LOW ACIDIC SPECIES COMPOSITIONS AND METHODS FOR PRODUCING AND USING THE SAME

RELATED APPLICATIONS

This application claims priority to International Patent Application Serial No. PCT/US2013/031485, filed on March 14, 2013 and International Patent Application Serial No. PCT/US2013/031681, filed on March 14, 2013, the contents of each of which is hereby incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

The production of compositions comprising proteins for biopharmaceutical applications involves the use of upstream process technologies (*e.g.*, cell culture) and downstream process technologies (*e.g.*, protein purification) that are known to produce proteins exhibiting varying levels of protein variants and impurities within the composition. Such protein variants include, but are not limited to, the presence of acidic species, including process-related impurities. For example, in monoclonal antibody (mAb) preparations, acidic species can be detected by various methods, such as ion exchange chromatography, for example, WCX-10 HPLC (a weak cation exchange chromatography) or IEF (isoelectric focusing). Because of their similar chemical characteristics to the antibody product molecules of interest, reduction of acidic species is a challenge in monoclonal antibody production.

Reduction of acidic species is particularly advantageous in the context of commercially produced recombinant biotherapeutics, as they have the potential to impact numerous product characteristics, including, but not limited to, product stability, product safety and product efficacy. Accordingly, there remains a need in the art for low acidic species compositions and high-efficiency methods of producing protein compositions, *e.g.*, antibodies, having low levels of acidic species.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclosure is based on the identification and optimization of upstream and downstream process technologies for protein production, *e.g.*, production of antibodies or antigen-binding portions thereof, resulting in the production of compositions comprising proteins that comprise low percentages of acidic species. As demonstrated herein, these low acidic species compositions have improved therapeutic efficacy and improved biological

properties, for example, increased cartilage tissue penetration, reduced cartilage destruction, reduced synovial proliferation, reduced bone erosion, increased protection against the development of arthritis as measured by arthritic scores and/or histopathology scores, reduced cell infiltration, reduced proteoglycan loss, reduced chondrocyte death, and/or increased TNFα affinity, as compared to a non-low acidic species composition.

Accordingly, in one embodiment, the present ~~invention~~ disclosure provides a low acidic species (low AR) composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 15% or less AR. In one aspect of this embodiment, the low AR composition comprises about 14% or less AR, 13% or less AR, 12% or less AR, 11% or less AR, 10% or less AR, 9% or less AR, 8% or less AR, 7% or less AR, 6% or less AR, 5% or less AR, 4.5% or less AR, 4% or less AR, 3.5% or less AR, 3% or less AR, 2.5% or less AR, 2% or less AR, 1.9% or less AR, 1.8% or less AR, 1.7% or less AR, 1.6% or less AR, 1.5% or less AR, 1.4% or less AR, 1.3% or less AR, 1.2% or less AR, 1.1% or less AR, 1% or less AR, 0.9% or less AR, 0.8% or less AR, 0.7% or less AR, 0.6% or less AR, 0.5% or less AR, 0.4% or less AR, 0.3% or less AR, 0.2% or less AR, 0.1% or less AR, or 0.0% AR, and ranges within one or more of the preceding. In one aspect of this embodiment, the present ~~invention~~ disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR, about 0.0% to about 5% AR, about 0.0% to about 4% AR, about 0.0% to about 3% AR, about 0.0% to about 2% AR, about 3% to about 5% AR, about 5% to about 8% AR, or about 8% to about 10% AR, or about 10% to about 15% AR, and ranges within one or more of the preceding.

In one embodiment, the low AR composition comprises a first acidic species region (AR1) and a second acidic species region (AR2). In one aspect of this embodiment, the low AR composition comprises about 0.1% or less AR1 and about 3% or less AR2, or about 0.0% AR1 and about 1.4% or less AR2. In a related embodiment, the low AR composition comprises about 15% or less AR1, 14% or less AR1, 13% or less AR1, 12% or less AR1, 11% or less AR1, 10% or less AR1, 9% or less AR1, 8% or less AR1, 7% or less AR1, 6% or less AR1, 5% or less AR1, 4.5% or less AR1, 4% or less AR1, 3.5% or less AR1, 3% or less AR1, 2.5% or less AR1, 2% or less AR1, 1.9% or less AR1, 1.8% or less AR1, 1.7% or less AR1, 1.6% or less AR1, 1.5% or less AR1, 1.4% or less AR1, 1.3% or less AR1, 1.2% or less AR1, 1.1% or less AR1, 1% or less AR1, 0.9% or less AR1, 0.8% or less AR1, 0.7% or less AR1, 0.6% or less AR1, 0.5% or less AR1, 0.4% or less AR1, 0.3% or less AR1, 0.2% or less AR1, 0.1% or less AR1, or 0.0% AR1, and ranges within one or more of the preceding. In

one aspect of this embodiment, the present ~~invention~~ disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR1, about 0.0% to about 5% AR1, about 0.0% to about 4% AR1, about 0.0% to about 3% AR1, about 0.0% to about 2% AR1, about 3% to about 5% AR1, about 5% to about 8% AR1, or about 8% to about 10% AR1, or about 10% to about 15% AR1, and ranges within one or more of the preceding.

In one aspect of this embodiment, the low AR composition comprises about 15% or less AR2, 14% or less AR2, 13% or less AR2, 12% or less AR2, 11% or less AR2, 10% or less AR2, 9% or less AR2, 8% or less AR2, 7% or less AR2, 6% or less AR2, 5% or less AR2, 4.5% or less AR2, 4% or less AR2, 3.5% or less AR2, 3% or less AR2, 2.5% or less AR2, 2% or less AR2, 1.9% or less AR2, 1.8% or less AR2, 1.7% or less AR2, 1.6% or less AR2, 1.5% or less AR2, 1.4% or less AR2, 1.3% or less AR2, 1.2% or less AR2, 1.1% or less AR2, 1% or less AR2, 0.9% or less AR2, 0.8% or less AR2, 0.7% or less AR2, 0.6% or less AR2, 0.5% or less AR2, 0.4% or less AR2, 0.3% or less AR2, 0.2% or less AR2, 0.1% or less AR2, or 0.0% AR2, and ranges within one or more of the preceding. In one aspect of this embodiment, the present ~~invention~~ disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR2, about 0.0% to about 5% AR2, about 0.0% to about 4% AR2, about 0.0% to about 3% AR2, about 0.0% to about 2% AR2, about 3% to about 5% AR2, about 5% to about 8% AR2, or about 8% to about 10% AR2, or about 10% to about 15% AR2, and ranges within one or more of the preceding.

In another embodiment, the low AR composition, *e.g.*, a low AR composition of adalimumab, comprises about 1.4% or less AR. For example, in one aspect of this embodiment, the low AR composition, *e.g.*, a low AR composition of adalimumab comprising about 1.4% or less AR can comprise about 0.0% AR1 and about 1.4% or less AR2.

In another aspect, the present ~~invention~~ disclosure provides compositions comprising an antibody, or antigen-binding portion thereof, wherein the composition is substantially free of acidic species and other process-related impurities, including, for example, host cell proteins (HCPs), host nucleic acids, chromatographic materials, and/or media components, as well as product related impurities such as aggregates.

In one embodiment, the antibody, or antigen-binding portion thereof, of the compositions disclosed herein is an anti-TNFα antibody, or antigen-binding portion thereof. For example, in one aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, dissociates from human TNFα with a K_d of about 1 x 10^{-8} M or less and a K_{off} rate constant of $1x10^{-3}$ S^{-1} or less. In another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4.

In still another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2. In yet another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

In one embodiment, the low AR composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of AR that is not the same as the percentage of AR present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008), the contents of which are hereby incorporated herein by reference.

In another embodiment, the low AR composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of AR that is lower than the percentage of AR present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008), the contents of which are hereby incorporated herein by reference.

In another embodiment, the present ~~invention~~ disclosure provides low AR compositions comprising an anti-TNFα antibody, or antigen-binding portion thereof, comprising a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5,

In still another aspect of this embodiment, the fragmentation variants in the low AR composition comprising an antibody, or antigen-binding portion thereof (*e.g.*, an anti-TNFα antibody, or antigen binding portion thereof, such as adalimumab), are AR1 species and comprise, for example, Fab fragment variants, C-terminal truncation variants or variants missing a heavy chain variable domain.

In another embodiment, the acidic species in the low AR composition comprising an antibody, or antigen-binding portion thereof (*e.g.*, an anti-TNFα antibody, or antigen binding portion thereof, such as adalimumab), are AR2 species, and comprise charge variants, such as deamidation variants or glycation variants. For example, when the low AR composition comprises adalimumab, the deamidation variants can result from deamidation occurring at asparagine residues comprising Asn393 and Asn329 of adalimumab and at glutamine residues comprising Gln3 and Gln6. In another aspect of this embodiment, when the low AR composition comprises adalimumab, the glycation variants result from glycation occurring at Lys98 and Lys151 of adalimumab.

In one embodiment, the percent of acidic species in a low AR composition is determined using ion exchange chromatography, for example WCX-10 HPLC. In another aspect of this embodiment, the percent acidic species in a low AR composition is determined using isoelectric focusing (IEF).

In one embodiment, the low AR compositions of the ~~invention~~ disclosure comprise product preparation-derived acidic species. For example, in one aspect of this embodiment, the acidic species are cell culture-derived acidic species. In another aspect of this embodiment, the acidic species of the low AR compositions are storage-derived acidic species which are primarily generated when stored under process, intermediate or shelf storage conditions prior to use..

In still another embodiment, the ~~invention~~ disclosure provides low AR compositions that further comprise a pharmaceutically acceptable carrier.

In another aspect, the present ~~invention~~ disclosure provides methods for treating a subject having a disorder in which TNFα is detrimental, by administering to the subject a low AR composition of the ~~invention~~disclosure, *e.g.*, a low AR adalimumab composition, thereby treating the subject having a disorder in which TNFα is detrimental. In one aspect of this embodiment, the disorder in which TNFα is detrimental is selected from the group consisting of

rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis (JIA), ulcerative colitis, and Crohn's Disease.

In one aspect, upstream methods for producing the low AR compositions of the ~~invention~~ disclosure are included. In one embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media comprising an increased concentration of an amino acid selected from the group consisting of arginine, lysine, ornithine and histidine, or a combination thereof, as compared to the amino acid concentration in cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof. In another aspect of this embodiment, the amino acid concentration in the culture media is between about 0.025 and 20 g/L.

In another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media comprising an increased concentration of calcium as compared to the calcium concentration in cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof. In one aspect of this embodiment, the calcium concentration is between about 0.005 and 5 mM. In another aspect of this embodiment, the cell culture media further comprises an increased concentration of an amino acid selected from the group consisting of arginine, lysine, ornithine and histidine, or a combination thereof, as compared to the amino acid concentration in cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof.

In still another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media comprising an increased concentration of niacinamide, calcium, and at least one amino acid, as compared to the concentration of niacinamide, calcium, and amino acid in the cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof. In one aspect of the embodiment, the at least one amino acid is selected from the group consisting of arginine, lysine, ornithine and histidine, and combinations thereof.

In yet another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media having a pH of between about 7.1-6.8.

In still another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media having an altered exchange rate as compared to the exchange rate of cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof.

In another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, extracting a clarified harvest from the cell culture, and adding one or more amino acids to the clarified harvest. In one aspect of this embodiment, the one or more amino acids are selected from the group consisting of arginine, histidine, lysine, aspartic acid, glutamic acid and leucine, and combinations thereof.

In yet another embodiment, a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprises culturing cells expressing the antibody, or antigen binding portion thereof, extracting a clarified harvest from the cell culture, and adjusting the pH of the clarified harvest to between about 4.5 and 6.5.

In another aspect of the ~~invention~~ disclosure, upstream methods for producing the low AR compositions of the ~~invention~~ disclosure are included. In one embodiment, the ~~invention~~ disclosure includes a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, comprising contacting a first sample comprising the antibody, or antigen binding portion thereof, to a chromatography media, wherein the contact occurs in the context of a loading buffer; washing the chromatography media with a wash buffer that is substantially the same as the loading buffer; and collecting a chromatography sample, wherein the chromatography sample comprises a composition of the antibody, or antigen binding portion thereof, which contains less than about 10% acidic species, thereby producing a low acidic species composition comprising an antibody, or antigen binding portion thereof. In one aspect of this embodiment, the bound antibody material is eluted with

species composition comprising an antibody, or antigen binding portion thereof. In one embodiment, the second eluted sample is contacted to a CEX chromatography at least one additional time. In one embodiment, the method further comprises performing viral filtration on the third eluted sample resulting in a filtered sample. In another embodiment, the method further comprises filtering the filtered sample using ultrafiltration/diafilteration (UF/DF).

In another aspect, the ~~invention~~ disclosure provides a method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, the method comprising contacting a sample comprising an antibody, or antigen binding portion thereof to one or more of the group consisting of: an anion exchange (AEX) chromatography adsorbent material, a cation exchange (CEX) chromatography adsorbent material, a mixed mode media, a cation exchange mixed mode media, and an anion exchange mixed mode media, in a load buffer, and eluting the sample from the AEX chromatography adsorbent material, the CEX chromatography adsorbent material, the mixed mode media, the cation exchange mixed mode media, or the anion exchange mixed mode media, wherein the eluted sample comprises a composition of the antibody, or antigen binding portion thereof, which contains less than about 3% acidic species, thereby producing a low acidic species composition comprising an antibody, or antigen binding portion thereof. In one aspect of this embodiment, the method further comprises contacting the eluted sample to a hydrophobic interaction chromatography (HIC) media.

In another aspect, the disclosure provides a use of the composition as disclosed herein in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental.

The present ~~invention~~ disclosure is further illustrated by the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the effect of total arginine concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2).

Figure 2 depicts the effect of total arginine concentration in adalimumab producing cell line 2, media 1 on viability (n=2).

Figure 3 depicts the effect of total arginine concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2).

Figure 4 depicts the effect of total arginine concentration in adalimumab producing cell line 2, media 1 on day 10 WCX-10 profile total acidic regions (n=2).

58. The composition of any one of claims 1-12, 14-18, 25-38 or 40-57, wherein said composition further comprises a pharmaceutically acceptable carrier.

59. The composition of any one of claims 1-12, 14-18, 25-38 or 40-57, wherein the % acidic species is determined using WCX-10 HPLC.

60. The composition of any one of claims 1-12, 14-18, 25-38 or 40-57, wherein the % acidic species is determined using isoelectric focusing (IEF).

61. The composition of any one of claims 1-12, 14-18, 25-38 or 40-57, wherein the acidic species are product preparation-derived acidic species.

62. The composition of claim 62, wherein the acidic species are cell culture-derived acidic species.

63. The composition of any one of claims 1-12, 14-18, 25-38 or 40-57, wherein the acidic species are storage-derived acidic species.

64. ~~A method for treating a subject having a disorder in which TNFα is detrimental, comprising administering to the subject~~ Use of the composition of any one of claims 1 or 34~~, thereby treating the subject having a disorder in which TNFα is detrimental~~ in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental.

65. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is rheumatoid arthritis (RA).

66. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is psoriasis.

67. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is psoriatic arthritis.

68. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is ankylosing spondylitis.

69. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is Crohn's Disease.

70. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is Ulcerative Colitis.

71. The ~~method~~ use of claim 64, wherein the disorder in which TNFα is detrimental is juvenile idiopathic arthritis.

72. A method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, the method comprising:

culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media comprising an increased concentration of an amino acid selected from the group consisting of arginine, lysine, ornithine and histidine, or a combination thereof, as compared to the amino acid concentration in cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof.

73. The method of claim 72, wherein the low acidic species composition comprises less than about 10% acidic species.

74. The method of claim 72, wherein the amino acid concentration in the culture media is between about 0.025 and 20 g/L.

75. A method for producing a low acidic species composition comprising an antibody, or antigen binding portion thereof, the method comprising:

culturing cells expressing the antibody, or antigen binding portion thereof, in a cell culture media comprising an increased concentration of calcium as compared to the calcium concentration in cell culture media used to produce a non-low acidic species composition comprising the antibody, or antigen binding portion thereof.

76. The method of claim 75, wherein the calcium concentration is between about 0.005 and 5 mM.