pulmonary inflammation, goblet cell hyperplasia, mucus hypersecretion, and airway fibrosis (Yang et al., 2005, J Pharmacol Exp Ther, 313, 8-15). IL- 13 is implicated in the pathogenesis of human asthma as elevated levels of IL- 13 mRNA and protein have been detected in lungs of asthmatic patients, which correlate with severity of the disease (Huang et al., 1995, J Immunol, 155, 2688-94). In addition, human IL- 3 genetic polymorphisms, which lead to elevated IL-13 levels, have been identified and are associated with asthma and atopy (Heinzmann et al., 2000, Hum Mol Genet, 9, 549-59; Hoerauf et al., 2002, Microbes Infect, 4, 37-42; Vercelli, 2002, Curr Opin Allergy Clin Immunol, 2, 389-93; Heinzmann et al., 2003, J Allergy Clin Immunol, 112, 735-9; Chen et al., 2004, J Allergy Clin Immunol, 114, 553-60; Vladich et al., 2005, J Clin Invest, 115, 747-54), and elevated IL- 13 levels have been detected in the lung of asthma patients (Huang et al., 1995, J Immunol, 155, 2688-94; Arima et al., 2002, J Allergy Clin Immunol, 109, 980-7; Berry et al., 2004, J Allergy Clin Immunol, 114, 1106-9). A genetic linkage between IL-13 and asthma has also been demonstrated as individuals with a polymorphism in the IL-13 gene which causes higher plasma IL-13 levels have an increased risk for atopy and asthma (Wills-Karp, 2000, Respir Res, 1, 19-23).

Due to the role of human IL-13 in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract IL-13 activity. In particular, antibodies that bind to, and neutralize, IL-13 have been sought as a means to inhibit IL-13 activity. However, there exists a need in the art for improved methods of producing and purifying such antibodies for pharmaceutical use. The present invention addresses this need.

SUMMARY OF THE ~~INVENTION~~DISCLOSURE

In certain embodiments, the present ~~invention~~disclosure is directed to purified, isolated antibodies and antibody fragments that bind to IL-13 as well as pharmaceutical compositions comprising such antibodies and fragments. In certain embodiments, the ~~invention~~disclosure pertains to isolated antibodies, or antigen-binding portions thereof, that bind to human IL-13. The isolated anti-IL-13 antibodies of the present ~~invention~~disclosure can be used in a clinical setting as well as in research and development. In certain embodiments, the present ~~invention~~disclosure is directed to an anti-IL-13 antibody comprising the heavy and light chain sequences identified in Figure 1.

Certain embodiments of the ~~invention~~disclosure are directed toward methods of purifying anti-IL-13 antibodies, or antigen-binding portions thereof, from a sample

matrix to render the antibodies substantially free of host cell proteins ("HCPs") and leached Protein A. In certain aspects, the sample matrix (or simply "sample") comprises a cell line employed to produce anti-IL-13 antibodies of the present ~~invention~~disclosure. In particular aspects, the sample comprises a cell line used to produce human anti-IL-13 antibodies.

In certain embodiments the present ~~invention~~disclosure provides for a method of purifying IL-13 antibodies that comprises a primary recovery step to, among other things, remove cells and cellular debris. In certain embodiments of the method, the primary recovery step includes one or more centrifugation or depth filtration steps. For example, and not by way of limitation, such centrifugation steps can be performed at approximately 7000 x g to approximately 11,000 x g. In addition, certain embodiments of the above-described method will include a depth filtration step, such as a delipid depth filtration step.

In certain embodiments, the primary recovery sample is subjected to an affinity chromatography step. The affinity chromatography step comprises subjecting the primary recovery sample to a column comprising a suitable affinity chromatographic support. Non-limiting examples of such chromatographic supports include, but are not limited to Protein A resin, Protein G resin, affinity supports comprising the antigen against which the antibody of interest was raised, and affinity supports comprising an Fc binding protein. Protein A resin is useful for affinity purification and isolation of antibodies (IgG). In one aspect, a Protein A column is equilibrated with a suitable buffer prior to sample loading. An example of a suitable buffer is a Tris/NaCl buffer, pH around 7.2. Following this equilibration, the sample can be loaded onto the column. Following the loading of the column, the column can be washed one or multiple times using, *e.g.*, the equilibrating buffer. Other washes including washes employing different buffers can be used before eluting the column. The Protein A column can then be eluted using an appropriate elution buffer. An example of a suitable elution buffer is an acetic acid/NaCl buffer, pH around 3.5. The eluate can be monitored using techniques well known to those skilled in the art. For example, the absorbance at OD_{280} can be followed. The eluated fraction(s) of interest can then be prepared for further processing

In certain embodiments of the present ~~invention~~disclosure, a low pH adjustment step follows Protein A affinity chromatography. In such embodiments, the Protein A eluate comprising the putative anti-IL-13 antibody, or antigen-binding portion thereof,

of a suitable buffer is the equilibration buffer itself. Flow-through collection can commence, *e.g.*, as the absorbance (OD_{280}) rises above about 0.2 AU.

In certain embodiments, a first and second ion exchange step is performed following primary recovery or otherwise in the absence of an affinity chromatography step. In certain of such embodiments, the ion exchange sample is subjected to an intermediate filtration step, either prior to the first ion exchange step, between the two ion exchange steps, or both. In certain aspects, this filtration step comprises capture ultrafiltration/diafiltration ("UF/DF"). Among other things, such filtration facilitates the concentration and buffer exchange of anti-IL-13 antibodies and antigen-binding portions thereof.

Certain embodiments of the ~~invention~~disclosure provide for a method comprising one or more hydrophobic interactive chromatography ("HIC") step. A suitable HIC column is one whose stationary phase comprises hydrophobic groups. A non-limiting example of such a column is a Phenyl HP Sepharose™ column. In certain circumstances anti-IL-13 antibodies will form aggregates during the isolation/purification process. Inclusion of one or more HIC step facilitates the reduction or elimination of such aggregations. HIC also assists in the removal of impurities. In certain embodiments the HIC step employs a high salt buffer to promote interaction of the anti-IL-13 antibodies (or aggregations thereof) with the hydrophobic column. The anti-IL-13 antibodies can then be eluted using lower concentrations of salt.

In certain embodiments, the HIC eluate is filtered using a viral removal filter such as, but not limited to, an Ultipor DV50™ filter (Pall Corporation, East Hills, N.Y.). Alternative filters, such as Viresolve™ filters (Millipore, Billerica, Mass.); Zeta Plus VR™ filters (CUNO; Meriden, Conn.); and Planova™ filters (Asahi Kasei Pharma, Planova Division, Buffalo Grove, Ill.), can also be used in such embodiments.

In certain embodiments, the ~~invention~~disclosure is directed to one or more pharmaceutical composition comprising an isolated anti-IL-13 antibody or antigen-binding portion thereof and an acceptable carrier. In one aspect, the composition further comprises one or more antibody or antigen-binding portion thereof in addition to the anti-IL-13 antibody. In another aspect, the compositions further comprise one or more pharmaceutical agents.

The purity of the antibodies of interest in the resultant sample product can be analyzed using methods well known to those skilled in the art, *e.g.*, size-exclusion chromatography, Poros™ A HPLC Assay, HCP ELISA, Protein A ELISA, and western blot analysis.

In an aspect, there is provided a method for producing a host cell-protein (HCP) reduced anti-IL-13 antibody, or antigen-binding portion thereof, preparation from a sample mixture comprising an anti-IL-13 antibody, or antigen-binding fragment thereof, and at least one HCP, said method comprising:

(a) contacting said sample mixture to a Protein A affinity chromatography resin, washing said affinity chromatography resin with a buffer comprising a buffer comprising 25 mM Tris, 100 mM NaCl, pH 7.2, followed by a buffer comprising 0.5 M NaCl, 20 mM Na citrate, at pH 6, and a buffer comprising 25 mM Tris, 100 mM NaCl, pH 7.2 and collecting an affinity chromatography sample;

(b) subjecting said affinity chromatography sample to a reduction in pH thus forming a reduced pH sample, wherein said reduction in pH is from about 3 to about 4;

(c) adjusting said reduced pH sample to a pH of about 4.5 to about 6.5 and contacting said adjusted pH sample to an ion exchange resin and collecting an ion exchange sample;

(d) contacting said ion exchange sample to a hydrophobic interactive chromatography (HIC) resin and collecting an HIC sample, wherein said HIC sample comprises said HCP-reduced antibody, or antigen binding portion thereof, preparation.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Figure 1 discloses the heavy and light chain variable region sequences of a non-limiting example of an anti-IL-13 antibody.

Figure 2 discloses an exemplary cell culture process flow diagram, including set points, in process control tests, and action limits.

Figure 3 discloses a comparison of alternative cell culture process flow strategies.

Figure 4 discloses a primary recovery capture chromatography process flow diagram, including set points, in process control tests, and action limits.

Figure 5 discloses a comparison of alternative primary recovery and capture flow strategies.

Figure 6 discloses a fine purification process flow diagram, including set points, in process control tests, and action limits.

Figure 7 discloses a comparison of alternative fine purification flow strategies.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to antibodies that bind to IL-13. In one aspect, the invention pertains to isolated antibodies, or antigen-binding portions thereof, that bind to human IL-13. The isolated anti-IL-13 antibody of the present invention can be used in a clinical setting as well as in research and development. The present invention also pertains to methods for purifying anti-IL-13 antibodies, or antigen-binding portions thereof. Suitable anti-IL-13 antibodies that may be purified in the context of the instant invention are disclosed in PCT Application No. PCT/US2007/019660, which is hereby incorporated by reference in its entirety, including the antibody that has subsequently been identified as ABT-308. Exemplary anti-IL-13 antibody heavy and light chain sequences are set forth in Figure 1. The present invention also relates to pharmaceutical compositions comprising the anti-IL-13 antibodies or antigen-binding portions thereof described herein.

CLAIMS

What is claimed is:

1. A method for producing a host cell-protein (HCP) reduced anti-IL-13 antibody, or antigen-binding portion thereof, preparation from a sample mixture comprising an anti-IL-13 antibody, or antigen-binding fragment thereof, and at least one HCP, said method comprising:

(a) contacting said sample mixture to a Protein A affinity chromatography resin, washing said affinity chromatography resin with a buffer comprising a buffer comprising 25 mM Tris, 100 mM NaCl, pH 7.2, followed by a buffer comprising 0.5 M NaCl, 20 mM Na citrate, at pH 6, and a buffer comprising 25 mM Tris, 100 mM NaCl, pH 7.2 and collecting an affinity chromatography sample;

(b) subjecting said affinity chromatography sample to a reduction in pH thus forming a reduced pH sample, wherein said reduction in pH is from about 3 to about 4;

(c) adjusting said reduced pH sample to a pH of about 4.5 to about 6.5 and contacting said adjusted pH sample to an ion exchange resin and collecting an ion exchange sample;

(d) contacting said ion exchange sample to a hydrophobic interactive chromatography (HIC) resin and collecting an HIC sample, wherein said HIC sample comprises said HCP-reduced antibody, or antigen binding portion thereof, preparation.

2. The method of claim 1, wherein said reduction in pH is accomplished by admixing a suitable acid with said sample mixture, and wherein said suitable acid is selected from the group consisting of citric acid, acetic acid, and caprylic acid.

3. The method of claim 1, wherein said Protein A resin comprises Protein A coupled to crosslinked agarose beads.

4. The method of claim 1, wherein said ion exchange sample is applied to a cation exchange resin and a cation exchange sample is collected prior to application to the hydrophobic interaction chromatography resin.