2401-6, 2001) or chronic condition (for example diabetes) (Ritz et al., Nephrol. Dial. Transplant. 11 Suppl 9:38-

44, 1996). The pathogenesis is typically characterized by an initial inflammatory response followed by sustained fibrogenesis of the glomerular filtration apparatus and tubular interstitium (Liu, Kidney Int. 69:213-7, 2006). Tubulointerstitial fibrosis has been shown to play a critical role in the pathogenesis of renal injury to end-stage renal failure and the proximal tubule cell has been revealed as a central mediator (Phillips and Steadman, Histol. Histopathol. 17:247-52, 2002; Phillips, Chang Gung Med. J. 30:2-6, 2007). Fibrogenesis in the tubulointerstitial compartment is mediated in part by activation of resident fibroblasts, which secrete pro-inflammatory cytokines that stimulate the proximal tubule epithelium to secrete local inflammatory and fibrogenic mediators. Additionally, chemotactic cytokines are secreted by fibroblasts and epithelial cells and provide a directional gradient guiding the infiltration of monocytes/macrophages and T-cells into the tubulointerstitium. The inflammatory infiltrate produces additional fibrogenic and inflammatory cytokines that further activate fibroblast and epithelial cytokine release while also stimulating the epithelium to undergo a phenotypic transition in which the cells deposit excess extracellular matrix components (Simonson, Kidney Int. 71:846-54, 2007). IL-17A has been shown to be upregulated during human renal allograft rejection (Van Kooten *et al.*, J. Am. Soc. Nephrol. 9:1526-34, 1998; Loong *et al.*, J. Path. 197:322-32, 2002). IL-17A stimulates the production of the pro-inflammatory mediators IL-6, IL-8, complement component C3, and RANTES by proximal tubular epithelium (Van Kooten *et al.*, J. Am. Soc. Nephrol. 9:1526-34, 1998; Woltman *et al.*, J. Am. Nephrol. 11:2044-55, 2000). These factors, in turn, mediate the recruitment of other inflammatory cell-types into the interstitium that contribute to the maintenance of the inflammatory/immune response and, if not suppressed, the onset of fibrosis and chronic allograft nephropathy (Racusen *et al.*, Kidney Int. 55:713-23, 1999; Mannon, Am. J. Transpl. 6:867-75, 2006).

Other exmplary fibrotic conditions may include liver fibrosis (including but not limited to alcohol-induced cirrhosis, viral-induced cirrhosis, autoimmune-induced hepatitis); lung fibrosis (including but not limited to scleroderma, idiopathic pulmonary fibrosis); kidney fibrosis (including but not limited to scleroderma, diabetic nephritis, glomerular nehpritis, lupus nephritis); dermal fibrosis (including but not limited to scleroderma, hypertrophic and keloid scarring, burns); myelofibrosis; neurofibromatosis; fibroma; intestinal fibrosis; and fibrotic adhesions resulting from surgical procedures. The fibrosis can be organ specific fibrosis or systemic fibrosis. The organ specific fibrosis can be associated with lung fibrosis, liver fibrosis, kidney fibrosis, heart fibrosis, vascular fibrosis, skin fibrosis, eye fibrosis or bone marrow fibrosis. The lung fibrosis can be associated with idiopathic pulmonary fibrosis, drug induced pulmonary fibrosis, asthma, sarcoidosis or chronic obstructive pulmonary disease. The liver fibrosis can be associated with cirrhosis, schistomasomiasis or cholangitis. The cirrhosis can be selected from alcoholic cirrhosis, post-hepatitis C cirrhosis, primary biliary cirrhosis. The cholangitis can be sclerosing cholangitis. The kidney fibrosis can be associated with diabetic nephropathy or lupus glomeruloschelerosis. The heart fibrosis can be associated with myocardial infarction. The vascular fibrosis can be associated with postangioplasty arterial restenosis or atherosclerosis. The skin fibrosis can be associated with burn scarring, hypertrophic scarring, keloid, or nephrogenic fibrosing dermatopathy. The eye fibrosis can be associated with retro-orbital fibrosis, postcataract surgery or proliferative vitreoretinopathy. The bone marrow fibrosis can be associated with idiopathic myelofibrosis or drug induced myelofibrosis. The systemic fibrosis can be systemic sclerosis or graft versus host disease.

Other inflammatory conditions and neuropathies, which may be prevented or treated by the methods of the invention are those caused by autoimmune diseases. These conditions and neuropathies

include multiple sclerosis, systemic lupus erythematous, and neurodegenerative and central nervous system (CNS) disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, bipolar disorder and Amyotrophic Lateral Sclerosis (ALS), liver diseases including primary biliary cirrhosis, primary sclerosing cholangitis, non-alcoholic fatty liver disease/steatohepatitis, fibrosis, hepatitis C virus (HCV) and hepatitis B virus (HBV), diabetes and insulin resistance, cardiovascular disorders including atherosclerosis, cerebral hemorrhage, stroke and myocardial infarction, arthritis, rheumatoid arthritis, psoriatic arthritis and juvenile rheumatoid arthritis (JRA), osteoporosis, osteoarthritis, pancreatitis, fibrosis, encephalitis, psoriasis, Giant cell arteritis, ankylosing spondolytis, autoimmune hepatitis, human immunodeficiency virus (HIV), inflammatory skin conditions, transplant, cancer, allergies, endocrine diseases, wound repair, other autoimmune disorders, airway hyperresponsiveness and cell, virus, or prion-mediated infections or disorders.

Administration/Pharmaceutical Compositions

The "therapeutically effective amount" of the agent effective in the treatment of conditions where suppression of IL-17A activity is desirable can be determined by standard research techniques. For example, the dosage of the agent that will be effective in the treatment of an inflammatory condition such as asthma, Crohn's Disease, ulcerative colitis or rheumatoid arthritis can be determined by administering the agent to relevant animal models, such as the models described herein.

In addition, *in vitro* assays can optionally be employed to help identify optimal dosage ranges. Selection of a particular effective dose can be determined (*e.g.*, via clinical trials) by those skilled in the art based upon the consideration of several factors. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled

artisan. The precise dose to be employed in the formulation will also depend on the route of administration, and the severity of disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The mode of administration for therapeutic use of the agent of the invention may be any suitable route that delivers the agent to the host. Pharmaceutical compositions of these agents are particularly useful for parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous or intranasal.

The agent of the invention may be prepared as pharmaceutical compositions containing an effective amount of the agent as an active ingredient in a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the active compound is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the agent of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 ml sterile buffered water, and between about 1 ng to about 100 mg, *e.g.* about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of a IL-17A antibody antagonist of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 mg to about 30 mg and preferably 5 mg to about 25 mg of an antagonist of the invention. Actual methods for preparing parenterally administrable compositions are well known and are described in more detail in, for example, "Remington's Pharmaceutical Science", 15th ed., Mack Publishing Company, Easton, PA.

The antibody antagonists of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and protein preparations and art-known lyophilization and reconstitution techniques can be employed.

The present invention will now be described with reference to the following specific, non-limiting examples.

Example 1

Identification of anti-human IL-17A antagonistic mAbs

The MorphoSys Human Combinatorial Antibody Library (HuCAL®) Gold phage display library (Morphosys AG, Martinsried, Germany) was used as a source of human antibody fragments and was panned in subpools in solution. In the first round of panning, the sublibraries were selected against biotinylated mature His6-tagged IL-17A A132Q and A70Q variant (IL-17Amut6) (SEQ ID NO: 106). In the second round, the amplified output of round 1 was selected against biotinylated His6-tagged IL-17Amut6 in the presence or absence of other IL-17A family members as a competitor to bias against antibodies that were specific to IL-17A. The amplified output of round 2 was divided in two pools.

The first pool was panned as in round 1. The clones in the second pool were further diversified in either HCDR2 or LCDR3, depending on the sublibrary used in the initial selections, and then carried through 2 additional rounds of panning against IL-17Amut6 to give a second source of clones for screening. Fabs from clone lysates were captured in ELISA plate wells coated with sheep anti-human Fd antibody and screened for binding to biotinylated IL-17Amut6. Crude lysates of positive clones were screened for inhibition of IL-17Amut6 binding to recombinant human IL-17RA receptor (SEQ ID NO: 107).

Select clones were chosen for further characterization as purified Fabs based on sequence scoring, affinity, and representation of all sequence families, and were designated with MOR numbers. Additional variants for MOR7708, MOR7785, MOR7706, MOR7775 and MOR7700 were generated to replace Trp or Met residing in HCDR2, HCDR3, or LCDR3. Table 3 shows the generated variants.

Table 3.

Family	Parent MOR#	Variant MOR#	Substitutions		
			HCDR2	HCDR3	LCDR3
2	MOR7708	MOR8297	W57T		
		MOR8298	W57Y		
	MOR7785	MOR8104	W57T		
		MOR8105	W57Y		
6b	MOR7706	MOR8299		M106I	
		MOR8300		M106L	
		MOR8301		M106T	
	MOR7775	MOR8101		M106I	
		MOR8102		M106L	
		MOR8103		M106T	
19a	MOR7700	MOR8095	M53I		
		MOR8096	M53L		
		MOR8097		M101L	
		MOR8098		M101T	
		MOR8141			M96L
		MOR8142			M96T
		MOR8143			M96Y
		MOR8160		M101T	M96L
		MOR8161		M101T	M96T
		MOR8162		M101T	M96Y
		MOR8302	M53L	M101T	
		MOR8303	M53L	M101T	M96T

The Fabs were tested for their inhibition of IL-17Amut6 and cynoIL-17A binding to recombinant human IL-17RA receptor, and their binding to IL-17Amut6. All tested Fabs inhibited both IL-17Amut6 and cynoIL-17A binding to the IL-17RA. Affninty of the Fabs to IL-17Amut6 was measured using the SET assay (Table 4). From the identified Fabs, candidates from families 2, 6a, 6b, 19a and 19b were seleced for further characterization.

Table 4.

Family	MOR#	Kd (SET) (pM)
2	7702	11
	7701	45
	7708	90
	7785	6
	8104	150
	8105	130
	7786	20
6b	7706	90
	7775	44
	8101	150
	8102	130
	8103	89

Family	MOR#	Kd (SET) (pM)
19a	7700	30
	8095	77
	8096	28
	8097	69
	8098	47
	8141	30
	8142	90
	8143	130
	8160	70

Example 2

Derivation, engineering and characterization of anti-IL-17A antagonistic mAbs

The selected MOR# Fabs were converted and expressed as mAbs in a human IgG1 format, and given corresponding MORmAb designation. The generated MORmAbs were tested for expression and aggregation, their ability to inhibit human and cyno IL-17A binding to human IL-17RA, and IL-8 secretion from NHDF cells. Table 5 shows IC50 values for select assays for the MORmAbs.

None of the tested MORmAbs (MORmAb#s 7702, 7708, 7785, 7786, 7706, 7775, 7700, 8095, 8096, 8097, 8098, 7768) cross-reacted with other IL-17 family members.

Table 5.

Family	MORmAb#	Human IL-17, IC50 (pM)		Cyno IL-17, IC50 (pM)
		IL-17RA Inhibition	IL-8 secretion	IL-17RA Inhibition
2	7702	297	6214	209
	7708	284	398	289
	7785	172	196	270
	8104	204	512	306
	8105	538	1168	498
	7786	140	368	59
6b	7706	378	402	961
	7775	138	2244	541
	8101	108	1907	845
	8102	186	24520	838
	8103	167	929	491
19a	7700	99	70	236
	8095	130	121	198
	8096	189	75	58
	8097	178	84	300
	8098	225	146	289
	8141	117	67	1435
	8142	129	79	139
	8143	191	61	252
	7768	456	229	388

Framework engineering of anti-IL-17A antagonistic mAbs

Based on activity and biophysical and biochemical properties, select MORmAbs were further engineered in their variable regions to change certain framework residues to those present in a matching human germline and to change codons to those most frequently occurring in highly expressed mammalian proteins. In family 2 VL, L11V and V85T (linear sequence) substitutions were made, converting the framework to an exact

match with the VK-1 germline Vb-L5 (IGKV1-12*01). An exemplary variable region with the V11V and V85T substitutions is the variable region having the amino acid sequence shown in SEQ ID NO: 76. In family 6a and 6b VL, D1E, V59I and T86V substitutions were made (linear sequence), converting the framework to exact match with the Vk-3 germline Vb-L6 (IGKV3-11*01). An exemplary variable region with the D1E, V59I and T86V substitutions is the variable region having the amino acid sequence shown in SEQ ID NO: 77. In family 6a and 6b VH, a G44S substitution was made (linear sequence) to match the Vh-6 germline Vb 6-01 (IGHV6-1*01). An exemplary variable region with the G44S substitution is the variable region having the amino acid sequence shown in SEQ ID NO: 81. In family 19a and 19b VL, amino acids 1-3 (DIE) were substituted with QSV to replace the artificial kappa N-terminus with that of a lambda chain. Exemplary variable region with the QSV substitution is a variable region having the amino acid sequence shown in SEQ ID NO: 79. In family 19a and 19b VH, a V5L substitution was made to give a close match the Vh-3 germline Vb 3-23 (IGHV3-23*01. Also, in this process the heavy chain constant region amino acid sequence residues 353-357 (REEMT) were substituted with RDELT. An exemplary variable region with the V5L substitution is a variable region having the amino acid sequence shown in SEQ ID NO: 86. An exemplary heavy chain with the constant region 353-357 REEMT -> RDELT substitutions is a heavy chain having the amino acid sequence shown in SEQ ID NO: 100. The engineered antibodies were given mAb numbers.

The corresponding designations and the sequence listings of the engineered and original variable regions and full length antibodies are listed in Table 2. The sequences of the CDRs within each family are shown in Figure 1.

The engineered mAbs were characterized as described above for the MORmAbs. The IC50 values (pM) measured using indicated assays are shown in Table 6.

Table 6.

Family	mAb#	IC50 (pM), human IL-17A			IC50 (pM), cynoIL-17A		
		IL-17RA inhibiton	IL-8 production	IL-6 production	IL-17RA inhibition	IL-8 production	IL-6 production
2	mAb624	78	687	234	51	192	687
	mAb3077	118	292	37	54	374	113
	mAb7024	185	693	412	117	2979	999
6b	mAb4538	229	1483	264	636	1754	847
	mAb3584	195	2388	370	489	1253	823
	mAb732	327	2607	560	463	12527	2017
	mAb4168	266	4878	732	764	3301	2172
19a	mAb1926	108	62	35	53	105	67
	mAb7146	143	71	40	67	140	139
	mAb6785	172	95	45	76	563	193

Affinity of select mAbs was assessed using Biacore. The results of the measurements are shown in Table 7.

Table 7.

Binding to human IL-17Amut6

Antibody	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)	Stoichiometry *
mAb7146	4.67×10^6	5.57×10^{-5}	12	2.1
mAb6785	3.80×10^6	6.98×10^{-5}	18	2.1
Fab6486	3.14×10^6	1.23×10^{-4}	39	1.1
mAb5548	3.63×10^6	1.45×10^{-4}	40	2.1
mAb1926	4.43×10^6	3.41×10^{-5}	8	2.1

Binding to cyno IL-17A

Antibody	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)	Stoichiometry*
mAb7146	2.23×10^6	1.12×10^{-4}	50	2
mAb6785	1.80×10^6	2.67×10^{-4}	148	2.2
Fab6486	1.60×10^6	3.28×10^{-4}	205	1.1
mAb5548	1.61×10^6	3.62×10^{-4}	225	1.9
mAb1926	2.77×10^6	5.11×10^{-5}	18	2.3

*dimers per anti-IL-17

Anti-IL-17 antibody inhibits cytokine secretion in NHBE cells

IL-17A has been shown to regulate neutrophilic inflammation in the lungs, a hallmark of severe asthma as well as COPD, owing to the capacity of IL-17A to induce factors important in neutrophil recruitment, survival and activation (e.g, IL-6, IL-8, GM-CSF). To determine whether anti-IL-17A antibodies of the invention can inhibit IL-17A-induced changes in lung resident cells, normal human bronchial epithelial (NHBE) cells were stimulated with human IL-17A for 48 hours in the presence of mAb6785. mAb6785 inhibited IL-17A-induced IL-6 and GM-CSF production by NHBE cells with IC50=619.0 ± 64.0 pM and 564 ± 86 pM, respectively.

Anti-IL-17 antibody inhibits the biological activity of IL-17A/F heterodimer

Normal Human Dermal Fibroblasts (NHDF; Lonza) cells were seeded into a 48-well flat bottom tissue culture plate at 10,000 cells per well in FGM-$_2$ medium (Lonza) and incubated overnight (37°, 5% CO_2). Following incubation, 50 ng/mL final concentration (1.47 nM) of rhIL-17A/F heterodimer (R&D Systems) was pre-incubated with a dilution series (30 µg/mL – 0.5 ng/mL) of mAb6785 or control antibodies for 10 minutes at room temperature, and added to cells. Cells were incubated for 48h (37°, 5% CO_2) and culture supernatants were collected and assayed by ELISA for IL-6 content using Human IL-6 Duo Sets (R&D Systems, Inc.) according to manufacturer's instructions. IC50 values were determined by non-linear regression using GraphPad Prism software (GraphPad Software, Inc). mAb6785 inhibited IL-17A/F heterodimer -induced IL-6 production by NHDF with EC50 2 ± 2.5 nM.

Methods

Determination of Picomolar Affinities Using Solution Equilibrium Titration (SET)

For K_D determination by solution equilibrium titration (SET), monomer fractions (at least 90% monomer content, analyzed

by analytical SEC; Superdex75 column, GE) of Fab protein were used.

Electrochemiluminescence (ECL) based affinity determination in solution and data evaluation were basically performed as described previously (Haenel *et al.*, Anal Biochem 339:182-4, 2005). A defined fixed concentration of purified Fab (~10-100 pM) was incubated with increasing concentrations of IL-17Amut6 (highest concentration of 5 nM) in solution until chemical equilibrium was achieved. To quantify the unbound Fab in solution the samples were transferred to a Streptavidin MSD 384-well microtiter plate (Meso Scale Discovery, Gaithersburg, MD) with coated biotinylated IL-17Amut6. For detection, a ruthenium complex-labeled anti-human Fab/IgG antibody was applied and the plates were read with the Sector™ Imager 6000 (MSD). Titration curves (concentration of free Fab as a function of antigen concentration) were plotted and fitted with Excel / XLfit software using the model described below.

For data evaluation for K_D determination of Fab molecules, the following fit model was used (modified according to Abraham *et al.* J Mol Recognit. 9:456-461, 1996):

y=Bmax-(Bmax/(2*cFab)*(x +cFab+KD-sqrt((x+cFab+ KD)*(x+cFab+ KD)-4*x*cFab)))

Whereas:

Bmax: maximum binding signal (at antigen concentration=0)

cFab: applied Fab concentration

x: applied total soluble antigen concentration (binding sites)

sqrt: square root

K_D: Equilibrium dissociation constant

Inhibition of IL-17A binding to IL-17RA (e.g. "IL-17RA inhibition" assay

Clear maxisorp plates were coated with 100 µl/well of 2.5 µg/ml human IL-17RA-Fc (R&D Systems, Minneapolis, MN) in 0.1 M sodium carbonate-bicarbonate buffer, pH 9.4 and incubated overnight at 4°C. After blocking and washes, 25 ng/ml of

biotinylated human IL-17mut6 (SEQ ID NO: 106) or cynomolgus IL-17A (SEQ ID NO: 108) was pre-incubated with tested mAbs or control mAbs (30 to 0 μg/ml final concentration) in a combined volume of 100 μl for 5-10 minutes, and then added to plates. The signal was detected with 100 μl of 1:10,000 dilution of 1 mg/ml SA-HRP (Jackson Immunoresearch, West Grove, PA) for 20 minutes at room temperature (RT) followed by 100 μl/well of OPD substrate (Sigma-Aldrich Corp., St. Louis, MO). The plates were read at 492 nm (Envision, PerkinElmer, Waltham, MA). Fab binding to IL-17RA was tested as described for mAbs.

Inhibition of IL-8 and IL-6 production from NHDF cells (e.g. "IL-8 production" and "IL-6 production" assays)

Effect of inhibition of anti-IL-17A mAbs on IL-8 and IL-6 production was assessed in normal human dermal fibroblasts (NHDF). Cells were plated in a 48-well flat bottom tissue culture plate at $0.1x10^5$ cells per well, 250 μl per well in FGM-2 medium and incubated overnight (37°, 5% CO_2). Following incubation, 0.1 ng/ml human TNF-α was added to all wells. 10 ng/ml IL-17mut6 or 25 ng/ml cynomolgus IL-17A was pre-incubated with tested mAbs or control mAbs (30-0 μg/ml final concentration) in a combined volume of 250 μl for 10 minutes at RT, and then added to 250 μl of cells. In the assays, IL-17mut6 samples with no added antibody were included as control samples, while samples consisting of TNF-α or culture medium only were included as negative controls. Cells were incubated for 24 hours (37°, 5% CO_2) and conditioned media were collected and assayed by ELISA for IL-6 and IL-8 using human IL-6 & IL-8 ELISA Duo Sets according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). The Fabs were assayed as described fro the mAbs.

Inhibition of IL-6 and G-CSF production from NHBE cells

Normal human bronchial epithelial (NHBE; Lonza) cells were seeded at 20,000 cells per well in BEGM media (Lonza) and incubated overnight (37°, 5% CO_2). Following incubation, cells were stimulated with IL-17Amut6 for 48 hours in the presence of the tested antibodies at a range of concentrations (30 µg/mL – 0.5 ng.mL). Supernatants were collected after the incubation and assayed for IL-6 or G-CSF content using a human IL-6- or G-CSF-specific ELISA (R&D Systems, Inc.). IC50 values were determined by non-linear regression using GraphPad Prism software (GraphPad Software, Inc).

Cross-reactivity with IL-17A family members

Clear maxisorp plates were coated with 100 µl/well of 5 µg/ml mAbs or isotype control mAbs in PBS, and incubated overnight at 4°C. The plates were blocked with 200 µl/well for 1 hour with ELISA block buffer (1% BSA, 5% Sucrose in PBS with 0.05% NaN_3) and washed three times with wash buffer (PBS, 0.01% Tween-20). Competing cytokines were titrated in Assay Diluent Buffer (1% BSA in PBS) at 2x final concentration, and biotinylated cytokine was prepared at 2x final concentration. 100 µl of cytokines at 2x final concentration were mixed (30-0 µg/ml final concentration) with 100 µl of biotinylated IL-17mut6 at 2x final concentration (25 ng/ml final concentration) in assay buffer. Recombinant human IL-23 (R&D Systems, Minneapolis, MN) was used as a negative control, buffer only sample as background control, and IL-17mut6 as positive control. 100 µl per well in duplicate of cytokine/biotinylated IL-17mut6 mixture was added to the plate and incubated for 1-2 hours. Plates were washed three times with wash buffer, and incubated with 100 µl of 1:10,000 dilution of 1 mg/ml SA-HRP (Jackson Immunoresearch, West Grove, PA) for 20 minutes at RT. Plates were washed three times with ELISA wash buffer. Following wash, 100 µl/well of OPD substrate

(Sigma-Aldrich Corp., St. Louis, MO) was added to each well and incubated until the appropriate color change was detected. The reaction was stopped with the addition of 50 µl of 2N sulfuric acid, and the plates were read 492 nm using the Envision instrument.

Affinity measurements - Biacore assay

Affinity measurements using Surface Plasmon Resonance (SPR) were performed using a Biacore 3000 optical biosensor (Biacore). Selected Fabs (~30 RU) or mAbs (~50 RU) were captured onto the sensor chip surface using a sheep anti-Fd antibody or an anti-human Fc antibody for Fab or mAb capture, respectively. Capture of Fab or mAb was followed by injection of huIL-17mut6 or cyno IL-17A in solution (0.2 to 49 nM).

Example 4

Epitope mapping

Antibody epitopes were deduced by a combination of competition binding, H/D exchange analysis, and antibody-IL-17A co-structure (see Example 5). The following antibodies were used: mAb1926, MORmAb7700, MORmAb7706, MORmAb7708, mAb7357 (a mouse anti-human IL-17A neutralizing antibody derived from hybridoma C1863), mAb2832 (a mouse/human chimeric anti-human IL-17A neutralizing antibody derived from hybridoma C1861), mAb317 (mouse anti- human IL-17A antibody, R&D Systems, Minneapolis, MN) and mAb3171 (mouse anti-human IL-17A antibody, R&D Systems, Minneapolis, MN), and mAbeBIO16-7178 (a mouse anti-human IL-17A antibody, e-Bioscience, San Diego, CA). The three commercial antibodies showed varying degrees of neutralizing activity.

Competitive Epitope Binding

For competitive ELISA, 5 µl (20 µg/ml) of IL-17Amut6 protein was coated on MSD HighBind plate (Meso Scale Discovery, Gaithersburg, MD) per well for 2 hr at room temperature. 150 µl

of 5% MSD Blocker A buffer (Meso Scale Discovery, Gaithersburg, MD) was added to each well and incubated for 2 hr at room temperature. Plates were washed with 0.1 M HEPES buffer (pH 7.4). Labeled antibody (MDS fluorescence dye), 10 nM, was incubated with increasing concentrations of competitor antibodies (1 nM - 2 μM), and 25 μl of the mixture was added to the designated wells. After 2-hour incubation with gentle shaking at RT, plates were washed as above, 150 μl diluted MSD Read Buffer T was added, and the plates were read with a MDS Sector Imager 6000.

Assays were carried out with labeled mAb1926, mAb317, mAb3171, or mAb7357 (Figure 4). Based on competition assays, anti-IL-17A antibodies were assigned to four different bins. Bin A: mAb1926, MORmAb7706, and MORmAb7708; Bin B: eBio16-7178 and mAb7357; Bin C: mAb317; Bin D: mAb3171.

H/D exchange analysis:

For H/D exchange, the procedure used to analyze the antibody perturbation was similar to that described previously (Hamuro *et al.*, J. Biomol. Techniques, 14:171-82, 2003; Horn *et al.*, Biochemistry, 45: 8488-98, 2006) with some modification. Recombinant IL-17Amut6 (expressed in HEK293E cells with C-terminal His-tag) was incubated in a deuterated water solution for pre-determined times resulting in deuterium incorporation at exchangeable hydrogen atoms. The deuterated IL-17Amut6 was captured on a column containing immobilized individual anti-IL-17A mAbs and then washed with aqueous buffer. The back-exchanged IL-17Amut6 protein was eluted from the column and localization of deuterium containing fragments was determined by protease digestion and mass spec analysis. Regions bound to the antibody were inferred to be those sites relatively protected from exchange and thus containing a higher fraction of deuterium, compared to IL-17Amut6 not complexed with antibody. H/D exchange

perturbation maps of IL-17Amut6 are shown in Figure 5. The numbers on top of the bars refer to IL-17Amut6 resiudes.

MORmAb7700, MORmAb7706 and MORmAb7708 showed varying degrees of differential exchange for three segments of IL-17A (SEQ ID NO: 105) $_{45}$NRSTSPWNLH$_{54}$ (SEQ ID NO: 159), $_{56}$NEDPERYPSVIWE$_{68}$ (SEQ ID NO: 157) and $_{100}$RREPPHCPNSFRLEKIL$_{116}$ (SEQ ID NO: 158), indicating protection by the antibodies. The $_{56}$NEDPERYPSVIWE$_{68}$ (SEQ ID NO: 157) fragment was strongly protected by MORmAb7708, weakly protected by MORmAb7700, and not protected by MORmAb7706. The overlap in the fragment protection patterns of these antibodies is consistent with their cross-inhibition in the competition assays described above.

For both mAb7357 and mAbeBio16-7178, strong protection was observed for $_{71}$CRHLGCINADGNVDYHM$_{87}$ (SEQ ID NO: 160) consistent with their cross-inhibition in the competition assays described above. Weak, and therefore inconclusive, differential exchange was observed for other fragments with mAb7357, mAb2832, mAb317 and mAb3171.

The H/D exchange studies localized the binding sites for two of the four competition groups defined above. Bin A antibodies (MORmAb7700, MORmAb7706 and MORmAb7708) bound in the region of peptide segments $_{45}$NRSTSPWNLH$_{54}$, (SEQ ID NO: 159), $_{56}$NEDPERYPSVIWE$_{68}$ (SEQ ID NO: 157) and $_{100}$RREPPHCPNSFRLEKIL$_{116}$ (SEQ ID NO: 158), of SEQ ID NO: 105, and Bin B antibodies (mAb7357 and mAbeBio16-7178) bound in region of peptide segment $_{71}$CRHLGCINADGNVDYHM$_{87}$ (SEQ ID NO: 160). mAb317 and mAb3171 bound to sites distinct from each other and from the bin A and bin B antibodies. However, the weak signals in the H/D exchange studies with both antibodies did not provide sufficient evidence to localize their epitopes on IL-17A.

Example 5

Co-crystal structure of IL-17A and anti-IL-17A antibody

The co-structure of IL-17Amut6 with the Fab6468, a

recombinant His6-tagged Fab of mAb6785, was determined by X-ray crystallography. The amino acid sequence of the light chain of Fab6468 is shown in SEQ ID NO: 90, and the heavy chain amino acid sequence is shown in SEQ ID NO: 111. In the Example 5, the IL-17A amino acid residues referred to indicate residues according to SEQ ID NO: 105, and the Fab6468 residues referred to indicate light chain variable region residues according to SEQ ID NO: 79 and heavy chain variable region residues according to SEQ ID NO: 86. The expression, refolding and purification of recombinant human IL-17Amut6 has been described (Wu et al., Cytokine, ePub ahead of print Jul29). Fab6468 was expressed in HEK-293F cells and purified using a similar method as described (Zhao *et al.*, Protein Expr Purif, 67:182-9, 2009).

Crystallization of IL-17A/Fab6468 complex

The IL-17A/Fab6468 complex was prepared by mixing IL-17Amut6 and Fab6468 in 1:1.1 molar ratio in 20 mM MES pH 6.5, 0.2 M NaCl, and 10% glycerol and incubated over night at 4°C. The complex was purified from excess un-complexed Fab using size exclusion chromatography (SEC) on a Superdex 200 10/300 GL column (GE Healthcare, Piscataway, NJ) in 20 mM MES pH 6.5, 0.2 M NaCl, and 10% glycerol. Fractions corresponding to the complex were pooled and concentrated with an Amicon Ultra 10000 MWCO device to 4.6 mg/ml.

Automated crystallization screening was performed using the Oryx4 automatic protein crystallization robot (Douglas Instruments, East Garston, UK) dispensing equal volumes of protein and reservoir solution in a sitting drop format using Corning plate 3550 (Corning Inc., Corning, NY). Initial screening was performed with Hampton Crystal Screen HT (HR2-130, Hampton Research) and produced needle-like crystals from several conditions containing ammonium sulfate, PEGs at pH 4.5-4.6. These small crystals were used to produce a seed stock for microseed-matrix screening (MMS) (D'Arcy *et al.*, Acta Crystallographica Section D, 63:550-4, 2007). Diffraction

quality crystals were obtained from the MMS screen in 0.1 M Sodium Acetate pH 5.5, 12% PEG MME 5000 and 0.2 M Lithium Sulfate.

X-ray data collection of IL-17A/Fab6468 complex

For X-ray data collection, the crystal was soaked for a few seconds in the mother liquor supplemented with 24% glycerol, and flash frozen in the stream of nitrogen at 95°K. X-ray diffraction data were collected and processed using a Rigaku MicroMax™-007HF microfocus X-ray generator equipped with an Osmic™ VariMax™ confocal optics, Saturn 944 CCD detector, and an X-stream™ 2000 cryocooling system (Rigaku, Woodlands, TX). Diffraction intensities were detected over a 254° crystal rotation with the exposure time of 3 min per half-degree image to the maximum resolution of 2.2 Å. The X-ray data were processed with the program D*TREK (Pflugrath, J., Acta Crystallographica Section D, 55:1718-25, 1999). The crystal belonged to the monoclinic space group $P2_1$ with a=73.40 Å, b=64.04 Å, c=145.61 Å and β=95.39°. The X-ray data statistics are given in Table 8.

Table 8.

Wavelength (Å)	1.5418	
Temperature (K)	95	
Rotation range (°)	254	
Space group	$P2_1$	
Unit cell axes (Å)	73.40, 64.04, 145.61	
Unit cell angles (°)	90, 95.39, 90	
Molecules/asymmetric unit	IL-17 dimer + 2 Fabs	
V_m ($Å^3$/Da)	2.76	
Solvent content (%)	55	
Resolution (Å)	73-2.2	(2.28-2.20)*
No.measured reflections	251,653	(16,276)
No.unique reflections	61,776	(5,947)
Completeness (%)	89.8	(86.9)
Redundancy	4.1	(2.7)
R-merge	0.151	(0.353)
<I/σ>	5.6	(1.9)
B-factor (Wilson) ($Å^2$)	33.3	

*Values for highest resolution shell are in ()'s.

Structure Determination

The crystal structure of IL-17A/Fab6468 was determined by molecular replacement using Phaser (Read, Acta Crystallogr D Biol Crystallogr, 57:1373-82, 2001). The search models were IL-17F (PDB ID 1JPY) (Hymowitz *et al.*, EMBO J., 20:5332-41, 2001) and a homology model for the Fv (VH/VL), which was constructed based upon the anti-IL-13 antibody CNTO607 (PDB ID 3G6A) (Teplyakov *et al.*, J. Mol. Biol. 389:115-23, 2009) for both the VH and VL, using Modeller (Accelrys, CA). The two constant domains CL/CH1 were taken from PDB ID 8FAB (Strong *et al.*, Biochemistry, 30:3739-48, 1991). The structure refinement was carried with PHENIX (Adams *et al.*, J. Synchrotron. Radiat. 11:53-5, 2004). The two-fold non-crystallographic symmetry was initially imposed in early stages of refinement but was relaxed in the final stages based upon R_{free}. Model adjustment and manual rebuilding were done using COOT (Emsley *et al.*, Acta Crystallogr. D. Biol. Crystallogr. 60:2126-32, 2004). The final R_{cryst} and R_{free} were 23.4% and 29.7%, respectively, for all 61,706 independent reflections to 2.2Å. The refinement statistics are given in Table 9.

Table 9.

Structure refinement	
Resolution (Å)	73-2.2 (2.234-2.2)
R_{cryst}/R_{free} (%)[b]	23.4/29.7 (27.2/37.7)
No. of reflections	
Working set	58,570
Test set (5% data)	3,136
Rmsd from ideal values	
Bond length (Å)	0.007
Bond angels (°)	1.1
Average B-factor (Å^2)	28.0
Number of protein atoms	7,994
Number of Solvent (water + ions)	864
Ramachandran plot[c]	
Most Favored regions (%)	90.5
Additional Allowed (%)	8.6
Generously allowed	0.2
Disallowed (%)	0.7

The IL-17A/Fab6468 complex structure

The structure of the complex was determined to high resolution (~ 2.2 Å). IL-17A was a nearly symmetrical homo-dimer in the crystal and bound two Fab molecules. The antibody-antigen interactions were largely hydrophobic and in contrast to most antibodies, the light chain CDRs made a number of the important contacts. The overall molecular structure of IL-17A/Fab6468 complex is shown in Figure 6A. The monomer of the IL-17A dimer adopted the overall topology of a cystine knot (Figure 6B). The two monomers were very similar with a Cα RMSD of 0.54 Å for 77 backbone Cα atoms. The overall architecture of IL-17A monomer cystine knot was very similar to that of IL-17F with an rmsd of 0.71 Å for 76 Cα atoms (Figure 6B). Each IL-17A monomer was stabilized by three disulfide bonds. For chain B, three intra-chain disulfide bonds were observed (C^{10}–C^{106}, C^{71}–C^{121}, C^{76}–C^{123}), whereas for chain A the C^{10}–C^{106} disulfide bond was not observed due to disorder in these segments of the monomer. The latter two disulfide bonds (C^{71}–C^{121}, C^{76}–C^{123}) stabilized the cystine knot architecture, analogous to IL-17F and NGF. The structural model for chain B of IL-17A included all residues 10-128 (residues 1-9 were disordered), whereas for chain A residues only residues 21-29, 41-104 and 109-127 were observed and the other residues 1-20, 30-40, 105-108 and 128 were missing due to disorder in the structure. For the two Fabs, residues 1-2 of both the light chains were disordered or had poor electron density. The C-terminal 3 residues of both the heavy and light chains, including the inter-chain disulfide bonds as well as the His tag on the heavy chain were disordered.

The ordered N-terminal segment of IL-17A (chain B) contained a short helical element (residues 8-12). It folded back towards the loop 3-4 of the same monomer and formed an intra-chain disulfide bond (C^{10}-C^{106}). In contrast, the equivalent segment of IL-17F reached over to the other monomer of the dimer and formed an inter-chain disulfide bond and linked the two

monomers covalently. The ordered parts of the segments 17-39 of the two IL-17A monomers were swapped, as in IL-17F. This swapping resulted in a cross-over for these parts of the IL-17A dimer. Combined with the intra-molecular disulfide bond (C^{10}-C^{106}), the two N-terminal segments of IL-17A formed two inter-locked monomers, which also gave rise to an apparent dimer of 26 kD on non-reducing SDS-PAGE.

The dimer of the IL-17A was nearly symmetrical for the four main β-strands (strands 1-4) (Figure 6C). The Cα rmsd for 76 residues is 0.71 Å. The slight asymmetry came from two sources. First, the chain A contained a number of disordered segments, mainly in N-terminus. Only a short β-strand (strand 0, residues 22-26) was apparently ordered, whereas residues 10-40 of chain B were ordered with a helical segment (residues 12-16) and a β-strand (strand 0, residues 21-25). Second, while the cystine knotted four main β-strands of the two monomers (40-128) of the IL-17A were related by a two-fold symmetry of rotation, the ordered parts of strand 0 did not superimpose well when the main body is overlaid (not shown). Whether this was an artifact of protein refolding or such an arrangement exists in nature is not clear without further investigation. The bioactivity of this species was similar to that of a reference IL-17A from a commercial source (also produced in E. coli) (R&D Systems, Minneapolis, MN) suggesting that the inter- or intra- chain disulfide linkage for the C^{10}-C^{106} is not important for its receptor binding. Nevertheless, the current structure suggests that the N-terminal segments (1-20) and (30-39) are very flexible and their structures do not impact the activity of the folded IL-17A dimer.

The epitope and the paratope

The residues involved in binding between IL-17A and Fab6468 are listed in Table 10. Due to the missing residues in protomer A in the IL-17A and the slight asymmetrical nature of the IL-17A dimer, all epitope residues from the two contact sites were not

identical (Table 10 and Figure 7). However, there was a core set of residues as well as their interactions that were identical. These residues were L26, R55, E57, P59, E60, R61, Y62, S64, V65, W67, R101, E102, P103 and F110 of IL-17A SEQ ID NO: 105 (highlighted black in Table 10), and they constitute the core epitope for Fab6468.

Table 10. Core epitope residues are highlighted in black. Core paratope residues are bolded. Extended paratope residues are shown in parenthesis.

VL	Il-17A epitope on monomer B	VH		VL	IL-17A epitope on monomer A	VH
(D25)	F18					
(F93)	P19					
(F93)	V22					
F93	L26			**F93**	L26	
				(N26)	N27	
				(D29), (F93)	I28	
(D29)	H29			(G28)	N30	
				(D50), (I51), (Y31)	N36	
				(I51), (D52), (D49), (Y31)	R39	
D49	R55	**T101, Q99**		(Y48), **D49**	R55	**T101, Q99**
	E57	**L100, Q99, T101**			E57	**L100, Q99, T101**
Y90, F92	P59	**Y59**, (L100)		**Y90, F92**	P59	(S52), **Y59**
F92	E60	**Y59, F57, S52, T54**		**F92**	E60	**Y59, F57, S52, T54**
F92	R61			**F92**	R61	
F92	Y62			**F92**, (F93), (D29)	Y62	
Y90	S64			**Y90**	S64	(L100)
Y31	V65			**Y31**, (D49)	V65	
D49, (Y31)	W67			**D49**, (D52), (Y48)	W67	
	L99			(F93)	L99	
F92, Y90	R101	**F57, Y59**		**Y90, F92**	R101	**F57, Y59**
	E102	**F57**			E102	**F57**
	P103	**F57**			P103	**F57**
	P104				P104	(G56)
F92	F110			**F92**, (F93)	F110	

Similarly, the contact residues from the antibodies in the two sites were not all identical. The residues involved in identical contacts to the core epitope residues are referred to as the "core paratope", which was composed of the following residues: Light Chain (LC): Y31, D49, Y90, F92, F93 (SEQ ID NO: 79); and heavy chain (HC): S52, T54, F57, Y59, Q99, L100 and T101 (SEQ ID NO: 86) (Table 10). The core paratope residues are shown

in bold in Table 10. The additional "extended paratope" residues identified in one monomer binding a specific IL-17A residue are shown in parenthesis.

The H/D protection data for the MORmAb7700 was in agreement with the co-crystal studies, as all core epitope residues identified in the co-crystal structure except L26 were within or at the borders of two of the protected segments identified by H/D exchange, $_{56}$NEDPERYPSVIWE$_{68}$ (SEQ ID NO: 157) and $_{100}$RREPPHCPNSFRLEKIL$_{116}$ (SEQ ID NO: 158) for the MORmAb7700. All of the MORmAb7700 antibody derivatives, including MORmAb8302 and mAb1926, are assumed to have the same binding specificity as Fab6468 since they differ at most by one residue in the N-terminal region of VH (see Example 1), 3 residues at the N-terminus of VL (see Example 1), and 3 CDR residues (one each in H2, H3 and L3, Table 1a), none of which are part of the antibody paratope.

The IL-17A structure characterized in this invention is very similar to the previously published structure, except that due to missing segments, the P2 pocket cavity (see below) was not identified in the previous work (structure 2VXS, available at the Protein DataBank http_//www_rcsb_org/pdb/home/home_do; Gerhardt *et al.*, J. Mol. Biol. 394:905-21, 2009).

The crystal structure of the human IL-17F in complex with IL-17RA has been reported (Ely *et al.*, Nat. Immunology, 10:1245-51, 2009). Because of the sequence and structural similarities between IL-17A and IL-17F, it is likely that IL-17A will interact with the IL-17RA in a similar manner to IL-17F. Molecular modeling by overlaying the IL-17A structure in complex with Fab6468 obtained in this study onto the IL-17F in the reported IL-17F/IL-17RA complex showed that segments of Fab6468 would have steric clashes with IL-17RA. One of these segments localize around the FF motif (residues 92 and 93 of SEQ ID NO: 79) in the light chain CDR3 of Fab6468. Thus, not wishing to be bound by any particular theory, it is suggested that Fab6468 would inhibit IL-17A function by blocking its interactions with IL-17RA and by

analogy, IL-17RC, though the mode of interaction between IL-17RC and IL-17A is not known at the molecular level.

The significant differences in the affinities of IL-17A and IL-17F for IL-17RA suggest there may be significant differences in the details of IL-17A and IL-17RA interactions, the extent of which will only be available when the co-crystal structure of IL-17A/IL-17RA is determined. This is implicated by the identification of the P2 pocket cavity in this study, which is only partially identified in IL-17F analogous region in the reported IL-17F/IL-17RA crystal structure (Ely *et al.*, Nat. Immunology, 10:1245-51, 2009).

Two deep, largely hydrophobic pockets were identified on the surface of IL-17A along the dimer interface (Figure 8A, 5B). The P1 pocket, which is analogous to a pocket first discovered in IL-17F (Hymowitz *et al.*, EMBO J, 20: 5332-41, 2001), is composed of residues Q94, E95, L97 and K114 of monomer A and L53, Y62, P63, V65, I66, W67, I96, V117 and V119 of monomer B, and vice versa. On one side of the dimer, the P1 pocket is partially covered by the segment 30-40, whereas on the other side it was completely open due to the segment being disordered. Since this segment appears to be flexible, the P1 pocket would be accessible by other molecules. The P2 pocket is also composed of residues from both chains: V24, L26, I28, Y62, L99, R101, F110 and L112 of monomer A and V22, V24 and L112 of monomer B, and vice versa.

Though the details of the P2 pockets are slightly different due to the asymmetry of the IL-17A dimer as described above, the overall geometry of the two P2 pockets is very similar. The two sets of residues lining the P1 and P2 pockets are very well conserved between IL-17A and IL-17F (Figure 8C). However, in the IL-17F structure, the P2 pocket is occupied by residues F10 and F11 ($F_{10}F_{11}$ motif) (Figure 8B) (Hymowitz *et al.*, EMBO J, 20: 5332-41, 2001). The FF motif is absent in human IL-17A; instead, the corresponding amino acid residues are I4 and P5 (residues 4 and 5 in SEQ ID NO: 105) (Figure 8C). These residues are not likely to bind in the P2 pocket as well as the FF motif

because they are much smaller than the phenylalanine residues, and most likely will not have sufficient affinity for the P2 pocket. Thus, the FF motif of IL-17F is likely a structural discriminant for human IL-17A and IL-17F interactions with receptors IL-17RA and IL-17RC. It is likely that both of these largely hydrophobic pockets (P1 and P2) are required for IL-17A binding to IL-17RA. The recent crystal structure of IL-17F/IL-17RA complex shows that the FF motif is displaced by IL-17RA (Ely *et al.*, Nat. Immunology 10:1245-51, 2009). The energetic penalty of FF motif eviction from P2 likely results in lower binding affinity. This is consistent with the observations that IL-17RA binds IL-17A with high affinity but IL-17F with low affinity in humans (Kuestner *et al.*, J Immunol, 179:5462-73, 2007), and potentially could explain the differences in IL-17A and IL-17F potencies. In mice, the FF motif is absent in both IL-17A and IL-17F, and is replaced by residues IP and AL, respectively. The AL and IP residue pairs are small and likely to have low affinity for the P2 pocket. Thus, P2 would be available in mouse IL-17A and IL-17F for IL-17RA binding. Both mouse IL-17A and IL-17F bind mouse IL-17RA with similar affinity (Kuestner *et al.*, J Immunol, 179:5462-73, 2007), consistent with the present suggestion that availability of P2 pocket for binding increases affinity of the ligands.

Overall, the structural differences observed between IL-17A and IL-17F provides a basis for dissecting their interactions with respective receptors. Furthermore, it is conceivable that peptides, peptidomimetics and small molecules can be designed to bind in either or both pockets to block IL-17A and/or IL-17F from interacting with their receptors. Since the FF motif present in Fab6468 (residues F92 and F93 in SEQ ID NO: 79) binds P2 pocket residues L26, R61, L99, R101 and R102, the Fab 6468 structure could be used to select and optimize additional IL-17A antagonists, such as peptides from randomized or designed peptide libraries using phage display.

The residues lining the P1 and P2 pockets are well conserved between IL-17A and IL-17F and molecular modeling suggests that an IL-17A/F heterodimer would adopt a nearly identical overall structure when compared to the IL-17A homodimer alone. Therfore, it is likely that the P1 and P2 pockets are present in the IL-17A/F heterodimer with similar overall topology and constitute its receptor binding sites. Thus IL-17A antagonists binding to the P2 pocket residues could bind and antagonize the IL-17A/F heterodimer.

Example 6

Cross-species binding specificity

To evaluate cross-species binding specificity of mAbtr1926, a binding ELISA was performed with different IL-17A proteins coated on micro-titer plates. Human, mouse and rat IL-17A proteins were coated on the micro-titer plates. Serial dilutions of labeled mAb1926 were incubated at 37^{0}C for 2 hours. Following incubation, micro-titer plates were washed thoroughly, and bound labeled mAb1926 was detected. mAb 1926 bound to human IL-17A much stronger than to rat or mouse IL-17A proteins (Figure 9). This reduced binding to rat and mouse IL-17A is consistent with these proteins both differing from human IL-17A at 7 positions of the Fab6468 extended epitope (Table 10). In addition, there is a one amino acid insertion in rat and mouse IL-17A between residues 40 and 41 of human IL-17A, a position close to part of the Fab 6468 epitope.

CLAIMS

1. An isolated antibody or fragment thereof that binds specifically to human IL-17A, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH region comprises the HCDR1, HCDR2 and HCDR3 having the amino acid sequences as shown in SEQ ID NOs:25, 46 and 61, respectively; and the VL region comprises the LCDR1, LCDR2, and LCDR3 having the amino acid sequences as shown in SEQ ID NOs:3, 6 and 22, respectively; and wherein

in SEQ ID NO:46,

Xaa1 may be Ala, Gly, Thr or Val;

Xaa3 may be Asn or Ser;

Xaa4 may be Gly, Met, Lys, Ile, Leu or His;

Xaa5 may be Leu, Asp, Ala, His, Thr, Gly or Ser;

Xaa6 may be Gly or Ser;

Xaa7 may be Thr, Gly, Tyr or Asp;

Xaa8 may be His, Trp, Tyr or Phe;

Xaa9 may be Lys, Thr or Ile; and

Xaa10 may be Tyr, Phe or Asn;

in SEQ ID NO:61,

Xaa may be Met, Leu or Thr; and

in SEQ ID NO:22,

Xaa is Met, Leu, Thr or Tyr.

2. The isolated antibody or fragment of claim **1**, wherein the antibody comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in:

a. SEQ ID NOs: 25, 37 and 58, respectively;
b. SEQ ID NOs: 25, 38 and 58, respectively;
c. SEQ ID NOs: 25, 39 and 58, respectively;
d. SEQ ID NOs: 25, 40 and 58, respectively;
e. SEQ ID NOs: 25, 41 and 58, respectively;
f. SEQ ID NOs: 25, 42 and 58, respectively;
g. SEQ ID NOs: 25, 43 and 58, respectively;
h. SEQ ID NOs: 25, 41 and 59, respectively;
i. SEQ ID NOs: 25, 41 and 60, respectively;
j. SEQ ID NOs: 25, 43 and 60, respectively;
k. SEQ ID NOs: 25, 44 and 58, respectively; or
l. SEQ ID NOs: 25, 45 and 58, respectively; and

the LCDR1, LCDR2, and LCDR3 amino acid sequences as shown in:

m. SEQ ID NOs: 3, 6 and 18, respectively;
n. SEQ ID NOs: 3, 6 and 19, respectively;

o. SEQ ID NOs: 3, 6 and 20, respectively; or
p. SEQ ID NOs: 3, 6 and 21, respectively.

3. An isolated monoclonal antibody or fragment thereof that binds specifically to human IL-17A that competes for human IL-17A binding with a monoclonal antibody comprising the heavy chain CDR 1, 2 and 3 (HCDR1, HCDR2 and HCDR3) amino acid sequences as shown in SEQ ID NOs: 25, 43 and 60, respectively, and the light chain CDR 1, 2 and 3 (LCDR1, LCDR2 and LCDR3) amino acid sequences as shown in SEQ ID NOs: 3, 6 and 18, respectively.

4. The isolated antibody or fragment of claim **3** or **1**, wherein the antibody is fully human.

5. The isolated antibody or fragment of claim **3** or **1**, wherein the antibody is conjugated to polyethylene glycol.

6. The isolated antibody or fragment of claim **3** or **1**, having an IgG1 or IgG4 isotype.

7. The isolated antibody or fragment of claim **3** or **1**, wherein the Fc domain comprises S229P, P235A or L236A mutations in the Fc domain.

8. A pharmaceutical composition comprising the isolated antibody or fragment of claim **3** or **1** and a pharmaceutically acceptable carrier.

9. An isolated antibody or fragment thereof that binds specifically to human IL-17A comprising a VH and a VL, wherein the antibody comprises:

a. the HCDR1, HCDR2 and HCDR3 amino acid sequences as shown in SEQ ID NOs: 25, 43 and 60, respectively and
the LCDR1, LCDR2, and LCDR3 amino acid sequences as shown in SEQ ID NOs: 3, 6 and 18, respectively; or

b. the VH of SEQ ID NO: 86; or

c. the VL of SEQ ID NO: 79; or

d. the VH of SEQ ID NO: 86 and the VL of SEQ ID NO: 79.

10. An isolated antibody or fragment thereof that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the VH having the amino acid sequence shown in SEQ ID NO: 86.

11. The isolated antibody or fragment of claim **10**, wherein the antibody comprises the VL having the amino acid sequence shown in SEQ ID NO: 79.

12. An isolated antibody or fragment thereof that binds specifically to human IL-17A, comprising a VH and a VL, wherein the antibody comprises the VL having the amino acid sequence shown in SEQ ID NO: 79.