IL-6 ANTAGONISTS AND USES THEREOF

RELATED APPLICATIONS

The present application claims priority to U.S. Application Serial No. 61/723,972, filed November 8, 2012 and U.S. Application Serial No. 61/831,699, filed June 6, 2013. The entire content of each of the foregoing applications is hereby incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 19, 2013, is named D2046-7044WO_SL.txt and is 46,819 bytes in size.

FIELD OF THE INVENTION

The field of the invention relates to IL-6. More particularly, the field relates to modulators of IL-6 and their uses in treating disease such diseases of the eye.

BACKGROUND

IL-6 is a pleiotropic cytokine with reported roles in inflammation, hematopoiesis, angiogenesis, cell differentiation, and neuronal survival.

SUMMARY

The invention relates to IL-6 antibodies and fragments, and derivatives of such antibodies and fragments that have certain features, including binding specificity to site II of IL-6, and methods of using such antibodies, fragments, and derivatives. Accordingly, the present invention relates to an antibody, fragment, or derivative thereof that can specifically bind to site II of an IL-6. In some embodiments, the antibody, fragment, or derivative can bind to IL-6 with a K_D of 240 pM or less. In some embodiments, the antibody, fragment, or derivative has a T_m of 70°C or greater. In some embodiments, the antibody, fragment, or derivative can bind to IL-6 with a K_D of 240 pM or less and has a T_m of 70°C or greater. The antibody or fragment or derivative thereof can, in some cases, bind to at least one of R24, K27, Y31, D34, S118, or V121 of human IL-6; in some cases the antibody or fragment or derivative thereof can bind to R24, K27, Y31, D34, S118, and V121 of human IL-6. In embodiments, the antibody or fragment or derivative thereof can bind to at least 1, at least 2, at least 3, at least 4, or at least 5 of R24, K27, Y31, D34, S118, and V121 of human IL-6.

In one aspect provided herein is an antibody or fragment thereof (e.g., an antigen binding fragment thereof) that can specifically bind to site II of a human IL-6.

To carry out the affinity maturation designed to restore or improve the affinity of the humanized h64IgG, the h64 antibody sequences were recloned to generate a Fab molecule in pYC2/CT-derived yeast vectors in which the FabH chain was fused to the anti-FITC scFv 4m5.3 through a (G4S)3 linker (SEQ ID NO: 29). A library of h64 variants was then generated by error prone PCR following the protocol of Chao et al. (2006, Nature Protocols, 1:755-768). H64 variants were expressed and surface captured by yeast labeled with FITC-PEG-NHS then incubated with biotinylated human IL-6. Bound IL-6 was detected with streptavidin-APC, and cells with the highest amount of bound IL-6 relative to the amount of displayed Fabs were selected on a BD FACSAria™ cell sorter. After four rounds of selection, a population of higher affinity variants was selected and sequenced. The sequence of the clone selected by affinity maturation (referred to as h64-1.4) is as follows with the selected mutations (i.e., mutated compared to the sequences of h64 VH and VL) in boldface and the CDRs are underlined. These are the variable domains of 018 (as well as the 020 and 029 IL-6a molecules described below). Note that the full Fabs include the CK and IgG1 CH1 domains. In the context of this application, reference to a "Fab" heavy chain or light chain amino acid sequence means that sequence can be part of a functioning Fab consisting of a light chain-derived sequence and a heavy chain-derived sequence.

h64-1.4 VH (018VH)(variable domain)
QVQLVQSGAEVKKPGSSVKVSCKASGYA**L**SNYLIEWVRQAPGQGLEWMGVITPGSGTI
NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT
TVTVSS (SEQ ID NO:17)

h64-1.4 VL (018VL) (variable domain)
DIVMTQSPDSLAVSLGERATINCRASESVDNYGI**P**FMNWYQQKPGQPPKLLIYAASN**R**G
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQS**E**EVPLTFGQGTKLEIKRTV (SEQ
ID NO:18)

The h64-1.4 variable domains were recloned into the pcDNA3.1 human IgG1 vector and expressed as a full length IgG1 in Freestyle™-HEK293 cells (Life Technologies). The resulting purified IgG was significantly more potent than the original h64 antibody in both binding and cellular antagonism studies. Testing affinity using the yeast system, the affinity increased from

primates. Human IL-6 differs from cynomolgus monkey and rhesus monkey IL-6 at seven sites, one of which is in site II (amino acid 28) and is the same at site II in African green monkey IL-6. This appears to decrease binding of an antibody comprising 018 sequences by only about 3-4 fold. The ability to bind to a non-human primate IL-6 is a useful feature of an IL-6 antagonist, facilitating development of the candidate as a drug, e.g., by enabling testing such as toxicology testing in non-human primates.

As with most IL-6 antibodies, anti-IL-6 antibodies described herein did not cross-react to rodent, rabbit, or canine IL-6 due to low sequence homology. However, in affinity studies, it was found that 018 Fab binds cynomolgus monkey and African green monkey IL-6 with approximately human affinity (Table 2).

Table 2: Monovalent affinity (018 Fab) for various IL-6 of various species

Species	K_D
Human	200 pM
African Green Monkey	280 pM
Cynomolgus monkey	840 Pm
Dog	> 1 μM
Mouse	> 1 μM
Rabbit	> 1 μM
Rat	> 1 μM

These data further demonstrate the ability of an IL-6a as described herein to specifically bind and the ability to develop a molecule having features permitting testing, e.g., for toxicology and reproductive studies, in a suitable animal.

Example 7: Increasing expression of an IL-6a

To increase expression of 018 Fab and 019 Fab polypeptides, constructs were made introducing five additional amino acids (DKTHT (SEQ ID NO: 30)) to the heavy chain in the CH1/hinge region using methods known in the art. The sequence of the altered 018Fab heavy chain is shown below as SEQ ID NO:24 . The altered 018 sequence is referred to herein as 020 and the altered 019

sequence is referred to herein as 021. The 020 molecule (the 020Fab heavy chain and the 018Fab light chain) had improved expression compared to the parent Fab that had 018Fab heavy and 018Fab light chains. The 019 molecule exhibited no significant affinity difference compared to the 020 molecule. Expression of both 020 and 019 was increased by about two fold, respectively, and the affinities were not affected by the alteration.

020 Heavy chain (Fab with DKTHT (SEQ ID NO: 30) at the carboxy terminus))
QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV
ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR
WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL
VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT
QTYICNVNHK PSNTKVDKKV EPKSCDKTHT (SEQ ID NO:24)

IL-6 antagonism using the 020Fab was measured in HEK-Blue™ IL-6 reporter cells (InvivoGen, San Diego, CA). Cells were incubated in a mixture of 10 pM IL-6 and varying concentrations of either 020 or IL-6Rα antibody (Cell Sciences, Canton, MA), with or without 50 nM IL-6Rα. After 20-24 hours of incubation, 20 μL of cell culture supernatant was mixed with 180 μL of QuantiBlue™ (InvivoGen) substrate and incubated for one hour; the absorbance was then measured at 655 nm. Fig. 3A and Fig. 3B show data from these experiments, demonstrating the ability of 020 to inhibit IL-6 activity in the presence or absence of IL-6R.

Example 8: IgG2 IL-6 antibodies

018 was reformatted into a human IgG2 isotype framework to reduce FcγR binding and reduce ADCC compared to the IgG1 formatted antibody using methods known in the art. In addition, reformatting 018 to a full-length format, e.g., an IgG2, is expected to decrease the rate of clearance from the vitreous due to the larger size of the molecule.

Construction/purification of anti-IL6 IgG2 antibodies

To construct human IgG2 antibodies using anti-IL-6 sequences described *supra*, a human IgG2 constant domain was PCR amplified from cDNA with NheI and MluI restriction sites at the N- and C-terminal ends, respectively. The PCR product was purified, digested with NheI and