(19) DANMARK

(10) DK/EP 3842456 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 07 K 16/24 (2006.01)* *G 01 N 33/53 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2024-10-28**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2024-09-18**

(86) Europæisk ansøgning nr.: **20194926.0**

(86) Europæisk indleveringsdag: **2012-07-05**

(87) Den europæiske ansøgnings publiceringsdag: **2021-06-30**

(30) Prioritet: **2011-07-21 US 201161510268 P**

(62) Stamansøgningsnr: **17168574.6**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Zoetis Services LLC, 10 Sylvan Way, Parsippany, NJ 07054, USA**

(72) Opfinder: **Bammert, Gary F., Zoetis, VMRD, 333 Portage Street, Kalamazoo, Michigan 49007, USA**
Dunham, Steven A., Zoetis, VMRD, 333 Portage Street, Kalamazoo, Michigan 49007, USA

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **Monoklonalt Interleukin-31-antistof**

(56) Fremdragne publikationer:
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DESCRIPTION

Description

FIELD OF THE INVENTION

[0001] The present invention provides isolated anti-IL31 antibodies in the form of veterinary compositions useful for treating a pruritic condition or an allergic condition in dogs.

BACKGROUND OF THE INVENTION

[0002] Atopic dermatitis has been defined by the American College of Veterinary Dermatology task force as "a genetically-predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features" (Olivry, et al. Veterinary Immunology and Immunopathology 2001; 81: 143-146). The task force also recognized that the disease in canines has been associated with allergen-specific IgE (Olivry, et al. 2001 *supra;* Marsella & Olivry Clinics in Dermatology 2003; 21: 122-133). Severe pruritus, along with secondary alopecia and erythema, are the most noticeable and concerning symptoms to pet owners.

[0003] The prevalence of atopic dermatitis is not known with precision due to poor and inconsistent epidemiological data, but is estimated to be 10% of the total canine population (Marsella & Olivry 2003 *supra;* Scott, et al. Canadian Veterinary Journal 2002; 43: 601-603; Hillier Veterinary Immunology and Immunopathology 2001; 81: 147-151). Globally, about 4.5 million dogs are affected with this chronic and lifelong condition. Incidence appears to be increasing. Breed and sex predilections have been suspected, but may vary greatly depending on geographical region (Hillier, 2001 *supra*; Picco, et al. Vet Dermatol. 2008; 19: 150-155).

[0004] The potential factors involved in allergic dermatitis are numerous and poorly understood. Components in food may trigger atopic dermatitis (Picco, 2008 *supra*), as well as environmental allergens such as fleas, dust mites, ragweed, plant extracts, etc. Genetic factors also play an important role. Although there is no confirmed breed predilection, some mode of inheritance is thought to increase predisposition to atopic dermatitis (Sousa & Marsella Veterinary Immunology and Immunopathology 2001; 81: 153-157; Schwartzman, et al. Clin. Exp. Immunol. 1971; 9: 549-569.

[0005] Interleukin-31 (IL-31) is a cytokine that was cloned in 2004. It is mainly produced by activated T helper (Th)2 cells (Dillon et al. Nat Immunol 2004; 5:752-60), but is also produced in mast cells and macrophages. IL-31 binds a co-receptor composed of IL-31 receptor A (IL-31

RA) and the oncostatin M receptor (OSMR) (Dillon et al. 2004 *supra* and Bilsborough et al. J Allergy Clin Immunol. 2006 117(2):418-25). Receptor activation results in phosphorylation of STAT through JAK receptor(s). Expression of the co-receptor has been shown in macrophages, keratinocytes and in dorsal root ganglia. Recently, it has been found that IL-31 is involved in dermatitis, pruritic skin lesions, allergy and airway hypersensitivity. See Fig. 1.

[0006] Stimulation of T cells with anti-CD3 and anti-CD28 antibodies immediately upregulates IL-31 mRNA expression (Dillon et al. 2004 *supra*). Microarray analysis has shown that IL-31 induces certain chemotactic genes, such as CXCL1, CLL17 (thymus and activation-regulated chemokine [TARC]), CCL19 (macrophage inflammatory protein [MIP]3β), CCL22 (monocyte-derived chemokine [MDC], CCL23 (MIP3), and CCL4 (MIPβ) (Dillon et al. 2004 *supra*).

[0007] Transgenic mice that over-express IL-31 show skin inflammation, pruritis, severe dermatitis, and alopecia (Dillon et al. 2004 *supra*). Subcutaneous injection of IL-31 into mice triggers infiltration by the inflammatory cells, neutrophils, eosinophils, lymphocytes, and macrophages, and results in epidermal thickening and dermal acanthosis. In NC/Nga mice, with atopic dermatitis (AD) due to natural causes, IL-31 is overexpressed in skin lesions and correlates with pruritus (Takaoka et al. Eur J. Pharmacol. 2005; 516, 180-181; Takaoka et al. Exp. Dermatol. 2006; 15, 161-167). Also, in murine models, IL-31 has been shown to induce rapid onset pruritus (Raap et al. J Allergy Clin Immunol. 2008;122(2):421-3)

[0008] Mizuno et al. (Veterinary Immunology and Immunopathology, vol. 131, no.1-2, page 140-143) discloses the cloning of canine IL-31.An anti-IL-31 antibody has been reported to ameliorate scratching behaviour in mice (Grimstad et al, Exp. Dermatol. 2009, 18:35-43) (US7,939,068 B2). WO2006/122079 and US2009/0252732 disclose general methodologies for creating anti-IL31 antibodies. US2010/0075996 discloses compounds that can be used as JAK inhibitors and pharmaceutical compositions containing these compounds. Olivry et al. (Veterinary Dermatology, 21, 233-248) reviews the treatment of canine atopic dermatitis. WO 03/060080 discloses caninized antibodies.

[0009] Further studies have indicated that IL-31 is associated with atopic-dermatitis-induced skin inflammation and pruritus in humans. In human AD patients, the expression of IL-31 mRNA is considerably higher in skin lesions than in non-lesional skin, and the expression in non-lesional skin is greater than that in normal skin from healthy patients (Sonkoly et al. J Allergy Clin Immunol 2006; 117:411-7). Another study has reported that CD45RO+ (memory) cutaneous lymphocyte antigen (CLA)-positive T cells in the skin of AD patients express IL-31 mRNA and protein (Bilsborough et al. 2006 *supra*). It has also been reported that IL-31 mRNA overexpression in the skin of patients or allergic contact dermatitis is correlated with IL-4 and IL-13 mRNA expression, but not with interferon (IFN)-γ mRNA expression (Neis et al. J. Allergy Clin. Immunol. 2006; 118, 930-937). Furthermore, IL-31 serum levels have been shown to be elevated in human patients with chronic spontaneous urticaria and even more so in patients with AD (Raap et al. Exp Dermatol. 2010;19(5):464-6). Also, a correlation of the severity of AD with serum IL-31 levels has been observed in humans (Rapp et al. 2008 *supra*). IL-31 secretion has also been shown to be enhanced in mast cells following IgE cross-linking and as

a response to *Staphylococcal* superantigen in atopic individuals. In addition, IL-31 has been shown to stimulate the production of several pro-inflammatory mediators including IL-6, IL-8, CXCL1, CC17 and multiple metalloproteinases in human colonic myofibroblasts (Yagi,et al. International Journal of Molecular Medicine 2007; 19(6): 941-946.

[0010] Type I hypersensitivity against environmental allergens is considered to be the main mechanism of canine AD, and the levels of Th2-mediated cytokines, such as IL-4 are increased in the skin lesions of dogs with AD (Nuttall, et al. Vet. Immunol. Immunopathol. 2002; 87, 379-384). Moreover, infiltration by inflammatory cells, lymphocytes and neutrophils, is an important mechanism underlying the aggravation of the skin lesions; the overexpression of chemotactic genes such as CCL17/TARC, CCR4, and CCL28/mucosae-associated epithelial chemokine (MEC) contributes to the aggravation of skin lesions in the dogs with AD (see, Maeda, et al. Vet. Immunol. Immunopathol. 2005; 103, 83-92; Maeda, et al. Vet. Immunol. Immunopathol.2002b; 90, 145-154; and Maeda, et al. J. Vet. Med. Sci. 2008; 70, 51-55).

[0011] Recent evidence has suggested that IL-31 might be involved in promoting allergic inflammation and an airway epithelial response characteristic of allergic asthma (Chattopadhyay, et al. J Biol Chem 2007; 282:3014-26; and Wai, et al. Immunology, 2007; 122, 532-541).

[0012] These observations support the hypothesis that IL-31 plays a significant role in both pruritic and allergic conditions. It would be desirable to provide a therapeutic antibody against IL-31 useful for treating a pruritc condition and/or an allergic condition in dogs or cats.

SUMMARY OF THE INVENTION

[0013] The present invention provides an antibody or antigen-binding portion thereof that that specifically binds to canine IL-31 protein encoded by the nucleoide sequence of SEQ ID NO:33, wherein saidthe antibody or antigen-binding portion thereof inhibits IL-31 activity in a dog and inhibits dog IL-31-mediated pSTAT signaling in a cell based assay. In some embodiments, the antibody is a monoclonal antibody. In one embodiment, the monoclonal antibody is chimeric. In another embodiment, the antibody is caninized

[0014] The antibody reduces, inhibits, or neutralizes IL-31 activity in a dog eat. In preferred embodiments, the antibody reduces, inhibits, or neutralizes a pruritic condition or an allergic condition. Pruritic conditions include, for example, atopic dermatitis, eczema, psoriasis, scleroderma, and pruritus. Allergic conditions include, for example, allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstructive pulmonary disease, and inflammatory processes resulting from autoimmunity, such as Irritable bowel syndrome (IBS). In one embodiment, the present invention provides an isolated antibody or antigen-binding portion thereof including at least one of the following:

a variable heavy (V_H) chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1), or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2), or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3), or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0015] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof including at least one of the following group:

a variable light (V_L) chain comprising a complementary determining region (CDR) 1 having the amino acid sequence RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) or KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);

a variable light chain CDR2 having the amino acid sequence RASNLES (SEQ ID NO: 13; 11E12-VL-CDR2), or RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);

a variable light chain CDR3 having the amino acid sequence QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3), or QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0016] In still other embodiments, an antibody having at least one of the variable light chain CDRs described above, can further include at least one of the following variable heavy chain CDRs:

a variable heavy chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1), or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2), or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3), or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of

CDR1, CDR2, or CDR3.

[0017] In some embodiments, the antibody can include at least one of the following:

1. a) a variable light chain comprising
   DIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMHWYQQKPGQPPKLLIYRASNLESGIPA RFSGSGSRTDFTLTINPVETDDVATYYCQQSNKDPLTFGAGTKLELK (SEQ ID NO: 19; MU-11E12-VL),

   DIVMTQTPLSLSVSPGEPASISCRASESVDNYGISFMHWYQQKPGQPPKLLIYRASNLESGVPD RFSGSGSGTDFTLRISRVEADDAGVYYCQQSNKDPLTFGAGTKLEIK (SEQ ID NO: 20; CAN-11E12-VL-cUn-FW2),

   DIVMTQTPLSLSVSPGEPASISCRASESVDNYGISFMHWFQQKPGQSPQLLIYRASNLESGVPD RFSGSGSGTDFTLRISRVEADDAGVYYCQQSNKDPLTFGAGTKLEIK (SEQ ID NO: 21; CAN-11E12-VL-cUn-13),

   DILLTQSPASLAVSLGQRAIISCKASQSVSFAGTGLMHWYQQKPGQQPKLLIYRASNLEAGVPT RFSGSGSRTDFTLNIHPVEEEDAATYFCQQSREYPWTFGGGTKLEIK (SEQ ID NO: 24; MU-34D03-VL),

   or

   EIVMTQSPASLSLSQEEKVTITCKASQSVSFAGTGLMHWYQQKPGQAPKLLIYRASNLEAGVPS RFSGSGSGTDFSFTISSLEPEDVAVYYCQQSREYPWTFGQGTKLEIK (SEQ ID NO: 25; CAN-34D03-VL-998-1);
2. b) a variable heavy chain comprising
   QVQLQQSGAELVKPGASVKLSCKASGYTFKYYDINWVRQRPEQGLEWIGWIFPGDGGTKYNE TFKGKATLTTDKSSSTAYMQLSRLTSEDSAVYFCARGGTSVIRDAMDYWGQGTSVTVSS (SEQ ID NO: 26; MU-11E12-VH),

   EVQLVQSGAEVKKPGASVKVSCKTSGYTFKYYDINWVRQAPGAGLDWMGWIFPGDGGTKYN ETFKGRVTLTADTSTSTAYMELSSLRAGDIAVYYCARGGTSVIRDAMDYWGQGTLVTVSS (SEQ ID NO: 27; CAN-11E12-VH-415-1),

   EVQLVESGGDLVKPGGSLKLSCAASGFSFSNYGMSWVRQTPDKRLEWVATISYGGSYTYYPD NIKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCVRGYGYDTMDYWGQGTSVTVSS (SEQ ID NO: 30; MU-34D03-VH), or

   EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYGMSWVRQAPGKGLQWVATISYGGSYTYYP DNIKGRFTISRDNAKNTLYLQMNSLRAEDTAMYYCVRGYGYDTMDYWGQGTLVTVSS (SEQ ID NO: 31; CAN-34D03-VH-568-1);

   and
3. c) variants thereof having one or more conservative amino acid substitutions.

[0018] In one embodiment, the present invention provides a monoclonal antibody that specifically binds to a region between about amino acid residues 95 and 125 of the canine IL-31 amino acid sequence of SEQ ID NO: 32. In some embodiments, the antibody specifically binds to a region between about amino acid residues 102 and 122 of the canine IL-31 amino acid sequence of SEQ ID NO: 32.

[0019] The present invention also provides a veterinary composition including a therapeutically effective amount of at least one antibody described above.

[0020] In other embodiments, the invention provides a host cell that produces an antibody described above.

[0021] In still further embodiments, the invention provides an isolated nucleic acid including a nucleic acid sequence encoding at least one of the following:

a variable heavy (V_H) chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1), or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2), or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3), or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0022] In further embodiments, the isolated nucleic acid described above may further include a nucleic acid sequence encoding at least one of the following:

a variable light (V_L) chain comprising a complementary determining (CDR) 1 having the amino acid sequence RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) or KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);

a variable light chain CDR2 having the amino acid sequence RASNLES (SEQ ID NO: 13; 11E12-VL-CDR2), or RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);

a variable light chain CDR3 having the amino acid sequence QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3) or QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of

CDR1, CDR2, or CDR3.

[0023] The present invention further provides a vector including at least one of the nucleic acids described above.

[0024] In other embodiments, the present invention provides a method of producing an antibody comprising culturing a host cell described above under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture medium of the host cell. **The invention provides an antibody or antigen-binding portion thereof as defined in the claims for use in** treating a pruritic condition or an allergic condition, including administering a therapeutically effective amount of an antibody described above. In some embodiments, the pruritic condition is selected from atopic dermatitis, eczema, psoriasis, scleroderma, and pruritus. In other embodiments, the allergic condition to be treated is selected from allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstructive pulmonary disease, and inflammatory processes resulting from autoimmunity, such as Irritable bowel syndrome (IBS).

[0025] The invention provides an antibody or antigen-binding portion thereof as defined in the claims for use in inhibiting IL-31 activity in a dog by administering an antibody described above.

[0026] Also provided is a method of detecting or quantitating IL-31 in a sample, the method including incubating a clinical or biological sample containing IL-31 in the presence of an antibody described above; and detecting the antibody which is bound to IL-31 in the sample. In one embodiment, the antibody is detectably labeled. In another embodiment, the antibody is may be unlabeled and is used in combination with a second antibody which is labeled.

Brief Description of the Drawings

[0027]

Figure 1 is a schematic representation of the IL-31 pathway.

Figure 2 is a schematic representation of the general structure of a mouse immunoglobulin G (IgG) molecule highlighting the antigen binding site.

Figure 3 is a schematic representation of the general structure of a mouse:canine chimeric IgG

Figure 4 is an illustration showing speciation or "caninization" of a mouse IgG, mouse CDRs are grafted onto canine frameworks identified from sequence databases

Figure 5 is an illustration of a "heterochimeric" monoclonal antibody pairing the chimeric light

chain with a fully caninized heavy chain.

Figure 6 ELISA Titers from IL-31 Immunized Mice (CF-1 MU#1 - 4) relative to pre-bleed and positive control mice.

Figure 7 is an illustration of antibody variable chains showing primers to constant regions and degenerate primers directed at mouse variable regions.

Figure 8 is a graph of the pilot efficacy of chimeric 11E12 in a placebo controlled, single dose, SC study (76A60).

Figure 9 is of a table showing the individual pruritic scores from dogs enrolled in study 76A60.

Figure 10 is of Western blots showing binding of chimeric (Blot #1), caninized (Blot#2), and heterochimeric (Blots #3 and 4) versions of 11E12 to canine IL-31. The heterochimera in Blot #3 has a caninized light chain paired with a chimeric heavy chain. The heterochimera in Blot #4 has the chimeric light chain paired with the caninized heavy chain. Each nitrocellulose blot contains- left lane, pre-stained protein standards (Seeblue plus 2, Invitrogen Corp., Carlsbad, CA) and right lane, 800 ng of canine IL-31.

Figure 11 is a schematic overview of caninized11E12 light chain framework substitution work.

Figure 12 is of Western blots showing binding of caninized versions of 11E12 with single backmutations to mouse framework 2 light chain residues. Each nitrocellulose blot contains- left lane, pre-stained protein standards (Seeblue plus 2, Invitrogen Corp., Carlsbad, CA) and right lane, 800 ng of canine IL-31.

Figure 13 is of Western blots with full length and truncated canine IL-31 proteins. Individual nitrocellulose blots were probed with A) anti-His B) 34D03 and C)11E12 antibodies. Lanes 1-9 of the blots correspond to the following: Lane 1-pre-stained protein standards (Seeblue plus 2, Invitrogen Corp., Carlsbad, CA); Lane 2- full-length canine IL-31; Lane 3- N-terminal truncation - 20N; Lane 4- N-terminal truncation -40N; Lane 5-N-terminal truncation -60N; Lane 6-C-terminal truncation -20C; Lane 7-C-terminal truncation -40C; Lane 8-C-terminal truncation -60C; and Lane 9- beta-galactosidase (lacZ). Note: full length IL-31 and proteins with C-terminal truncations (-20, -40C, and -60C) showed no detectable expression under these conditions.

Figure 14 is of Western blots with truncated canine IL-31 proteins. Individual nitrocellulose blots were probed with A) anti-His B) 11E12 and C) 34D03 antibodies. Lanes 1-5 of the blots correspond to the following: Lane 1-pre-stained protein standards (Seeblue plus 2, Invitrogen Corp., Carlsbad, CA); Lane 2-C-terminal truncations at positions 20-122; Lane 3-C-terminal truncations at positions 20-100; Lane 4-C-terminal truncations at positions 20-80; and Lane 5-beta-galactosidase (lacZ).

Figure 15 is a section of Western blots with lysates of *E. coli* strains expressing canine IL-31 with alanine substituted for each amino acid position (76-122). Individual nitrocellulose blots were probed with anti-His, 11E12 and, 34D03 antibodies, as shown in the Figure.

Figure 16 is a section of Western blots with double and triple mutations in canine IL-31. -20N protein lysate was run as a positive control.

Figure 17 is a graph showing the pruritic scores for dogs injected subcutaneously with caninized 34D03 antibody (1.0 mg/kg). Pruritic scores were measured on each study day prior to (baseline response) and following (2 h response) challenge with 1.5 µg/kg canine IL-31.

Figure 18 is a 4-12 % Bis Tris SDS PAGE with purified canine and feline IL-31 proteins. Panel A shows coomassie staining of proteins run under reducing conditions. Panel B shows coomassie staining of proteins run under non-reducing conditions Panels. Panels C and D are the Western blots of gels identical to A and B respectively, probed with an anti-His antibody. Lane 1-canine IL-31; Lane 2-feline IL-31; Lane 3- pre-stained protein standards (Seeblue plus 2, Invitrogen Corp., Carlsbad, CA); Lane 4-canine IL-31; and Lane 5-feline IL-31.

Figure 19 is a graph of pSTAT signaling in canine DH-82 monocytes induced by canine and feline IL-31 produced in *E. coli.* Canine IL-31 (CHO) is the reference protein used for all previous cell-based assays, dog pruritus model, and as the immunogen for initial identification of antibodies.

Figure 20 is an alignment showing the sequence conservation between feline and canine IL-31 in the region of the protein involved in binding of 11E12 and 34D03 antibodies (annotated with a plus sign).

Figure 21 is of Western blots with IL-31 proteins. Individual nitrocellulose blots were probed with A) anti-His B) 11E12 and C) 34D03 antibodies. Note- Canine IL-31 (CHO) does not contain a 6-His tag.

Figure 22 is a graph showing the inhibition of canine IL-31 induced pSTAT signaling in canine DH82 monocytes comparing felinized and caninized antibody 34D03.

Figure 23 is a Western blot of feline and canine IL-31 under reducing conditions probed with felinized antibody 34D03.

BRIEF DESCRIPTION OF THE SEQUENCES

[0028]

SEQ ID NO: 1 is a variable heavy chain CDR1 referred to herein as 11E12-VH-CDR1;

SEQ ID NO: 2 is a variable heavy chain CDR1 referred to herein as 19D07-VH-CDR1;

SEQ ID NO: 3 is a variable heavy chain CDR1 referred to herein as 34D03-VH-CDR1;

SEQ ID NO: 4 is a variable heavy chain CDR2 referred to herein as 11E12-VH-CDR2;

SEQ ID NO: 5 is a variable heavy chain CDR2 referred to herein as 19D07-VH-CDR2;

SEQ ID NO: 6 is a variable heavy chain CDR2 referred to herein as 34D03-VH-CDR2;

SEQ ID NO: 7 is a variable heavy chain CDR3 referred to herein as 11E12-VH-CDR3;

SEQ ID NO: 8 is a variable heavy chain CDR3 referred to herein as 19D07-VH-CDR3;

SEQ ID NO: 9 is a variable heavy chain CDR3 referred to herein as 34D03-VH-CDR3;

SEQ ID NO: 10 is a variable light chain CDR1 referred to herein as 11E12-VL-CDR1;

SEQ ID NO: 11 is a variable light chain CDR1 referred to herein as 19D07-VL-CDR1;

SEQ ID NO: 12 is a variable light chain CDR1 referred to herein as 34D03-VL-CDR1;

SEQ ID NO: 13 is a variable light chain CDR2 referred to herein as 11E12-VL-CDR2;

SEQ ID NO: 14 is a variable light chain CDR2 referred to herein as 19D07-VL-CDR2;

SEQ ID NO: 15 is a variable light chain CDR2 referred to herein as 34D03-VL-CDR2;

SEQ ID NO: 16 is a variable light chain CDR3 referred to herein as 11E12-VL-CDR3;

SEQ ID NO: 17 is a variable light chain CDR3 referred to herein as 19D07-VL-CDR3;

SEQ ID NO: 18 is a variable light chain CDR3 referred to herein as 34D03-VL-CDR3;

SEQ ID NO: 19 is a variable light chain sequence referred to herein as MU-11E12-VL;

SEQ ID NO: 20 is a variable light chain sequence referred to herein as CAN-11E12-VL-cUn-FW2;

SEQ ID NO: 21 is a variable light chain sequence referred to herein as CAN-11E12-VL-cUn-13;

SEQ ID NO: 22 is a variable light chain sequence referred to herein as MU-19D07-VL;

SEQ ID NO: 23 is a variable light chain sequence referred to herein as CAN-19D07-VL-998-1;

SEQ ID NO: 24 is a variable light chain sequence referred to herein as MU-34D03-VL;

SEQ ID NO: 25 is a variable light chain sequence referred to herein as CAN-34D03-VL-998-1;

SEQ ID NO: 26 is a variable heavy chain sequence referred to herein as MU-11E12-VH;

SEQ ID NO: 27 is a variable heavy chain sequence referred to herein as CAN-11E12-VH-415-1;

SEQ ID NO: 28 is a variable heavy chain sequence referred to herein as MU-19D07-VH;

SEQ ID NO: 29 is a variable heavy chain sequence referred to herein as CAN-19D07-VH-400-1;

SEQ ID NO: 30 is a variable heavy chain sequence referred to herein as MU-34D03-VH;

SEQ ID NO: 31 is a variable heavy chain sequence referred to herein as CAN-34D03-VH-568-1;

SEQ ID NO: 32 is the amino acid sequence corresponding to GenBank Accession No. C7G0W1 and corresponds to Canine IL-31 full-length protein;

SEQ ID NO: 33 is the nucleotide sequence corresponding to GenBank Accession No. C7G0W1 and corresponds to the nucleotide sequence encoding Canine IL-31 full-length protein;

SEQ ID NO: 34 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU-11E12-VL;

SEQ ID NO: 35 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU-11E12-VH;

SEQ ID NO: 36 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU-19D07-VL;

SEQ ID NO: 37 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU-19D07-VH;

SEQ ID NO: 38 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU-34D03-VL;

SEQ ID NO: 39 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU-34D03-VH;

SEQ ID NO: 40 is the amino acid sequence for the canine heavy chain constant region referred to herein as HC-64 (GenBank accession no. AF354264);

SEQ ID NO: 41 is the nucleotide sequence encoding the canine heavy chain constant region referred to herein as HC-64 (GenBank accession no. AF354264);

SEQ ID NO: 42 is the amino acid sequence for the canine heavy chain constant region referred to herein as HC-65 (GenBank accession no. AF354265);

SEQ ID NO: 43 is the nucleotide sequence encoding the canine heavy chain constant region referred to herein as HC-65 (GenBank accession no. AF354265);

SEQ ID NO: 44 is the amino acid sequence for the canine light chain constant region referred to herein as kappa (GenBank Accession No. XP_532962);

SEQ ID NO: 45 is the nucleotide sequence encoding the canine light chain constant region referred to as kappa (GenBank Accession No. XP_532962);

SEQ ID NO: 46 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN-19D07-VL-998-1;

SEQ ID NO: 47 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN-19D07-VH-998-1;

SEQ ID NO: 48 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN-34D03-VL-998-1;

SEQ ID NO: 49 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN-34D03-VH-568-1;

SEQ ID NO: 50 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN-11E12-VL-cUn-FW2;

SEQ ID NO: 51 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN-11E12-VH-415-1;

SEQ ID NO: 52 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN-11E12-VL-cUn-13;

SEQ ID NO: 53 is a variable light chain sequence referred to herein as CAN-11E12 VL cUn_1;
SEQ ID NO: 54 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN-11E12-VL-cUn-1;

SEQ ID NO: 55 corresponds to the amino acid sequence of the canine IL-31 full-length construct used herein for *E.coli* expression;

SEQ ID NO: 56 is the nucleotide sequence corresponding to the canine IL-31 full-length construct used herein for *E.coli* expression;

SEQ ID NO: 57 is the amino acid sequence of the canine IL-31 -20N construct for *E.coli* expression;

SEQ ID NO: 58 is the nucleotide sequence corresponding to the canine IL-31 -20N construct for *E.coli* expression;

SEQ ID NO: 59 is the amino acid sequence of the canine IL-31 -40N construct for *E.coli* expression;

SEQ ID NO: 60 is the nucleotide sequence corresponding to the canine IL-31 -40N construct for *E.coli* expression;

SEQ ID NO: 61 is the amino acid sequence of the canine IL-31 -60N construct for *E.coli* expression;

SEQ ID NO: 62 is the nucleotide sequence corresponding to the canine IL-31 -60N construct for *E.coli* expression;

SEQ ID NO: 63 is the amino acid sequence of the canine IL-31 20-122 construct for *E.coli* expression;

SEQ ID NO: 64 is the nucleotide sequence corresponding to the canine IL-31 20-122 construct for *E.coli* expression;

SEQ ID NO: 65 is the amino acid sequence of the canine IL-31 20-100 construct for *E.coli* expression;

SEQ ID NO: 66 is the nucleotide sequence corresponding to the canine IL-31 20-100 construct for *E.coli* expression;

SEQ ID NO: 67 is the is the amino acid sequence of the canine IL-31 20-80 construct for *E.coli* expression;

SEQ ID NO: 68 is the nucleotide sequence corresponding to the canine IL-31 20-80 construct for *E.coli* expression;

SEQ ID NO: 69 is the nucleotide sequence corresponding to the feline IL-31 full-length construct for *E.coli* expression;

SEQ ID NO: 70 is the amino acid sequence corresponding to the feline IL-31 full-length construct for *E.coli* expression;

SEQ ID NO: 71 is a variable light chain sequence referred to herein as FEL-34D03-VL-021-1;

SEQ ID NO: 72 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL-34D03-VL-021-1;

SEQ ID NO: 73 is a variable heavy chain sequence referred to herein as FEL-34D03-VH-035-1;

SEQ ID NO: 74 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL-34D03-VH-035-1;

SEQ ID NO: 75 is the amino acid sequence for the feline heavy chain constant region referred to herein as HC-A Feline (GenBank accession no. AB016710.1);

SEQ ID NO: 76 is the nucleotide sequence encoding the feline heavy chain constant region referred to herein as HC-A Feline (GenBank accession no. AB016710.1);

SEQ ID NO: 77 is the amino acid sequence for the feline light chain constant region referred to herein as LC-Kappa Feline (GenBank accession no. AF198257.1);

SEQ ID NO: 78 is the nucleotide sequence encoding the feline light chain constant region referred to herein as LC-Kappa Feline (GenBank accession no. AF198257.1);

DEFINITIONS

[0029] Before describing the present invention in detail, several terms used in the context of the present invention will be defined. In addition to these terms, others are defined elsewhere in the specification, as necessary. Unless otherwise expressly defined herein, terms of art used in this specification will have their art-recognized meanings.

[0030] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, reference to "an antibody" includes a plurality of such antibodies.

[0031] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others.

[0032] Epitope, as used herein, refers to the antigenic determinant recognized by the CDRs of the antibody. In other words, epitope refers to that portion of any molecule capable of being recognized by, and bound by, an antibody. Unless indicated otherwise, the term "epitope" as used herein, refers to the region of IL-31 to which an anti-IL-31 agent is reactive to.

[0033] An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of being recognized by, and bound by, an antibody (the corresponding antibody binding region may be referred to as a paratope). In general, epitopes consist of chemically active surface groupings of molecules, for example, amino acids or sugar side chains, and have specific three-dimensional structural characteristics as well as specific charge characteristics.

[0034] The term "specifically" in the context of antibody binding, refers to high avidity and/or high affinity binding of an antibody to a specific antigen, i.e., a polypeptide, or epitope. In many embodiments, the specific antigen is an antigen (or a fragment or subfraction of an antigen) used to immunize the animal host from which the antibody-producing cells were isolated. Antibody specifically binding an antigen is stronger than binding of the same antibody to other antigens. Antibodies which bind specifically to a polypeptide may be capable of binding other polypeptides at a weak, yet detectable level (e.g., 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to a subject polypeptide, e.g. by use of appropriate controls. In general, specific antibodies bind to an antigen with a binding affinity with a K_D of 10^{-7} M or less, e.g., 10^{-8} M or less (e.g., 10^{-9} M or less, 10^{-10} or less, 10^{-11}or less, 10^{-12} or less, or 10^{-13} or less, etc.).

[0035] As used herein, the term "antibody" refers to an intact immunoglobulin having two light and two heavy chains. Thus a single isolated antibody or fragment may be a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a recombinant antibody, a chimeric antibody, a heterochimeric antibody, a caninized antibody, or a felinized antibody. The term "antibody" preferably refers to monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof that can bind to the IL-31 protein and fragments thereof. The term

antibody is used both to refer to a homogeneous molecular, or a mixture such as a serum product made up of a plurality of different molecular entities.

[0036] "Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains. FIG. 2 is an example of the general structure of a native mouse immunoglobulin G (IgG) highlighting the antigen binding site.

[0037] The term "antibody fragment" refers to less than an intact antibody structure, including, without limitation, an isolated single antibody chain, an Fv construct, a Fab construct, an Fc construct, a light chain variable or complementarity determining region (CDR) sequence, etc.

[0038] The term "variable" region comprises framework and CDRs (otherwise known as hypervariables) and refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise multiple FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the α-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0039] The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (Kabat, et al. (1991), above) and/or those residues from a "hypervariable loop" (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987). "Framework" or "FR" residues are those variable domain residues

other than the hypervariable region residues as herein defined.

[0040] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0041] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and - binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0042] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH 1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0043] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0044] Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. Presently there are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2 (as defined by mouse and human designation). The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called *alpha*, *delta*, *epsilon*, *gamma*, and *mu*, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in multiple species. The prevalence of individual isotypes and functional activities associated with these constant domains are species-specific and must be experimentally defined.

[0045] "Monoclonal antibody" as defined herein is an antibody produced by a single clone of cells (specifically, a single clone of hybridoma cells) and therefore a single pure homogeneous type of antibody. All monoclonal antibodies produced from the same clone are identical and have the same antigen specificity. The term "monoclonal" pertains to a single clone of cells, a single cell, and the progeny of that cell.

[0046] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. Typically, chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to canine constant segments. FIG. 3 is a schematic representation of the general structure of one embodiment of a mouse:canine IgG. In this embodiment, the antigen binding site is derived from mouse while the F_C portion is canine.

[0047] "Caninized" forms of non-canine (e.g., murine) antibodies are genetically engineered antibodies that contain minimal sequence derived from non-canine immunoglobulin. Caninized antibodies are canine immunoglobulin sequences (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-canine species (donor antibody) such as mouse having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the canine immunoglobulin sequences are replaced by corresponding non-canine residues. Furthermore, caninized antibodies may include residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the caninized antibody will include substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-canine immunoglobulin sequence and all or substantially all of the FRs are those of a canine immunoglobulin sequence. The caninized antibody optionally also will comprise a complete, or at least a portion of an immunoglobulin constant region (Fc), typically that of a canine immunoglobulin sequence. FIG. 4 is an illustration of one embodiment showing speciation or caninization of a mouse IgG. In this embodiment, mouse CDRs are grafted onto canine frameworks.

[0048] "Felinized" forms of non-feline (e.g., murine) antibodies are genetically engineered antibodies that contain minimal sequence derived from non-feline immunoglobulin. Felinized antibodies are feline immunoglobulin sequences (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-feline species (donor antibody) such as mouse having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the feline immunoglobulin sequences are replaced by corresponding non-feline residues. Furthermore, felinized antibodies may include residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the felinized antibody will include substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-feline immunoglobulin sequence and all or substantially all of the FRs are those of a feline

immunoglobulin sequence. The felinized antibody optionally also will comprise a complete, or at least a portion of an immunoglobulin constant region (Fc), typically that of a feline immunoglobulin sequence.

[0049] The term "heterochimeric" as defined herein, refers to an antibody in which one of the antibody chains (heavy or light) is caninized while the other is chimeric. FIG. 5 depicts one embodiment of a heterochimeric molecule. In this embodiment, a caninized variable heavy chain (where all of the CDRs are mouse and all FRs are canine) is paired with a chimeric variable light chain (where all of the CDRs are mouse and all FRs are mouse. In this embodiment, both the variable heavy and variable light chains are fused to a canine constant region.

[0050] A "variant" anti-IL-31 antibody, refers herein to a molecule which differs in amino acid sequence from a "parent" anti-IL-31 antibody amino acid sequence by virtue of addition, deletion, and/or substitution of one or more amino acid residue(s) in the parent antibody sequence and retains at least one desired activity of the parent anti-IL-31-antibody. Desired activities can include the ability to bind the antigen specifically, the ability to reduce, inhibit or neutralize IL-31 activity in an animal, and the ability to inhibit IL-31-mediated pSTAT signaling in a cell-based assay. In one embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable and/or framework region(s) of the parent antibody. For example, the variant may comprise at least one, e.g. from about one to about ten, and preferably from about two to about five, substitutions in one or more hypervariable and/or framework regions of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 50% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, more preferably at least 65%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95% sequence identity. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind an IL-31 and preferably has desired activities which are superior to those of the parent antibody. For example, the variant may have a stronger binding affinity, enhanced ability to reduce, inhibit or neutralize IL-31 activity in an animal, and/or enhanced ability to inhibit IL-31-mediated pSTAT signaling in a cell-based assay.

[0051] A "variant" nucleic acid, refers herein to a molecule which differs in sequence from a "parent" nucleic acid. Polynucleotide sequence divergence may result from mutational changes such as deletions, substitutions, or additions of one or more nucleotides. Each of these changes may occur alone or in combination, one or more times in a given sequence.

[0052] The "parent" antibody herein is one that is encoded by an amino acid sequence used for the preparation of the variant. Preferably, the parent antibody has a canine framework

region and, if present, has canine antibody constant region(s). For example, the parent antibody may be a caninized or canine antibody. As another example, the parent antibody may be a felinized or feline antibody. As yet another example, the parent antibody is a murine monoclonal antibody.

[0053] The term "isolated" means that the material (e.g., antibody or nucleic acid) is separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the material, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. With respect to nucleic acid, an isolated nucleic acid may include one that is separated from the 5' to 3' sequences with which it is normally associated in the chromosome. In preferred embodiments, the material will be purified to greater than 95% by weight of the material, and most preferably more than 99% by weight. Isolated material includes the material *in situ* within recombinant cells since at least one component of the material's natural environment will not be present. Ordinarily, however, isolated material will be prepared by at least one purification step.

[0054] The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody or nucleic acid. The label may itself be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

[0055] The terms "nucleic acid", "polynucleotide", "nucleic acid molecule" and the like may be used interchangeably herein and refer to a series of nucleotide bases (also called "nucleotides") in DNA and RNA. The nucleic acid may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. The term "nucleic acid" includes, for example, single-stranded and double-stranded molecules. A nucleic acid can be, for example, a gene or gene fragment, exons, introns, a DNA molecule (e.g., cDNA), an RNA molecule (e.g., mRNA), recombinant nucleic acids, plasmids, and other vectors, primers and probes. Both 5' to 3' (sense) and 3' to 5' (antisense) polynucleotides are included.

[0056] A "subject" or "patient" refers to an animal in need of treatment that can be affected by molecules of the invention. Animals that can be treated in accordance with the invention include vertebrates, with mammals such as canine, feline, and equine animals being particularly preferred examples.

[0057] A "therapeutically effective amount" (or "effective amount") refers to an amount of an active ingredient, e.g., an agent according to the invention, sufficient to effect beneficial or desired results when administered to a subject or patient. An effective amount can be administered in one or more administrations, applications or dosages. A therapeutically effective amount of a composition according to the invention may be readily determined by one of ordinary skill in the art. In the context of this invention, a "therapeutically effective amount" is one that produces an objectively measured change in one or more parameters associated with

treatment of a pruritic condition or an allergic condition including clinical improvement in symptoms. Of course, the therapeutically effective amount will vary depending upon the particular subject and condition being treated, the weight and age of the subject, the severity of the disease condition, the particular compound chosen, the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which can readily be determined by one of ordinary skill in the art.

[0058] As used herein, the term "therapeutic" encompasses the full spectrum of treatments for a disease or disorder. A "therapeutic" agent of the invention may act in a manner that is prophylactic or preventive, including those that incorporate procedures designed to target animals that can be identified as being at risk (pharmacogenetics); or in a manner that is ameliorative or curative in nature; or may act to slow the rate or extent of the progression of at least one symptom of a disease or disorder being treated.

[0059] "Treatment", "treating", and the like refers to both therapeutic treatment and prophylactic or preventative measures. Animals in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. The term "treatment" or "treating" of a disease or disorder includes preventing or protecting against the disease or disorder (that is, causing the clinical symptoms not to develop); inhibiting the disease or disorder (i.e., arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder (i.e., causing the regression of clinical symptoms). As will be appreciated, it is not always possible to distinguish between "preventing" and "suppressing" a disease or disorder since the ultimate inductive event or events may be unknown or latent. Accordingly, the term "prophylaxis" will be understood to constitute a type of "treatment" that encompasses both "preventing" and "suppressing." The term "treatment" thus includes "prophylaxis".

[0060] The term "allergic condition" is defined herein as a disorder or disease caused by an interaction between the immune system and a substance foreign to the body. This foreign substance is termed "an allergen". Common allergens include aeroallergens, such as pollens, dust, molds, dust mite proteins, injected saliva from insect bites, etc. Examples of allergic conditions include, but are not limited to, the following: allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstructive pulmonary disease, and inflammatory processes resulting from autoimmunity, such as Irritable bowel syndrome (IBS).

[0061] The term "pruritic condition" is defined herein as a disease or disorder characterized by an intense itching sensation that produces the urge to rub or scratch the skin to obtain relief. Examples of pruritic conditions include, but are not limited to the following: atopic dermatitis, eczema, psoriasis, scleroderma, and pruritus.

[0062] As used herein, the terms "cell", "cell line", and "cell culture" may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or

inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell (e.g., bacterial cells, yeast cells, mammalian cells, and insect cells) whether located *in vitro* or *in vivo.* For example, host cells may be located in a transgenic animal. Host cell can be used as a recipient for vectors and may include any transformable organism that is capable of replicating a vector and/or expressing a heterologous nucleic acid encoded by a vector.

[0063] A "composition" is intended to mean a combination of active agent and another compound or composition which can be inert (e.g., a label), or active, such as an adjuvant.

[0064] As defined herein, pharmaceutically acceptable carriers suitable for use in the invention are well known to those of skill in the art. Such carriers include, without limitation, water, saline, buffered saline, phosphate buffer, alcoholic/aqueous solutions, emulsions or suspensions. Other conventionally employed diluents, adjuvants and excipients, may be added in accordance with conventional techniques. Such carriers can include ethanol, polyols, and suitable mixtures thereof, vegetable oils, and injectable organic esters. Buffers and pH adjusting agents may also be employed. Buffers include, without limitation, salts prepared from an organic acid or base. Representative buffers include, without limitation, organic acid salts, such as salts of citric acid, e.g., citrates, ascorbic acid, gluconic acid, histidine-HCl, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid, Tris, trimethanmine hydrochloride, or phosphate buffers. Parenteral carriers can include sodium chloride solution, Ringer's dextrose, dextrose, trehalose, sucrose, and sodium chloride, lactated Ringer's or fixed oils. Intravenous carriers can include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose and the like. Preservatives and other additives such as, for example, antimicrobials, antioxidants, chelating agents (e.g., EDTA), inert gases and the like may also be provided in the pharmaceutical carriers. The preparation of these pharmaceutically acceptable compositions, from the above-described components, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art. See, e.g., texts such as Remington: The Science and Practice of Pharmacy, 20th ed, Lippincott Williams & Wilkins, publ., 2000; and The Handbook of Pharmaceutical Excipients, 4.sup.th edit., eds. R. C. Rowe et al, APhA Publications, 2003.

[0065] The term "conservative amino acid substitution" indicates any amino acid substitution for a given amino acid residue, where the substitute residue is so chemically similar to that of the given residue that no substantial decrease in polypeptide function (e.g., enzymatic activity) results. Conservative amino acid substitutions are commonly known in the art and examples thereof are described, e.g., in U.S. Pat. Nos. 6,790,639, 6,774,107, 6,194,167, or 5,350,576. In a preferred embodiment, a conservative amino acid substitution will be any one that occurs within one of the following six groups

- 1. Small aliphatic, substantially non-polar residues: Ala, Gly, Pro, Ser, and Thr;
- 2. Large aliphatic, non-polar residues: Ile, Leu, and Val; Met;
- 3. Polar, negatively charged residues and their amides: Asp and Glu;
- 4. Amides of polar, negatively charged residues: Asn and Gln; His;

- 5. Polar, positively charged residues: Arg and Lys; His; and
- 6. Large aromatic residues: Trp and Tyr; Phe.

In a preferred embodiment, a conservative amino acid substitution will be any one of the following, which are listed as Native Residue (Conservative Substitutions) pairs: Ala (Ser); Arg (Lys); Asn (Gln; His); Asp (Glu); Gln (Asn); Glu (Asp); Gly (Pro); His (Asn; Gln); lie (Leu; Val); Leu (Ile; Val); Lys (Arg; Gln; Glu); Met (Leu; Ile); Phe (Met; Leu; Tyr); Ser (Thr); Thr (Ser); Trp (Tyr); Tyr (Trp; Phe); and Val (Ile; Leu).

[0066] Just as a polypeptide may contain conservative amino acid substitution(s), a polynucleotide hereof may contain conservative codon substitution(s). A codon substitution is considered conservative if, when expressed, it produces a conservative amino acid substitution, as described above. Degenerate codon substitution, which results in no amino acid substitution, is also useful in polynucleotides according to the present invention. Thus, e.g., a polynucleotide encoding a selected polypeptide useful in an embodiment of the present invention may be mutated by degenerate codon substitution in order to approximate the codon usage frequency exhibited by an expression host cell to be transformed therewith, or to otherwise improve the expression thereof.

DETAILED DESCRIPTION OF THE INVENTION

[0067] The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[0068] Unless otherwise defined, scientific and technical terms used in connection with the antibodies described herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art.

[0069] Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transfection (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification, See e.g., Sambrook et al. MOLECULAR CLONING: LAB. MANUAL (3rd ed., Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y., 2001) and Ausubel et al. Current Protocols in Molecular Biology (New York: Greene Publishing Association/Wiley Interscience), 1993. The nomenclatures utilized in connection with, and the

laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0070] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about."

[0071] The present invention provides for recombinant monoclonal antibodies as defined in the claims and their uses in clinical and scientific procedures, including diagnostic procedures.

[0072] With the advent of methods of molecular biology and recombinant technology, it is possible to produce antibody and antibody-like molecules by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with assembly of the synthesized chains to form active tetrameric (H_2L_2) structures with affinity for specific epitopes and antigenic determinants. This has permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

[0073] Regardless of the source of the antibodies, or how they are recombinantly constructed, or how they are synthesized, *in vitro* or *in vivo,* using transgenic animals, large cell cultures of laboratory or commercial size, using transgenic plants, or by direct chemical synthesis employing no living organisms at any stage of the process, all antibodies have a similar overall 3 dimensional structure. This structure is often given as H_2L_2 and refers to the fact that antibodies commonly comprise two light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity. The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets.

[0074] As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody binding region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues.

[0075] Within the variable regions of the H or L chains that provide for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the

basic specificity of the antibody for a particular antigenic determinant structure.

[0076] The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have three CDR regions, each non-contiguous with the others.

[0077] In all mammalian species, antibody peptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

[0078] Regarding the antigenic determinate recognized by the CDR regions of the antibody, this is also referred to as the "epitope." In other words, epitope refers to that portion of any molecule capable of being recognized by, and bound by, an antibody (the corresponding antibody binding region may be referred to as a paratope).

[0079] An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce an antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0080] The term "antibody" is meant to include both intact immunoglobulin molecules as well as portions, fragments, peptides and derivatives thereof such as, for example, Fab, Fab', $F(ab')_2$, Fv, Fse, CDR regions, paratopes, or any portion or peptide sequence of the antibody that is capable of binding an antigen or epitope. An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody.

[0081] Antibody also includes chimeric antibodies, heterochimeric antibodies, caninized antibodies, or felinized antibodies, as well as fragments, portions, regions, peptides or derivatives thereof, provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis, or recombinant techniques. Such antibodies of the present invention are capable of specifically binding canine IL-31. Antibody fragments or portions may lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Examples of antibody fragments may be produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). See, e.g., Wahl et al., 24 J. Nucl. Med. 316-25 (1983). Portions of antibodies may be made by any of the above methods, or may be made by expressing a portion of the recombinant molecule. For example, the CDR region(s) of a recombinant

antibody may be isolated and subcloned into the appropriate expression vector. See, e.g., U.S. Pat. No. 6,680,053.

Clones 11E12, 34D03 and 19D07 Nucleotide and Amino Acid Sequences

[0082] The present invention provides for novel monoclonal antibodies that specifically bind to canine IL-31 encoded by the nucleotide sequence of SEQ ID NO:33, wherein said antibody or antigen-binding portion thereof inhibits IL-31 activity in a dog and inhibits dog IL-31-mediated pSTAT signaling in a cell based assay. In one embodiment, a monoclonal antibody of the invention binds to canine IL-31 and prevents its binding to, and activation of, its co-receptor complex comprising IL-31 receptor A (IL-31Ra) and Oncostatin-M-specific receptor (OsmR or IL-31Rb). The monoclonal antibodies of the present invention are identified herein as "11E12", "34D03" and "19D07", which refers to the number assigned to its hybridoma clone. Herein, "11E12", "34D03", or "19D07" also refers to the portion of the monoclonal antibody, the paratope or CDRs, that bind specifically with an IL-31 epitope identified as 11E12, 34D03, or 19D07 because of its ability to bind the 11E12, 34D03, or 19D07 antibodies, respectively. The several recombinant, chimeric, heterochimeric, caninized and/or felinized forms of 11E12, 34D03 and 19D07 described herein may be referred to by the same name.

[0083] In one embodiment, the present invention provides an isolated antibody or antigen-binding portion thereof including at least one of the following:

a variable heavy (V_H) chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1), or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2), or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3), or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0084] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof including at least one of the following group:

a variable light (V_L) chain comprising a complementary determining region (CDR) 1 having the amino acid sequence RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) or KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);

a variable light chain CDR2 having the amino acid sequence RASNLES (SEQ ID NO: 13;

11E12-VL-CDR2), or RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);

a variable light chain CDR3 having the amino acid sequence QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3), or QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0085] In still other embodiments, an antibody having at least one of the variable light chain CDRs described above, can further include at least one of the following variable heavy chain CDRs:

a variable heavy chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1) or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2) or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3) or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0086] In some embodiments, the antibody can include at least one of the following:

1. a) a variable light chain comprising
   DIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMHWYQQKPGQPPKLLIYRASNLESGIPA
   RFSGSGSRTDFTLTINPVETDDVATYYCQQSNKDPLTFGAGTKLELK (SEQ ID NO: 19; MU-11E12-VL),

   DIVMTQTPLSLSVSPGEPASISCRASESVDNYGISFMHWYQQKPGQPPKLLIYRASNLESGVPD
   RFSGSGSGTDFTLRISRVEADDAGVYYCQQSNKDPLTFGAGTKLEIK (SEQ ID NO: 20; CAN-11E12-VL-cUn-FW2),

   DIVMTQTPLSLSVSPGEPASISCRASESVDNYGISFMHWFQQKPGQSPQLLIYRASNLESGVPD
   RFSGSGSGTDFTLRISRVEADDAGVYYCQQSNKDPLTFGAGTKLEIK (SEQ ID NO: 21; CAN-11E12-VL-cUn-13),

   DILLTQSPASLAVSLGQRAIISCKASQSVSFAGTGLMHWYQQKPGQQPKLLIYRASNLEAGVPT
   RFSGSGSRTDFTLNIHPVEEEDAATYFCQQSREYPWTFGGGTKLEIK (SEQ ID NO: 24; MU-34D03-VL),

or
EIVMTQSPASLSLSQEEKVTITCKASQSVSFAGTGLMHWYQQKPGQAPKLLIYRASNLEAGVPS
RFSGSGSGTDFSFTISSLEPEDVAVYYCQQSREYPWTFGQGTKLEIK (SEQ ID NO: 25; CAN-34D03-VL-998-1);

2. b) a variable heavy chain comprising
QVQLQQSGAELVKPGASVKLSCKASGYTFKYYDINWVRQRPEQGLEWIGWIFPGDGGTKYNE
TFKGKATLTTDKSSSTAYMQLSRLTSEDSAVYFCARGGTSVIRDAMDYWGQGTSVTVSS
(SEQ ID NO: 26; MU-11E12-VH),

EVQLVQSGAEVKKPGASVKVSCKTSGYTFKYYDINWVRQAPGAGLDWMGWIFPGDGGTKYN
ETFKGRVTLTADTSTSTAYMELSSLRAGDIAVYYCARGGTSVIRDAMDYWGQGTLVTVSS
(SEQ ID NO: 27; CAN-11E12-VH-415-1),

EVQLVESGGDLVKPGGSLKLSCAASGFSFSNYGMSWVRQTPDKRLEWVATISYGGSYTYYPD
NIKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCVRGYGYDTMDYWGQGTSVTVSS (SEQ ID NO: 30; MU-34D03-VH),

or
EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYGMSWVRQAPGKGLQWVATISYGGSYTYYP
DNIKGRFTISRDNAKNTLYLQMNSLRAEDTAMYYCVRGYGYDTMDYWGQGTLVTVSS (SEQ ID NO: 31; CAN-34D03-VH-568-1);

and

3. c) variants thereof having one or more conservative amino acid substitutions.

[0087] In other embodiments, the invention provides a host cell that produces an antibody described above.

[0088] The present invention also includes, within its scope, nucleotide sequences encoding the variable regions of the light and heavy chains of the anti-IL-31 antibody of the present invention. Included also within the scope of the invention is any nucleotide sequence that encodes the or amino acid sequence of 11E12 or 34D03

[0089] In some embodiments, the invention provides an isolated nucleic acid including a nucleic acid sequence encoding at least one of the following:

a variable heavy (V_H) chain complementary determining region (CDR)1 having the amino acid sequence YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1) or NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);

a variable heavy chain CDR2 having the amino acid sequence WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2) or TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

a variable heavy chain CDR3 having the amino acid sequence ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3), or VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0090] In further embodiments, the isolated nucleic acid described above may further include a nucleic acid sequence encoding at least one of the following:

a variable light (V_L) chain comprising a complementary determining (CDR) 1 having the amino acid sequence RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) or KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);

a variable light chain CDR2 having the amino acid sequence RASNLES (SEQ ID NO: 13; 11E12-VL-CDR2),or RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);

a variable light chain CDR3 having the amino acid sequence QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3), or QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); and

a variant thereof having one or more conservative amino acid substitutions in at least one of CDR1, CDR2, or CDR3.

[0091] The present invention further provides a vector including at least one of the nucleic acids described above.

[0092] Because the genetic code is degenerate, more than one codon can be used to encode a particular amino acid. Using the genetic code, one or more different nucleotide sequences can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual XXX-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an anti-IL-31 antibody or portion. Such "codon usage rules" are disclosed by Lathe, et al., 183 J. Molec. Biol. 1-12 (1985). Using the "codon usage rules" of Lathe, a single nucleotide sequence, or a set of nucleotide sequences, that contains a theoretical "most probable" nucleotide sequence capable of encoding anti-IL-31 sequences can be identified. It is also intended that the antibody coding regions for use in the present invention could also be provided by altering existing antibody genes using standard molecular biological techniques that result in variants (agonists) of the antibodies and peptides described herein. Such variants include, but are not limited to deletions, additions and substitutions in the amino acid sequence of the anti-IL-31 antibodies or peptides.

[0093] For example, one class of substitutions is conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in an anti-IL-31 antibody peptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the

replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg, replacements among the aromatic residues Phe, Tyr, and the like. Guidance concerning which amino acid changes are likely to be phenotypically silent is found in Bowie et al., 247 Science 1306-10 (1990).

[0094] Variant or agonist anti-IL-31 antibodies or peptides may be fully functional or may lack function in one or more activities. Fully functional variants typically contain only conservative variations or variations in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

[0095] Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. Cunningham et al., 244 Science 1081-85 (1989). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as epitope binding or in vitro ADCC activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallography, nuclear magnetic resonance, or photoaffinity labeling. Smith et al., 224 J. Mol. Biol. 899-904 (1992); de Vos et al., 255 Science 306-12 (1992).

[0096] Moreover, polypeptides often contain amino acids other than the twenty "naturally occurring" amino acids. Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques well known in the art. Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, *gamma* carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0097] Such modifications are well known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as Proteins--Structure and Molecular Properties (2nd ed., T. E. Creighton, W.H. Freeman & Co., NY, 1993). Many

detailed reviews are available on this subject, such as by Wold, Posttranslational Covalent Modification of proteins, 1-12 (Johnson, ed., Academic Press, NY, 1983); Seifter et al. 182 Meth. Enzymol. 626-46 (1990); and Rattan et al. 663 Ann. NY Acad. Sci. 48-62 (1992).

[0098] Accordingly, the antibodies and peptides of the present invention also encompass derivatives or analogs in which a substituted amino acid residue is not one encoded by the genetic code.

[0099] Similarly, the additions and substitutions in the amino acid sequence as well as variations, and modifications just described may be equally applicable to the amino acid sequence of the IL-31 antigen and/or epitope or peptides thereof, and are thus encompassed by the present invention. As mentioned above, the genes encoding a monoclonal antibody according to the present invention is specifically effective in the recognition of IL-31.

Antibody Derivatives

[0100] Included within the scope of this invention are antibody derivatives. A "derivative" of an antibody contains additional chemical moieties not normally a part of the protein. Covalent modifications of the protein are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. For example, derivatization with bifunctional agents, well-known in the art, is useful for cross-linking the antibody or fragment to a water-insoluble support matrix or to other macromolecular carriers.

[0101] Derivatives also include radioactively labeled monoclonal antibodies that are labeled. For example, with radioactive iodine (^{125}I,^{131}I), carbon (^{14}C), sulfur (^{35}S), indium (^{111}In), tritium (^{3}H) or the like; conjugates of monoclonal antibodies with biotin or avidin, with enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-D-galactosidase, glucose oxidase, glucoamylase, carboxylic acid anhydrase, acetylcholine esterase, lysozyme, malate dehydrogenase or glucose 6-phosphate dehydrogenase; and also conjugates of monoclonal antibodies with bioluminescent agents (such as luciferase), chemoluminescent agents (such as acridine esters) or fluorescent agents (such as phycobiliproteins).

[0102] Another derivative bifunctional antibody of the present invention is a bispecific antibody, generated by combining parts of two separate antibodies that recognize two different antigenic groups. This may be achieved by crosslinking or recombinant techniques. Additionally, moieties may be added to the antibody or a portion thereof to increase half-life *in vivo* (e.g., by lengthening the time to clearance from the blood stream. Such techniques include, for example, adding PEG moieties (also termed pegilation), and are well-known in the art. See U.S. Patent. Appl. Pub. No. 20030031671.

Recombinant Expression of Antibodies

[0103] In some embodiments, the nucleic acids encoding a subject monoclonal antibody are introduced directly into a host cell, and the cell is incubated under conditions sufficient to induce expression of the encoded antibody. After the subject nucleic acids have been introduced into a cell, the cell is typically incubated, normally at 37°C, sometimes under selection, for a period of about 1-24 hours in order to allow for the expression of the antibody. In one embodiment, the antibody is secreted into the supernatant of the media in which the cell is growing.

[0104] Traditionally, monoclonal antibodies have been produced as native molecules in murine hybridoma lines. In addition to that technology, the present invention provides for recombinant DNA expression of monoclonal antibodies. This allows the production of caninized antibodies, as well as a spectrum of antibody derivatives and fusion proteins in a host species of choice.

[0105] A nucleic acid sequence encoding at least one anti-IL-31 antibody, portion or polypeptide of the present invention may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Maniatis et al., MOLECULAR CLONING, LAB. MANUAL, (Cold Spring Harbor Lab. Press, NY, 1982 and 1989), and Ausubel et al. 1993 *supra,* may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule or antigen binding region thereof.

[0106] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as anti-IL-31 peptides or antibody portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook et al., 2001 *supra;* Ausubel et al., 1993 *supra.*

[0107] The present invention accordingly encompasses the expression of an anti-IL-31 antibody or peptide, in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and mammalian cells either *in vivo,* or *in situ*, or host cells of mammalian, insect, bird or yeast origin. The mammalian cell or tissue may be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used.

[0108] In one embodiment, the introduced nucleotide sequence will be incorporated into a

plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. See, e.g., Ausubel et al., 1993 *supra.* Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0109] Example prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, .pi.VX). Such plasmids are, for example, disclosed by Maniatis et al., 1989 *supra;* Ausubel et al, 1993 *supra.* Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, in THE MOLEC. BIO. OF THE BACILLI 307-329 (Academic Press, NY, 1982). Suitable Streptomyces plasmids include plJ101 (Kendall et al., 169 J. Bacteriol. 4177-83 (1987)), and Streptomyces bacteriophages such as .phi.C31 (Chater et al., in SIXTH INT'L SYMPOSIUM ON ACTINOMYCETALES BIO. 45-54 (Akademiai Kaido, Budapest, Hungary 1986). Pseudomonas plasmids are reviewed in John et al., 8 Rev. Infect. Dis. 693-704 (1986); Izaki, 33 Jpn. J. Bacteriol. 729-42 (1978); and Ausubel et al., 1993 *supra.*

[0110] Alternatively, gene expression elements useful for the expression of cDNA encoding anti-IL-31antibodies or peptides include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter (Okayama et al., 3 Mol. Cell. Biol. 280 (1983)), Rous sarcoma virus LTR (Gorman et al., 79 Proc. Natl. Acad. Sci., USA 6777 (1982)), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885 (1985)); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayarea et al., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983).

[0111] Immunoglobulin cDNA genes can be expressed as described by Weidle et al., 51 Gene 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

[0112] For immunoglobulin genes comprised of part cDNA, part genomic DNA (Whittle et al., 1 Protein Engin. 499 (1987)), the transcriptional promoter can be human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions can be the native chromosomal immunoglobulin sequences.

[0113] In one embodiment, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the

immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[0114] Each fused gene can be assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the chimeric immunoglobulin chain gene product are then transfected singly with an anti-IL-31 peptide or chimeric H or chimeric L chain-encoding gene, or are co-transfected with a chimeric H and a chimeric L chain gene. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

[0115] In one embodiment, the fused genes encoding the anti-IL-31 peptide or chimeric H and L chains, or portions thereof are assembled in separate expression vectors that are then used to co-transfect a recipient cell. Alternatively the fused genes encoding the chimeric H and L chains can be assembled on the same expression vector.

[0116] For transfection of the expression vectors and production of the chimeric antibody, the recipient cell line may be a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid. Other suitable recipient cells include lymphoid cells such as B lymphocytes of human or non-human origin, hybridoma cells of human or non-human origin, or interspecies heterohybridoma cells.

[0117] The expression vector carrying a chimeric, or caninized antibody construct or anti-IL-31 polypeptide of the present invention can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment. Johnston et al., 240 Science 1538 (1988).

[0118] Yeast can provide substantial advantages over bacteria for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides). Hitzman et al., 11th Int'l Conference on Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982).

[0119] Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of anti-IL-31 peptides, antibody and assembled murine and chimeric,

heterochimeric, caninized, or felinized antibodies, fragments and regions thereof. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast. See Vol. II DNA Cloning, 45-66, (Glover, ed.,) IRL Press, Oxford, UK 1985).

[0120] Bacterial strains can also be utilized as hosts for the production of antibody molecules described by this invention. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of murine, chimeric, heterochimeric, caninized or felinized antibodies, fragments and regions or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, 1985 *supra;* Ausubel, 1993 *supra;* Sambrook, 2001 *supra;* Colligan et al., eds. Current Protocols in Immunology, John Wiley & Sons, NY, NY (1994-2001); Colligan et al., eds. Current Protocols in Protein Science, John Wiley & Sons, NY, NY (1997-2001).

[0121] Host mammalian cells may be grown *in vitro* or *in vivo.* Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

[0122] Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61) cells.

[0123] Many vector systems are available for the expression of cloned anti-IL-31 peptides H and L chain genes in mammalian cells (see Glover, 1985 *supra).* Different approaches can be followed to obtain complete H_2L_2 antibodies. It is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H_2L_2 antibodies and/or anti-IL-31 peptides. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and L chains and/or anti-IL-31 peptides can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an H chain plasmid containing a second selectable marker. Cell lines producing anti-IL-31 peptides and/or H_2L_2 molecules *via* either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties,

such as higher production of assembled H_2L_2 antibody molecules or enhanced stability of the transfected cell lines.

[0124] For long-term, high-yield production of recombinant antibodies, stable expression may be used. For example, cell lines, which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with immunoglobulin expression cassettes and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and grow to form foci which in turn can be cloned and expanded into cell lines. Such engineered cell lines may be particularly useful in screening and evaluation of compounds/components that interact directly or indirectly with the antibody molecule.

[0125] Once an antibody of the invention has been produced, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In many embodiments, antibodies are secreted from the cell into culture medium and harvested from the culture medium.

Pharmaceutical Applications

[0126] The anti-IL-31 antibodies or peptides of the present invention can be used for example in the treatment of pruritic and/or allergic conditions in More specifically, the invention further provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody according to the invention. The antibody can be a chimeric, heterochimeric, or caninized antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are also envisioned. The antibody and pharmaceutical compositions thereof of this invention are useful for parenteral administration, e.g., subcutaneously, intramuscularly or intravenously.

[0127] Anti-IL-31 antibodies of the present invention can be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

[0128] Administration of the antibodies disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), orally, or by topical administration of the antibodies (typically carried in a pharmaceutical formulation) to an airway surface. Topical administration to an airway surface

can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler). Topical administration of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed. Oral administration may be, for example, in the form of an ingestable liquid or solid formulation.

[0129] In some desired embodiments, the antibodies are administered by parenteral injection. For parenteral administration, anti-IL-31 antibodies or peptides can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. For example the vehicle may be a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, such as an aqueous carrier such vehicles are water, saline, Ringer's solution, dextrose solution, trehalose or sucrose solution, or 5% serum albumin, 0.4% saline, 0.3% glycine and the like. Liposomes and nonaqueous vehicles such as fixed oils can also be used. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example 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 fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

[0130] Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, REMINGTON'S PHARMA. SCI. (15th ed., Mack Pub. Co., Easton, Pa., 1980).

[0131] The antibodies of this 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 immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss and that use levels may have to be adjusted to compensate.

[0132] The compositions containing the present antibodies or a cocktail thereof can be administered for prevention of recurrence and/or therapeutic treatments for existing disease. Suitable pharmaceutical carriers are described in the most recent edition of REMINGTON'S PHARMACEUTICAL SCIENCES, a standard reference text in this field of art.

[0133] In therapeutic application, compositions are administered to a subject already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose" or a "therapeutically effective amount". Amounts effective for this use will depend upon the severity of the disease and the general state of the subject's own immune system, but generally range from about 0.1 mg antibody per kg body weight to about 10 mg antibody per kg body weight, preferably about 0.3 mg antibody per kg of body weight to about 5 mg of antibody per kg of body weight. In view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present canine-like antibodies of this invention, it may be possible to administer substantial excesses of these antibodies.

[0134] The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms kind of concurrent treatment, frequency of treatment, and the effect desired.

[0135] As a non-limiting example, treatment of IL-31-related pathologies in dogs can be provided as a biweekly or monthly dosage of anti-IL-31 antibodies of the present invention in the dosage range described above.

[0136] Example antibodies for canine therapeutic use are high affinity (these may also be high avidity) antibodies, and fragments, regions and derivatives thereof having potent *in vivo* anti-IL-31 activity, according to the present invention.

[0137] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating veterinarian. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the subject.

Diagnostic Applications

[0138] The present invention also provides the above anti-IL-31 antibodies for use in diagnostic methods for detecting IL-31 in companion animals known to be or suspected of having a puritic and/or allergic condition.

[0139] Anti-IL-31 antibodies of the present invention are useful for immunoassays which detect or quantitate IL-31, or anti-IL-31 antibodies, in a sample. An immunoassay for IL-31 typically comprises incubating a clinical or biological sample in the presence of a detectably labeled high affinity (or high avidity) anti-IL-31 antibody or polypeptide of the present invention capable of selectively binding to IL-31, and detecting the labeled peptide or antibody which is bound in a sample. Various clinical assay procedures are well known in the art. See, e.g.,

IMMUNOASSAYS FOR THE 80'S (Voller et al., eds., Univ. Park, 1981). Such samples include tissue biopsy, blood, serum, and fecal samples, or liquids collected from animal subjects and subjected to ELISA analysis as described below.

[0140] In some embodiments, the binding of antigen to antibody is detected without the use of a solid support. For example, the binding of antigen to antibody can be detected in a liquid format.

[0141] In other embodiments, an anti-IL-31 antibody can, for example, be fixed to nitrocellulose, or another solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled IL-31-specific peptide or antibody. The solid phase support can then be washed with the buffer a second time to remove unbound peptide or antibody. The amount of bound label on the solid support can then be detected by known method steps.

[0142] "Solid phase support" or "carrier" refers to any support capable of binding peptide, antigen, or antibody. Well-known supports or carriers, include glass, polystyrene, polypropylene, polyethylene, polyvinylidenefluoride (PVDF), dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material can have virtually any possible structural configuration so long as the coupled molecule is capable of binding to IL-31 or an anti-IL-31 antibody. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface can be flat, such as a sheet, culture dish, test strip, etc. For example, supports may include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody, peptide or antigen, or can ascertain the same by routine experimentation.

[0143] Well known method steps can determine binding activity of a given lot of anti-IL-31 peptide and/or antibody. Those skilled in the art can determine operative and optimal assay conditions by routine experimentation.

[0144] Detectably labeling an IL-31-specific peptide and/or antibody can be accomplished by linking to an enzyme for use in an enzyme immunoassay (EIA), or enzyme-linked immunosorbent assay (ELISA). The linked enzyme reacts with the exposed substrate to generate a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the IL-31-specific antibodies of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

[0145] By radioactively labeling the IL-31-specific antibodies, it is possible to detect IL-31 through the use of a radioimmunoassay (RIA). See Work et al., LAB. TECHNIQUES & BIOCHEM. 1N MOLEC. Bio. (No. Holland Pub. Co., NY, 1978). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention include: ^{3}H, ^{125}I, ^{131}I, ^{35}S, ^{14}C, and ^{125}I.

[0146] It is also possible to label the IL-31-specific antibodies with a fluorescent compound. When the fluorescent labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

[0147] The IL-31-specific antibodies can also be delectably labeled using fluorescence-emitting metals such a ^{125}Eu, or others of the lanthanide series. These metals can be attached to the IL-31-specific antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediamine-tetraacetic acid (EDTA).

[0148] The IL-31-specific antibodies also can be detectably labeled by coupling to a chemiluminescent compound. The presence of the chemiluminescently labeled antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0149] Likewise, a bioluminescent compound can be used to label the IL-31-specific antibody, portion, fragment, polypeptide, or derivative of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

[0150] Detection of the IL-31-specific antibody, portion, fragment, polypeptide, or derivative can be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorometric methods which employ a substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0151] For the purposes of the present invention, the IL-31 which is detected by the above assays can be present in a biological sample. Any sample containing IL-31 may be used. For example, the sample is a biological fluid such as, for example, blood, serum, lymph, urine, feces, inflammatory exudate, cerebrospinal fluid, amniotic fluid, a tissue extract or homogenate, and the like.

[0152] *In situ* detection can be accomplished by removing a histological specimen from an animal subject, and providing the combination of labeled antibodies of the present invention to such a specimen. The antibody (or portion thereof) may be provided by applying or by overlaying the labeled antibody (or portion) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of IL-31 but also the distribution of IL-31 in the examined tissue. Those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

[0153] The antibody, fragment or derivative of the present invention can be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support that is insoluble in the fluid being tested and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantification of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

[0154] The antibodies may be used to quantitatively or qualitatively detect the IL-31 in a sample or to detect presence of cells that express the IL-31. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with fluorescence microscopy, flow cytometric, or fluorometric detection. For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the antibody, such as antibodies specific for canine or feline immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays, such as those discussed previously are available and are well known to those skilled in the art.

[0155] In one embodiment, the diagnostic method for detecting IL-31 is a lateral flow immunoassay test. This is also known as the immunochromatographic assay, Rapid ImmunoMigration (RIM™) or strip test. Lateral flow immunoassays are essentially immunoassays adapted to operate along a single axis to suit the test strip format. A number of variations of the technology have been developed into commercial products, but they all operate according to the same basic principle. A typical test strip consists of the following components: (1) sample pad - an absorbent pad onto which the test sample is applied; (2) conjugate or reagent pad - this contains antibodies specific to the target analyte conjugated to colored particles (usually colloidal gold particles, or latex microspheres); (3) reaction membrane - typically a hydrophobic nitrocellulose or cellulose acetate membrane onto which anti-target analyte antibodies are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing antibodies specific for the conjugate antibodies); and (4) wick or waste reservoir - a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it. The components of the strip are usually fixed to an inert backing material and may be presented in

a simple dipstick format or within a plastic casing with a sample port and reaction window showing the capture and control zones.

[0156] There are two main types of lateral flow immunoassay used in microbiological testing: double antibody sandwich assays and competitive assays. In the double antibody sandwich format, the sample migrates from the sample pad through the conjugate pad where any target analyte present will bind to the conjugate. The sample then continues to migrate across the membrane until it reaches the capture zone where the target/conjugate complex will bind to the immobilized antibodies producing a visible line on the membrane. The sample then migrates further along the strip until it reaches the control zone, where excess conjugate will bind and produce a second visible line on the membrane. This control line indicates that the sample has migrated across the membrane as intended. Two clear lines on the membrane is a positive result. A single line in the control zone is a negative result. Competitive assays differ from the double antibody sandwich format in that the conjugate pad contains antibodies that are already bound to the target analyte, or to an analogue of it. If the target analyte is present in the sample it will therefore not bind with the conjugate and will remain unlabelled. As the sample migrates along the membrane and reaches the capture zone an excess of unlabelled analyte will bind to the immobilized antibodies and block the capture of the conjugate, so that no visible line is produced. The unbound conjugate will then bind to the antibodies in the control zone producing a visible control line. A single control line on the membrane is a positive result. Two visible lines in the capture and control zones is a negative result. However, if an excess of unlabelled target analyte is not present, a weak line may be produced in the capture zone, indicating an inconclusive result. There are a number of variations on lateral flow technology. The capture zone on the membrane may contain immobilized antigens or enzymes - depending on the target analyte - rather than antibodies. It is also possible to apply multiple capture zones to create a multiplex test. For example, commercial test strips able to detect both EHEC Shiga toxins ST1 and ST2 separately in the same sample have been developed.

[0157] Importantly, the antibodies of the present invention may be helpful in diagnosing a pruritic and/or allergic in dogs. More specifically, the antibody of the present invention may identify the overexpression of IL-31 in companion animals. Thus, the antibody of the present invention may provide an important immunohistochemistry tool.

[0158] The antibodies of the present invention may be used on antibody arrays, highly suitable for measuring gene expression profiles.

Kits

[0159] Also included within the scope of the present invention are kits for practicing the subject methods. The kitslat least include one or more of the antibodies of the present invention, a nucleic acid encoding the same, or a cell containing the same. In one embodiment, an antibody of the present invention may be provided, usually in a lyophilized form, in a container. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are typically

included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like. Generally, these materials will be present in less than 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1% to 99% wt. of the total composition. Where a second antibody capable of binding to the primary antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above. The kit will generally also include a set of instructions for use.

[0160] In one embodiment, a kit according to the present invention is a test strip kit (lateral flow immunoassay kit) useful for detecting canine IL-31 protein in a sample. Such a test strip will typically include a sample pad onto which the test sample is applied; a conjugate or reagent pad containing an antibody specific to canine IL-31, wherein the antibody is conjugated to colored particles (usually colloidal gold particles); a reaction membrane onto which anti-IL-31 antibodies are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing antibodies specific for the conjugate antibodies); and a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it. The test strip kit will generally also include directions for use.

[0161] The invention will now be described further by the non-limiting examples below.

EXAMPLES

Example 1 Identification of mouse monoclonal antibodies recognizing canine Interleukin 31 (IL-31)

[0162] Recombinant canine IL-31 was created in CHO cells using the CHROMOS ACE (Artificial Chromosome Expression) system (Chromos Molecular Systems, Inc., Burnaby, British Columbia) to generate the secreted canine IL-31 protein having the sequence of SEQ ID NO: 32. This protein is encoded by the nucleotide sequence of SEQ ID NO: 33. Conditioned medium from 400 ml of cell culture (CHO cell line) was obtained and dialyzed against 10 volumes ofQA buffer (20 mM Tris pH 8.0, 20 mM NaCl) for 4.5 hours. Dialyzed medium was 0.2 um filtered and loaded at 1 ml/min onto a SOURCE™ Q column (GE Healthcare, Uppsala, Sweden) pre-equilibrated with QA buffer. Protein was eluted using a multi step linear gradient. The majority of IL-31 remained in the flow through (FT) fraction, a small amount of IL-31 eluted early in the gradient. Identity of the protein was previously confirmed by Western immunoblotting, and Mass-Spectro (MS) analysis of a tryptic digest. Protein in the FT fraction was concentrated 4-5 fold and dialyzed overnight against Phosphate Buffered Saline (PBS) at 4°C. Stability of the protein was checked following dialysis into PBS. No precipitation was

observed, and no proteolysis was observed after several days at 4°C. De-glycosylation experiments using N-glycosidase F resulted in the protein condensing down to a single band of ~15 kDa on SDS-PAGE. Protein concentration was determined using a bicinchoninic assay (BCA assay) with Bovine Serum Albumin (BSA) as a standard (ThermoFisher Scientific, Inc., Rockford, IL). The protein solution was split into aliquots, snap frozen (liquid N_2) and stored at -80°C.

[0163] Mouse monoclonal antibodies were identified using standard immunizations of female CF-1 mice with recombinant canine IL-31 produced in CHO cells. Titers from immunized mice were determined using an enzyme linked immunosorbent assay (ELISA). Canine IL-31 (50 ng/well) was immobilized to polystyrene microplates and used as a capture antigen. Serum from immunized mice was diluted in phosphate buffered saline with 0.05% tween-20 (PBST). The presence of mouse anti-canine IL-31 antibodies was detected with a Horse Radish Peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Kirkegard & Perry Laboratories, Inc. (KPL, Inc.), Gaithersburg, MD). Following addition of a chromogenic substrate (SureBlue Reserve TMB 1-Component Microwell Peroxidase Substrate, KPL, Inc., Gaithersburg, MD) and a ten minute incubation at room temperature (RT) the reaction was stopped with the addition of 100 µL of 0.1 N HCl. The absorbance of each well was determined at an optical density (OD) of 450 nm. Figure 6 summarizes the antibody response of individual mice immunized with canine IL-31. A pool of donor splenocytes from mice 3 and 4 were used for fusion. Following fusion and screening for anti IL-31 binding *via* direct ELISA, 100 wells were chosen for expansion and secondary screening of anti IL-31 activity. Secondary screening confirmed that 81 fusions retained the ability to produce anti IL-31 antibodies. Frozen cell stocks and supernatants from these 81 candidates were preserved for further evaluation.

[0164] To identify candidates with inhibitory activity, all 81 supernatants were assessed for their ability to affect IL-31-mediated pSTAT signaling in a cell-based assay. This cell-based assay measures pSTAT signaling in canine DH-82 monocyte cells pre-treated for 24 hours with canine gamma interferon (R&D Systems, Minneapolis, MN) at 10 ng/mL and serum starved for 2 hours prior to IL-31 treatment to increase IL-31 receptor expression. Following this pre-treatment, recombinant canine IL-31 is added at 1 µg/mL for 5 minutes and STAT phosphorylation is evaluated using the Alpha Screen technology (Perkin Elmer, Waltham, MA). Since antibody concentrations and purity are unknown in hybridoma supernatants, these supernatants were qualitatively measured for their ability to inhibit STAT phosphorylation following a 1 hour co-incubation with 1 µg/ml IL-31 using 1:2 or 1:20 dilutions of the supernatants. This experiment identified 31 supernatants that inhibited >50% of the STAT phosphorylation relative to untreated wells thereby justifying purification and further characterization.

[0165] Following purification and quantitation of each monoclonal antibody (mAb), the IC_{50} values of all 31 antibodies were evaluated in the DH-82 cell assay. Based on the resulting IC_{50} values and competitive ELISAs to define antibody classes based on epitope bins, three antibodies described in Table 1 were moved forward for further characterization, 11E12,

19D07, and 34D03.

TABLE 1

Antibody	HC isotype	LC isotype
11E12	G1/2b	kappa
19D07	2b	kappa
34D03	G1	kappa

Example 2 DNA Sequences Encoding 11E12, 19D07 and 34D03 Antibodies

[0166] Ribonucleic acid (RNA) was isolated from hybridoma cells 11E12, 19D07, and 34D03 using the Rneasy -mini kit (Qiagen, Inc., Germantown, MD) as described by the manufacturer. One million frozen cells from each hybridoma were harvested by centrifugation and RNA was purified from cell lysates using the Rneasy spin column according to method described in the protocol. RNA was eluted from each column and used immediately for quantitation and cDNA preparation. The RNA was analyzed for yield and purity by measuring it's absorbance at 260 nm and 280 nm using a GeneQuant pro spectrophotometer (GE Healthcare, Uppsala, Sweden). Following isolation, the remaining RNA was stored at -80° C for further use.

[0167] Oligonucletide primers designed for amplification of the mouse immunoglobulin (Ig) variable domains were used according to the manufacturer's instructions (EMD Chemicals, Inc., Gibbstown, NJ). cDNA was prepared from total hybridoma RNA by reverse transcription (RT) using the thermoscript RT kit (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. 200-400 ng of RNA from each hybridoma was added to an individual reaction tube containing a 3' Ig constant region primer. The 3' constant Ig primer is positioned proximal to the variable Ig region and will transcribe first strand cDNA representing the variable region of the mouse antibody. For each hybridoma RNA, an individual RT reaction was performed using a 3' constant heavy chain and 3' constant kappa light chain primer.

[0168] cDNA from each hybridoma were used as a template in a polymerase chain reaction (PCR) to amplify the variable IgG heavy and kappa light chain cDNA for the purpose of sequence determination. Multiple reactions were performed for each PCR using a degenerate 5' primer or primer pools designed to anneal to the signal sequence-coding regions of the mouse Ig variable domain. Separate PCR reactions were performed with a degenerate primer or primer pools for amplification of murine variable heavy and variable light chain regions (Figure 7). PCR was performed with 1 µl of the cDNA reaction using the Expand High Fidelity DNA polymerase kit (Roche Diagnostics Corp., Indianapolis, IN) according to the manufacturers protocol. Thermocycling parameters for the PCR were as follows; 94° C for 2 min., 35 cycles (94° C 15 sec., 55° C 30 sec., 72 ° C 1 min.), 72 ° C 7 min. Fragments amplified from the PCR were separated by gel electrophoresis on a 1 % agarose gel and purified using Qiagen gel extraction kit (Qiagen, Inc., Germantown, MD). Forward primers for the heavy and light chain variable region incorporate EcoRI or SalI (New England Biolabs

(NEB), Inc., Ipswich, MA) sites and reverse heavy and light chain variable, HindIII (NEB Inc., Ipswich, MA) to facilitate cloning into the pUC19 plasmid. Purified PCR fragments and pUC19 plasmid were digested with the above restriction endonucleases at 37° C for 1-2 hrs. Following digestion, PCR fragments were purified using a Qiaquick PCR cleanup kit (Qiagen, Inc., Germantown, MD). Digested plasmid was separated by gel electrophoresis on a 1 % agarose gel and purified using Qiagen gel extraction kit. Purified PCR fragments representing variable IgG heavy and kappa light chain DNA were ligated into pUC19 plasmid using T4 DNA ligase and ligation buffer (NEB, Inc., Ipswich, MA) at 4° C overnight. 3 µl of each ligation reaction was used to transform *E. coli* TOP10 cells (Invitrogen Corp., Carlsbad, CA).

[0169] Plasmids were isolated from positive clones representing the variable regions of each hybridoma using a Qiagen mini prep kit (Qiagen 27106) according to the manufacturer's protocol. M13 forward and reverse primers were used to amplify DNA sequence for each cloned insert using the BigDye sequencing reaction (Applied Biosystems by Life Technologies Corp., Carlsbad, CA) according to manufacturer's protocol. Sequencing reactions were purified using a 96 well purification kit (Zymo Research, Irvine, CA) according to the manufacturer's protocol. Samples were loaded onto an ABI-3730 capillary sequencer and resulting sequence traces were analyzed using Sequencher (GeneCodes v. 4.2) for presence of complete open reading frames. The murine anti canine IL-31 variable sequences determined for each antibody are as follows, 11E12 variable light chain (Seq ID NO:19 MU-11E12-VL, the corresponding nucleotide sequence for which is SEQ ID NO: 34), 11E12 variable heavy chain (Seq ID NO: 26 MU-11E12-VH, the corresponding nucleotide sequence for which is SEQ ID NO: 35), 19D07 variable light chain (Seq ID NO:22 MU-19D07-VL, the corresponding nucleotide sequence for which is SEQ ID NO: 36), 19D07 variable heavy chain (Seq ID NO: 28 MU-19D07-VH, the corresponding nucleotide sequence for which is SEQ ID NO: 37), 34D03 variable light chain (Seq ID NO:24 MU-34D03-VL, the corresponding nucleotide sequence for which is SEQ ID NO: 38), and 34D03 variable heavy chain (Seq ID NO:30 MU-34D03-VH, the corresponding nucleotide sequence for which is SEQ ID NO: 39).

[0170] To confirm the validity of cDNA sequence derived from each antibodies variable heavy and light chains, N-terminal sequence analysis was carried out on purified mAb protein using Edman degradation on an Applied Biosystems model 494 gas phase protein sequencer. Table 2 below describes the confirmation of variable light chain sequences for antibodies 11E12 and 34D03 and the variable heavy sequence of 34D03. The N-terminal amino acid of the variable heavy chain of antibody 11E12, derived by translation of the cDNA sequence, was determined to be glutamine. Glutamine, as amino terminal residue of a protein, can spontaneously undergoe cyclization to pyroglutamic acid preventing sequence determination by Edman degradation (Chelius et al., Anal Chem. 2006 78(7):2370-6).

TABLE 2*

Variable Light Chain		
Antibody	Translated cDNA Sequence	N-terminal Sequence
11E12	DIVLT	DIVLT
19D07	DIVMS	not tested

Variable Light Chain		
Antibody	Translated cDNA Sequence	N-terminal Sequence
34D03	DILLT	DILLT
Variable Heavy Chain		
Antibody	Translated cDNA Sequence	N-terminal Sequence
11E12	QVQLQ	blocked
19D07	EVKLV	not tested
34D03	EVQLV	EVQLV
* In Table 2, "DIVLT" corresponds to residues 1-5 of SEQ ID NO: 19, "DIVMS" corresponds to residues 1-5 of SEQ ID NO: 22, "DILLT" corresponds to residues 1-5 of SEQ ID NO: 24, "QVQLQ" corresponds to residues 1-5 of SEQ ID NO: 26, "EVKLV" corresponds to residues 1-5 of SEQ ID NO: 28, and "EVQLV" corresponds to residues 1-5 of SEQ ID NO: 30.		

Example 3 Construction of 11E12, 19007 and 34003 Chimeric Antibodies

[0171] As described above, antibodies are composed of a homodimer pairing of two heterodimeric proteins. Each protein chain (one heavy and one light) of the heterodimer consists of a variable domain and a constant domain. Each variable domain contains three complementary determining regions (CDRs) which contribute to antigen binding. CDRs are separated in the variable domain by framework regions which provide a scaffold for proper spatial presentation of the binding sites on the antibody. Together, the CDR and framework regions contribute to the antibodies ability to bind its cognate antigen (Figure 2).

[0172] As further described above, a chimeric antibody consists of the variable sequence (both CDR and framework) from the mouse antibody (as determined from the above sequence analysis) grafted onto the respective heavy and light constant regions of a canine IgG molecule (Figure 3). As the variable domain is responsible for antigen binding, grafting of the fully mouse variable domain onto canine constant region is expected to have little or no impact on the antibody's ability to bind the IL-31 immunogen.

[0173] To simultaneously confirm that the correct sequence of the heavy and light chain variable regions were identified and to produce recombinant, homogenous material, expression vectors to produce the chimeric antibodies in mammalian expression systems were generated. Forward and reverse primers were designed to amplify the mouse heavy and light chain variable region of antibody sequence derived from hybridomas 11E12, 19D07, and 34D03. A unique restriction endonuclease site, Kozak consensus sequence and, secretion leader sequence were incorporated into each forward primer to facilitate expression and secretion of the recombinant antibody from a mammalian cell line. Each reverse primer was designed to amplify each respective variable heavy and light chain and included a unique

restriction site to facilitate cloning. PCR was performed to amplify each heavy and light chain using cloned hybridoma variable chain antibody DNA as a template for each reaction. Each PCR product was cloned into a mammalian expression plasmid containing either the canine IgG heavy (referred to herein as HC-64 or HC-65) or light chain (referred to herein as kappa) constant regions based on sequences from GenBank accession numbers AF354264 or AF354265 and XP_532962 respectively. The amino acid and nucleotide sequences of HC-64 are represented by SEQ ID NOs: 40 and 41, respectively. The amino acid and nucleotide sequences of HC-65 are represented by SEQ ID NOs: 42 and 43, respectively. The amino acid and nucleotide sequences of kappa are represented by SEQ ID NOs: 44 and 45, respectively. The plasmids encoding each heavy and light chain, under the control of the CMV promoter, were co-transfected into HEK 293 cells using standard lipofectamine methods. Following six days of expression, chimeric mAbs were purified from 30ml of transiently transfected HEK293FS cell supernatants using MabSelect SuRe protein A resin (GE Healthcare, Uppsala, Sweden) according to standard methods for protein purification. Eluted fractions were pooled, concentrated to ~500ul using a 10,000 nominal MW cutoff Nanosep Omega centrifugal device (Pall Corp., Port Washington, NY), dialyzed overnight at 4° C in 1x PBS, pH7.2 and stored at 4° C. for further use.

[0174] Expression of chimeric canine IgG was assessed using SDS polyacrylamide electrophoresis (SDS PAGE) under native and denaturing conditions. Monoclonal antibodies (mAbs) from each transfection were separated on a 4-12% Bis Tris gel using SDS MES running buffer according to the manufacturers protocol (Invitrogen Corp., Carlsbad, CA). Following electrophoresis, proteins were visualized with Simply Blue Coomassie Stain (Invitrogen Corp., Carlsbad, CA) to ensure proper pairing had occurred and provide a crude assessment of protein homogeneity. To evaluate whether the recombinant mAbs retained the ability to bind canine IL-31, mAbs were assessed for their ability to bind canine IL-31 *via* Western Blots. Protein standards and recombinant canine IL-31 (800 ng) were resolved on SDS PAGE transferred to a nitrocellulose membrane using the Invitrogen iBlot device (Invitrogen Corp., Carlsbad, CA). Following transfer, membranes were washed with distilled deionized water and blocked with 5 % nonfat dried milk (NFDM) in phosphate buffered saline containing 0.05% tween-20 (PBST) for 1 hour at room temperature (RT). Following blocking, membranes were washed in PBST and incubated with either diluted supernatant from the transient expression or purified chimeric antibodies. Binding of the chimeric antibodies was evaluated using Goat anti-Dog IgG antibody-peroxidase conjugated (Bethyl Laboratories Inc., Montgomery, TX or Rockland, Immunochemicals, Inc., Gilbertsville, PA) at a 1:5000 dilution in PBST for 1 hour at RT. Confirmation of IL-31 binding was determined by the presence of a colorimetric band (apparent molecular weight 15 kDa) corresponding to the glycosylated form of canine IL-31 following addition of TMB substrate to the blot (KPL, Inc., Gaithersburg, MD).

[0175] Chimeric mAbs showing expression from HEK 293 cells and binding to the recombinant canine IL-31 immunogen by Western blot were further analyzed for affinity and functionality. To characterize the affinity with which candidate mAbs bind IL-31, surface plasmon resonance (SPR) was evaluated using a Biacore system (Biocore Life Sciences (GE Healthcare), Uppsala, Sweden). To avoid affinity differences associated with differential surface preparation that can

occur when immobilizing antibodies to surfaces; a strategy was employed where IL-31 was directly conjugated to the surface. Immobilization was obtained by amine coupling 5 µg/mL IL-31 using N-hydroxysuccinimide (NHS)/1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry. Chips were quenched with ethanolamine and the affinity with which all candidate mAbs bound to the immobilized IL-31 was evaluated. All curves were fit to a 1:1 model. Affinities <E-11 are below the lower limit of quantitation of detection for the instrument.

[0176] All candidate mAbs were also evaluated for their ability to inhibit IL-31 signaling in the cell-based assay in two independent formats. In the co-incubation format, mAb:IL-31 complexes were pre-incubated for one hour to ensure complex formation prior to cell addition. To increase the potential for differentiation between mAbs, a second set of experiments were performed that lacked co-incubation and mAbs were added directly to cells for 5 minutes followed by IL-31 addition. In both cases, IL-31 stimulation occurred for 5 minutes. As outlined in table 3 below, conversion of mouse monoclonals 11E12, 19D07 and 34D03 to a canine chimeric form, had little impact on their ability to bind IL-31 or inhibit cell-mediated signaling. The results also verify the correct variable heavy and variable light chain sequences derived from each mouse hybridoma.

TABLE 3

	DH82 pSTAT Assay		Biacore Affinity
Antibody	Co-incubation IC_{50} µg/ml	Pre-treatment IC_{50} µg/ml	K_D (M)
Mouse 11E12	1.61	2.28	8.93E-13
▪Chimeric 11E12	1.48	1.57	2.68E-13
Mouse 19D07	1.76	3.46	7.24E-12
*Chimeric 19D07	1.92	1.33	5.15E-13
Mouse 34D03	1.73	2.28	1.01E-12
◘ Chimeric 34D03	1.28	1.08	4.65E-12

▪Chimeric 11E12-for the heavy chain, MU-11E12-VH was paired with HC-64, and for the light chain, MU-11E12-VL was paired with kappa.
*Chimeric 19D07-for the heavy chain, MU-19D07-VH was paired with HC-64, and for the light chain, MU-19D07-VL was paired with kappa.

◘ Chimeric 34D03-for the heavy chain, MU-34D03-VH was paired with HC-64, and for the light chain, MU-34D03-VL was paired with kappa.

Example 4 *In vivo* evaluation of chimeric mAbs

[0177] To confirm that the inhibition of IL-31-mediated cell signaling, observed in the DH-82

assay, correlates with inhibition of IL-31-mediated pruritus in the dog, the chimeric 11E12 monoclonal antibody described above in Table 3 (Chimeric 11E12-64) was evaluated in the IL-31 dog pruritus model. In this model, canine IL-31, when given intraveneously (IV) at a dose of 1 to 1.5 µg/kg, produces a fast onset consistent pruritic response that can be quantitated over a two hour period of observation. To evaluate pruritic responses, dogs were placed in single housed pens and pruritic activity measurements were performed using video surveillance. Following an acclimation period of ≥ 1 hour, pruritic baseline scores were determined for each dog using real-time video surveillance using a categorical scoring system. Specifically, at consecutive 1 minute intervals, "yes/no" decisions were made in regards to whether pruritic behavior was displayed by each dog. Display of pruritic behavior such actions as licking/chewing of paws, flank, and/or anal regions, scratching of flanks, neck, and/or flooring, head-shaking, and scooting of their bottom across the cage flooring was sufficient to elicit a "yes" response over the designated time interval. At the end of this period, the numbers of yes determinations were added together to come up with a cumulative Pruritic Score Index (PSI). Pruritic scores were determined twice for each animal, with the first measurement being a 30 minute baseline score measured immediately prior to the start of the test-article treatment period. After completion of each scheduled observation period, the dogs were returned to their normal housing locations.

[0178] To evaluate whether subcutaneous (SC) administration of chimeric 11E12-64 can inhibit IL-31-mediated pruritus, a pilot study (76A03) was performed that included both a treated and a placebo group (N = 4/group). In this study, baseline responses were performed with all 8 dogs and dogs were randomized into groups and housed based on their PSI. Importantly, each group consisted of two high responders (PSI >55) and two moderate responders (PSI = 30 to 55). These dogs were then administered chimeric 11E12-64 on day 7 and IL-31 challenges were performed at day 8, 14 and 22. The results of this study are presented in Figure 8. These results demonstrate that the administration of chimeric 11E12-64 resulted in a greater than 75% reduction in mean PSI for day 8 and 14, relative to day 1, for chimeric 11E12-64 treated animals. This is in contrast to a 37 - 51% increase in PSI scores for untreated animals. The PSI had returned to baseline two weeks following mAb treatment, suggesting a duration of efficacy between one and two weeks for the 0.3 mg/kg dose administered.

[0179] A particular challenge when assessing PSI is the day to day variation associated with dog pruritic behavior. To help control for this variation, the 30 minute baseline PSI was determined for each dog, on each day prior to IL-31 challenge. Figure 9 shows the individual pruritc scores from doges enrolled in this study (76A60). The data in Figure 9 illustrates that the day 8 and day 14 baseline PSI was approximately 25% of the post IL-31 challenge in the chimeric 11E12-64 treated group. This observation is consistent with a complete abrogation of IL-31 related pruritus since the baseline observation time period (0.5 h) is 25% of the post IL-31 observation time period (2h). Taken together, these *in vivo* data provide very strong evidence that; 1) the chimeric 11E12 monoclonal can neutralize the ability of IL-31 to induce pruritus in dogs, 2) inhibition of IL-31 mediated signaling in the cell based assay correlates with *in vivo* efficacy and 3) the parameters necessary to utilize this IL-31 model for mAb evaluation are established for the evaluation of other candidate antibodies.

Example 5 Caninization Strategy

[0180] The generation of anti-drug antibodies (ADAs) can been associated with loss of efficacy for any biotherapeutic protein including monoclonal antibodies. Comprehensive evaluation of the literature has shown that speciation of monoclonal antibodies can reduce the propensity for mAbs to be immunogenic although examples of immunogenic fully human mAbs and non-immunogenic chimeric mAbs can be found. To help mitigate risks associated with ADA formation for the mouse anti IL-31 monoclonal antibodies provided herein, a caninization strategy was employed. This caninization strategy is based on identifying the most appropriate canine germline antibody sequence for CDR grafting (Figure 4). Following extensive analysis of all available canine germline sequences for both the heavy and light chain, germline candidates were selected based on their homology to the mouse mAbs, and the CDRs from the mouse progenitor mAbs were used to replace native canine CDRs. The objective was to retain high affinity and cell-based activity using fully canine frameworks to minimize the potential of immunogenicity *in vivo.* Caninized mAbs were expressed and characterized for their ability to bind IL-31 *via* Western blotting. These results are described below in Example 8. Only mAbs that retained the ability to bind IL-31 following caninization were advanced for further characterization. Those mAbs that lost the ability to bind IL-31 were systematically dissected to identify; 1) the chain responsible for the loss of function, 2) the framework responsible for the loss of function and 3) the amino acid(s) responsible for the loss function.

Example 6 Caninization of 11E12, 19007, and 34003 antibodies

[0181] Synthetic nucleotide constructs representing the caninized variable heavy and light chains of mAbs 11E12, 19D07, and 34D03 were made. Following subcloning of each variable chain into plasmids containing the respective canine heavy or kappa constant region, plasmids were co-transfected for antibody expression in HEK 293 cells. In summary, both the 19D07 and 34D03 mAbs retained IL-31 binding upon caninization. The caninized anti-canine IL-31 variable sequences determined for each antibody are as follows, 19D07 variable light chain (Seq ID NO: 23 CAN-19D07-VL-998-1, the corresponding nucleotide sequence for which is SEQ ID NO: 46), 19D07 variable heavy chain (Seq ID NO: 29 CAN-19D07-VH-400-1, the corresponding nucleotide sequence for which is SEQ ID NO: 47), 34D03 variable light chain (Seq ID NO: 25 CAN-34D03-VL-998-1, the corresponding nucleotide sequence for which is SEQ ID NO: 48), and 34D03 variable heavy chain (Seq ID NO: 31 CAN-34D03-VH-568-1, the corresponding nucleotide sequence for which is SEQ ID No: 49).

[0182] In contrast, the germline sequences used for the 11E12 caninization efforts resulted in certain non-functional mAbs. With reference to Figure 10, chimeric, heterochimeric, and caninized versions of mAb 11E12 were expressed and characterized for their ability to bind canine IL-31 *via* Western blotting. These results demonstrated that the caninized 11E12 antibody did not bind canine IL-31 (Blot #2). Also, with respect to the heterochimeras, the

chimeric heavy chain paired with the caninized light lost IL-31 binding (Blot #3), while the caninized heavy chain paired with the chimeric light retained IL-31 binding activity (Blot #4). Based on the results obtained from the heterochimeras, it was deduced that the caninized light chain was responsible for the loss of activity.

[0183] In an effort to restore the binding of caninized versions of 11E12 to canine IL-31, the caninized light chain was modified by swapping framework sequences. Figure 11 provides an overview of the 11 E12 light chain framework substitution work. This work identified an antibody replacing the canine framework II (FWII) with mouse framework II and restoring binding to canine IL-31 (11E12 variable light chain (Seq ID NO: 20 CAN-11E12-VL-cUn-FW2, the corresponding nucleotide sequence for which is SEQ ID NO: 50), 11E12 variable heavy chain (Seq ID NO: 27 CAN-11E12-VH-415-1, the corresponding nucleotide sequence for which is SEQ ID NO: 51)).

[0184] Further refinement of these back mutations identified an antibody with a single arginine to leucine back mutation (R50L) in framework II could restore IL-31 binding *via* Western blot analysis (11E12 variable light chain (Seq ID NO: 21 CAN-11E12-VL-cUn-13, the corresponding nucleotide sequence for which is SEQ ID NO: 52), 11E12 variable heavy chain (Seq ID NO: 27 CAN-11E12-VH-415-1)) (Figure 12). Once 'caninized' versions of each potential candidate were identified the mAbs were purified and dialyzed into PBS for further evaluation.

[0185] Table 4 summarizes the results of both the affinity measurements and cell-based inhibition data. These data demonstrate that the caninized derivatives of both 11E12 and 34D3 both retain excellent inhibitory activity in the cell based assay and affinity to IL-31 as measured by Biacore. Also worth noting is the observation that while the original caninized 19D7 molecule retains excellent potency as measured by Biacore, the ability to inhibit cell based IL-31 signaling appears compromised relative to its mouse progenitor. Little to no affinity loss was incurred when converting mAbs from their mouse isotype to the canine derivative.

TABLE 4

Antibody	DH82 pSTAT Assay		Biacore Affinity
	Co-incubation IC_{50} µg/ml	Pre-treatment IC_{50} µg/ml	K_D (M)
Mouse 11E12	1.61	2.28	8.93E-13
Caninized 11E12	not active	not active	5.06E-07
11E12 Heterochimera	2.67	3.35	4.97E-12
Caninized 11E12 FW2	2.7	5.31	1.47E-10
Caninized 11E12 13	5.49	5.18	5.16E-12
Mouse 19D07	1.76	3.46	7.24E-12
Caninized 19D07	inc. curve	inc. curve	9.23E-10
Mouse 34D03	1.73	2.28	1.01E-12

Antibody	DH82 pSTAT Assay		Biacore Affinity
	Co-incubation IC_{50} µg/ml	Pre-treatment IC_{50} µg/ml	K_D (M)
Caninized 34D03	2.42	2.25	2.91E-11
Antibody	Variable Chain		
	Heavy	Light	
Caninized 11E12	CAN-11E12-VH-415-1	CAN-11E12-VL-cUn-1	
11E12 Heterochimera	CAN-11E12-VH-415-1	Chimeric 11E12	
Caninized 11E12 FW2	CAN-11E12-VH-415-1	CAN-11E12-VL-cUn-FW2	
Caninized 11E12 13	CAN-11E12-VH-415-1	CAN-11E12-VL-cUn-13	
Caninized 19D07	CAN-19D07-VH-400-1	CAN-19D07-VL-998-1	
Caninized 34D03	CAN-34D03-VH-568-1	CAN-34D03-VL-998-1	

Heavy chains: All Caninized and heterochimeric forms of 11E12 included the V_H sequence of CAN-11E12-VH-415-1 (SEQ ID NO: 27) and the constant region termed HC-64 (SEQ ID NO: 40); Caninized 19D07 included the V_H sequence of CAN-19D07-VH-400-1 (SEQ ID NO: 29) and HC-64; Caninized 34D03 included the V_H sequence of CAN-34D03-VH-568-1 (SEQ ID NO: 31) and HC-64.
Light Chains: Caninized 11E12 included the V_L sequence of CAN-11E12-VL-cUn-1 (SEQ ID NO: 53) and the constant region termed kappa (SEQ ID NO: 44); Heterochimeric 11E12 included the V_L sequence of MU-11E12-VL (SEQ ID NO: 19) and kappa; Caninized 11E12 FW2 included the V_L sequence of CAN-11E12-VL-cUn-FW2 (SEQ ID NO: 20) and kappa; Caninized 11E12 13 included the V_L sequence of CAN-11E12-VL-cUn-13 (SEQ ID NO: 21) and kappa; Caninized 19D07 included the V_L sequence of CAN-19D07-VL-998-1 (SEQ ID NO: 23) and kappa; Caninized 34D03 included the V_L sequence of CAN-34D03-VL-998-1 (SEQ ID NO: 25) and kappa.

Example 7 Characterization of canine IL-31 binding to antibodies 11E12 and 34D03

[0186] To determine the amino acid residues involved with binding of canine IL-31 to antibodies 11E12 and 34D03, a mutational strategy was used that involved 1) truncation of the IL-31 protein from both the N and C terminus and 2) replacement of individual amino acids with alanine (ala scan) to determine the impact on mAb binding. PCR primers were designed to amplify a canine IL-31 gene that was codon optimized for expression in an *E. coli* host. The

sequence of this codon-optimized canine IL-31 full-length construct for *E.coli* expression is represented by SEQ ID NO: 55, the corresponding nucleotide sequence for which is SEQ ID NO: 56. Primers were designed to amplify the full length gene and to create 20 amino acid truncations of the protein moving inward from the N and C termini. For the purpose of these N-terminal truncations, position 1 corresponded to the glycine residue immediately following the N-terminal 6-His tag in the codon-optimized construct. PCR amplification products were cloned into pET101D (Invitrogen Corp., Carlsbad, CA) according to the manufacturers protocol. The pET101D plasmid allows fusion of the recombinant protein to an N-terminal 6-His epitope tag for confirmation of expression. Sequence confirmed plasmids were used to transform BL21 Star TOP10 *E.coli* cells (Invitrogen Corp., Carlsbad, CA) and expression of the recombinant protein was induced using 1mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) under standard culture conditions. Following inductions, cells were pelleted and lysed using Bacterial Protein Extraction Reagents (abbreviated B-PER, ThermoFisher Scientific Inc., Rockford, IL). Crude lysates were subjected to SDS-PAGE and Western blotting was carried out as described previously. All Western blotting for mutational analysis was performed using the mouse versions of 11E12 and 34D03 due to the availability of necessary purified antibodies and reagents. Each antibody was tested for its ability to bind the crude protein lysate blot representing full length and truncated IL-31. Control blots were also probed with the anti-His mAb to confirm expression of each protein. Proteins with an N-terminal truncation (-20N, -40N, and -60N) all showed robust expression in *E. coli* and were capable of binding to 11E12 and 34D03 (Figure 13). The amino acid and nucleotide sequences corresponding to the -20N construct are SEQ ID NOs: 57 and 58, respectively. The amino acid and nucleotide sequences corresponding to the -40N construct are SEQ ID NOs: 59 and 60, respectively. The amino acid and nucleotide sequences corresponding to the -60N construct are SEQ ID NOs: 61 and 62, respectively. However, full length IL-31 and proteins with C-terminal truncations (-20C, -40C, and -60C) failed to express under these conditions.

[0187] It was observed that the full length IL-31 protein was expressed very poorly. However, the construct with the first 20 amino acids removed (-20N) from the N-terminus showed robust expression. Antibodies 11E12 and 34D03 all bound to the -20N protein. Therefore, further work was carried out using this -20 N construct. Constructs representing C-terminal truncations at positions 20-122 (MW 15.3 with his tag), 20-100 (MW 12.9 with his tag), and 20-80 (MW 10.4 with his tag), were made to assess mAb binding to these areas on the IL-31 protein. The amino acid and nucleotide sequences corresponding to the 20-122 construct are SEQ ID NOs: 63 and 64, respectively. The amino acid and nucleotide sequences corresponding to the 20-100 construct are SEQ ID NOs: 65 and 66, respectively. The amino acid and nucleotide sequences corresponding to the 20-80 construct are SEQ ID NOs: 67 and 68, respectively. Figure 14 shows Western blots of crude protein lysates of these truncated proteins that were probed with mAbs 11E12 (Blot B) and 34D03 (Blot C). As shown in this Figure, mAbs 11E12 and 34D03 bound to IL-31 truncated proteins 20-122 and 20-100, but failed to bind to 20-80. These results indicated that amino acids between positions 80 and 100 of the canine IL-31 full-length construct of SEQ ID NO: 55 (using "SSHMA" as the N-terminus) were involved with binding of these antibodies. This region corresponds to amino acid Nos. between amino acid residues 102 and 122 of the canine IL-31 full-length protein sequence of SEQ ID NO: 32. A

control blot using the anti-His mAb (Blot A) showed that all truncated proteins were being expressed. In addition, the pET101D-lacZ protein was used as a control to confirm the lack of non-specific binding of mAbs to host proteins.

[0188] To further identify the amino acids in canine IL-31 involved with binding to mAbs 11E12 and 34D03, alanine-scanning mutagenesis was performed according to known methods. Individual constructs were made (in the -20N plasmid) substituting alanine for each position on canine IL-31 from amino acids 76 through 122. Following sequence confirmation, protein expression was carried out and crude protein lysates were subjected to Western blot analysis. Figure 15 shows a summary of results indicating positions on canine IL-31 that, when mutated to alanine, impact binding by mAbs 11E12 and 34D03. As shown in this Figure, positions 77, 78, 81 and 85 of the full-length IL-31 construct all impact binding of 11E12 or 34D03 antibodies. These correspond to amino acid residues 99, 100, 103 and 107, respectively, of the canine IL-31 full-length protein sequence of SEQ ID NO: 32.

[0189] To examine the impact of multiple mutations in the region of IL-31 important for binding to the 11E12 and 34D03 antibodies, expression plasmids were constructed with double (D82A, I85A) and triple (I81A, D82A, I85A) alanine substitutions. *E. coli* lysates expressing canine IL-31 with these double and triple mutations in addition to the -20N control were blotted with 11E12 and 34D03 antibodies (Figure 16). It is apparent that these three amino acids on canine IL-31 are involved with recognition of 11E12 and 34D03 as complete abrogation of binding is observed when these sites are changed to alanine. These three amino acids correspond to amino acid residues 103, 104 and 107 of the canine IL-31 full-length protein sequence of SEQ ID NO: 32.

[0190] In summary, truncation analysis of canine IL-31 revealed amino acid residues (annotated in Figure 15 between positions 80 and 122) are involved in binding 11E12 and 34D03 antibodies. Further, fine mutational analysis using alanine scanning revealed that ASP77, LYS78, ILE81, ASP82, and ILE85 of the full-length IL-31 construct all impact binding of 11E12 or 34D03 indicating this region most likely defines the epitope responsible for recognition by these antibodies. Interestingly, this region of the human IL-31 protein was shown to be involved with binding to the GPL subunit of its co-receptor (Le Saux S et al. Biol Chem. 2010 Jan 29;285(5):3470-7. Epub 2009 Nov 17). These observations, along with the ability of mAbs 11E12 and 34D03 to neutralize IL-31 mediated pSTAT activity in monocytes, support the hypothesis that these mAbs bind to residues on canine IL-31 that are essential for binding of this cytokine to its receptor, thereby inhibiting its ability to induce signaling.

Example 8 Production of caninized 34003 antibodies from Glutamine synthetase (GS) plasmids

[0191] The genes encoding the caninized 34D03 mAb (heavy and light chains, Table 4 above) were cloned into GS plasmids pEE 6.4 and pEE 12.4, respectively (Lonza, Basel, Switzerland). The resulting plasmids were digested according to the manufacturer's protocol and ligated

together to form a single mammalian expression plasmid. Each plasmid was used to transfect HEK 293 cells and expression was carried out in 20L of culture media. Protein was isolated from conditioned HEK medium using Protein A affinity chromatography according to standard protein purification methods. Medium was loaded onto chromatographic resin and eluted by pH shift. Eluted protein was pH adjusted, dialyzed, and sterile filtered prior to use. The resulting antibody was greater than 99 percent monomeric by analytical size exclusion chromatography with no high molecular weight aggregates observed. This antibody was subsequently used for evaluation in the dog pruritus model to evaluate *in vivo* efficacy.

Example 9 Evaluation of the caninized 34003 antibody in the dog pruritus model

[0192] The anti-pruritic activity of caninized 34D03 (CAN 34D03-65 represented by SEQ ID NO 31 (VH) paired with SEQ ID NO 25 (VL) on SEQ ID NO 42 (HC-65) and SEQ ID NO 44 (LC-Kappa)) was evaluated using a canine model of IL-31-induced pruritus. With this model, a 1.5 µg/kg intravenous challenge dose of recombinant canine IL-31 known to induce a transient period of pruritic behavior in beagle dogs (IL-31 challenge, pruritus duration <24 hour) was repeatedly delivered to animals before and up to 63 days after a single 1.0 mg/kg SC dose of CAN 34D03-65. At each IL-31 challenge period, real-time video surveillance was used to obtain a measure of pruritic behavior for 0.5 hours prior to cytokine delivery (pre-IL-31 baseline period) followed by a similar 2 hour measurement beginning 20 minutes after cytokine injection (2h post-IL-31 challenge period). Pruritic scores were generated at each time period under evaluation by making "yes/no" determinations as to whether a pruritic behavior was displayed over consecutive 1 minute time-intervals (maximal pruritic score = 30 for each baseline period; 120 for the post-IL-31 challenge period). Figure 17 summarizes the pruritic scores obtained before and after CAN 34 D03-65 treatment, which was given on day 0 of the study. Seven days prior to mAb treatment, the mean post-IL-31 challenge pruritic score of the dogs was 68 ± 13 (S.E., n=4). By comparison, on study days 7, 14, 21, the mean post-IL-31 challenge pruritic scores had lowered to 5 ± 2, 8 ± 4, and 9 ± 5, respectively. These changes in pruritic score between day -7 and days 7-21 represent a ≥85% decrease in overall pruritic reactivity to IL-31.

[0193] The degree of inhibition of IL-31-induced pruritus may actually have been closer to 100% over this time-frame if one considers that between days 0 and 21, the 0.5h pre-IL-31 baseline scores averaged 1.6 ± 0.6-- a level that would extrapolate to a pruritic score of 6-7 over a 2h period. The pruritic reactivity of the treated dogs to exogenous IL-31 did gradually recover over time. By day 63 post-CAN D03-65 treatment, the mean 2h post-IL-31 challenge pruritic score had increased to 57 ± 8 or roughly 84% of the pre-mAb IL-31 challenge responses observed on day -7. Thus, in a model of IL-31-induced pruritus, a single bolus SC injection of CAN 34D03-65 did provide weeks of anti-pruritic protection to treated dogs.

Example 10 Characterization of feline IL-31

[0194] The sequence of feline IL-31 was identified by a similarity searching of the feline genome with canine IL-31 using the NCBIs genome resources (www.ncbi.nlm.nih.gov). The gene representing feline IL-31 was synthesized for optimal expression in E. *coli.* Expression constructs were created with full length canine and feline IL-31 genes containing an N-terminal 6-His tag for detection and purification. The feline full-length construct used for expression in *E.coli* is represented by the nucleotide sequence of SEQ ID NO: 69 and the protein sequence of SEQ ID NO: 70. Sequence confirmed plasmids were used to transform E. *coli* BL21 Star™ (Invitrogen Corp., Carlsbad, CA) and expression was carried out at 30 C for 5 hours. Following lysis of cell pellets immunoreactive reactive protein was found to be highly enriched in the insoluble lysate. These cell pellets were solubilized in 6M urea and purification of the recombinant proteins was carried out under denaturing conditions using a nickel cobalt resin (Thermo Fisher Scientific Inc., Rockford, IL). Pooled eluted fractions, shown to be positive for the presence of the His tag, were step dialyzed against 0.8 M urea PBS followed by PBS, and analyzed by SDS PAGE (Figure 18). As was previously observed, the yield of recombinant canine IL-31 from *E. coli* induction was low. However, protein was recovered post purification that migrated according to expected molecular mass via SDS-PAGE.

[0195] To examine the biological activity of canine and feline IL-31 produced from *E. coli,* each protein was analyzed for its ability to induce pSTAT signaling in the DH82 cell assay. As recombinant IL-31 from mammalian cells (canine IL-31(CHO)) is highly glycosylated, it was unclear whether the unglycosylated form would retain biological activity. Figure 19 shows that feline IL-31 has comparable bioactivity to the reference reference IL-31 produced in CHO cells.

[0196] Alanine-scanning mutagenesis of canine IL-31 defined a region within the protein that is necessary for binding to the 11E12 and 34D03 antibodies. It was hypothesized, due to sequence conservation in this region (Figure 20), that these mAbs would cross-react with feline IL-31.

[0197] Figure 21 shows that mAbs 11E12 and 34D03 are capable of binding to canine IL-31 (*E. coli*) and are also capable of cross-reacting with the feline IL-31 protein. Based on these data, speciation of the 34D03 antibody to feline (felinization) was pursued.

Example 11 Felinization on antibody 34003

[0198] Similar to the caninization strategy described, appropriate germline antibody sequences were identified from all available feline sequences for CDR grafting from mAb 34D03. Variable light chain (SEQ ID NO: 71 FEL-34D03-VL-021-1, the corresponding nucleotide sequence for which is SEQ ID NO: 72) and variable heavy chain (SEQ ID NO: 73 FEL-34D03-VH-035-1, the corresponding nucleotide sequence for which is SEQ ID NO: 74) were selected based on the highest homology to their respective canine frameworks in caninized 34D03. Recombinant felinized 34D03 was produced using the selected variable regions joined to their respective constant heavy IgG1 (SEQ ID NO: 75 HC-A Feline, the corresponding nucleotide sequence for

which is SEQ ID NO: 76 GenBank accession No. AB016710.1) and kappa constant light (SEQ ID NO: 77 LC-Kappa Feline, the corresponding nucleotide sequence for which is SEQ ID NO: 78 GenBank accession No. AF198257.1) chain sequence. Antibody was produced from HEK cells and purified as previously described. Figure 22 shows the ability of feline 34D03 to neutralize pSTAT signaling with a comparable IC50 to the canine version.

[0199] Felinized 34D03 was assessed for its ability to bind both feline and canine IL-31. Figure 23 shows Western blots with felinized 34D03 using purified protein from both mammalian and *E. coli* sources. Conclusive binding was observed to both canine and feline proteins indicating full cross-reactivity of the felinized form of 34D03 and verification of binding to the feline protein. Taken together, these results suggest a conserved epitope on feline IL-31 may be a suitable target for inhibition of this cytokine in cats.

Example 12 Detection of IL-31 cytokine in dogs with naturally occurring atopic dermatitis

[0200] In the present example, the level of IL-31 protein in serum collected from populations of dogs, including those with atopic dermatitis, was evaluated using a quantitative immunoassay technique.

[0201] Serum was collected from the following populations of dogs and frozen prior to IL-31 serum measurements.

1. 1) Twenty four purpose bred beagles (Marshall BioResources, North Rose, NY) prior to and after sensitization to house dust mite allergen (*Dermatophagoides farina,* Greer Labs). All animals were approximately 9 months in age. The two sexes were represented approximately equally.
2. 2) Thirty flea allergic dogs (Youngs Veterinary Research Services, Turlock, CA) prior to flea infestation or approximately one week after infestation with adult cat fleas *(Ctenocephalides felis).* The majority of the dogs in this colony were of mixed breed. The average age was approximately 10.5 years. The two sexes were represented approximately equally.
3. 3) Eighty seven client-owned dogs with sub-clinical periodontal disease but otherwise determined to be in good health. Samples were collected across 18 US veterinary clinics. Animals were representative of the US canine population in terms of gender and breed and were between the age of two and five years.
4. 4) Two hundred and twenty four client-owned animals diagnosed with chronic, non-seasonal atopic dermatitis of at least 1-year duration (based on modified Willemse's criteria, and Prelaud (Willemse T. J small Anim Pract 1986; 27:771-778 and
   Prelaud et al. Revue de Medecine Veterinaire 1998;149: 1057-1064) with a minimum of "moderate itching" as assessed by the Owner, and a minimum skin lesion score of 25 on the CADESI-02) as assessed by a veterinarian. Samples were collected from 14 US veterinary practices with expertise in veterinary dermatology. Approximately 75% of the

dogs were purebred and ~25% of the total population were retrievers (Labrador (17.3%) and Golden (8.2%)). Dogs tended to be middle-aged (~6 years old). The two sexes were represented approximately equally.

[0202] A sandwich immunoassay was used to quantitate cIL-31 levels in canine serum. Serum samples were diluted 1:2 in Rexxip buffer (Gyrolab, Warren, NJ) and run on Bioaffy 1000 nL CDs (Gyrolab) using the Gyrolab xP workstation. cIL-31 was captured with a biotin-labeled anti-IL-31 monoclonal antibody according to the present invention and detected with an Alexaflour 647 labeled anti-IL-31 monoclonal antibody according to the present invention. Sample concentrations of cIL-31 were extrapolated from an 8-point standard curve with a dynamic range of 0.013-250 ng/mL using a 5-parmameter fit model with Gyrolab Evaluator software.

[0203] Levels of cIL-31 were detectable in serum samples of 57% of dogs with naturally occurring atopic dermatitis (≥13 pg/mL) but were not detectable (< 13 pg/mL) in the serum from purpose-bred beagles +/- sensitized to HDM, mixed breed dogs +/- flea infestation, or client-owned dogs with periodontal disease but otherwise considered in good health, regardless of breed. In the dogs with naturally occurring atopic dermatitis, 53% of the samples analyzed showed serum IL-31 levels between 13-1000 pg/mL, and 4% showed levels above 1000 pg/mL (Table 5).

Table 5: Serum IL-31 Levels in Various Canine Populations

Canine Populations	Number of Animals Evaluated	Number of Animals with Detectable IL-31 in Serum[a]	Percent of Animals with Detectable IL-31 in Serum
Purpose-bred beagles	24	0	0%
Purpose-bred beagles sensitized to HDM	24	0	0%
Mixed breed dogs - no fleas	30	0	0%
Mixed breed dogs - infested with fleas	30	0	0%
Healthy client owned animals - multiple breeds	87	0	0%
Naturally occurring atopic dermatitis in client owned animals - multiple breeds	224	128	57%
[a] Less than 13 pg/mL is below limits of quantitiation.			

[0204] The results of the present example demonstrate that IL-31 protein is elevated in a significant number of dogs with canine atopic dermatitis. Without wishing to be bound by any

one theory, it is believed that the IL-31 pathway plays a role in the pathobiology of pruritic allergic skin conditions such as, but not limited to, canine atopic dermatitis and represents a novel pathway for therapeutic intervention with an IL-31 antagonist, such as including, but not limited to, olacitnib and/or an anti-IL-31 antibody that specifically binds to canine IL-31.

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This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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Patentkrav

1. Antistof eller antigen-bindende del deraf, der specifikt binder til hunde-IL-31-protein, der kodes af nukleotidsekvensen af SEQ ID NO:33, hvor antistoffet eller den antigen-bindende del deraf inhiberer IL-31-aktivitet i en hund og inhiberer hunde-IL- 31-medieret pSTAT-signalering i et cellebaseret assay.

2. Antistof eller antigenbindende del deraf ifølge krav 1, hvor antistoffet eller den antigenbindende del deraf er et monoklonalt antistof.

3. Antistof eller antigenbindende del deraf ifølge krav 1 eller krav 2, hvor antistoffet eller den antigenbindende del deraf er kimærisk.

4. Antistof eller antigenbindende del deraf ifølge krav 1 eller krav 2, hvor antistoffet eller den antigenbindende del deraf er kaniniseret.

5. Antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 4, omfattende mindst én af gruppen bestående af:
en komplementaritetsbestemmende region (CDR)1 af den variable tunge (V_H) kæde med aminosyresekvensen YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1) eller NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);
en variabel tung kæde CDR2 med aminosyresekvensen WIFPGDGGTKYNETFKG (SEQ ID NO: 4; 11E12-VH-CDR2) eller TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);
en variabel tung kæde CDR3 med aminosyresekvensen ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3) eller VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); og
en variant deraf med en til ti aminosyresubstitutioner i en eller flere af CDR1, CDR2 og CDR3.

6. Antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 5, omfattende mindst én af gruppen bestående af:
en komplementaritetsbestemmende region (CDR)1 af den variable lette (V_L) kæde med aminosyresekvensen RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) eller KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);
en variabel let kæde CDR2 med aminosyresekvensen RASNLES (SEQ ID NO: 13; 11E12-VL-CDR2) eller RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);
en variabel let kæde CDR3 med aminosyresekvensen QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3) eller QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); og
en variant deraf med en til ti aminosyresubstitutioner i en eller flere af CDR1, CDR2 og CDR3.

7. Veterinærsammensætning omfattende en terapeutisk effektiv mængde af et antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 6.

8. Værtscelle, der producerer et antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 6.

9. Isoleret nukleinsyre, der koder for et antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 6, omfattende en nukleinsyresekvens, der koder for mindst én af gruppen bestående af:
en komplementaritetsbestemmende region (CDR)1 af den variable tunge (V_H) kæde med aminosyresekvensen YYDIN (SEQ ID NO: 1; 11E12-VH-CDR1) eller NYGMS (SEQ ID NO: 3; 34D03-VH-CDR1);
en variabel tung kæde CDR2 med aminosyresekvensen WIFPGDGGTKY-NETFKG (SEQ ID NO: 4; 11E12-VH-CDR2) eller TISYGGSYTYYPDNIKG (SEQ ID NO: 6; 34D03-VH-CDR2);

en variabel tung kæde CDR3 med aminosyresekvensen ARGGTSVIRDAMDY (SEQ ID NO: 7; 11E12-VH-CDR3) eller VRGYGYDTMDY (SEQ ID NO: 9; 34D03-VH-CDR3); og

en variant deraf med en til ti aminosyresubstitutioner i en eller flere af CDR1, CDR2 og CDR3,

og yderligere omfattende en nukleinsyresekvens, der koder for mindst én af gruppen bestående af:

en komplementaritetsbestemmende region (CDR)1 af den variable lette (V_L) kæde med aminosyresekvensen RASESVDNYGISFMH (SEQ ID NO: 10; 11E12-VL-CDR1) eller KASQSVSFAGTGLMH (SEQ ID NO: 12; 34D03-VL-CDR1);

en variabel let kæde CDR2 med aminosyresekvensen RASNLES (SEQ ID NO: 13; 11E12-VL-CDR2) eller RASNLEA (SEQ ID NO: 15; 34D03-VL-CDR2);

en variabel let kæde CDR3 med aminosyresekvensen QQSNKDPLT (SEQ ID NO: 16; 11E12-VL-CDR3) eller QQSREYPWT (SEQ ID NO: 18; 34D03-VL-CDR3); og

en variant deraf med en til ti aminosyresubstitutioner i en eller flere af CDR1, CDR2 og CDR3.

10. Vektor omfattende nukleinsyren ifølge krav 9.

11. Fremgangsmåde til fremstilling af et antistof eller antigenbindende del deraf omfattende dyrkning af værtscellen ifølge krav 8 under betingelser, der resulterer i produktion af antistoffet eller den antigenbindende del deraf, og isolering af antistoffet eller den antigenbindende del deraf fra værtscellen eller dyrkningsmedium af værtscellen.

12. Antistof eller antigenbindende del deraf ifølge et hvilket som helst af kravene 1 til 6 til anvendelse ved behandling af en kløetilstand eller en allergisk tilstand hos en hund.

13. Antistof eller antigenbindende del deraf til anvendelse ifølge krav 12, hvor kløetilstanden er valgt fra gruppen bestående af atopisk dermatitis, eksem, psoriasis, sklerodermi og pruritis, fortrinsvis hvor kløetilstanden er atopisk dermatitis.

14. Antistof eller antigen-bindende del deraf til anvendelse ifølge krav 12, hvor den allergiske tilstand er valgt fra gruppen bestående af allergisk dermatitis, sommereksem, nældefeber, engbrystighed, inflammatorisk luftvejssygdom, tilbagevendende luftvejsobstruktion, luftvejshyperresponsivitet, kronisk obstruktiv lungesygdom og inflammatoriske processer som følge af autoimmunitet.

15. Antistof eller antigenbindende del deraf til anvendelse ifølge krav 12 eller 13, hvor

(i) behandlingen er en lindrende behandling af en kløetilstand hos en hund;

(ii) behandlingen lindrer den kløende tilstand hos hunden; eller

(iii) behandlingen forårsager regression af kliniske symptomer.

DRAWINGS

Drawing

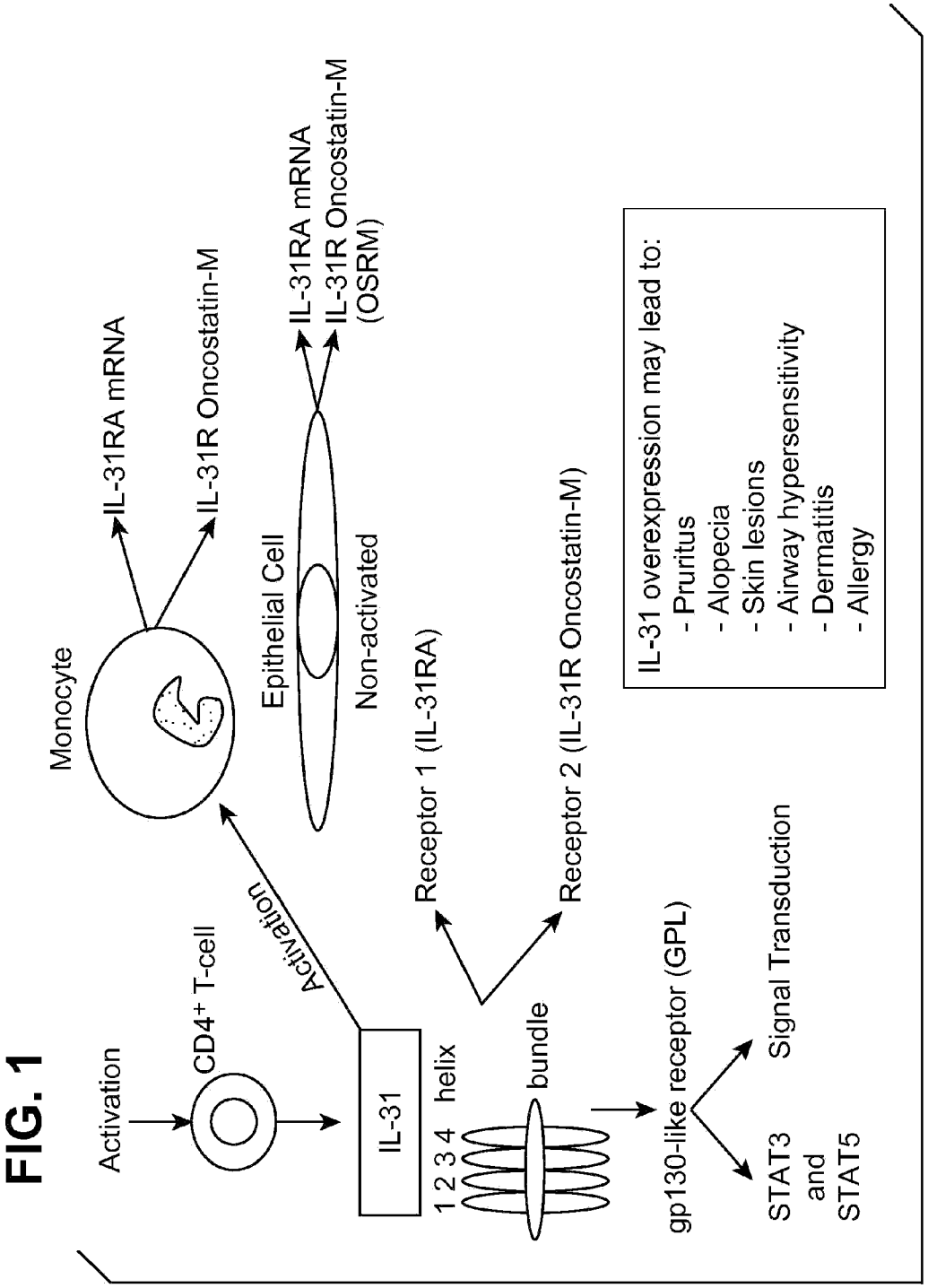


FIG. 1

FIG. 2

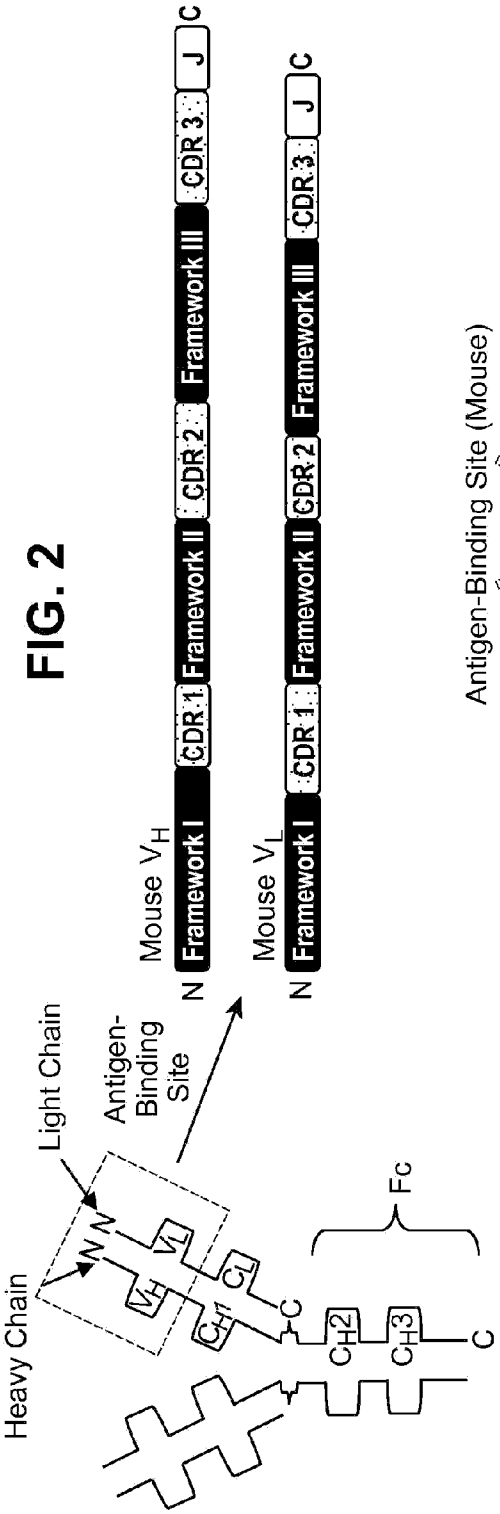

Heavy Chain
Light Chain
Antigen-Binding Site
Mouse V_H
Mouse V_L
N
Framework I
CDR 1
Framework II
CDR 2
Framework III
CDR 3
J
C
Fc
C_H2
C_H3

FIG. 3

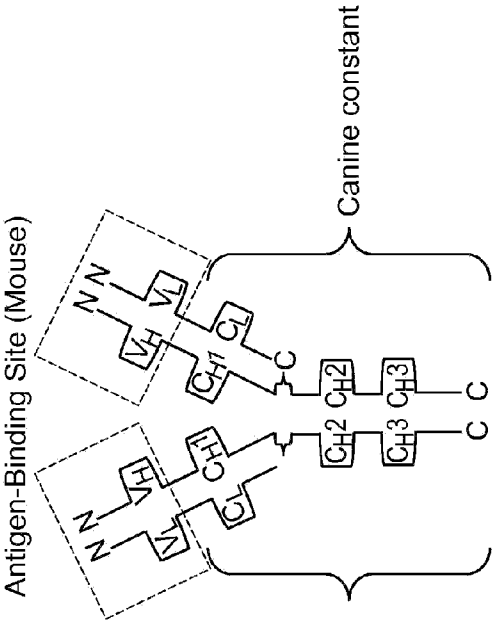

Antigen-Binding Site (Mouse)
Canine constant
V_H
V_L
C_H1
C_L
C_H2
C_H3
C
N

FIG. 4

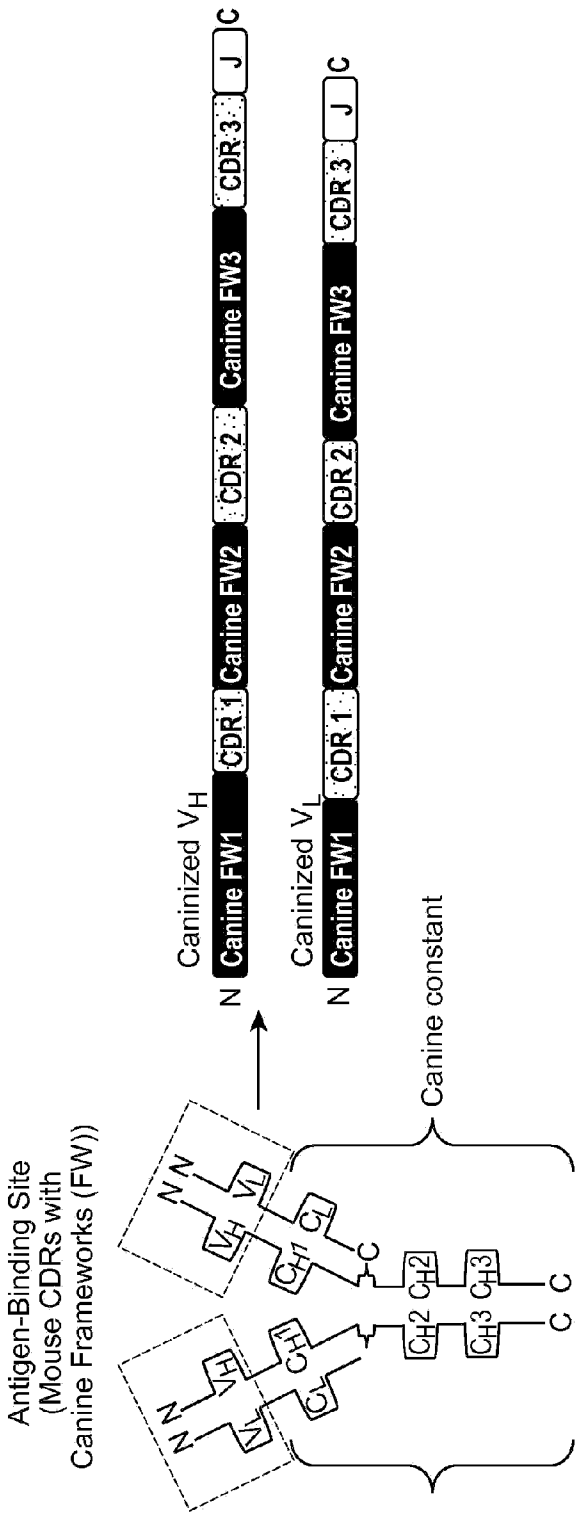

Caninized V_H
N
Canine FW1
CDR 1
Canine FW2
CDR 2
Canine FW3
CDR 3
J
C
Caninized V_L
N
Canine FW1
CDR 1
Canine FW2
CDR 2
Canine FW3
CDR 3
J
C
Antigen-Binding Site
(Mouse CDRs with
Canine Frameworks (FW))
Canine constant
N
N
V_H
V_L
C_H1
C_L
C
C_H2
C_H3
C C

FIG. 5

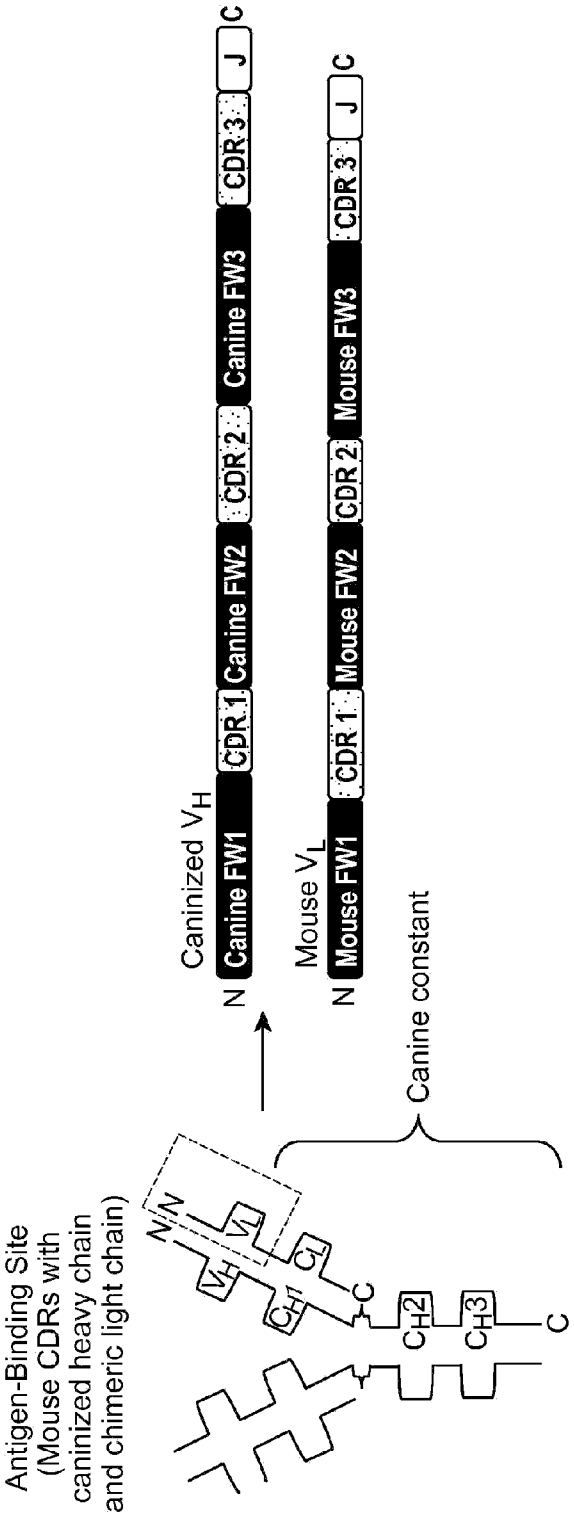

Antigen-Binding Site
(Mouse CDRs with
caninized heavy chain
and chimeric light chain)
N
C
Caninized V_H
Canine FW1
CDR 1
Canine FW2
CDR 2
Canine FW3
CDR 3
J
C
Mouse V_L
N
Mouse FW1
CDR 1
Mouse FW2
CDR 2
Mouse FW3
CDR 3
J
C
Canine constant
N
N
V_H
V_L
C_H1
C_L
C
C_H2
C_H3
C

FIG. 6

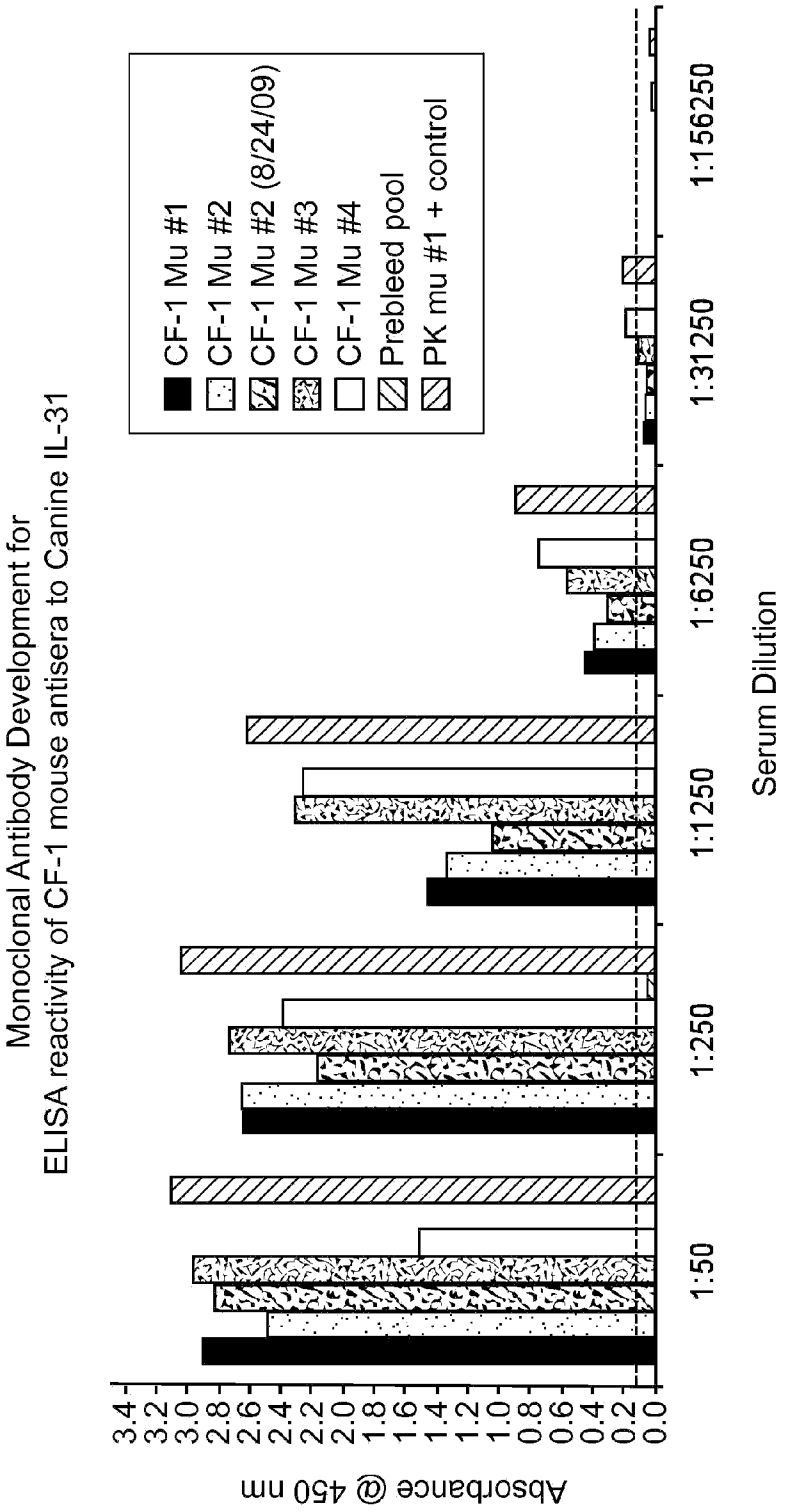

Monoclonal Antibody Development for
ELISA reactivity of CF-1 mouse antisera to Canine IL-31
CF-1 Mu #1
CF-1 Mu #2
CF-1 Mu #2 (8/24/09)
CF-1 Mu #3
CF-1 Mu #4
Prebleed pool
PK mu #1 + control
Absorbance @ 450 nm
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1:50
1:250
1:1250
1:6250
1:31250
1:156250
Serum Dilution

FIG. 7

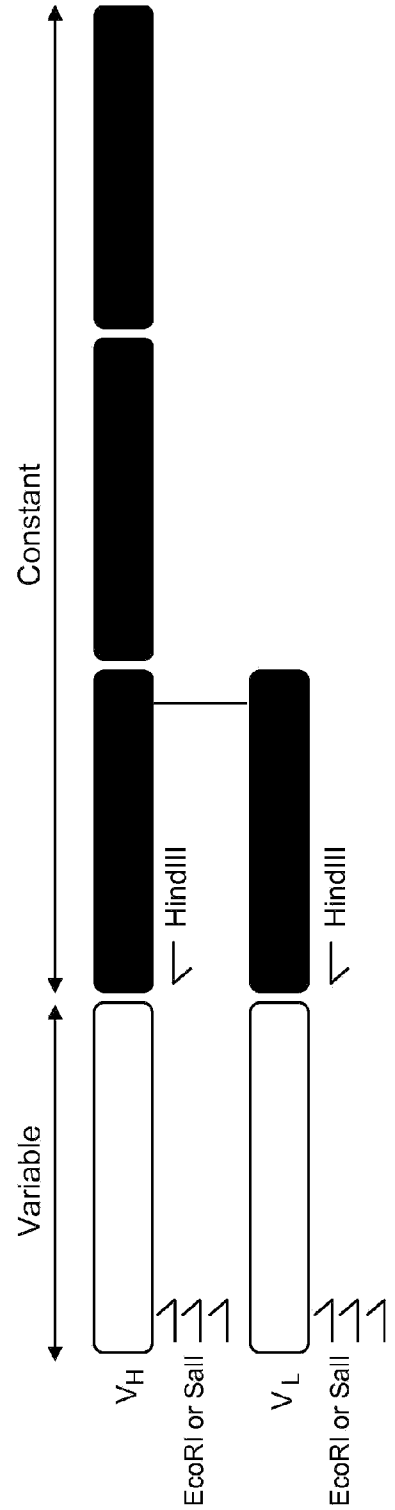

Constant
Variable
HindIII
HindIII
V_H
V_L
EcoRI or SalI
EcoRI or SalI

FIG. 8

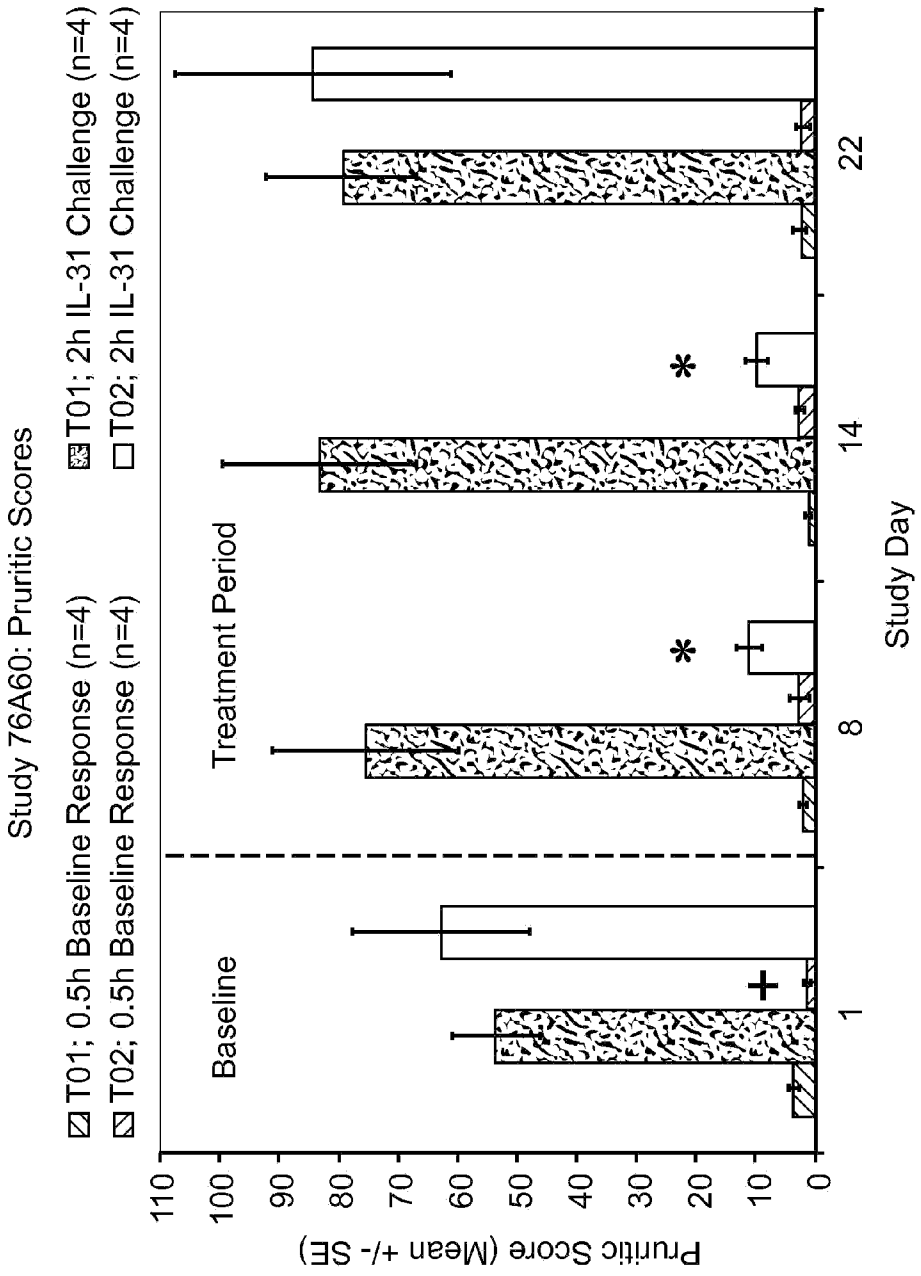

Study 76A60: Pruritic Scores
T01; 0.5h Baseline Response (n=4)
T02; 0.5h Baseline Response (n=4)
T01; 2h IL-31 Challenge (n=4)
T02; 2h IL-31 Challenge (n=4)
Baseline
Treatment Period
Pruritic Score (Mean +/- SE)
Study Day
0
10
20
30
40
50
60
70
80
90
100
110
1
8
14
22
+
*
*

FIG. 9

T01-Placebo @ 1X/d, SC on d0 and d7 (n=4)

									2h IL-31 Challenge Response Comparisons		
Date	26-Oct-10		2-Nov-10		8-Nov-10		15-Nov-10		Day 8 vs. Day 1	Day 14 vs. Day 1	Day 21 vs. Day 1
Day of Study	1	1	8	8	14	14	22	22			
Dog ID	30' BL	2h IL-31	30' BL	2h IL-31	30' BL	2h IL-31	30' BL	2h IL-31	% Change	% Change	% Change
4265068	4	58	0	77	0	102	0	82	32.8	75.86	41.4
4804228	5	71	3	114	2	114	4	112	60.6	60.56	57.7
4271963	4	37	2	39	0	41	3	52	5.4	10.81	40.5
4549040	1	48	3	72	2	76	3	72	50.0	58.33	50.0
Mean	3.5	54	2.0	76	1.0	83	2.5	80	37.2	51.4	47.4
StError	0.9	7	0.7	15	0.6	16	0.9	13	12.0	14.1	4.1

T02-Placebo @ 1X/d, SC on d0; Chim 11E12 @ 0.3mg/kg, SC on d7 (n=4)

									2h IL-31 Challenge Response Comparisons		
Date	26-Oct-10		2-Nov-10		8-Nov-10		15-Nov-10		Day 8 vs. Day 1	Day 14 vs. Day 1	Day 21 vs. Day 1
Day of Study	1		8		14		22				
Dog ID	30' BL	2h IL-31	30' BL	2h IL-31	30' BL	2h IL-31	30' BL	2h IL-31	% Change	% Change	% Change
5427193	0	55	1	11	1	7	1	92	(80.0)	(87.27)	67.3
5853010	1	32	8	16	4	14	1	17	(50.0)	(56.25)	(46.9)
5353556	3	61	0	7	2	10	5	120	(88.5)	(83.61)	96.7
5044073	1	103	2	10	3	8	1	109	(90.3)	(92.23)	5.8
Mean	1.3	63	2.8	11	2.5	10	2.0	85	(77.2)	(79.8)	30.7
StError	0.6	15	1.8	2	0.6	2	1.0	23	9.3	8.1	32.1

FIG. 12

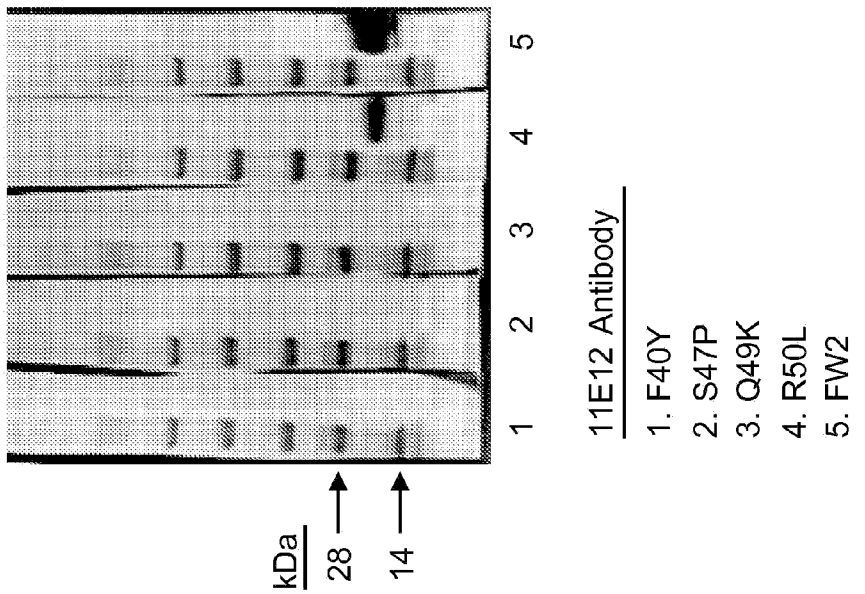

kDa
28
14
1
2
3
4
5
11E12 Antibody
1. F40Y
2. S47P
3. Q49K
4. R50L
5. FW2

FIG. 10

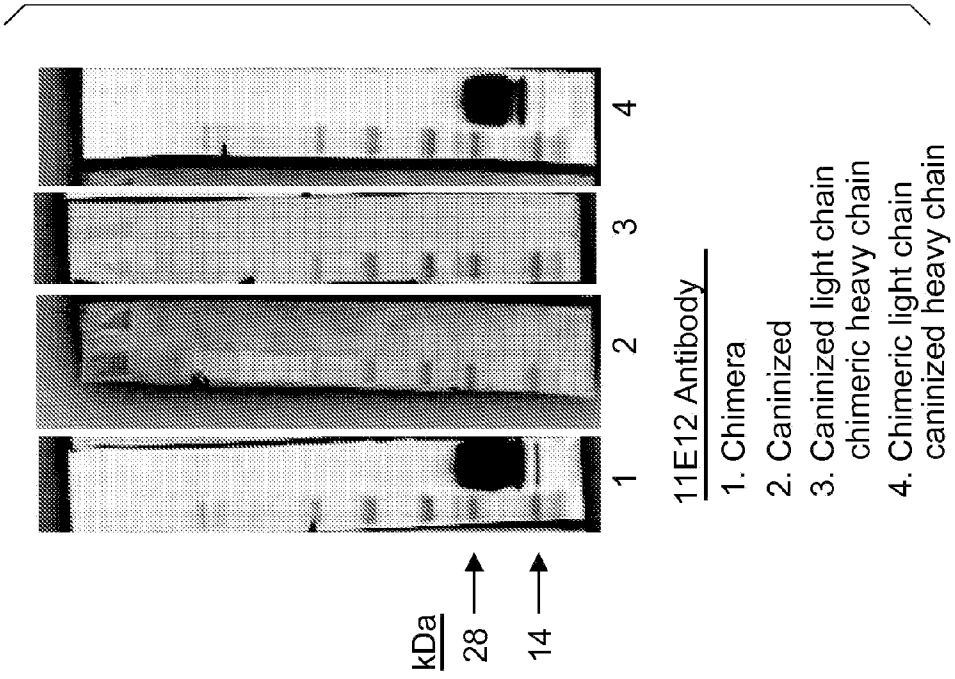

kDa
28
14
1
2
3
4
11E12 Antibody
1. Chimera
2. Caninized
3. Caninized light chain
chimeric heavy chain
4. Chimeric light chain
caninized heavy chain

FIG. 11

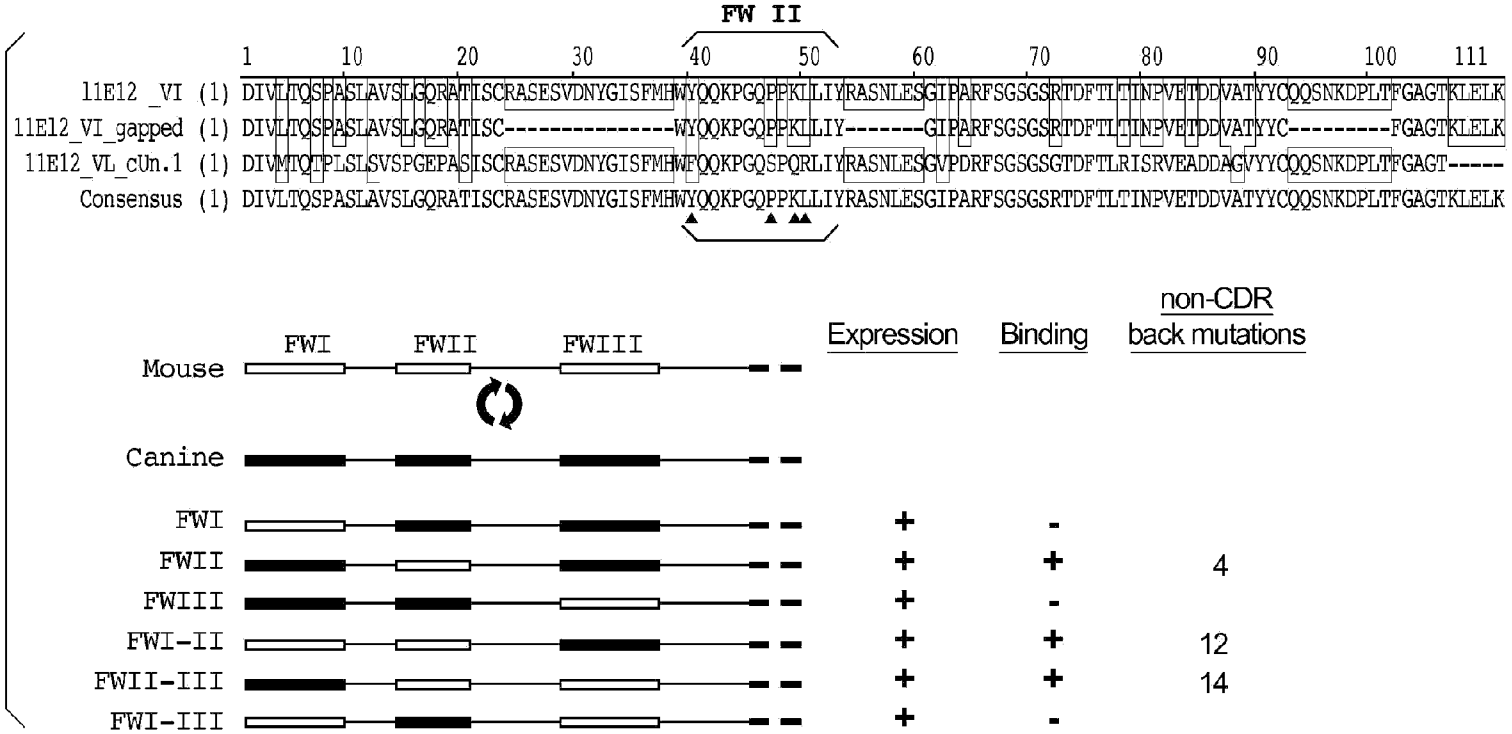


FIG. 13

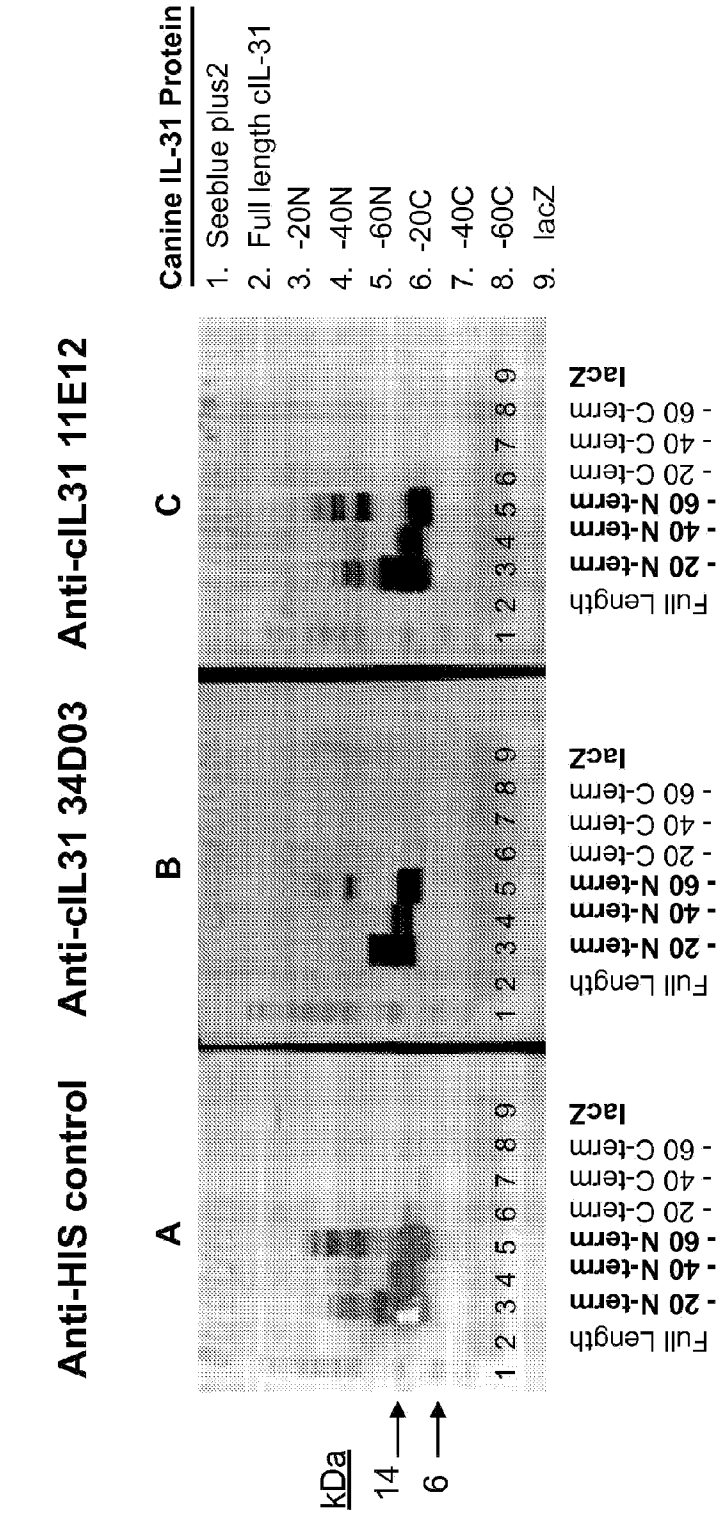

Anti-HIS control
Anti-cIL31 34D03
Anti-cIL31 11E12
A
B
C
Canine IL-31 Protein
1. Seeblue plus2
2. Full length cIL-31
3. -20N
4. -40N
5. -60N
6. -20C
7. -40C
8. -60C
9. lacZ
kDa
14
6
Full Length
- 20 N-term
- 40 N-term
- 60 N-term
- 20 C-term
- 40 C-term
- 60 C-term
lacZ
1 2 3 4 5 6 7 8 9

FIG. 14

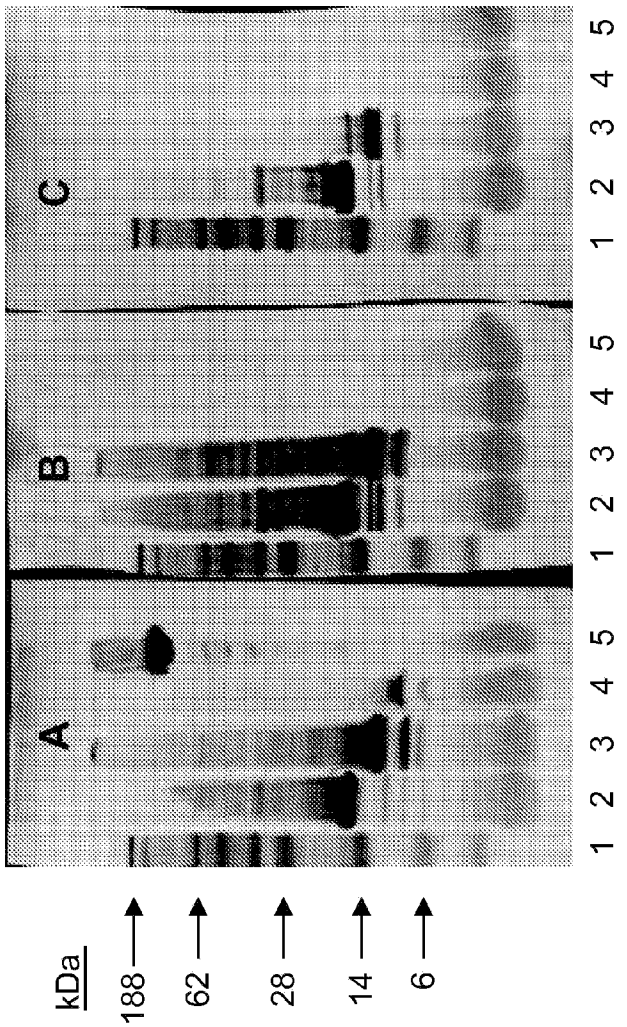

α His
11E12
34D03
A
B
C
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
kDa
188
62
28
14
6
Canine IL-31 Protein
1. Seeblue plus2
2. 20-122
3. 20-100
4. 20-80
5. lacZ

FIG. 15

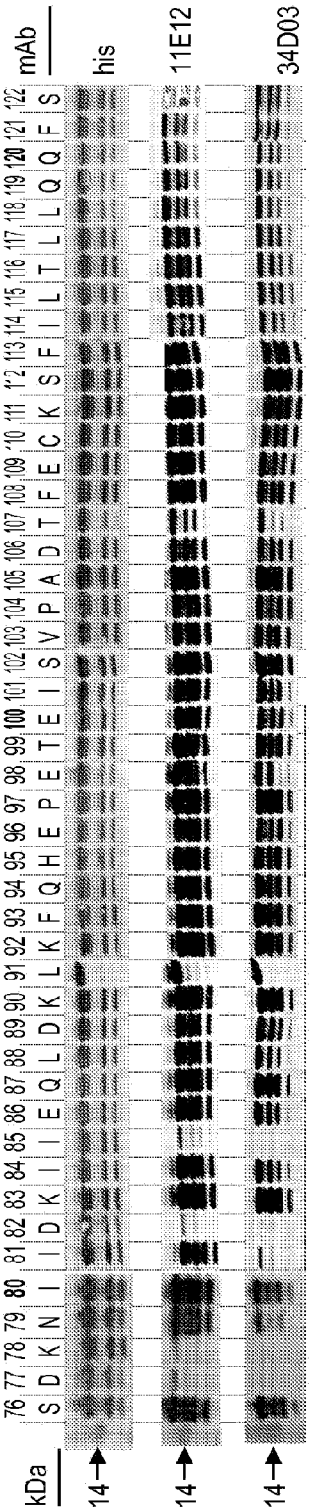

Canine IL-31with Alanine Substitution
kDa
mAb
his
11E12
34D03
14
14
14

FIG. 16

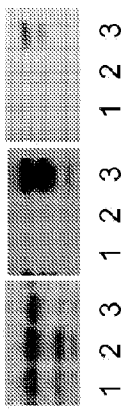

his
11E12
34D03
1 2 3 1 2 3 1 2 3
Canine IL-31 Protein
1. D82A, I85A
2. I81A, D82A, I85A
3. -20N

FIG. 17

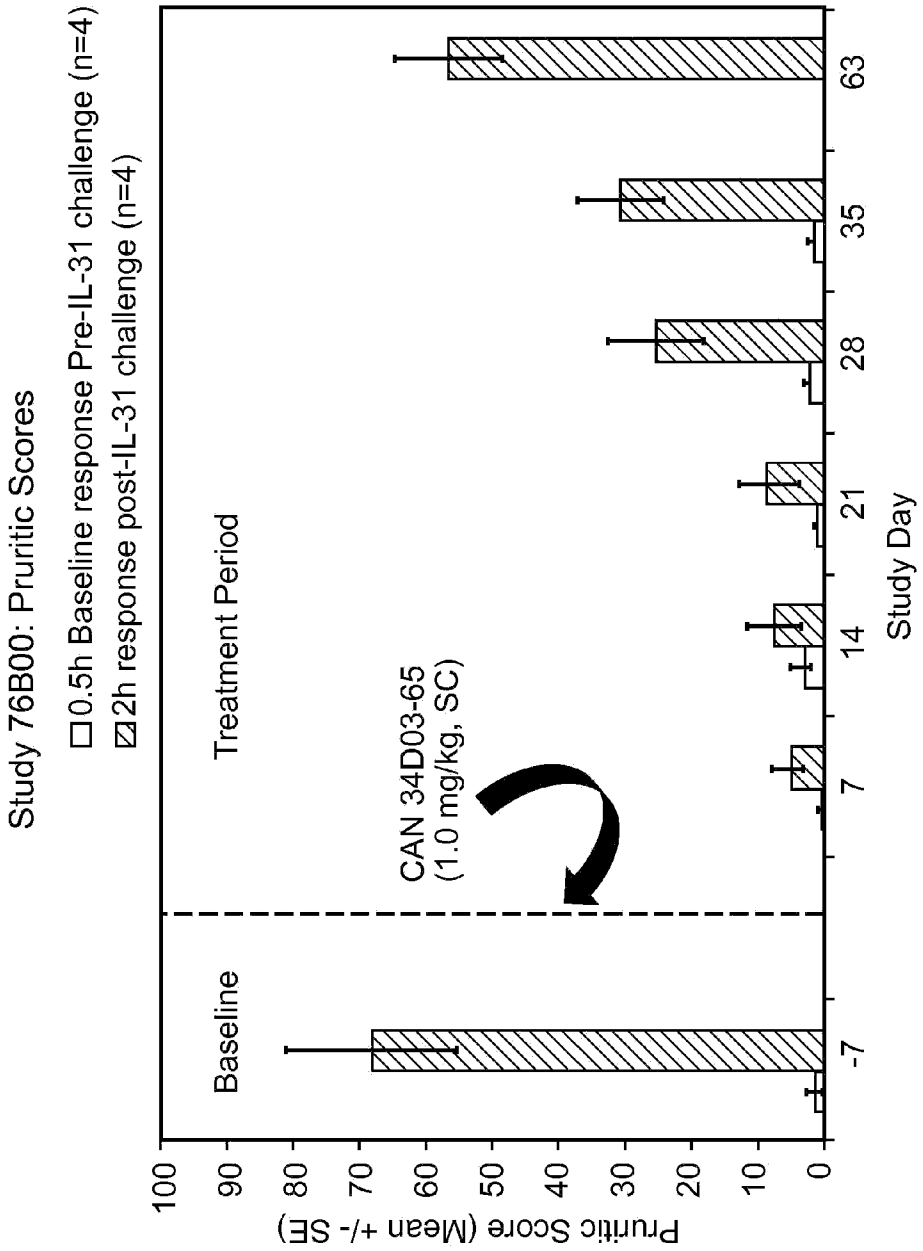

Study 76B00: Pruritic Scores
0.5h Baseline response Pre-IL-31 challenge (n=4)
2h response post-IL-31 challenge (n=4)
Baseline
Treatment Period
CAN 34D03-65
(1.0 mg/kg, SC)
Pruritic Score (Mean +/- SE)
0
10
20
30
40
50
60
70
80
90
100
-7
7
14
21
28
35
63
Study Day

FIG. 18

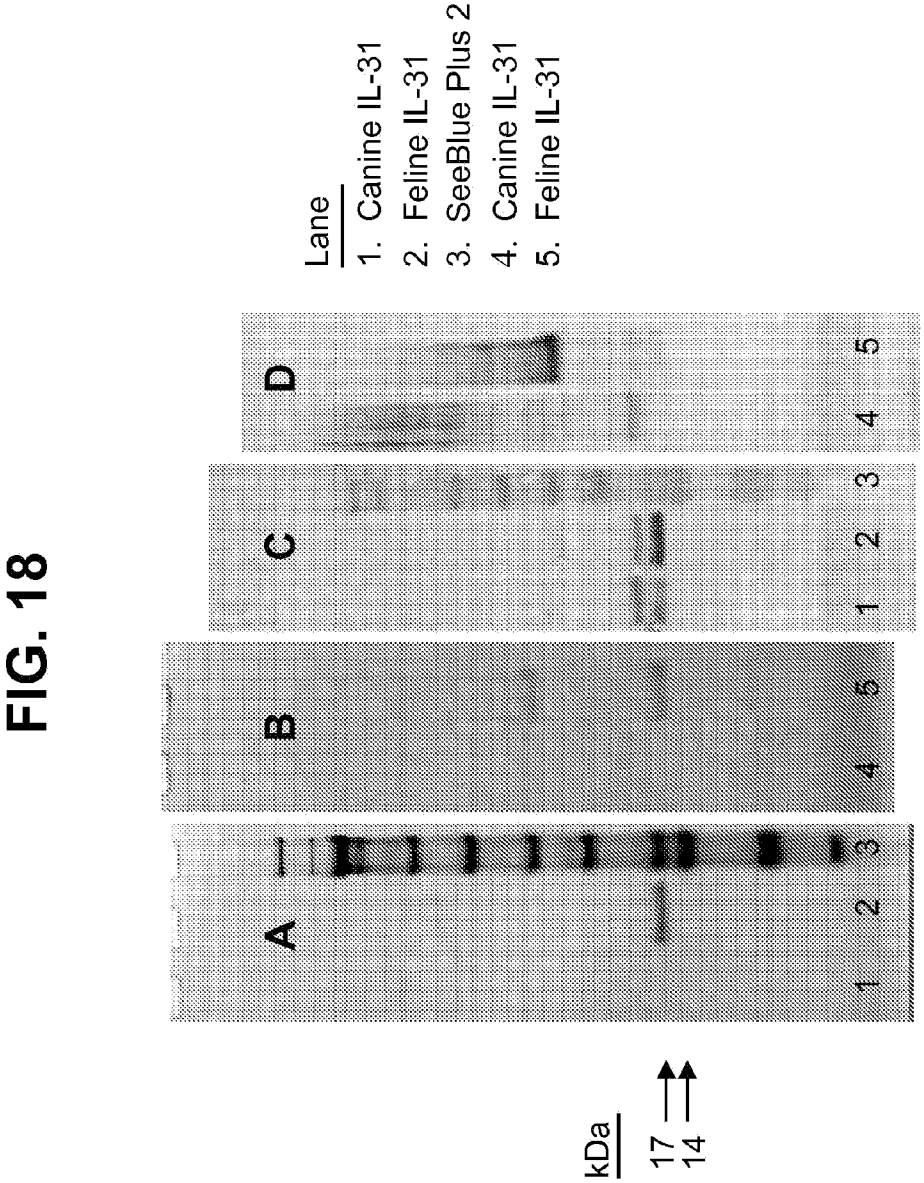

Lane
1. Canine IL-31
2. Feline IL-31
3. SeeBlue Plus 2
4. Canine IL-31
5. Feline IL-31
A
B
C
D
1 2 3
4 5
1 2 3
4 5
kDa
17
14

FIG. 19

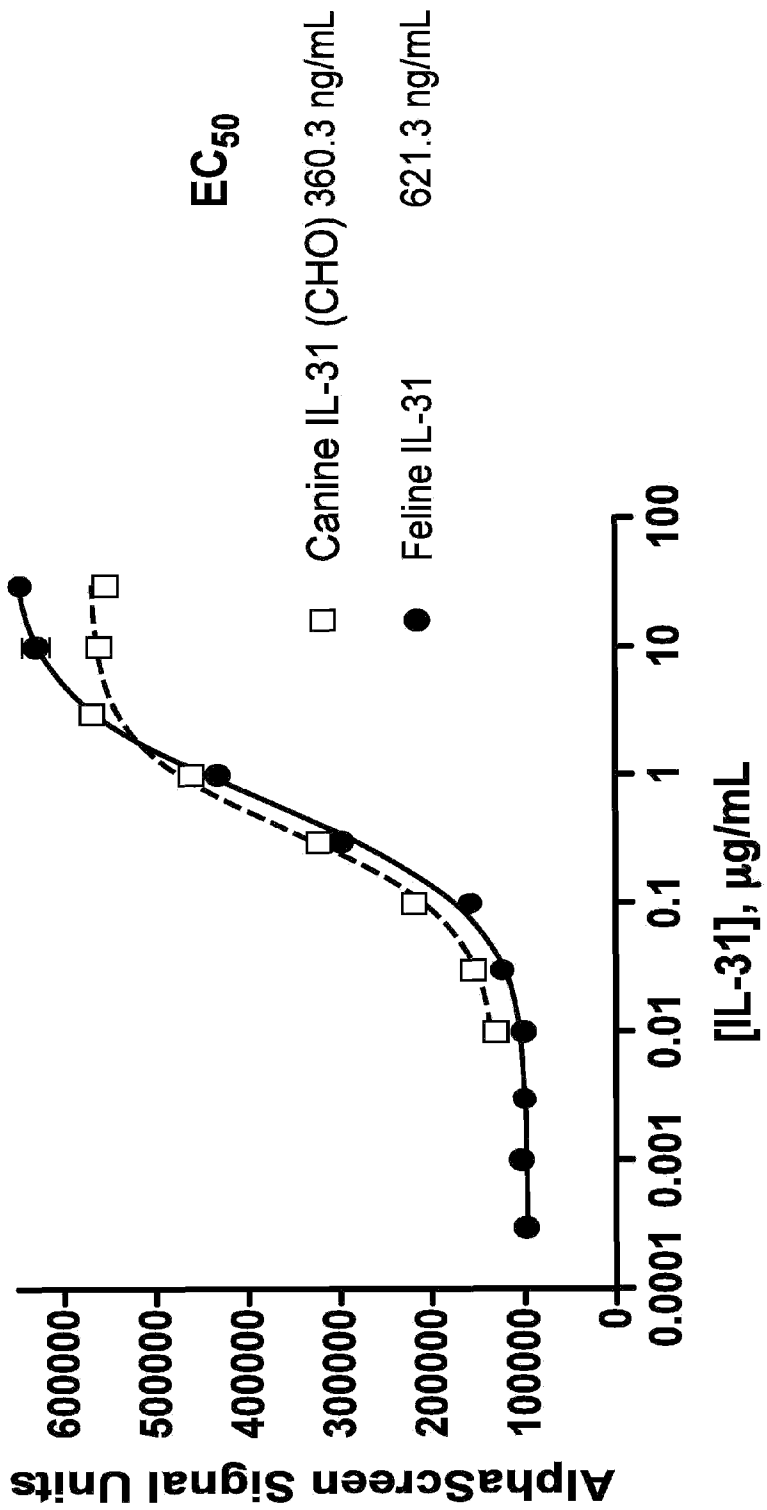
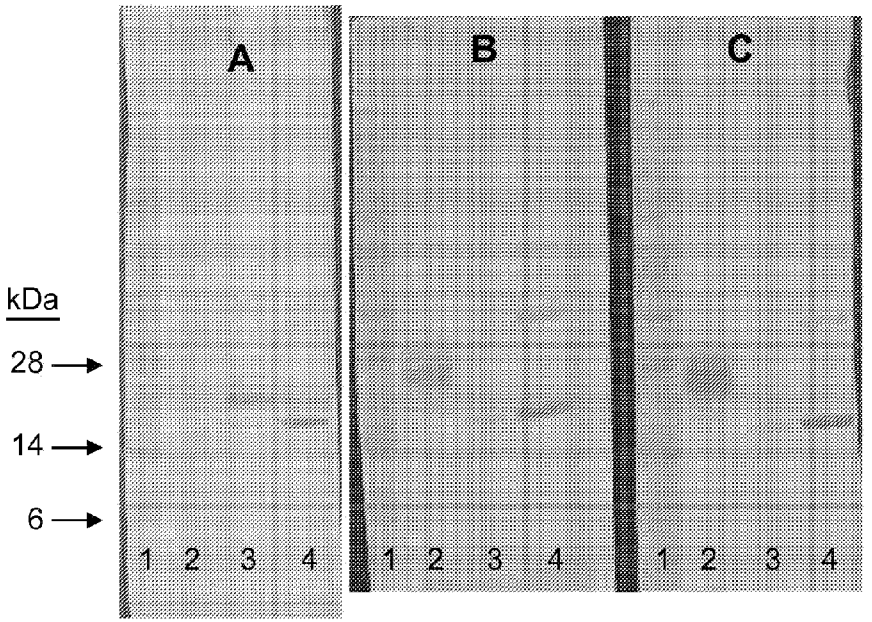

EC50
Canine IL-31 (CHO) 360.3 ng/mL
Feline IL-31 621.3 ng/mL
AlphaScreen Signal Units
600000
500000
400000
300000
200000
100000
0
0.0001 0.001 0.01 0.1 1 10 100
[IL-31], µg/mL

FIG. 20

					80											90	
Canine IL-31	S	D	K	N	I	I	D	K	I	I	E	Q	L	D	K	L	
Feline IL-31	S	D	K	N	T	I	D	K	I	I	E	Q	L	D	K	L	
	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	

FIG. 21



Lane

1. Seeblue plus2
2. Canine IL-31 (CHO), 400ng
3. Canine IL-31 (*E. coli*), ~150 ng
4. Feline IL-31 (*E. coli*), 400 ng

FIG. 22

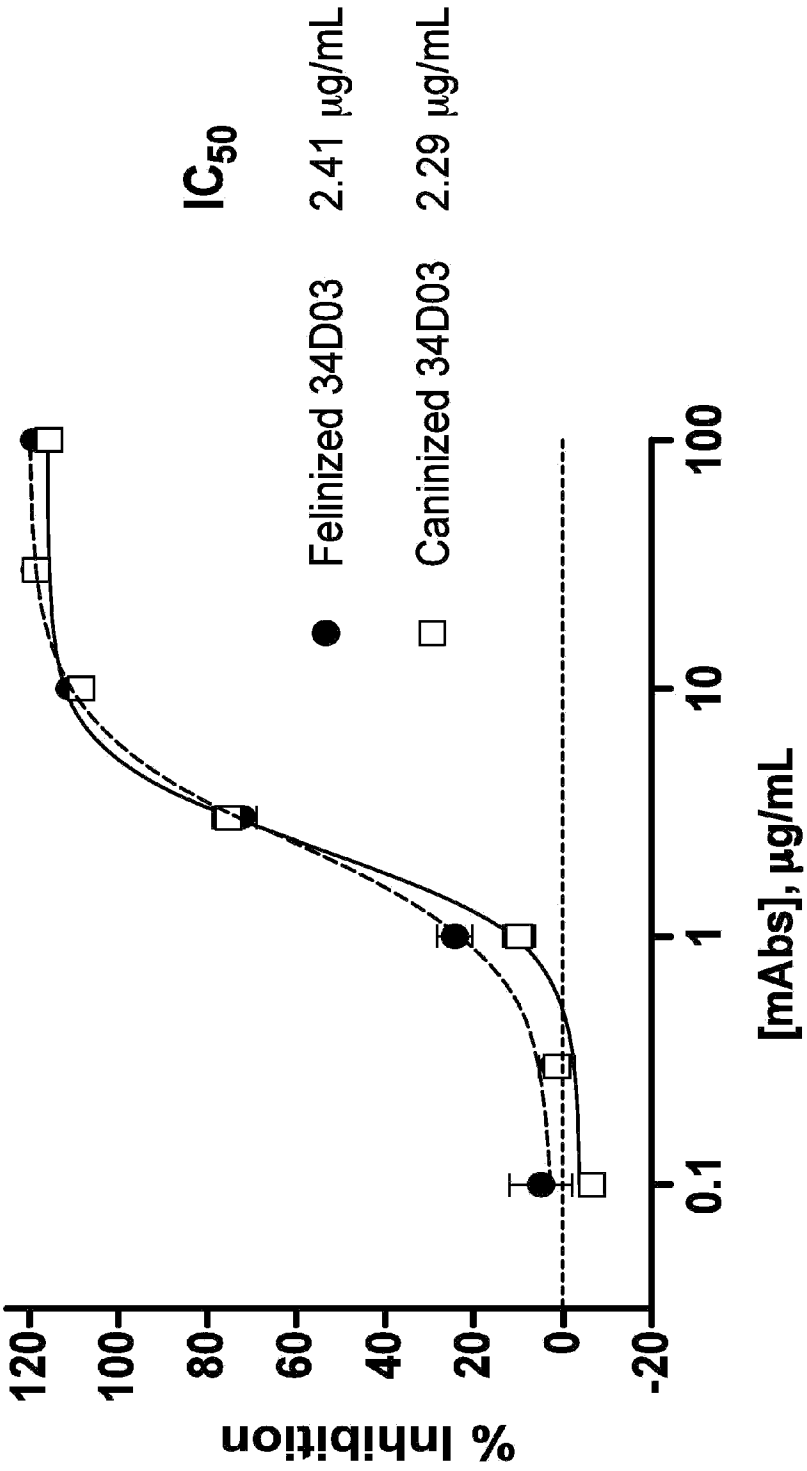
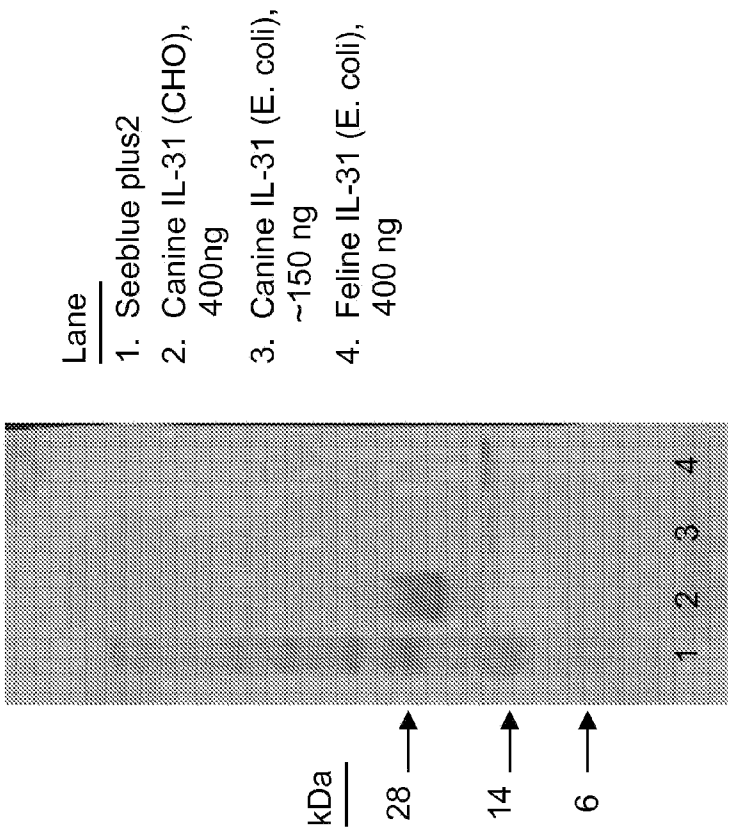

% Inhibition
120
100
80
60
40
20
0
-20
0.1
1
10
100
[mAbs], µg/mL
IC50
Felinized 34D03 2.41 µg/mL
Caninized 34D03 2.29 µg/mL

FIG. 23


Lane
1. Seeblue plus2
2. Canine IL-31 (CHO), 400ng
3. Canine IL-31 (E. coli), ~150 ng
4. Feline IL-31 (E. coli), 400 ng
kDa
28
14
6
1
2
3
4

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.