belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

**[0049]** Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

**Definitions**

**[0050]** The expressions "interleukin-33," "IL-33," and the like, as used herein, refer to a human IL-33 protein having the amino acid sequence of SEQ ID NO:307. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species (*e.g.*, "mouse IL-33," "monkey IL-33," etc.).

**[0051]** As used herein, "an antibody that binds IL-33" or an "anti-IL-33 antibody" includes antibodies, and antigen-binding fragments thereof, that bind a soluble fragment of an IL-33 protein. Soluble IL-33 molecules include natural IL-33 proteins as well as recombinant IL-33 protein variants such as, *e.g.*, monomeric and dimeric IL-33 constructs.

**[0052]** The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, IL-33). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three domains, $C_H1$, $C_H2$ and $C_H3$. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or $V_L$) and a light chain constant region. The light chain constant region comprises one domain ($C_L1$). The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-IL-33 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

**[0053]** The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring,

**Example 3. Antibody Binding to Human IL-33 as Determined by Surface Plasmon Resonance**

**[0137]** Equilibrium dissociation constants ($K_D$ values) for IL-33 binding to purified anti-IL-33 monoclonal antibodies were determined using a real-time surface plasmon resonance biosensor using a Biacore 4000 instrument. The Biacore sensor surface was first derivatized by amine coupling with either a polyclonal rabbit anti-mouse antibody (GE, # BR-1008-38) or with a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39) to capture anti-IL-33 monoclonal antibodies expressed with mouse or with human IgG4 constant regions, respectively. All Biacore binding studies were performed in 0.01M ADA pH 7.4, 0.15M NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20 (ABS-ET running buffer). Different concentrations of human IL-33 (hIL-33; R&D Systems, # 3625-IL-010/CF) or cynomolgus monkey IL-33 expressed with a C-terminal hexahistidine tag (MfIL-33-6His; SEQ ID NO:xx) prepared in ABS-ET running buffer (ranging from 100nM to 3.7nM, 3-fold dilutions) were injected over the anti-IL-33 monoclonal antibody captured surface at a flow rate of 30µL/minute. Association of either hIL-33 or MfIL-33-6His to the captured monoclonal antibody was monitored for 4 minutes and their dissociation in ABS-ET running buffer was monitored for 10 minutes. The effect of reduced pH on the binding of each anti-IL-33 antibody to either hIL-33 or MfIL-33-6His was studied using an in-line pH chase assay format in 0.01M ADA pH 6.0, 0.15M NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20 (ABS-ET pH6 buffer). To achieve this, association of either hIL-33 or MfIL-33-6His to the captured monoclonal antibody was monitored for 4 minutes in ABS-ET running buffer. Following a 30 second dissociation of either hIL-33 or MfIL-33-6His in ABS-ET running buffer, ABS-ET pH6 buffer was injected for 3 minutes, and the analyte dissociation under the low-pH conditions was measured. All the binding kinetic experiments were performed at both 25°C and 37°C. Kinetic association ($k_a$) and dissociation ($k_d$) constants were determined by fitting the real-time sensorgrams to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants ($K_D$) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

**[0138]** $K_D$ (M) = $k_d/k_a$ and $t_{1/2}$ (min) = ln(2)/(60*$k_d$)

**[0139]** Binding kinetic parameters for hIL-33 and MfIL-33-6His binding to different anti-IL-33 monoclonal antibodies at 25°C and 37°C are shown in Tables 2 through 5. At 25°C, hIL-33 bound to the anti-IL-33 antibodies with $K_D$ values ranging from 78pM to 757pM, as shown in Table 2. At 37°C, hIL-33 bound to the anti-IL-33 antibodies with $K_D$ values ranging from 411pM to 2.03nM, as shown in Table 3. At both 25°C and 37°C, one anti-IL-33 antibody demonstrated weak binding and therefore its binding kinetic parameters could not be fit using an 1:1 binding model. At 25°C, MfIL-33-6His bound to the anti-IL-33 antibodies with $K_D$ values ranging from 333pM to 38nM, as shown in Table 4. At 37°C, MfIL-33-6His bound to the anti-IL-33 antibodies

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| H4H9665P | | | 9.0E-11 | | | | 6.6E-10 |
| H4H9666P | | | 3.5E-11 | | | | 7.8E-08 |
| H4H9667P | | | 7.1E-11 | | | | 1.2E-08 |
| H4H9670P | | | 1.2E-10 | | | | 1.7E-08 |
| H4H9671P | | | | 2.5E-11 | | | 4.8E-09 |
| H4H9672P | | | 2.5E-11 | | | | 2.0E-08 |
| H4H9675P | | | | 7.5E-12 | | | 4.1E-09 |
| H4H9676P | | | 3.5E-11 | | | | 8.4E-09 |

**[0145]** Eighteen of the 20 anti-IL33 antibodies blocked human IL-33 stimulation of the HEK293/hST2/NFkB-luciferase cells with $IC_{50}$ values ranging from 7.5pM to 29nM, as shown in Table 8. Two of the antibodies tested, H1M9566N and H1M9568N, partially inhibited hIL-33 with a maximum inhibition of 48% and 66%, with $IC_{50}$ values of 950 pM and 250 pM, respectively. Eighteen of the 20 anti-IL33 antibodies blocked MfIL-33-6His stimulation of HEK293/hST2/NFkB-luciferase cells with $IC_{50}$ values ranging from 660pM to 130nM as shown in Table 8. Two of the antibodies tested, H1M9566N and H1M9568N, partially inhibited MfIL-33-6His with a maximum inhibition of 61% and 34%, with $IC_{50}$ values of 1.5 nM and 3.5 nM, respectively.

**Example 7. Inhibition of IL-33-Induced Degranulation of Human Basophils by Anti-IL-33 Antibodies**

**[0146]** To further assess the *in vitro* characteristics of select anti-IL-33 antibodies of the invention, their ability to block IL-33-induced basophil degranulation was measured. Peripheral blood mononuclear cells (PBMC) were purified from fresh whole blood from two different human donors by density gradient centrifugation. K2 EDTA whole blood was diluted 1:1 in RPMI 1640, carefully layered over Ficoll-Paque (GE Healthcare, # 17-1440-03) and centrifuged to separate PBMC. The interphase layer containing the PBMC was aspirated, transferred to a new tube, and washed twice with MACS buffer that was comprised of a 1:20 dilution of the MACS BSA solution (Militenyi Biotec, #130-091-376) in MACS rinsing solution (Militenyi Biotec, #130-091-222). The purified PBMC were then plated in a v-bottom 96-well plate at a final concentration of ~$3.0x10^6$ cells/mL in 100 µL of MACS buffer. To prime the basophils contained within the PBMC population, 1 ng of IL-3 (Sigma, # H7166-10UG) in 50 µL Dulbecco's Phosphate-Buffered Saline without $Ca^{++}$ or $Mg^{++}$ (DPBS) was added to the cell suspension, and then incubated at 37°C for 10 minutes.

**[0147]** Serial dilutions (1:3) of two different exemplary anti-IL-33 antibodies of the invention (H4H9675P and H4H9659P) or an isotype control antibody were made, ranging from 10 nM to 4.6 pM, plus a control with no antibody. The solutions were mixed with a fixed concentration of 100 pM (final concentration) of human IL-33 (R&D Systems, # 6325-IL/CF) or no IL-33 negative control prior to adding to the PBMC. All conditions were tested in duplicate.

monoclonal antibody with a mouse Fc or with a human Fc, respectively. Finally, the Octet biosensors were immersed for 4 minutes into wells containing the pre-mixed samples of 100nM of human IL-33 and 500nM of mAb-2. At the end of each cycle, the non-covalently captured anti-IL-33 antibodies along with the bound pre-complex of human IL-33 and mAb-2 were removed from the biosensors using three alternate 20 second immersions into 10mM HCl followed by submerging into HBS-ET kinetics buffer. The biosensors were washed in HBS-ET kinetics buffer in between every step of the experiment. The real-time binding response was monitored during the binding events, and the binding response (in units of nm) at the end of every step was recorded. During the analysis, the self-self background binding signal for a given mAb-2 (where mAb-1 = mAb-2, i.e., along the diagonal of the matrix) was subtracted from the observed signal for all mAb-2 binding events (across a column in the cross-competition matrix), and the background-corrected results are shown in Figure 1. The response of mAb-1 binding to the pre-complex of human IL-33 and each of the different mAb-2 samples was measured to determine the competitive/non-competitive behavior of different anti-IL-33 monoclonal antibodies with respect to each other.

**[0156]** As shown in Figure 1 light grey boxes with black font represent binding response for self-competition. Antibodies competing with each other in both directions, independent of the order of binding, are represented with black boxes and white font. Cells highlighted in dark grey with black font represent the anti-IL-33 monoclonal antibody that binds weakly to human IL-33, resulting in an observed unidirectional cross-competition. The isotype controls used in the experiment are represented by dark grey boxes with white font. White boxes with black font represent no competition between antibodies, which suggests each antibody has a distinct binding epitope.

**Example 10. Monkey IL-33 cross-competition using bio-layer interferometry**

**[0157]** Binding competition between a panel of different anti-IL-33 monoclonal antibodies was determined using a real time, label-free bio-layer interferometry assay on an Octet® HTX biosensor (ForteBio, A Division of Pall Life Sciences). The experiment was performed at 25°C using a buffer of 0.01M HEPES pH7.4, 0.15M NaCl, 3mM EDTA, 0.05% v/v Surfactant Tween-20, and 0.1mg/ml BSA (HBS-ET kinetics buffer) with the plate shaking at a speed of 1000rpm. To assess whether two antibodies were able to compete with one another for binding to recombinant monkey IL-33 expressed with a C-terminal hexahistidine tag (MfIL-33-6His; SEQ ID: xx), approximately 0.15nm binding units of MfIL-33-6His was first captured onto anti-penta-His antibody coated Octet biosensors (Fortebio Inc, # 18-5079) by submerging the biosensors for 85 seconds into wells containing 2µg/mL of MfIL-33-6His. The antigen-captured biosensors were then saturated with a first anti-IL-33 monoclonal antibody (subsequently referred to as mAb-1) by immersion into wells containing 50µg/mL solution of mAb-1 for 5 minutes. The

belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1 %. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

**[0049]** Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

**Definitions**

**[0050]** The expressions "interleukin-33," "IL-33," and the like, as used herein, refer to a human IL-33 protein as obtained from, for example, R & D Systems, catalogue #3625-IL-010/CF. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species (*e.g.*, "mouse IL-33," "monkey IL-33," etc.).

**[0051]** As used herein, "an antibody that binds IL-33" or an "anti-IL-33 antibody" includes antibodies, and antigen-binding fragments thereof, that bind a soluble fragment of an IL-33 protein. Soluble IL-33 molecules include natural IL-33 proteins as well as recombinant IL-33 protein variants such as, e.g., monomeric and dimeric IL-33 constructs.

**[0052]** The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (e.g., IL-33). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (e.g., IgM).

Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three domains, $C_H1$, $C_H2$ and $C_H3$. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or $V_L$) and a light chain constant region. The light chain constant region comprises one domain ($C_L1$ ). The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1 , CDR1 , FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-IL-33 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

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**[0139]** Binding kinetic parameters for hl L-33 and Mfl L-33-6His binding to different anti-IL-33 monoclonal antibodies at 25°C and 37°C are shown in Tables 2 through 5. At 25°C, hl L-33 bound to the anti-I L-33 antibodies with $K_D$ values ranging from 78pM to 757pM, as shown in Table 2. At 37°C, hl L-33 bound to the anti-I L-33 antibodies with $K_D$ values ranging from 411pM to 2.03nM, as shown in Table 3. At both 25°C and 37°C, one anti-I L-33 antibody demonstrated weak binding and therefore its binding kinetic parameters could not be fit using an 1:1 binding model. At 25°C, Mfl L-33-6His bound to the anti-I L-33 antibodies with $K_D$ values ranging from 333pM to 38nM, as shown in Table 4. At 37°C, Mfl L-33-6His bound to the anti-I L-33 antibodies

| | | | | | | | |
|---|---|---|---|---|---|---|---|
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| H4H9667P | | | 7.1E-11 | | | | 1.2E-08 |
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**[0147]** Serial dilutions (1:3) of two different exemplary anti-IL-33 antibodies of the invention (H4H9675P and H4H9659P) or an isotype control antibody were made, ranging from 10 nM to 4.6 pM, plus a control with no antibody. The solutions were mixed with a fixed concentration of 100 pM (final concentration) of human IL-33 (R&D Systems, # 3625-IL/CF) or no IL-33 negative control prior to adding to the PBMC. All conditions were tested in duplicate.

monoclonal antibody with a mouse Fc or with a human Fc, respectively. Finally, the Octet biosensors were immersed for 4 minutes into wells containing the pre-mixed samples of 100nM of human IL-33 and 500nM of mAb-2. At the end of each cycle, the non-covalently captured anti-IL-33 antibodies along with the bound pre-complex of human IL-33 and mAb-2 were removed from the biosensors using three alternate 20 second immersions into 10mM HCI followed by submerging into HBS-ET kinetics buffer. The biosensors were washed in HBS-ET kinetics buffer in between every step of the experiment. The real-time binding response was monitored during the binding events, and the binding response (in units of nm) at the end of every step was recorded. During the analysis, the self-self background binding signal for a given mAb-2 (where mAb-1 = mAb-2, i.e., along the diagonal of the matrix) was subtracted from the observed signal for all mAb-2 binding events (across a column in the cross-competition matrix), and the background-corrected results are shown in Figure 1. The response of mAb-1 binding to the pre-complex of human IL-33 and each of the different mAb-2 samples was measured to determine the competitive/non-competitive behavior of different anti-IL-33 monoclonal antibodies with respect to each other.

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