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(54) **Titre : ANTICORPS CONTRE LE RECEPTEUR ALPHA DE L'INTERLEUKINE 4 CANINE**
(54) **Title: ANTIBODIES TO CANINE INTERLEUKIN-4 RECEPTOR ALPHA**

(57) **Abrégé/Abstract:**
The present invention discloses antibodies and blocking antibodies to canine IL-4 receptor alpha that have specific sequences and a high binding affinity for canine IL-4 receptor a. The present invention also discloses the use of the antibodies of the present invention in the treatment of atopic dermatitis in dogs. The present invention further discloses unique epitopes that bind to the antibodies to canine IL-4 receptor alpha.

Abstract

The present invention discloses antibodies and blocking antibodies to canine IL-4 receptor alpha that have specific sequences and a high binding affinity for canine IL-4 receptor a. The present invention also discloses the use of the antibodies of the present invention in the treatment of atopic dermatitis in dogs. The present invention further discloses unique epitopes that bind to the antibodies to canine IL-4 receptor alpha.

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ANTIBODIES TO CANINE INTERLEUKIN-4 RECEPTOR *ALPHA*

FIELD OF THE INVENTION

The present invention relates to antibodies to canine IL-4 receptor *alpha* that have specific sequences, a high binding affinity for canine IL-4 receptor *alpha*, including some that can block the binding of canine IL-4 to canine IL-4 receptor *alpha*. The present invention further relates to unique epitopes that bind to the antibodies to canine IL-4 receptor *alpha*. The present invention also relates to use of the antibodies and the epitopes of the present invention in the treatment of atopic dermatis in dogs.

BACKGROUND OF THE INVENTION

The immune system comprises a network of resident and recirculating specialized cells that function collaboratively to protect the host against infectious diseases and cancer. The ability of the immune system to perform this function depends to a large extent on the biological activities of a group of proteins secreted by leukocytes and collectively referred to as interleukins. Among the well-studied interleukins are two important molecules identified as interleukin-4 (IL-4) and interleukin-13 (IL-13). IL-4 and IL-13 are two closely related proteins that can be secreted by many cell types including $CD4^+$ Th2 cells, natural killer T cells (NKT), macrophages, mast cells, and basophils. IL-4 and IL-13 display many overlapping functions and are critical to the development of T cell-dependent humoral immune responses. Despite their similarities in overall structure, cell sources and biological functions, each of these cytokines mediates certain specialized functions, which has stimulated considerable research aimed at identifying the receptors and the downstream signaling pathways through which these interleukins mediate both their common and unique biological activities.

It is now known that IL-4 binds with high affinity to two receptors *i.e.*, type-I and type-II IL-4 receptors. The type I IL-4 receptor consists of the IL-4 receptor α chain and the common γ C chain, which is also part of the receptor for several other interleukins including IL-2, IL-7, IL-9, and IL-15. The Type II IL-4 receptor consists of the IL-4 receptor α chain and the IL-13 receptor α1 chain. On other hand, IL-13 binds to the type-II IL-4 receptor, and to a unique receptor designated IL-13 receptor α2. The binding of IL-13 to the IL-13 receptor α2 does not transduce a signal and this receptor is also secreted in a soluble form. Accordingly the IL-13 receptor α2 has often been referred to as a decoy receptor.

The genes encoding the IL-4 protein from various species have been cloned and expressed in bacterial and mammalian cells. For example, the cDNA encoding human IL-4 shows that the mature human IL-4 is a secreted polypeptide of 129 amino acids with a predicted molecular weight of 15 Kd [Yokota *et al., Proc Natl Acad Sci U S A.* 83(16): 5894–5898 (1986)]. The cDNA encoding the canine IL-4 protein has also been identified and shown to encode a 132 amino acid polypeptide that shares 40% identity with human IL-4 [van der Kaaij *et al.*, *Immunogenetics* 49:142–143(1999)]. The gene encoding human IL-13 has been cloned and expressed in a variety of host systems [Minty *et al.*, *Nature* 362:248-50 (1993)]. A cDNA encoding human IL-13 shows that the mature IL-13 is a secreted polypeptide with a 12.4 Kd apparent molecular weight. A cDNA encoding canine IL-13 also has been identified [Yang *et al.*, *J. Interferon and Cytokine Research* 20:779–785 (2000)]. The predicted canine IL-13 mature polypeptide consists of 111 amino acids and shares 61.8% identity with human IL-13.

The genes encoding the human and mouse IL-4 receptor α chains have been cloned and expressed in a variety of host systems. For example, the cDNA encoding the human IL-4 receptor α chain has been described by *Galizzi et al.*, [*International Immunology* 2(7):669-675 (1990)] and the cDNA encoding the murine IL-4 receptor α chain has been described by *Mosley et al.*, [*Cell*, 59(2):335-348 (1989)]. The cDNA for human IL-4 receptor α chain encodes for 825 amino acid residues including a 24 amino acid residue signal sequence. Although the murine protein is 15 amino acid residues shorter than the human receptor, both proteins are closely related with an overall sequence identity of 50% at the amino acid level.

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Genes encoding equine, canine, and feline IL-4 receptor α chains have also been disclosed [*see*, US 7,208,579 B2]. In addition, a cDNA predicted to be corresponding to one isoform of canine IL-4 receptor α can be found in Genbank database (SEQ ID NO: 1). The present invention therefore undertook to determine the IL-4 receptor α chain cDNA and to definitively determine its encoded polypeptide sequence.

Although IL-4 and IL-13 are critical cytokines for the development of Th2 immune responses that are required for protection against extracellular pathogens (*e.g.*, tissue or lumen dwelling parasites), both cytokines have been implicated in the pathogenesis of a variety of allergic diseases in humans and animals, including asthma and atopic dermatitis. Asthma is a common respiratory disease in humans. The disease is characterized by lung inflammation, hyper-responsiveness of bronchial airways to external stimuli, and structural modifications of the bronchial wall tissues. The pathophysiology of allergic asthma has been reviewed by Vatrella *et al.*, [*Journal of Asthma and Allergy* 7:123-130 (2014)]. Asthma is sustained by $CD4^+$ Th2 cells which produce large amounts of IL-4 and IL-13 and orchestrate the immune inflammatory response in the allergic airways. Recent progress in understanding the asthmatic response highlights the important roles played by both IL-4 and IL-13 in the disease pathogenesis. For example, both cytokines stimulate immunoglobulin isotype switch in B cells from IgM to IgE, and this allergen-specific IgE contribute to mast cell degranulation and release of inflammatory mediators in the airways. In addition, both IL-4 and IL-13 increase bronchial smooth muscle contraction and stimulate airway recruitment of eosinophils which can also degranulate in response to crosslinking of allergen-bound IgE to its receptor on eosinophils. In addition, IL-13 also stimulates mucus secretion and promotes airway remodeling by stimulating goblet cell hyperplasia, deposition of collagen, and proliferation of airway smooth muscle cells. Thus it is now clear that IL-4 and IL-13 are intimately involved in the pathological changes that lead to expression of asthmatic episodes including bronchial constriction and increased airway hyperactivity.

Atopic dermatitis (AD) is a relapsing pruritic inflammatory skin disease that is characterized by immune system dysregulation and epidermal barrier abnormalities. The pathological and immunological attributes of AD have been the subject of extensive investigations [reviewed in Rahman *et al. Inflammation & Allergy-drug target* 10:486-496 (2011) and Harskamp *et al.*,

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Seminar in Cutaneous Medicine and Surgery 32:132-139 (2013)]. AD is the most common skin disease in man affecting 2-10% of the adult population in the United States and about 25% of children worldwide. In man, AD skin lesions are characterized by infiltrations with Th2 cells, eosinophils, mast cells and dendritic cells. In the acute phase of AD, these lesions display a predominant expression of Th2-type cytokines including IL-4 and IL-13. AD is also characterized by elevated circulating levels of IgE and is positively correlated with IL-4 and IL-13 expression in $CD4^+$ Th2 cells in the skin. Although AD has been classified as a Th2 disease, other T cell subsets such as Th1, Th22 and Th17 might also contribute to disease pathogenesis. Despite the increasing incidence of AD worldwide, treatment options available to patients whose symptoms are not adequately controlled by topical agents are limited to oral corticosteroids, oral cyclosporine and narrow band UVB phototherapy. These therapies are not always effective and their use is associated with a variety of safety effects. Recently, monoclonal antibodies specific to human IL-4 R_α have been developed and some of these antibodies have been tested extensively for their therapeutic utilities in man for treatment of atopic dermatitis [*see, e.g.,* US20150017176 A1].

AD is also a common disease in companion animals, especially dogs, where its prevalence has been estimated to be approximately 10-15% of the canine population. The pathogenesis of AD in dogs and cats [*reviewed in* Nuttall *et al., Veterinary Records* 172(8):201-207 (2013)] bears significant similarities to that of AD in man including skin infiltration by a variety of immune cells and $CD4^+$ Th2 polarized cytokine milieu including preponderance of IL-4 and IL-13 cytokines. As in humans, current therapies for atopic dermatitis in dogs and cats rely on palliative therapy such as shampoos and moisturizers or symptomatic therapy *via* the use of oral or systemic corticosteroids and oral cyclosporine. As with human AD, these therapies do not address the underlying mechanism of disease and have significant safety and efficacy issues. Thus, there is an unmet medical need for a safe and effective treatment option for AD in companion animals. Such treatment should preferably interfere with the underlying mechanism of disease.

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

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SUMMARY OF THE INVENTION

The present invention relates to anti-canine interleukin-4 receptor *alpha* (IL-4R$_\alpha$) antibodies that have a high binding affinity for canine IL-4R$_\alpha$. In more particular embodiments, the anti-canine interleukin-4 receptor *alpha* (IL-4R$_\alpha$) antibodies also have the ability to block the binding of canine IL-4 and canine IL-13 to the type-I or type II IL-4 receptors and subsequently inhibit the signaling from both canine IL-4 and IL-13. In particular embodiments such anti-canine IL-4R$_\alpha$ antibodies are murine anti-canine IL-4R$_\alpha$ antibodies. In more particular embodiments the anti-canine IL-4R$_\alpha$ antibodies have a high binding affinity to canine IL-4R$_\alpha$, as well as have the ability to block the binding of canine IL-4 and canine IL-13 to the type-I and type II IL-4 receptors.

Moreover, the present invention relates to the complementary determining regions (CDRs) comprised by these antibodies and the combination of these CDRs (*e.g.*, obtained from murine anti-canine IL-4R$_\alpha$ antibodies) into canine frames to form caninized anti-canine IL-4R$_\alpha$ antibodies. The present invention also relates to use of such antibodies in the treatment of conditions such as atopic dermatitis and/or other adverse conditions due to the downstream effects of the signaling from the binding of canine IL-4 and/or canine IL-13 to the type-I and/or type II IL-4 receptors.

Accordingly, the present invention provides unique sets of CDRs from fourteen (14) exemplified murine anti-canine IL-4R$_\alpha$ antibodies. The 14 exemplified murine anti-canine IL-4R$_\alpha$ antibodies have unique sets of CDRs, *i.e.*, three light chain CDRs: CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3) and three heavy chain CDRs CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3). As detailed below, there is substantial sequence homology within each group of CDRs, and even some redundancy *e.g.*, *see*, the set of CDRL1s below. Therefore, the present invention not only provides the amino acid sequences of the six CDRs from the 14 exemplified murine anti-canine IL-4R$_\alpha$ antibodies, but further provides conservatively modified variants of these CDRs, as well as variants that comprise (*e.g.*, share) the same canonical structure and/or bind to one or more (*e.g.*, 1 to 4, or more) amino acid residues of canine IL-4R$_\alpha$ that are comprised by an epitope of canine IL-4R$_\alpha$.

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Therefore, the present invention provides an antibody or antigen binding fragment thereof that binds IL-4R$_\alpha$ with specificity comprising a light chain complementary determining region 1 (VL CDR1) that comprises the amino acid sequence of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131, and/or a light chain complementary determining region 2 (VL CDR2) comprising the amino acid sequence of SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 132, SEQ ID NO: 133, or SEQ ID NO: 134, and/or a light chain complementary determining region 3 (VL CDR3) comprising the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139, and/or a heavy chain complementary determining region 1 (VH CDR1) in which the CDRH1 comprises the amino acid sequence of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, or SEQ ID NO: 143, and/or a heavy chain complementary determining region 2 (VH CDR2) comprising the amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, or SEQ ID NO: 148 and/or a heavy chain complementary determining region 3 (VH CDR3) comprising the amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, or SEQ ID NO: 153. In particular embodiments the antibody is a mammalian antibody. In more particular embodiments the antibody is a caninized antibody.

Accordingly, a caninized antibody of the present invention or antigen binding fragment thereof comprises one or more of the heavy chain complementary determining region 1 (VH CDR1) with an amino acid sequence of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, or SEQ ID NO: 143. In another embodiment, the

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heavy chain complementary determining region 2 (VH CDR2) comprises an amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, or SEQ ID NO: 148. In still another embodiment the heavy chain complementary determining region 3 (VH CDR3) comprises an amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, or SEQ ID NO: 153. In a particular embodiment of this type, the caninized antibody or antigen binding fragment comprises both a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, or SEQ ID NO: 143 and a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, or SEQ ID NO: 148. In another such embodiment, the caninized antibody or antigen binding fragment comprises both a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, or SEQ ID NO: 143, and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, or SEQ ID NO: 100, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, or SEQ ID NO: 153. In yet another such embodiment, the caninized antibody or antigen binding fragment comprises both a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, or SEQ ID NO: 148 and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, or SEQ ID NO: 100, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, or SEQ ID NO: 153. In still another such embodiment, the caninized antibody or antigen binding fragment comprises a VH CDR1 comprising an amino acid

sequence of SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, or SEQ ID NO: 143,, a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, or SEQ ID NO: 148 and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, or SEQ ID NO: 100, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, or SEQ ID NO: 153.

In particular embodiments, the caninized antibody or antigen binding fragment also comprises a light chain complementary determining region 1 (VL CDR1) comprising an amino acid sequence of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131. In related embodiments the light chain complementary determining region 2 (VL CDR2) comprises an amino acid sequence of SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 132, SEQ ID NO: 133, or SEQ ID NO: 134. In still another embodiment the light chain complementary determining region 3 (VL CDR3) comprises an amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139. In a particular embodiment of this type, the caninized antibody or antigen binding fragment comprises both a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131 and a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 132, SEQ ID NO: 133, or SEQ ID NO: 134.

In other such embodiments, the caninized antibody or antigen binding fragment comprises both a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131 and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139. In yet another such embodiments, the caninized antibody or antigen binding fragment comprises both a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 132, SEQ ID NO: 133, or SEQ ID NO: 134 and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139.

In still other such embodiments, the caninized antibody or antigen binding fragment comprises a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131, a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 132, SEQ ID NO: 133, or SEQ ID NO: 134, and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139.

In particular embodiments the caninized anti-canine IL-4R$_\alpha$ antibody comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-3A, and H3-12, respectively for CDR1, CDR2, and CDR3 of the heavy chain, *i.e.*, CDR1 of the heavy chain has the canonical structure class 1, CDR2 of the heavy chain has the canonical structure class 3A, and CDR3 of the heavy chain has the canonical structure class 12. In even more

particular embodiments, the CDRs for the corresponding light chains have canonical structures of: L1-1, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2A, and H3-7, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-2A, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In still other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2B, and H3-15, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-4, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In yet other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-1, and H3-15, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-3, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In still other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2B, and H3-6, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-2A, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain.

In yet other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2B, and H3-4, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-6, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In still other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-1, and H3-13, respectively for CDR1, CDR2, and CDR3 of the heavy

chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-1, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In yet other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2A, and H3-6, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-2A, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain.

In still other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-3A, and H3-15 or alternatively H3-13, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-6, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In yet other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-2A, and H3-10, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-6, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In still other embodiments the caninized anti-canine IL-4R$_\alpha$ antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-3A, and H3-9, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-3, L2-1, and L3-3, respectively for CDR1, CDR2, and CDR3 of the light chain.

The present invention also provides an isolated caninized antibody or antigen binding fragment thereof that specifically binds IL-4R$_\alpha$ comprising a canine IgG heavy chain and a canine *kappa* or *lambda* light chain. In particular embodiments of this type, the canine *kappa* or *lambda* light chain that comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and the canine IgG heavy chain

comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3) is obtained from the murine anti-canine IL-4R$_\alpha$ antibodies. Particular embodiments of the caninized antibodies and antigen binding fragments thereof of the present invention bind canine IL-4R$_\alpha$ and/or block the binding of canine IL-4R$_\alpha$ to canine IL-4.

In specific embodiments, the present invention provides an isolated mammalian antibody or antigen binding fragment thereof that binds canine interleukin-4 receptor *alpha* (IL-4R$_\alpha$) with specificity comprising three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3). In certain embodiments the CDRL1 comprises the amino acid sequence of SEQ ID NO: 47, a variant of SEQ ID NO: 47, a conservatively modified variant of SEQ ID NO: 47, a variant of SEQ ID NO: 47 that comprises the canonical structure class of 1, SEQ ID NO: 48, a variant of SEQ ID NO: 48, a conservatively modified variant of SEQ ID NO: 48, a variant of SEQ ID NO: 48 that comprises the canonical structure class of 2A, SEQ ID NO: 49, a variant of SEQ ID NO: 49, a conservatively modified variant of SEQ ID NO: 49, a variant of SEQ ID NO: 49 that comprises the canonical structure class of 4, SEQ ID NO: 50, a variant of SEQ ID NO: 50, a conservatively modified variant of SEQ ID NO: 50, a variant of SEQ ID NO: 50 that comprises the canonical structure class of 3, SEQ ID NO: 51, a variant of SEQ ID NO: 51, a conservatively modified variant of SEQ ID NO: 51, a variant of SEQ ID NO: 51 that comprises the canonical structure class of 3, SEQ ID NO: 52, a variant of SEQ ID NO: 52, a conservatively modified variant of SEQ ID NO: 52, a variant of SEQ ID NO: 52 that comprises the canonical structure class of 2A, SEQ ID NO: 53, a variant of SEQ ID NO: 53, a conservatively modified variant of SEQ ID NO: 53, a variant of SEQ ID NO: 53 that comprises the canonical structure class of 6, SEQ ID NO: 54, a variant of SEQ ID NO: 54, a conservatively modified variant of SEQ ID NO: 54, a variant of SEQ ID NO: 54 that comprises the canonical structure class of 1, SEQ ID NO: 55, a variant of SEQ ID NO: 55, a conservatively modified variant of SEQ ID NO: 55, a variant of SEQ ID NO: 55 that comprises the canonical structure class of 2A, SEQ ID NO: 129, a variant of SEQ ID NO: 129, a conservatively modified variant of SEQ ID NO: 129, a variant of SEQ ID NO: 129 that comprises the canonical structure class of 6, SEQ ID NO: 130, a variant of SEQ ID NO: 130, a conservatively modified variant of SEQ ID NO: 130, a variant of SEQ ID NO: 130

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that comprises the canonical structure class of 6, SEQ ID NO: 131, a variant of SEQ ID NO: 131, a conservatively modified variant of SEQ ID NO: 131, or a variant of SEQ ID NO: 131 that comprises the canonical structure class of 3.

The corresponding CDRL2 comprises the amino acid sequence of SEQ ID NO: 56, a variant of SEQ ID NO: 56, a conservatively modified variant of SEQ ID NO: 56, a variant of SEQ ID NO: 56 that comprises the canonical structure class of 1, SEQ ID NO: 57, a variant of SEQ ID NO: 57, a conservatively modified variant of SEQ ID NO: 57, a variant of SEQ ID NO: 57 that comprises the canonical structure class of 1, SEQ ID NO: 58, a variant of SEQ ID NO: 58, a conservatively modified variant of SEQ ID NO: 58, a variant of SEQ ID NO: 58 that comprises the canonical structure class of 1, SEQ ID NO: 59, a variant of SEQ ID NO: 59, a conservatively modified variant of SEQ ID NO: 59, a variant of SEQ ID NO: 59 that comprises the canonical structure class of 1, SEQ ID NO: 60, a variant of SEQ ID NO: 60, a conservatively modified variant of SEQ ID NO: 60, a variant of SEQ ID NO: 60 that comprises the canonical structure class of 1, SEQ ID NO: 61, a variant of SEQ ID NO: 61, a conservatively modified variant of SEQ ID NO: 61, a variant of SEQ ID NO: 61 that comprises the canonical structure class of 1, SEQ ID NO: 62, a variant of SEQ ID NO: 62, a conservatively modified variant of SEQ ID NO: 62, a variant of SEQ ID NO: 62 that comprises the canonical structure class of 1, SEQ ID NO: 63, a variant of SEQ ID NO: 63, a conservatively modified variant of SEQ ID NO: 63, a variant of SEQ ID NO: 63 that comprises the canonical structure class of 1, SEQ ID NO: 64, a variant of SEQ ID NO: 64, a conservatively modified variant of SEQ ID NO: 64, or a variant of SEQ ID NO: 64 that comprises the canonical structure class of 1, SEQ ID NO: 132, a variant of SEQ ID NO: 132, a conservatively modified variant of SEQ ID NO: 132, a variant of SEQ ID NO: 132 that comprises the canonical structure class of 1, SEQ ID NO: 133, a variant of SEQ ID NO: 133, a conservatively modified variant of SEQ ID NO: 133, a variant of SEQ ID NO: 133 that comprises the canonical structure class of 1, SEQ ID NO: 134, a variant of SEQ ID NO: 134, a conservatively modified variant of SEQ ID NO: 134, or a variant of SEQ ID NO: 134 that comprises the canonical structure class of 1.

The corresponding CDRL3 comprises the amino acid sequence of SEQ ID NO: 65, a variant of SEQ ID NO: 65, a conservatively modified variant of SEQ ID NO: 65, a variant of SEQ ID

NO: 65 that comprises the canonical structure class of 1, SEQ ID NO: 66, a variant of SEQ ID NO: 66, a conservatively modified variant of SEQ ID NO: 66, a variant of SEQ ID NO: 66 that comprises the canonical structure class of 1, SEQ ID NO: 67, a variant of SEQ ID NO: 67, a conservatively modified variant of SEQ ID NO: 67, a variant of SEQ ID NO: 67 that comprises the canonical structure class of 1, SEQ ID NO: 68, a variant of SEQ ID NO: 68, a conservatively modified variant of SEQ ID NO: 68, a variant of SEQ ID NO: 68 that comprises the canonical structure class of 1, SEQ ID NO: 69, a variant of SEQ ID NO: 69, a conservatively modified variant of SEQ ID NO: 69, a variant of SEQ ID NO: 69 that comprises the canonical structure class of 1, SEQ ID NO: 70, a variant of SEQ ID NO: 70, a conservatively modified variant of SEQ ID NO: 70, a variant of SEQ ID NO: 70 that comprises the canonical structure class of 1, SEQ ID NO: 71, a variant of SEQ ID NO: 71, a conservatively modified variant of SEQ ID NO: 71, a variant of SEQ ID NO: 71 that comprises the canonical structure class of 1, SEQ ID NO: 72, a variant of SEQ ID NO: 72, a conservatively modified variant of SEQ ID NO: 72, a variant of SEQ ID NO: 72 that comprises the canonical structure class of 1, SEQ ID NO: 73, a variant of SEQ ID NO: 73, a conservatively modified variant of SEQ ID NO: 73, a variant of SEQ ID NO: 73 that comprises the canonical structure class of 1,

SEQ ID NO: 135, a variant of SEQ ID NO: 135, a conservatively modified variant of SEQ ID NO: 135, a variant of SEQ ID NO: 135 that comprises the canonical structure class of 1, SEQ ID NO: 136, a variant of SEQ ID NO: 136, a conservatively modified variant of SEQ ID NO: 136, a variant of SEQ ID NO: 136 that comprises the canonical structure class of 1, SEQ ID NO: 137, a variant of SEQ ID NO: 137, a conservatively modified variant of SEQ ID NO: 137, a variant of SEQ ID NO: 137 that comprises the canonical structure class of 1, SEQ ID NO: 138, a variant of SEQ ID NO: 138, a conservatively modified variant of SEQ ID NO: 138, a variant of SEQ ID NO: 138 that comprises the canonical structure class of 3, SEQ ID NO: 139, a variant of SEQ ID NO: 139, a conservatively modified variant of SEQ ID NO: 139, or a variant of SEQ ID NO: 139 that comprises the canonical structure class of 1.

The corresponding CDRH1 comprises the amino acid sequence of SEQ ID NO: 74, a variant of SEQ ID NO: 74, a conservatively modified variant of SEQ ID NO: 74, a variant of SEQ ID NO: 74 that comprises the canonical structure class of 1, SEQ ID NO: 75, a variant of SEQ ID

NO: 75, a conservatively modified variant of SEQ ID NO: 75, a variant of SEQ ID NO: 75 that comprises the canonical structure class of 1, SEQ ID NO: 76, a variant of SEQ ID NO: 76, a conservatively modified variant of SEQ ID NO: 76, or a variant of SEQ ID NO: 76 that comprises the canonical structure class of 1, SEQ ID NO: 77, a variant of SEQ ID NO: 77, a conservatively modified variant of SEQ ID NO: 77, or a variant of SEQ ID NO: 77 that comprises the canonical structure class of 1, SEQ ID NO: 78, a variant of SEQ ID NO: 78, a conservatively modified variant of SEQ ID NO: 78, a variant of SEQ ID NO: 78 that comprises the canonical structure class of 1, SEQ ID NO: 79, a variant of SEQ ID NO: 79, a conservatively modified variant of SEQ ID NO: 79, a variant of SEQ ID NO: 79 that comprises the canonical structure class of 1, SEQ ID NO: 80, a variant of SEQ ID NO: 80, a conservatively modified variant of SEQ ID NO: 80, a variant of SEQ ID NO: 80 that comprises the canonical structure class of 1, SEQ ID NO: 81, a variant of SEQ ID NO: 81, a conservatively modified variant of SEQ ID NO: 81, a variant of SEQ ID NO: 81 that comprises the canonical structure class of 1, SEQ ID NO: 82, a variant of SEQ ID NO: 82, a conservatively modified variant of SEQ ID NO: 82, or a variant of SEQ ID NO: 82 that comprises the canonical structure class of 1, SEQ ID NO: 140, a variant of SEQ ID NO: 140, a conservatively modified variant of SEQ ID NO: 140, a variant of SEQ ID NO: 140 that comprises the canonical structure class of 1, SEQ ID NO: 141, a variant of SEQ ID NO: 141, a conservatively modified variant of SEQ ID NO: 141, a variant of SEQ ID NO: 141 that comprises the canonical structure class of 1, SEQ ID NO: 142, a variant of SEQ ID NO: 142, a conservatively modified variant of SEQ ID NO: 142, a variant of SEQ ID NO: 142 that comprises the canonical structure class of 1, SEQ ID NO: 143, a variant of SEQ ID NO: 143, a conservatively modified variant of SEQ ID NO: 143, or a variant of SEQ ID NO: 143 that comprises the canonical structure class of 1.

The corresponding CDRH2 comprises the amino acid sequence of SEQ ID NO: 83, a variant of SEQ ID NO: 83, a conservatively modified variant of SEQ ID NO: 83, a variant of SEQ ID NO: 83 that comprises the canonical structure class of 3A, SEQ ID NO: 84, a variant of SEQ ID NO: 84, a conservatively modified variant of SEQ ID NO: 84, a variant of SEQ ID NO: 84 that comprises the canonical structure class of 2A, SEQ ID NO: 85, a variant of SEQ ID NO: 85, a conservatively modified variant of SEQ ID NO: 85, or a variant of SEQ ID NO: 85 that comprises the canonical structure class of 2B, SEQ ID NO: 86, a variant of SEQ ID NO: 86, a

conservatively modified variant of SEQ ID NO: 86, SEQ ID NO: 87, a variant of SEQ ID NO: 87, a conservatively modified variant of SEQ ID NO: 87, a variant of SEQ ID NO: 87 that comprises the canonical structure class of 1, SEQ ID NO: 88, a variant of SEQ ID NO: 88, a conservatively modified variant of SEQ ID NO: 88, a variant of SEQ ID NO: 88 that comprises the canonical structure class of 2B, SEQ ID NO: 89, a variant of SEQ ID NO: 89, a conservatively modified variant of SEQ ID NO: 89, a variant of SEQ ID NO: 89 that comprises the canonical structure class of 2B, SEQ ID NO: 90, a variant of SEQ ID NO: 90, a conservatively modified variant of SEQ ID NO: 90, a variant of SEQ ID NO: 90 that comprises the canonical structure class of 1, SEQ ID NO: 91, a variant of SEQ ID NO: 91, a conservatively modified variant of SEQ ID NO: 91, a variant of SEQ ID NO: 91 that comprises the canonical structure class of 2A, SEQ ID NO: 144, a variant of SEQ ID NO: 144, a conservatively modified variant of SEQ ID NO: 144, a variant of SEQ ID NO: 144 that comprises the canonical structure class of 3A, SEQ ID NO: 145, a variant of SEQ ID NO: 145, a conservatively modified variant of SEQ ID NO: 145, a variant of SEQ ID NO: 145 that comprises the canonical structure class of 2A, SEQ ID NO: 146, a variant of SEQ ID NO: 146, a conservatively modified variant of SEQ ID NO: 146, a variant of SEQ ID NO: 146 that comprises the canonical structure class of 3A, SEQ ID NO: 147, a variant of SEQ ID NO: 147, a conservatively modified variant of SEQ ID NO: 147, a variant of SEQ ID NO: 147 that comprises the canonical structure class of 3A, SEQ ID NO: 148, a variant of SEQ ID NO: 148, a conservatively modified variant of SEQ ID NO: 148, or a variant of SEQ ID NO: 148 that comprises the canonical structure class of 3A.

The corresponding CDRH3 comprises the amino acid sequence of SEQ ID NO: 92, a variant of SEQ ID NO: 92, a conservatively modified variant of SEQ ID NO: 92, a variant of SEQ ID NO: 92 that comprises the canonical structure class of 12, SEQ ID NO: 93, a variant of SEQ ID NO: 93, a conservatively modified variant of SEQ ID NO: 93, a variant of SEQ ID NO: 93 that comprises the canonical structure class of 7, SEQ ID NO: 94, a variant of SEQ ID NO: 94, a conservatively modified variant of SEQ ID NO: 94, or a variant of SEQ ID NO: 94 that comprises the canonical structure class of 15, SEQ ID NO: 95, a variant of SEQ ID NO: 95, a conservatively modified variant of SEQ ID NO: 95, or a variant of SEQ ID NO: 95 that comprises the canonical structure class of 11, SEQ ID NO: 96, a variant of SEQ ID NO: 96, a conservatively modified variant of SEQ ID NO: 96, a variant of SEQ ID NO: 96 that comprises the canonical structure

class of 15, SEQ ID NO: 97, a variant of SEQ ID NO: 97, a conservatively modified variant of SEQ ID NO: 97, a variant of SEQ ID NO: 97 that comprises the canonical structure class of 6, SEQ ID NO: 98, a variant of SEQ ID NO: 98, a conservatively modified variant of SEQ ID NO: 98, a variant of SEQ ID NO: 98 that comprises the canonical structure class of 4, SEQ ID NO: 99, a variant of SEQ ID NO: 99, a conservatively modified variant of SEQ ID NO: 99, a variant of SEQ ID NO: 99 that comprises the canonical structure class of 13, SEQ ID NO: 100, a variant of SEQ ID NO: 100, a conservatively modified variant of SEQ ID NO: 100, or a variant of SEQ ID NO: 100 that comprises the canonical structure class of 6, SEQ ID NO: 149, a variant of SEQ ID NO: 149, a conservatively modified variant of SEQ ID NO: 149, a variant of SEQ ID NO: 149 that comprises the canonical structure class of 15, SEQ ID NO: 150, a variant of SEQ ID NO: 150, a conservatively modified variant of SEQ ID NO: 150, a variant of SEQ ID NO: 150 that comprises the canonical structure class of 10, SEQ ID NO: 151, a variant of SEQ ID NO: 151, a conservatively modified variant of SEQ ID NO: 151, a variant of SEQ ID NO: 151 that comprises the canonical structure class of 15, SEQ ID NO: 152, a variant of SEQ ID NO: 152, a conservatively modified variant of SEQ ID NO: 152, a variant of SEQ ID NO: 152 that comprises the canonical structure class of 9, SEQ ID NO: 153, a variant of SEQ ID NO: 153, a conservatively modified variant of SEQ ID NO: 153, or a variant of SEQ ID NO: 153 that comprises the canonical structure class of 13.

In particular embodiments the mammalian antibodies (including chimeric mammalian antibodies) and/or antigen binding fragments thereof of the present invention bind the canine interleukin-4 receptor *alpha* (IL-4R$_\alpha$) and/or block the binding of canine IL-4R$_\alpha$, to canine IL-4 and/or canine IL-13. In related embodiments the mammalian antibodies and/or antigen binding fragments thereof of the present invention block the binding of canine IL-4 and/or canine IL-13 to the IL-4 Type I receptor and/or the IL-4 Type II receptor. In particular embodiments the mammalian antibodies (whether isolated or not) are caninized antibodies.

Accordingly, in certain embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 47, a variant of SEQ ID NO: 47, a conservatively modified variant of SEQ ID NO: 47, or a variant of SEQ ID NO: 47 that comprises the canonical structure class of 1; the CDRL2 comprises the amino acid sequence of

SEQ ID NO: 56, a variant of SEQ ID NO: 56, a conservatively modified variant of SEQ ID NO: 56, or a variant of SEQ ID NO: 56 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 65, a variant of SEQ ID NO: 65, a conservatively modified variant of SEQ ID NO: 65, or a variant of SEQ ID NO: 65 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 74, a variant of SEQ ID NO: 74, a conservatively modified variant of SEQ ID NO: 74, or a variant of SEQ ID NO: 74 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 83, a variant of SEQ ID NO: 83, a conservatively modified variant of SEQ ID NO: 83, and a variant of SEQ ID NO: 83 that comprises the canonical structure class of 3A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 92, a variant of SEQ ID NO: 92, a conservatively modified variant of SEQ ID NO: 92, or a variant of SEQ ID NO: 92 that comprises the canonical structure class of 12.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 48, a variant of SEQ ID NO: 48, a conservatively modified variant of SEQ ID NO: 48, or a variant of SEQ ID NO: 48 that comprises the canonical structure class of 2A; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 57, a variant of SEQ ID NO: 57, a conservatively modified variant of SEQ ID NO: 57, or a variant of SEQ ID NO: 57 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 66, a variant of SEQ ID NO: 66, a conservatively modified variant of SEQ ID NO: 66, or a variant of SEQ ID NO: 66 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 75, a variant of SEQ ID NO: 75, a conservatively modified variant of SEQ ID NO: 75, or a variant of SEQ ID NO: 75 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 84, a variant of SEQ ID NO: 84, a conservatively modified variant of SEQ ID NO: 84, and a variant of SEQ ID NO: 84 that comprises the canonical structure class of 2A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93, a variant of SEQ ID NO: 93, a conservatively modified variant of SEQ ID NO: 93, or a variant of SEQ ID NO: 93 that comprises the canonical structure class of 7.

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In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 49, a variant of SEQ ID NO: 49, a conservatively modified variant of SEQ ID NO: 49, or a variant of SEQ ID NO: 49 that comprises the canonical structure class of 4; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 58, a variant of SEQ ID NO: 58, a conservatively modified variant of SEQ ID NO: 58, or a variant of SEQ ID NO: 58 that comprises the canonical structure class of 4; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 67, a variant of SEQ ID NO: 67, a conservatively modified variant of SEQ ID NO: 67, or a variant of SEQ ID NO: 67 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 76, a variant of SEQ ID NO: 76, a conservatively modified variant of SEQ ID NO: 76, or a variant of SEQ ID NO: 76 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 85, a variant of SEQ ID NO: 85, a conservatively modified variant of SEQ ID NO: 85, and a variant of SEQ ID NO: 85 that comprises the canonical structure class of 2B, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 94, a variant of SEQ ID NO: 94, a conservatively modified variant of SEQ ID NO: 94, or a variant of SEQ ID NO: 94 that comprises the canonical structure class of 15.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 51, a variant of SEQ ID NO: 51, a conservatively modified variant of SEQ ID NO: 51, or a variant of SEQ ID NO: 51 that comprises the canonical structure class of 3; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 60, a variant of SEQ ID NO: 60, a conservatively modified variant of SEQ ID NO: 60, or a variant of SEQ ID NO: 60 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 69, a variant of SEQ ID NO: 69, a conservatively modified variant of SEQ ID NO: 69, or a variant of SEQ ID NO: 69 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 78, a variant of SEQ ID NO: 78, a conservatively modified variant of SEQ ID NO: 78, or a variant of SEQ ID NO: 78 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 87, a variant of SEQ ID NO: 87, a conservatively modified variant of SEQ ID NO: 87, and a variant of SEQ ID NO: 87 that comprises the canonical structure class of 1, the CDRH3 comprises the amino acid sequence of

SEQ ID NO: 96, a variant of SEQ ID NO: 96, a conservatively modified variant of SEQ ID NO: 96, or a variant of SEQ ID NO: 96 that comprises the canonical structure class of 15.

In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 52, a variant of SEQ ID NO: 52, a conservatively modified variant of SEQ ID NO: 52, or a variant of SEQ ID NO: 52 that comprises the canonical structure class of 2A; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 61, a variant of SEQ ID NO: 61, a conservatively modified variant of SEQ ID NO: 61, or a variant of SEQ ID NO: 61 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 70, a variant of SEQ ID NO: 70, a conservatively modified variant of SEQ ID NO: 70, or a variant of SEQ ID NO: 70 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 79, a variant of SEQ ID NO: 79, a conservatively modified variant of SEQ ID NO: 79, or a variant of SEQ ID NO: 79 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 88, a variant of SEQ ID NO: 88, a conservatively modified variant of SEQ ID NO: 88, and a variant of SEQ ID NO: 88 that comprises the canonical structure class of 2B, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 97, a variant of SEQ ID NO: 97, a conservatively modified variant of SEQ ID NO: 97, or a variant of SEQ ID NO: 97 that comprises the canonical structure class of 6.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 53, a variant of SEQ ID NO: 53, a conservatively modified variant of SEQ ID NO: 53, or a variant of SEQ ID NO: 53 that comprises the canonical structure class of 6; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 62, a variant of SEQ ID NO: 62, a conservatively modified variant of SEQ ID NO: 62, or a variant of SEQ ID NO: 62 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 71, a variant of SEQ ID NO: 71, a conservatively modified variant of SEQ ID NO: 71, or a variant of SEQ ID NO: 71 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 80, a variant of SEQ ID NO: 80, a conservatively modified variant of SEQ ID NO: 80, or a variant of SEQ ID NO: 80 that comprises the canonical structure class of 1; the CDRH2

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comprises the amino acid sequence of SEQ ID NO: 89, a variant of SEQ ID NO: 89, a conservatively modified variant of SEQ ID NO: 89, and a variant of SEQ ID NO: 89 that comprises the canonical structure class of 2B, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 98, a variant of SEQ ID NO: 98, a conservatively modified variant of SEQ ID NO: 98, or a variant of SEQ ID NO: 98 that comprises the canonical structure class of 4.

In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 54, a variant of SEQ ID NO: 54, a conservatively modified variant of SEQ ID NO: 54, or a variant of SEQ ID NO: 54 that comprises the canonical structure class of 1; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 63, a variant of SEQ ID NO: 63, a conservatively modified variant of SEQ ID NO: 63, or a variant of SEQ ID NO: 63 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 72, a variant of SEQ ID NO: 72, a conservatively modified variant of SEQ ID NO: 72, or a variant of SEQ ID NO: 72 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 81, a variant of SEQ ID NO: 81, a conservatively modified variant of SEQ ID NO: 81, or a variant of SEQ ID NO: 81 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 90, a variant of SEQ ID NO: 90, a conservatively modified variant of SEQ ID NO: 90, and a variant of SEQ ID NO: 90 that comprises the canonical structure class of 1, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 99, a variant of SEQ ID NO: 99, a conservatively modified variant of SEQ ID NO: 99, or a variant of SEQ ID NO: 99 that comprises the canonical structure class of 13. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 157, or SEQ ID NO: 158, or within both SEQ ID NO: 157 and SEQ ID NO: 158.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 55, a variant of SEQ ID NO: 55, a conservatively modified variant of SEQ ID NO: 55, or a variant of SEQ ID NO: 55 that comprises

the canonical structure class of 2A; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 64, a variant of SEQ ID NO: 64, a conservatively modified variant of SEQ ID NO: 64, or a variant of SEQ ID NO: 64 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 73, a variant of SEQ ID NO: 73, a conservatively modified variant of SEQ ID NO: 73, or a variant of SEQ ID NO: 73 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 82, a variant of SEQ ID NO: 82, a conservatively modified variant of SEQ ID NO: 82, or a variant of SEQ ID NO: 82 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 91, a variant of SEQ ID NO: 91, a conservatively modified variant of SEQ ID NO: 91, and a variant of SEQ ID NO: 91 that comprises the canonical structure class of 2A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 100, a variant of SEQ ID NO: 100, a conservatively modified variant of SEQ ID NO: 100, or a variant of SEQ ID NO: 100 that comprises the canonical structure class of 6.

In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 129, a variant of SEQ ID NO: 129, a conservatively modified variant of SEQ ID NO: 129, or a variant of SEQ ID NO: 129 that comprises the canonical structure class of 6; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 132, a variant of SEQ ID NO: 132, a conservatively modified variant of SEQ ID NO: 132, or a variant of SEQ ID NO: 132 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 135, a variant of SEQ ID NO: 135, a conservatively modified variant of SEQ ID NO: 135, or a variant of SEQ ID NO: 135 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 140, a variant of SEQ ID NO: 140, a conservatively modified variant of SEQ ID NO: 140, or a variant of SEQ ID NO: 140 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 144, a variant of SEQ ID NO: 144, a conservatively modified variant of SEQ ID NO: 144, and a variant of SEQ ID NO: 144 that comprises the canonical structure class of 3A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 149, a variant of SEQ ID NO: 149, a conservatively modified variant of SEQ ID NO: 149, or a variant of SEQ ID NO: 149 that comprises the canonical structure class of 15. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof)

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binds canine interleukin-4 receptor α (IL-4$R_α$) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 127, or SEQ ID NO: 128, or within both SEQ ID NO: 127 and SEQ ID NO: 128.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 130, a variant of SEQ ID NO: 130, a conservatively modified variant of SEQ ID NO: 130, or a variant of SEQ ID NO: 130 that comprises the canonical structure class of 6; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 133, a variant of SEQ ID NO: 133, a conservatively modified variant of SEQ ID NO: 133, or a variant of SEQ ID NO: 133 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 136, a variant of SEQ ID NO: 136, a conservatively modified variant of SEQ ID NO: 136, or a variant of SEQ ID NO: 136 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 141, a variant of SEQ ID NO: 141, a conservatively modified variant of SEQ ID NO: 141, or a variant of SEQ ID NO: 141 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 145, a variant of SEQ ID NO: 145, a conservatively modified variant of SEQ ID NO: 145, and a variant of SEQ ID NO: 145 that comprises the canonical structure class of 2A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 150, a variant of SEQ ID NO: 150, a conservatively modified variant of SEQ ID NO: 150, or a variant of SEQ ID NO: 150 that comprises the canonical structure class of 10. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4$R_α$) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 158, or SEQ ID NO: 162, or within both SEQ ID NO: 158 and SEQ ID NO: 162.

In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 129, a variant of SEQ ID NO: 129, a conservatively modified variant of SEQ ID NO: 129, or a variant of SEQ ID NO: 129 that comprises the canonical structure class of 6; the CDRL2 comprises the amino acid sequence of

SEQ ID NO: 134, a variant of SEQ ID NO: 134, a conservatively modified variant of SEQ ID NO: 134, or a variant of SEQ ID NO: 134 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 137, a variant of SEQ ID NO: 137, a conservatively modified variant of SEQ ID NO: 137, or a variant of SEQ ID NO: 137 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 140, a variant of SEQ ID NO: 140, a conservatively modified variant of SEQ ID NO: 140, or a variant of SEQ ID NO: 140 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 146, a variant of SEQ ID NO: 146, a conservatively modified variant of SEQ ID NO: 146, and a variant of SEQ ID NO: 146 that comprises the canonical structure class of 3A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 151, a variant of SEQ ID NO: 151, a conservatively modified variant of SEQ ID NO: 151, or a variant of SEQ ID NO: 151 that comprises the canonical structure class of 15. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 125 or SEQ ID NO: 126, or within both SEQ ID NO: 125 and SEQ ID NO: 126.

In yet other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 131, a variant of SEQ ID NO: 131, a conservatively modified variant of SEQ ID NO: 131, or a variant of SEQ ID NO: 131 that comprises the canonical structure class of 3; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 60, a variant of SEQ ID NO: 60, a conservatively modified variant of SEQ ID NO: 60, or a variant of SEQ ID NO: 60 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 138, a variant of SEQ ID NO: 138, a conservatively modified variant of SEQ ID NO: 1385, or a variant of SEQ ID NO: 138 that comprises the canonical structure class of 3, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 142, a variant of SEQ ID NO: 142, a conservatively modified variant of SEQ ID NO: 142, or a variant of SEQ ID NO: 142 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 147, a variant of SEQ ID NO: 147, a conservatively modified variant of SEQ ID NO: 147, and a variant of SEQ ID NO: 147 that

comprises the canonical structure class of 3A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 152, a variant of SEQ ID NO: 152, a conservatively modified variant of SEQ ID NO: 152, or a variant of SEQ ID NO: 152 that comprises the canonical structure class of 9. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or any combination thereof.

In still other embodiments of mammalian antibodies (including caninized antibodies) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 129, a variant of SEQ ID NO: 129, a conservatively modified variant of SEQ ID NO: 129, or a variant of SEQ ID NO: 129 that comprises the canonical structure class of 6; the CDRL2 comprises the amino acid sequence of SEQ ID NO: 132, a variant of SEQ ID NO: 132, a conservatively modified variant of SEQ ID NO: 132, or a variant of SEQ ID NO: 132 that comprises the canonical structure class of 1; the CDRL3 comprises the amino acid sequence of SEQ ID NO: 139, a variant of SEQ ID NO: 139, a conservatively modified variant of SEQ ID NO: 139, or a variant of SEQ ID NO: 139 that comprises the canonical structure class of 1, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 143, a variant of SEQ ID NO: 143, a conservatively modified variant of SEQ ID NO: 143, or a variant of SEQ ID NO: 143 that comprises the canonical structure class of 1; the CDRH2 comprises the amino acid sequence of SEQ ID NO: 148, a variant of SEQ ID NO: 148, a conservatively modified variant of SEQ ID NO: 148, and a variant of SEQ ID NO: 148 that comprises the canonical structure class of 3A, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 153, a variant of SEQ ID NO: 153, a conservatively modified variant of SEQ ID NO: 153, or a variant of SEQ ID NO: 153 that comprises the canonical structure class of 13. In particular embodiments of this type, when the antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or any combination thereof.

The present invention includes antibodies and antigen binding fragments thereof that bind canine interleukin-4 receptor *alpha* (IL-4R$_\alpha$) with specificity. In particular embodiments of this type, the antibodies and antigen binding fragments thereof bind canine IL-4R$_\alpha$ and block the binding of canine IL-4R$_\alpha$ to canine IL-4 and/or IL-13. As indicated above, the isolated mammalian antibodies or antigen binding fragments thereof can be caninized antibodies or caninized antigen binding fragments thereof. In other embodiments, the isolated mammalian antibodies or antigen binding fragments thereof can be murine antibodies or murine antigen binding fragments thereof.

The caninized antibodies or caninized antigen binding fragments thereof of the present invention can comprise a hinge region. In a particular embodiment of this type, the hinge region comprises the amino acid sequence of SEQ ID NO: 101. In another embodiment the hinge region comprises the amino acid sequence of SEQ ID NO: 102. In still another embodiment the hinge region comprises the amino acid sequence of SEQ ID NO: 103. In yet another embodiment the hinge region comprises the amino acid sequence of SEQ ID NO: 104.

In certain embodiments the caninized antibody or antigen binding fragment thereof, comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 164. In particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 163. In other embodiments the caninized antibody or antigen binding fragment thereof, comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 166. In particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 165. In still other embodiments, the caninized antibody or antigen binding fragment thereof, comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 168. In particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 167. In specific embodiments of such types, when the caninized antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R$_\alpha$) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or any combination thereof.

In related embodiments the caninized antibody or antigen binding fragment thereof, comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 170. In particular embodiments of this type, the light chain is encoded by the nucleotide sequence of SEQ ID NO: 169. In other embodiments the caninized antibody or antigen binding fragment thereof, comprises a light chain comprising the amino acid sequence of SEQ ID NO: 172. In particular embodiments of this type, the light chain is encoded by the nucleotide sequence of SEQ ID NO: 171. In yet other embodiments the caninized antibody or antigen binding fragment thereof, comprises a light chain comprising the amino acid sequence of SEQ ID NO: 174. In particular embodiments of this type, the light chain is encoded by the nucleotide sequence of SEQ ID NO: 173. In particular embodiments of such types, when the caninized antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or any combination thereof.

The present invention further provides antibodies comprising a combination of such heavy chains and light chains. In particular embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 164 and the light chain comprises the amino acid sequence of SEQ ID NO: 170. In more particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 163 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 169. In other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 166 and the light chain comprises the amino acid sequence of SEQ ID NO: 172. In more particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 165 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 171. In still other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 168 and the light chain comprises the amino acid sequence of SEQ ID NO: 174. In more particular embodiments of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 167 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 173.

In related embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 164 and the light chain comprises the amino acid sequence of SEQ ID NO: 172. In other

embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 164 and the light chain comprises the amino acid sequence of SEQ ID NO: 174. In still other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 166 and the light chain comprises the amino acid sequence of SEQ ID NO: 170. In yet other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 166 and the light chain comprises the amino acid sequence of SEQ ID NO: 174. In still other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 168 and the light chain comprises the amino acid sequence of SEQ ID NO: 170. In other embodiments the heavy chain comprises the amino acid sequence of SEQ ID NO: 168 and the light chain comprises the amino acid sequence of SEQ ID NO: 172.

In particular embodiments of such types, when the caninized antibody (or antigen binding fragment thereof) binds canine interleukin-4 receptor α (IL-4R_α) the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight or more amino acid residues within the amino acid sequence of SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or any combination thereof.

Accordingly, the present invention further provides isolated mammalian antibodies or antigen binding fragments thereof (including caninized antibodies or antigen binding fragments thereof) that bind canine interleukin-4 receptor α (IL-4R_α) with specificity, and when bound to canine IL-4R_α the antibody binds to at least one amino acid residue, preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162, or any combination thereof. In particular embodiments, the antibody or antigen binding fragment thereof binds canine IL-4R_α and blocks the binding of canine IL-4R_α to canine interleukin-4.

The present invention further provides mammalian antibodies or antigen binding fragments thereof that bind to canine IL-4R_α with a dissociation constant (Kd) that is lower (*e.g.*, 1 X

10^{-13} M, or lower) than 1 X 10^{-12} M. In particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$with a dissociation constant of 1 X 10^{-5} M to 1 X 10^{-12} M. In more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with a dissociation constant of 1 X 10^{-7} M to 1 X 10^{-11} M. In still more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with a dissociation constant of 1 X 10^{-8} M to 1 X 10^{-11} M. In yet more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$with a dissociation constant of 1 X 10^{-8} M to 1 X 10^{-10} M.

The present invention also provides mammalian antibodies or antigen binding fragments thereof that bind to canine IL-4R$_\alpha$ with an on rate (k_{on}) that is greater than 1 X 10^7 $M^{-1}s^{-1}$. In particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an on rate of 1 X 10^2 $M^{-1}s^{-1}$ to 1 X 10^7 $M^{-1}s^{-1}$. In more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an on rate of 1 X 10^3 $M^{-1}s^{-1}$ to 1 X $10^6$$M^{-1}s^{-1}$. In still more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an on rate of 1 X 10^3 $M^{-1}s^{-1}$ to 1 X $10^5$$M^{-1}s^{-1}$. In yet more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ on rate of 1 X 10^4 $M^{-1}s^{-1}$ to 1 X $10^5$$M^{-1}s^{-1}$.

The present invention further provides mammalian antibodies or antigen binding fragments thereof that bind to canine IL-4R$_\alpha$ with an off rate (k_{off}) slower than 1 X 10^{-7} s^{-1}. In particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an off rate of 1 X 10^{-3} s^{-1} to 1 X 10^{-8} s^{-1}. In more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an off rate of 1 X 10^{-4} s^{-1} to 1 X 10^{-7} s^{-1}. In still more particular embodiments the mammalian antibodies or antigen binding fragments thereof bind to canine IL-4R$_\alpha$ with an off rate of 1 X 10^{-5} s^{-1} to 1 X 10^{-7} s^{-1}.

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In particular embodiments, a mammalian antibody of the present invention (including chimeric antibodies) blocks the binding of canine IL-4 with IL-4R$_\alpha$. In more particular embodiments the antibody blocks the binding of canine IL-4 to IL-4R$_\alpha$ with a minimum EC50 of 1 X10^{-8} M to 1 X10^{-9} M or an even lower concentration. In still more particular embodiments the EC50 is 5 X10^{-9} M to 5 X10^{-13} M. In still more particular embodiments the EC50 is between 5 X10^{-9} M and 5 X10^{-11} M.

In related embodiments, the mammalian antibodies or antigen binding fragments thereof negatively attenuate, *e.g.*, inhibit, the cell signaling pathway(s) mediated by IL-4 and/or IL-13 binding to type I and/or type II IL-4 receptors. In particular embodiments, the mammalian antibodies or antigen binding fragments thereof ameliorate a pruritic inflammatory skin disease, *e.g.*, atopic dermatitis, in an animal subject. In more specific embodiments the animal subject is a canine. In a related embodiment, the animal subject is a feline.

Accordingly, any of the antibodies of the present invention can exhibit one, two, three, four, or all these properties, *i.e.*, the aforesaid dissociation constants with canine IL-4R$_\alpha$, the aforesaid on rates for binding with canine IL-4R$_\alpha$, the aforesaid off rates for dissociating from the antibody-canine IL-4R$_\alpha$ binding complex, inhibiting the cell signaling pathway(s) mediated by IL-4 and/or IL-13 binding to type I and/or type II IL-4 receptors, or ameliorating a pruritic inflammatory skin disease, *e.g.*, atopic dermatitis, in an animal subject.

As indicated above, the antibodies (and antigen binding fragments thereof) of the present invention, including the aforesaid antibodies (and antigen binding fragments thereof), can be monoclonal antibodies (and antigen binding fragments thereof), mammalian antibodies (and antigen binding fragments thereof), *e.g.*, murine (mouse) antibodies (and antigen binding fragments thereof), caninized antibodies (and antigen binding fragments thereof) including caninized murine antibodies (and antigen binding fragments thereof), and in certain embodiments the antibodies (and antigen binding fragments thereof) are isolated.

The present invention further provides nucleic acids (including isolated nucleic acids) that encode any one of the light chains of the caninized antibody of the present invention. Similarly, the

present invention provides isolated nucleic acids that encode any one of the heavy chains of the caninized antibody of the present invention.

The present invention further provides expression vectors that comprise one or more of the nucleic acids (including isolated nucleic acids) of the present invention. The present invention further provides host cells that comprise one or more expression vectors of the present invention.

In particular embodiments, the antibody is a recombinant antibody or an antigen binding fragment thereof. In related embodiments, the variable heavy chain domain and variable light chain domain are connected by a flexible linker to form a single-chain antibody.

In particular embodiments, the antibody or antigen binding fragment is a Fab fragment. In other embodiments, the antibody or antigen binding fragment is a Fab' fragment. In other embodiments, the antibody or antigen binding fragment is a $(Fab')_2$ fragment. In still other embodiments, the antibody or antigen binding fragment is a diabody. In particular embodiments, the antibody or antigen binding fragment is a domain antibody. In particular embodiments, the antibody or antigen binding fragment is a single domain antibody.

In particular embodiments, a caninized murine anti-canine IL-$4R_\alpha$ antibody or antigen binding fragment negatively attenuates the cell signaling pathway(s) mediated by IL-4 and/or IL-13 binding to type I and/or type II IL-4 receptors in an animal subject (*e.g.*, canine) being treated. In more particular embodiments, administration of a caninized murine anti-canine IL-$4R_\alpha$ antibody or antigen binding fragment of the present invention serves to ameliorate one or more symptom of atopic dermatitis in the animal subject (*e.g.*, canine) being treated.

The present invention further provides isolated nucleic acids that encode caninized murine anti-canine IL-$4R_\alpha$ antibodies or portions thereof. In related embodiments such antibodies or antigen binding fragments can be used for the preparation of a medicament to treat atopic dermatitis in a canine subject. Alternatively, or in conjunction, the present invention provides for the use of any of the antibodies or antibody fragments of the present invention for diagnostic use. In yet

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additional embodiments, a kit is provided comprising any of the caninized antibodies or antigen binding fragments disclosed herein.

In yet additional embodiments, an expression vector is provided comprising an isolated nucleic acid encoding any of the caninized murine anti-canine IL-4R$_\alpha$ antibodies or antigen binding fragments of the invention. The invention also relates to a host cell comprising any of the expression vectors described herein. In particular embodiments, these nucleic acids, expression vectors or polypeptides of the invention are useful in methods of making an antibody.
The present invention further provides peptides (including isolated antigenic peptides) that consist of 80 or fewer amino acid residues that comprise the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162. In related embodiments, the peptides (including isolated antigenic peptides) consist of 60 or fewer amino acid residues that comprise the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162. In related embodiments, the peptides (including isolated antigenic peptides) consist of 10 to 45 amino acid residues that comprise the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162. In yet other embodiments the peptides (including isolated antigenic peptides) consist of 5 to 25 amino acid residues from the, or that comprise the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162.

The present invention further provides antigenic peptides (including isolated peptides) that consist of 80 or fewer amino acid residues that comprise an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or

SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162 and binds to an isolated mammalian antibody or antigen binding fragment thereof the present invention. In related embodiments, the antigenic peptides (including isolated antigenic peptides) consist of 60 or fewer amino acid residues that comprise an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162 and binds to an isolated mammalian antibody or antigen binding fragment thereof. In other embodiments the peptides consist of 5 to 25 amino acid residues from the, or that comprise an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 125, or SEQ ID NO: 126, or SEQ ID NO: 127, or SEQ ID NO: 128, or SEQ ID NO: 154, or SEQ ID NO: 155, or SEQ ID NO: 156, or SEQ ID NO: 157, or SEQ ID NO: 158, or SEQ ID NO: 159, or SEQ ID NO: 160, or SEQ ID NO: 161, or SEQ ID NO: 162 and binds to an isolated mammalian antibody or antigen binding fragment thereof. In particular embodiments the mammalian antibody comprises the CDRs of 4D8. In other embodiments the mammalian antibody comprises the CDRs of 11H2. In yet other embodiments the mammalian antibody comprises the CDRs of 4H3. In still other embodiments the mammalian antibody comprises the CDRs of 11B6. In yet other embodiments the mammalian antibody comprises the CDRs of 2E2. In still other embodiments the mammalian antibody comprises the CDRs of 6C12.

The present invention further provides fusion proteins that comprise any of the aforesaid peptides. In a particular embodiment, the fusion protein comprises such an antigenic peptide and an Fc region of a non-canine mammalian IgG antibody. In a more particular embodiment the fusion protein comprises an Fc region of a non-canine mammalian IgG antibody. In certain embodiments the non-canine mammalian IgG antibody is a murine IgG. In alternative embodiments the non-canine mammalian IgG antibody is a human IgG. In other embodiments the non-canine mammalian IgG antibody is an equine IgG. In still other embodiments the non-canine mammalian IgG antibody is a porcine IgG. In yet other embodiments the non-canine mammalian IgG antibody is a bovine IgG.

In particular embodiments the non-canine mammalian IgG antibody is an IgG1. In other embodiments the non-canine mammalian IgG antibody is an IgG2a. In still other embodiments the non-canine mammalian IgG antibody is an IgG3. In yet other embodiments the non-canine mammalian IgG antibody is an IgG4. In other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and maltose-binding protein. In yet other embodiments, the fusion protein comprises any of the aforesaid antigenic peptides and *beta*-galactosidase. In still other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and glutathione S-transferase. In yet other embodiments, the fusion protein comprises any of the aforesaid antigenic peptides and thioredoxin. In still other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and Gro EL. In yet other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and NusA.

The present invention further provides nucleic acids (including isolated nucleic acids) that encode the antigenic peptides and the corresponding fusion proteins of the present invention. The present invention also provides expression vectors that comprise these nucleic acids and host cells that comprise one or more expression vectors of the present invention.

In addition, the present invention includes pharmaceutical compositions comprising anti-canine IL-4R$_\alpha$ antibodies or antigen binding fragments thereof of the present invention, antigenic peptides (including isolated antigenic peptides) from canine IL-4R$_\alpha$, fusion proteins comprising the antigenic peptides from canine IL-4R$_\alpha$ of the present invention, nucleic acids (including isolated nucleic acids) encoding the antigenic fragments and/or fusion proteins of the present invention, the expression vectors comprising such nucleic acids, or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

In addition, the present invention provides methods of negatively attenuating the activity of IL-4 and/or IL-13 comprising administering to an animal subject in need thereof a therapeutically effective amount of such pharmaceutical compositions. In certain embodiments the method is used for the treatment of atopic dermatitis in a canine.

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These and other aspects of the present invention will be better appreciated by reference to the following Brief Description of the Drawings and the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the reactivity of purified mouse anti-canine IL-4R$_\alpha$ monoclonal antibodies (mAbs) against the extracellular domain of canine IL-4R$_\alpha$. Various mouse mAbs were tested for their binding to the extracellular domain of canine IL-4R$_\alpha$ by ELISA. The mAbs tested are designated as: 1A3(•), 1A9(■), 1B12 (▲), 10C12(▼), 10F2(♦), 10E10(●), 10 G8(■), 11B6(▲), 11D3(▼), and the control antibody(◆). The abscissa depicts the log concentration of the mAB (nM) being added, the ordinate depicts the optical density obtained by the ELISA.

Figure 2A shows the dose response curve for the binding of canine IL-4 to canine IL-4R$_\alpha$ expressed on the surface of CHO cells, using a cell-based CHO-cIL-4R$_\alpha$ binding assay. The abscissa depicts the log concentration of IL-4 being added, the ordinate depicts the mean fluorescence intensity (MFI) employing FACS.

Figure 2B depicts the dose response curves for CHO-cIL-4R$_\alpha$ by the mouse anti-canine IL-4R$_\alpha$ monoclonal antibodies (mAbs): 11B6(•), 4D8(■), 4H3(▲), 2E2(▼), 11H2(♦), and 6C12(**0**). The abscissa depicts the log concentration of the mAb (nM) being added, the ordinate depicts the mean fluorescence intensity (MFI) employing FACS. The half maximal effective concentrations (EC50) for each of the antibodies is provided in Table 2 below.

Figures 3A and 3B show the results of the addition of successively diluted individual mouse anti-canine IL-4R$_\alpha$ monoclonal antibodies (mAbs) on the binding of IL-4 with the cell-based CHO-cIL-4R$_\alpha$. Figure 3A depicts the concentration-dependent ability of the monoclonal antibodies 11B6(◆), 4D8(■), 4H3(▲), 2E2(▼), and 11H2(♦) to individually block the binding of IL-4 with the cell-based CHO-cIL-4R$_\alpha$. Figure 3B depicts the concentration-dependent ability of monoclonal antibodies 11H2(◆), and 6C12(■) to individually block the binding of IL-4 with the

cell-based CHO-cIL-4R$_\alpha$. The abscissa depicts the log concentration of the mAb (nM) being added, the ordinate depicts the mean fluorescence intensity (MFI) employing FACS.

Figure 4 depicts the binding of chimeric and caninized monoclonal antibodies to canine IL-4R$_\alpha$ as evaluated by ELISA. The dose-dependent reactivity of caninized monoclonal antibodies against canine IL-4 receptor *alpha* chain is as follows: 4H3 M-C (●); 2G9 M-C (◇); c4H3 H1-L1 (■); c4H3 H2-L2(▲); c4H3 H3-L3 (o).

DETAILED DESCRIPTION

A variety of approaches for treatment of human AD are now under investigation in many clinical trials [reviewed in *Malajian et al., New pathogenic and therapeutic paradigms in atopic dermatitis Cytokine,* (2014)]. Some of these approaches aim to interfere with one or more of the signaling molecules/events leading to the development and activation of Th2 cells. One line of investigation in this area encompasses approaches for blockade of the actions of key interleukin drivers of the Th2 pathway. Based on the observations that AD is largely a Th2 dominated disease and the accumulating data supporting a key role for the combined actions of both IL-4 and IL-13 as key drivers of Th2 cell development, and based on the data indicating that IL-4 receptor α chain is a requisite receptor for signaling from both cytokines, the present invention describes the generation and characterization of monoclonal antibodies that block the binding of canine IL-4 and canine IL-13 to the type-I and type II IL-4 receptors and subsequently inhibit the signaling from both canine IL-4 and IL-13. These antibodies have utilities in treatment of atopic dermatitis and other diseases in companion animals as disclosed herein.

Abbreviations

Throughout the detailed description and examples of the invention the following abbreviations will be used:

ADCC	Antibody-dependent cellular cytotoxicity
CDC	Complement-dependent cyotoxicity
CDR	Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system

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CHO	Chinese hamster ovary
EC50	concentration resulting in 50% efficacy or binding
ELISA	Enzyme-linked immunosorbant assay
FR	Antibody framework region: the immunoglobulin variable regions excluding the CDR regions.
HRP	Horseradish peroxidase
IFN	interferon
IC50	concentration resulting in 50% inhibition
IgG	Immunoglobulin G
Kabat	An immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat [*Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]
mAb	Monoclonal antibody (also Mab or MAb)
MES	2-(N-morpholino)ethanesulfonic acid
MOA	Mechanism of action
NHS	Normal human serum
PCR	Polymerase chain reaction
PK	Pharmacokinetics
SEB	Staphylococcus Enterotoxin B
TT	Tetanus toxoid
V region	The segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.
VH	Immunoglobulin heavy chain variable region
VL	Immunoglobulin light chain variable region
VK	Immunoglobulin *kappa* light chain variable region

DEFINITIONS

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other

technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

"Activation" as it applies to cells or to receptors refers to the activation or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. "Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compounds derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors.

"Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton. "Activity" can also mean specific activity, *e.g.*, [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], concentration in a biological compartment, or the like. "Activity" may refer to modulation of components of the innate or the adaptive immune systems.

"Administration" and "treatment," as it applies to an animal, *e.g.*, a canine experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal *e.g.*, a canine subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic,

binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (*e.g.*, canine, feline, or human) and most preferably a canine.

As used herein, a "substitution of an amino acid residue" with another amino acid residue in an amino acid sequence of an antibody for example, is equivalent to "replacing an amino acid residue" with another amino acid residue and denotes that a particular amino acid residue at a specific position in the amino acid sequence has been replaced by (or substituted for) by a different amino acid residue. Such substitutions can be particularly designed *i.e.*, purposefully replacing an alanine with a serine at a specific position in the amino acid sequence by *e.g.*, recombinant DNA technology. Alternatively, a particular amino acid residue or string of amino acid residues of an antibody can be replaced by one or more amino acid residues through more natural selection processes *e.g.*, based on the ability of the antibody produced by a cell to bind to a given region on that antigen, *e.g.*, one containing an epitope or a portion thereof, and/or for the antibody to comprise a particular CDR that retains the same canonical structure as the CDR it is replacing. Such substitutions/replacements can lead to "variant" CDRs and/or variant antibodies.

"Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies or antigen binding fragments of the present invention, internally or externally to a canine subject or patient having one or more disease symptoms, or being suspected of having a disease, for which the agent has therapeutic activity.

Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the patient (*e.g.*, canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veterinarians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (*e.g.*, a treatment method or article

of manufacture) may not be effective in alleviating the target disease symptom(s) in every subject, it should alleviate the target disease symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a human, veterinary (*e.g.*, canine) or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a human, veterinary (*e.g.*, canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies or antigen binding fragments of the present invention to a canine or other animal subject, a cell, tissue, physiological compartment, or physiological fluid.

As used herein, the term "canine" includes all domestic dogs, Canis lupus familiaris or Canis familiaris, unless otherwise indicated.

As used herein, the term "feline" refers to any member of the *Felidae* family. Members of this family include wild, zoo, and domestic members, such as any member of the subfamilies *Felinae*, *e.g.*, cats, lions, tigers, pumas, jaguars, leopards, snow leopards, panthers, North American mountain lions, cheetahs, lynx, bobcats, caracals or any cross breeds thereof. Cats also include domestic cats, pure-bred and/or mongrel companion cats, show cats, laboratory cats, cloned cats, and wild or feral cats.

As used herein the term "canine frame" refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in the majority of embodiments the amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse antibody) in both chains. Optionally the heavy and/or light chains of the canine antibody may contain some foreign non-CDR residues, *e.g.*, so as to preserve the conformation of the foreign CDRs within the canine antibody, and/or to modify the Fc function, as exemplified below.

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Canine IL-4R$_\alpha$ has been found to comprise the amino acid sequence of SEQ ID NO: 2 [SEQ ID NO: 4, without the signal sequence]. In a specific embodiment canine IL-4R$_\alpha$ is encoded by a nucleic acid that comprises the nucleotide sequence of SEQ ID NO: 1 [SEQ ID NO: 3, without the signal sequence]. Canine IL-4R$_\alpha$ sequences may differ by having, for example, conserved variations in non-conserved regions, but the canine IL-4R$_\alpha$ will have substantially the same biological function as the canine IL-4R$_\alpha$ comprising the amino acid sequence of SEQ ID NO: 2 [SEQ ID NO: 4, without the signal sequence].

The cytokines IL-4 and IL-13 have been implicated in the pathogenesis of a variety of allergic diseases in humans and animals, including asthma and atopic dermatitis. Because the IL-4 receptor α chain is a requisite receptor for the signaling from either of these cytokines, the present invention describes the generation and characterization of monoclonal antibodies that block the binding of canine IL-4 and canine IL-13 to IL-4R$_\alpha$ and thereby inhibits the signaling from both canine IL-4 and IL-13. These antibodies therefore have utility in treatment of atopic dermatitis and other diseases in companion animals as disclosed herein. In addition, a biological function of canine IL-4R$_\alpha$ may be having, for example, an epitope in the extracellular domain that is specifically bound by an antibody of the instant disclosure.

A particular canine IL-4R$_\alpha$ amino acid sequence will generally be at least 90% identical to the canine IL-4R$_\alpha$ comprising the amino acid sequence of SEQ ID NO: 4. In certain cases, a canine IL-4R$_\alpha$, may be at least 95%, or even at least 96%, 97%, 98% or 99% identical to the canine IL-4R$_\alpha$ comprising the amino acid sequence of SEQ ID NO: 4. In certain embodiments, a canine IL-4R$_\alpha$ amino acid sequence will display no more than 10 amino acid differences from the canine IL-4R$_\alpha$ comprising the amino acid sequence of SEQ ID NO: 4. In certain embodiments, the canine IL-4R$_\alpha$ amino acid sequence may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the canine IL-4R$_\alpha$ comprising the amino acid sequence of SEQ ID NO: 4. Percent identity can be determined as described herein below.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to,

destruction of, or elimination from the mammalian body (*e.g.*, canine body) of cancerous cells, cells or tissues infected with pathogens, or invading pathogens.

Anti-canine IL-4R$_\alpha$ antibodies

The present invention provides isolated antibodies (particularly murine anti-canine IL-4R$_\alpha$ antibodies and caninized antibodies thereof) or antigen binding fragments thereof that bind canine IL-4R$_\alpha$ and uses of such antibodies or fragments thereof. In specific embodiments murine anti-canine IL-4R$_\alpha$ CDRs from murine anti-canine IL-4R$_\alpha$ antibodies are provided that have been shown to both bind canine IL-4R$_\alpha$ and to block the binding of canine IL-4R$_\alpha$ to one or more of its ligands, canine IL-4 or IL-13. These CDRs can be inserted into a modified canine frame of a canine antibody to generate a caninized murine anti-canine IL-4R$_\alpha$ antibody.

As used herein, an "anti-canine IL-4R$_\alpha$ antibody" refers to an antibody that was raised against canine IL-4R$_\alpha$ (*e.g.*, in a mammal such as a mouse or rabbit) and that specifically binds to canine IL-4R$_\alpha$. An antibody that "specifically binds to canine IL-4R$_\alpha$," and in particular canine IL-4R$_\alpha$, or an antibody that "specifically binds to a polypeptide comprising the amino acid sequence of canine IL-4R$_\alpha$", is an antibody that exhibits preferential binding to canine IL-4R$_\alpha$ as compared to other antigens, but this specificity does not require absolute binding specificity. An anti-canine IL-4R$_\alpha$ antibody is considered "specific" for canine IL-4R$_\alpha$ if its binding is determinative of the presence of canine IL-4R$_\alpha$ in a sample, or if it is capable of altering the activity of canine IL-4R$_\alpha$ without unduly interfering with the activity of other molecules in a canine sample, *e.g.* without producing undesired results such as false positives in a diagnostic context or side effects in a therapeutic context. The degree of specificity necessary for an anti-canine IL-4R$_\alpha$ antibody may depend on the intended use of the antibody, and at any rate is defined by its suitability for use for an intended purpose. The antibody, or binding compound derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, preferably at least ten-times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with any other antigen.

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As used herein, an antibody is said to bind specifically to a polypeptide *comprising* a given antigen sequence (in this case a portion of the amino acid sequence of canine IL-4R$_\alpha$) if it binds to polypeptides comprising the portion of the amino acid sequence of canine IL-4R$_\alpha$, but does not bind to other canine proteins lacking that portion of the sequence of canine IL-4R$_\alpha$. For example, an antibody that specifically binds to a polypeptide comprising canine IL-4R$_\alpha$, may bind to a FLAG®-tagged form of canine IL-4R$_\alpha$, but will not bind to other FLAG®-tagged canine proteins. An antibody, or binding compound derived from the antigen-binding site of an antibody, binds to its canine antigen, or a variant or mutein thereof, "with specificity" when it has an affinity for that canine antigen or a variant or mutein thereof which is at least ten-times greater, more preferably at least 20-times greater, and even more preferably at least 100-times greater than its affinity for any other canine antigen tested.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), canonized antibodies, fully canine antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as caninization of an antibody for use as a canine therapeutic antibody.

As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, *i.e.* antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, *e.g.* fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')$_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, *e.g.*, sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

A "Fab fragment" is comprised of one light chain and the C$_H$1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab fragment" can be the product of papain cleavage of an antibody.

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A “fragment crystallizable” ("Fc") region contains two heavy chain fragments comprising the C_H3 and C_H2 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains.

A "Fab' fragment" contains one light chain and a portion or fragment of one heavy chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form a $F(ab')_2$ molecule.
A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains. An "$F(ab')_2$ fragment" can be the product of pepsin cleavage of an antibody.

The "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant regions.

The term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. [*See*, Pluckthun, THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113 Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); WO 88/01649; and U.S. 4,946,778 and U.S. 5,260,203.]

As used herein, the term “canonical structure” refers to the local conformation that can be adopted by each of the hypervariable regions of the heavy and light chain of an antibody within the framework that they reside. For each hypervariable region, there are a small number of canonical structures (generally denoted by simple integers such as 1 or 2 etc.), which can be predicted with great accuracy from the amino acid sequences of the corresponding hypervariable region

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[particularly within the context of the amino acid sequence of its framework, as provided below for the corresponding anti-canine IL-4R$_\alpha$ variable domains (*see,* Table 3 below)]. These canonical structures can be determinative regarding whether a modification of the amino acid sequence of a given CDR will result in the retention or loss of the ability to bind to its antigen binding partner [*See, Chothia and Lesk, Canonical Structures for the hypervariable regions of immunoglobulins, J. Mol. Biol.* 196:901-917(1987)*;* Chothia *et al., Conformation of immunoglobulin hypervaribale regions, Nature,* 34:877-883(1989)*;* and Al-Lazikani *et al., Standard Conformations for the canonical structures of immunoglobulins, J. Mol. Biol.* 273:927-948 (1997)].

A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

A "bivalent antibody" comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific (see below).

In certain embodiments, monoclonal antibodies herein also include camelized single domain antibodies. [*See, e.g.,* Muyldermans *et al., Trends Biochem. Sci.* 26:230 (2001); Reichmann *et al., J. Immunol. Methods* 231:25 (1999); WO 94/04678; WO 94/25591; U.S. 6,005,079]. In one embodiment, the present invention provides single domain antibodies comprising two V_H domains with modifications such that single domain antibodies are formed.

As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. [*See,*

EP 0 404 097 B1; WO 93/11161; and Holliger *et al., Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)]. For a review of engineered antibody variants [generally *see* Holliger and Hudson *Nat. Biotechnol.* 23:1126-1136 (2005)].

Typically, an antibody or antigen binding fragment of the invention retains at least 10% of its canine IL-4R$_\alpha$ binding activity (when compared to the parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the canine IL-4R$_\alpha$ binding affinity as the parental antibody. It is also intended that an antibody or antigen binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

"Isolated antibody" refers to the purification status and in such context means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

As used herein, a "chimeric antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, where the first and second antibodies are from different species. [U.S. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81: 6851-6855 (1984)]. Typically the variable domains are obtained from an antibody from an experimental animal (the "parental antibody"), such as a rodent, and the constant domain sequences are obtained from the animal subject antibodies, *e.g.*, human or canine so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a canine or human subject respectively, than the parental (*e.g.*, rodent) antibody.

As used herein, the term "caninized antibody" refers to forms of antibodies that contain sequences from both canine and non-canine (*e.g.*, murine) antibodies. In general, the caninized antibody

will comprise substantially all of at least one or more typically, two variable domains in which all or substantially all of the hypervariable loops correspond to those of a non-canine immunoglobulin (*e.g.*, comprising 6 murine anti-canine IL-4R$_\alpha$ CDRs as exemplified below), and all or substantially all of the framework (FR) regions (and typically all or substantially all of the remaining frame) are those of a canine immunoglobulin sequence. As exemplified herein, a caninized antibody comprises both the three heavy chain CDRs and the three light chain CDRS from a murine anti-canine IL-4R$_\alpha$ antibody together with a canine frame or a modified canine frame. A modified canine frame comprises one or more amino acids changes as exemplified herein that further optimize the effectiveness of the caninized antibody, *e.g.*, to increase its binding to canine IL-4R$_\alpha$ and/or its ability to block the binding of canine IL-4 and/or canine IL-13 to the type-I and/or type II IL-4 receptors.

The term "fully canine antibody" refers to an antibody that comprises canine immunoglobulin protein sequences only. A fully canine antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" refers to an antibody that comprises mouse immunoglobulin sequences only. Alternatively, a fully canine antibody may contain rat carbohydrate chains if produced in a rat, in a rat cell, or in a hybridoma derived from a rat cell. Similarly, "rat antibody" refers to an antibody that comprises rat immunoglobulin sequences only.

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa*.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions,

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enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2 , CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of *Sequences of Proteins of Immunological Interest*, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978); Kabat, *et al., J. Biol. Chem.* 252:6609-6616 (1977); Chothia, *et al., J. Mol. Biol.* 196:901-917 (1987) or Chothia, *et al., Nature* 342:878-883 (1989)].

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 in the heavy chain variable domain). [*See* Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), defining the CDR regions of an antibody by sequence; *see also* Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987) defining the CDR regions of an antibody by structure]. As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

Besides binding and activating of canine immune cells, a canine or caninized antibody against IL-4R$_\alpha$ optimally has two attributes:

1. Lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
2. be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has high level of ADCC activity. On the other hand, IgG-A binds weakly to protein A, but displays undesirable ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D display no ADCC activity. (IgG-C has considerable ADCC activity). One way the present invention overcomes this

difficulty is by providing mutant canine IgG-B antibodies specific to IL-4R$_\alpha$; such antibodies lack effector functions such as ADCC and can be easily of purified using industry standard protein A chromatography.

"Homology" refers to sequence similarity between two polynucleotide sequences or between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared ×100. For example, if 6 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The phrase "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to use promoters, polyadenylation signals, and enhancers.

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A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that not all progeny will have precisely identical DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

As used herein, "germline sequence" refers to a sequence of unrearranged immunoglobulin DNA sequences. Any suitable source of unrearranged immunoglobulin sequences may be used. Human germline sequences may be obtained, for example, from JOINSOLVER® germline databases on the website for the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the United States National Institutes of Health. Mouse germline sequences may be obtained, for example, as described in Giudicelli *et al.* [*Nucleic Acids Res.* 33:D256-D261 (2005)].

Properties of Murine Anti-Canine IL-4R$_\alpha$ and Caninized Murine Anti-Canine IL-4R$_\alpha$ Antibodies

The present invention provides isolated murine anti-canine IL-4R$_\alpha$ antibodies and caninized antibodies thereof, methods of use of the antibodies or antigen binding fragments thereof in the

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treatment of disease *e.g.*, the treatment of atopic dermatitis in canines. In canine, there are four IgG heavy chains referred to as A, B, C, and D. These heavy chains represent four different subclasses of dog IgG, which are referred to as IgGA, IgGB, IgGC and IgGD. Each of the two heavy chains consists of one variable domain (VH) and three constant domains referred to as CH-1, CH-2, and CH-3. The CH-1 domain is connected to the CH-2 domain *via* an amino acid sequence referred to as the "hinge" or alternatively as the "hinge region".

The DNA and amino acid sequences of these four heavy chains were first identified by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001)]. The amino acid and DNA sequences for these heavy chains are also available from the GenBank data bases. For example, the amino acid sequence of IgGA heavy chain has accession number AAL35301.1, IgGB has accession number AAL35302.1, IgGC has accession number AAL35303.1, and IgGD has accession number (AAL35304.1). Canine antibodies also contain two types of light chains, *kappa* and *lambda*. The DNA and amino acid sequence of these light chains can be obtained from GenBank Databases. For example the *kappa* light chain amino acid sequence has accession number ABY 57289.1 and the *lambda* light chain has accession number ABY 55569.1.

In the present invention, the amino acid sequence for each of the four canine IgG Fc fragments is based on the identified boundary of CH1 and CH2 domains as determined by Tang *et al, supra.* Caninized murine anti-canine IL-4R$_\alpha$ antibodies that bind canine IL-4R$_\alpha$ include, but are not limited to: antibodies that comprise canine IgG-A, IgG-B, and IgG-D heavy chains and/or canine *kappa* light chains together with murine anti-canine IL-4R$_\alpha$ CDRs. Accordingly, the present invention provides isolated murine anti-canine IL-4R$_\alpha$ and/or caninized murine anti-canine IL-4R$_\alpha$ antibodies or antigen binding fragments thereof that bind to canine IL-4R$_\alpha$ and block the binding of canine IL-4 and canine IL-13 to the type-I or type II IL-4 receptors.

The present invention further provides full length canine heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized murine anti-canine antigen antibodies (including isolated caninized murine anti-canine IL-4R$_\alpha$ antibodies) and methods of use of the antibodies or antigen binding fragments thereof in the treatment of disease *e.g.*, the treatment of atopic dematitis in canines.

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The present invention also provides caninized murine anti-canine-IL-4Rα antibodies that comprise a canine fragment crystallizable region (cFc region) in which the cFc has been genetically modified to augment, decrease, or eliminate one or more effector functions. In one aspect of the present invention, the genetically modified cFc decreases or eliminates one or more effector functions. In another aspect of the invention the genetically modified cFc augments one or more effector function. In certain embodiments, the genetically modified cFc region is a genetically modified canine IgGB Fc region. In another such embodiment, the genetically modified cFc region is a genetically modified canine IgGC Fc region. In a particular embodiment the effector function is antibody-dependent cytotoxicity (ADCC) that is augmented, decreased, or eliminated. In another embodiment the effector function is complement-dependent cytotoxicity (CDC) that is augmented, decreased, or eliminated. In yet another embodiment, the cFc region has been genetically modified to augment, decrease, or eliminate both the ADCC and the CDC.

In order to generate variants of canine IgG that lack effector functions, a number of mutant canine IgGB heavy chains were generated. These variants may include one or more of the following single or combined substitutions in the Fc portion of the heavy chain amino acid sequence: P4A, D31A, N63A, G64P, T65A, A93G, and P95A. Variant heavy chains (*i.e.*, containing such amino acid substitutions) were cloned into expression plasmids and transfected into HEK 293 cells along with a plasmid containing the gene encoding a light chain. Intact antibodies expressed and purified from HEK 293 cells were evaluated for binding to $Fc_{\gamma}RI$ and C1q to assess their potential for mediation of immune effector functions. [*see*, U.S. provisional patent application 62/030,812, filed July 30, 2014, and U.S. provisional patent application 62/092,496, filed December 16, 2014].

The present invention also provides modified canine IgGDs which in place of its natural IgGD hinge region they comprise a hinge region from:

IgGA: FNECRCTDTPPCPVPEP,	SEQ ID NO: 101;
IgGB: PKRENGRVPRPPDCPKCPAPEM,	SEQ ID NO: 102; or
IgGC: AKECECKCNCNNCPCPGCGL,	SEQ ID NO: 103.

Alternatively, the IgGD hinge region can be genetically modified by replacing a serine residue with a proline residue, *i.e.*, PKESTCKCI**P**PCPVPES, SEQ ID NO: 104 (with the proline residue (P) underlined and in bold substituting for the naturally occurring serine residue). Such modifications can lead to a canine IgGD lacking fab arm exchange. The modified canine IgGDs can be constructed using standard methods of recombinant DNA technology [*e.g.*, Maniatis *et al.*, *Molecular Cloning, A Laboratory Manual* (1982)]. In order to construct these variants, the nucleic acids encoding the amino acid sequence of canine IgGD can be modified so that it encodes the modified IgGDs. The modified nucleic acid sequences are then cloned into expression plasmids for protein expression.

The antibody or antigen binding fragment thereof that binds canine IL-4R$_\alpha$ can comprise one, two, three, four, five, or six of the complementarity determining regions (CDRs) of the murine anti-canine antibody as described herein. The one, two, three, four, five, or six CDRs may be independently selected from the CDR sequences of those provided below. In a further embodiment, the isolated antibody or antigen-binding fragment thereof that binds canine IL-4R$_\alpha$ comprises a canine antibody *kappa* light chain comprising a murine light chain CDR-1, CDR-2 and/or CDR-3 and a canine antibody heavy chain IgG comprising a murine heavy chain CDR-1, CDR-2 and/or CDR-3.

In other embodiments, the invention provides antibodies or antigen binding fragments thereof that specifically binds IL-4R$_\alpha$ and have canine antibody *kappa* light chains comprising one to six different CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequences of SEQ ID NOs: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and/or 73 and canine antibody heavy chain IgG comprising one to six different CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequences of SEQ ID NOs: 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, and/or 100, while still exhibiting the desired binding and functional properties. In another embodiment the antibody or antigen binding fragment of the present invention comprises a canine frame comprising a combination of IgG heavy chain sequence with a *kappa* light chain having one or more of the above-mentioned

CDR amino acid sequences with 0, 1, 2, 3, 4, or 5 conservative or non-conservative amino acid substitutions, while still exhibiting the desired binding and functional properties.

Sequence identity refers to the degree to which the amino acids of two polypeptides are the same at equivalent positions when the two sequences are optimally aligned. As used herein one amino acid sequence is 100% "identical" to a second amino acid sequence when the amino acid residues of both sequences are identical. Accordingly, an amino acid sequence is 50% "identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.,* a protein, or a portion of the polypeptide being compared. In a particular embodiment, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account.

Sequence similarity includes identical residues and nonidentical, biochemically related amino acids. Biochemically related amino acids that share similar properties and may be interchangeable are discussed

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity [*see, e.g.*, Watson *et al., Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.; 1987)]. In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table 1 directly below.

TABLE 1
Exemplary Conservative Amino Acid Substitutions

Original residue	Conservative substitution
Ala (A)	Gly; Ser;
Arg (R)	Lys; His

Original residue	Conservative substitution
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala; Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants," as used herein, refers to antibodies or fragments in which one or more amino acid residues have been changed without altering a desired property, such an antigen affinity and/or speficity. Such variants include, but are not limited to, replacement of an amino acid with one having similar properties, such as the conservative amino acid substitutions of Table 1 above.

Nucleic Acids

The present invention further comprises the nucleic acids encoding the immunoglobulin chains of murine anti-canine IL-4R$_\alpha$ and/or caninized murine anti-canine IL-4R$_\alpha$ antibodies and antigen binding fragments thereof disclosed herein (see Examples below).

Also included in the present invention are nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the amino acid sequences of the CDRs and antibodies provided herein when the comparison is performed by a BLAST algorithm

wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. The present invention further provides nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences when the comparison is performed with a BLAST algorithm, wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

As used herein, nucleotide and amino acid sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.,* using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

The following references relate to BLAST algorithms often used for sequence analysis: BLAST ALGORITHMS: Altschul, S.F., *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish, W., *et al.*, *Nature Genet.* 3:266-272 (1993); Madden, T.L., *et al.*, *Meth. Enzymol.* 266:131-141(1996); Altschul, S.F., *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang, J., *et al.*, *Genome Res.* 7:649-656 (1997); Wootton, J.C., *et al.*, *Comput. Chem.* 17:149-163 (1993); Hancock, J.M. *et al.*, *Comput. Appl. Biosci.* 10:67-70 (1994); ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, (1978); *Natl. Biomed. Res. Found.*, Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, vol. 5, suppl. 3." (1978), M.O. Dayhoff (ed.), pp. 353-358 (1978), *Natl. Biomed. Res. Found.*, Washington, DC; Altschul, S.F., *J. Mol. Biol.* 219:555-565 (1991); States, D.J., *et al.*, *Methods* 3:66-70(1991); Henikoff, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 89:10915-10919

(1992); Altschul, S.F., *et al.*, *J. Mol. Evol.* 36:290-300 (1993); ALIGNMENT STATISTICS: Karlin, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 87:2264-2268 (1990); Karlin, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 90:5873-5877 (1993); Dembo, A., *et al.*, *Ann. Prob.* 22:2022-2039 (1994); and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in *Theoretical and Computational Methods in Genome Research* (S. Suhai, ed.), pp. 1-14, Plenum, New York (1997).

This present invention also provides expression vectors comprising the isolated nucleic acids of the invention, wherein the nucleic acid is operably linked to control sequences that are recognized by a host cell when the host cell is transfected with the vector. Also provided are host cells comprising an expression vector of the present invention and methods for producing the antibody or antigen binding fragment thereof disclosed herein comprising culturing a host cell harboring an expression vector encoding the antibody or antigen binding fragment in culture medium, and isolating the antigen or antigen binding fragment thereof from the host cell or culture medium.

Epitope Binding and Binding Affinity

The present invention further provides antibodies or antigen binding fragments thereof that bind to amino acid residues of the same epitope of canine IL-4R$_\alpha$ as the murine anti-canine IL-4R$_\alpha$ antibodies disclosed herein. In particular embodiments the murine anti-canine IL-4R$_\alpha$ antibodies or antigen binding fragments thereof are also capable of inhibiting/blocking the binding of canine IL-4 and canine IL-13 to the type-I and/or type II IL-4 receptors.

A caninized murine anti-canine IL-4R$_\alpha$ antibody can be produced recombinantly by methods that are known in the field. Mammalian cell lines available as hosts for expression of the antibodies or fragments disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other

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cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion or fragment thereof, the light chain and/or antigen-binding fragment thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In general, glycoproteins produced in a particular cell line or transgenic animal will have a glycosylation pattern that is characteristic for glycoproteins produced in the cell line or transgenic animal. Therefore, the particular glycosylation pattern of an antibody will depend on the particular cell line or transgenic animal used to produce the antibody. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein, comprise the instant invention, independent of the glycosylation pattern that the antibodies may have. Similarly, in particular embodiments, antibodies with a glycosylation pattern comprising only non-fucosylated *N*-glycans may be advantageous, because these antibodies have been shown to typically exhibit more potent efficacy than their fucosylated counterparts both *in vitro* and *in vivo* [*See* for example, Shinkawa *et al.*, *J. Biol. Chem.* 278: 3466-3473 (2003); U.S. Patent Nos. 6,946,292 and 7,214,775].

The present invention further includes antibody fragments of the murine anti-canine IL-4R$_\alpha$ antibodies disclosed herein. The antibody fragments include F(ab)$_2$ fragments, which may be produced by enzymatic cleavage of an IgG by, for example, pepsin. Fab fragments may be

produced by, for example, reduction of $F(ab)_2$ with dithiothreitol or mercaptoethylamine. A Fab fragment is a V_L-C_L chain appended to a V_H-C_{H1} chain by a disulfide bridge. A $F(ab)_2$ fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an $F(ab)_2$ molecule includes a portion of the F_c region between which disulfide bridges are located. An F_V fragment is a V_L or V_H region.

In one embodiment, the antibody or antigen binding fragment comprises a heavy chain constant region, *e.g.*, a canine constant region, such as IgG-A, IgG-B, IgG-C and IgG-D canine heavy chain constant region or a variant thereof. In another embodiment, the antibody or antigen binding fragment comprises a light chain constant region, *e.g.*, a canine light chain constant region, such as *lambda* or *kappa* canine light chain region or variant thereof. By way of example, and not limitation, the canine heavy chain constant region can be from IgG-B and the canine light chain constant region can be from *kappa*.

Antibody Engineering

Caninized murine anti-canine IL-4R$_\alpha$ antibodies of the present invention can be engineered to include modifications to canine framework and/or canine frame residues within the variable domains of a parental (i.*e.*, canine) monoclonal antibody, *e.g.* to improve the properties of the antibody.

Experimental and diagnostic uses

Murine anti-canine IL-4R$_\alpha$ and/or caninized murine anti-canine IL-4R$_\alpha$ antibodies or antigen-binding fragments thereof of the present invention may also be useful in diagnostic assays for canine IL-4R$_\alpha$ protein, *e.g.*, detecting its expression in conjunction with and/or relation to atopic dermatitis.

For example, such a method comprises the following steps:

(a) coat a substrate (*e.g.*, surface of a microtiter plate well, *e.g.*, a plastic plate) with a murine anti-canine IL-4R$_\alpha$ antibody or an antigen-binding fragment thereof;

(b) apply a sample to be tested for the presence of canine IL-4R$_\alpha$ to the substrate;

(c) wash the plate, so that unbound material in the sample is removed;

(d) apply detectably labeled antibodies (*e.g.*, enzyme-linked antibodies) which are also specific to the IL-4R$_\alpha$ antigen;

(e) wash the substrate, so that the unbound, labeled antibodies are removed;

(f) if the labeled antibodies are enzyme linked, apply a chemical which is converted by the enzyme into a fluorescent signal; and

(g) detect the presence of the labeled antibody.

In a further embodiment, the labeled antibody is labeled with peroxidase which react with ABTS [*e.g.*, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] or 3,3',5,5'-Tetramethylbenzidine to produce a color change which is detectable. Alternatively, the labeled antibody is labeled with a detectable radioisotope (*e.g.*, ^{3}H) which can be detected by scintillation counter in the presence of a scintillant. Murine anti-canine IL-4R$_\alpha$ antibodies of the invention may be used in a Western blot or immuno protein blot procedure.

Such a procedure forms part of the present invention and includes for example:

(i) contacting a membrane or other solid substrate to be tested for the presence of bound canine IL-4R$_\alpha$ or a fragment thereof with a murine anti-canine IL-4R$_\alpha$ antibody or antigen-binding fragment thereof of the present invention. Such a membrane may take the form of a nitrocellulose or vinyl-based [*e.g.*, polyvinylidene fluoride (PVDF)] membrane to which the proteins to be tested for the presence of canine IL-4R$_\alpha$ in a non-denaturing PAGE (polyacrylamide gel electrophoresis) gel or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gel have been transferred (*e.g.*, following electrophoretic separation in the gel). Before contact of membrane with the murine anti-canine IL-4R$_\alpha$ antibody or antigen-binding fragment thereof, the membrane is optionally blocked, *e.g.*, with non-fat dry milk or the like so as to bind non-specific protein binding sites on the membrane.

(ii) washing the membrane one or more times to remove unbound murine anti-canine IL-4R$_\alpha$ antibody or an antigen-binding fragment thereof and other unbound substances; and

(iii) detecting the bound murine anti-canine IL-4R$_\alpha$ antibody or antigen-binding fragment thereof.

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Detection of the bound antibody or antigen-binding fragment may be by binding the antibody or antigen-binding fragment with a secondary antibody (an anti-immunoglobulin antibody) which is detectably labeled and, then, detecting the presence of the secondary antibody.

The murine anti-canine IL-4R$_\alpha$ antibodies and antigen-binding fragments thereof disclosed herein may also be used for immunohistochemistry. Such a method forms part of the present invention and comprises, *e.g.*, (1) contacting a cell to be tested for the presence of canine IL-4R$_\alpha$ with a murine anti-canine IL-4R$_\alpha$ antibody or antigen-binding fragment thereof of the present invention; and (2) detecting the antibody or fragment on or in the cell. If the antibody or antigen-binding fragment itself is detectably labeled, it can be detected directly. Alternatively, the antibody or antigen-binding fragment may be bound by a detectably labeled secondary antibody which is detected.

Imaging techniques include SPECT imaging (single photon emission computed tomography) or PET imaging (positron emission tomography). Labels include *e.g.*, iodine-123 (^{123}I) and technetium-99m (^{99m}Tc), *e.g.*, in conjunction with SPECT imaging or ^{11}C, ^{13}N, ^{15}O or ^{18}F, *e.g.*, in conjunction with PET imaging or Indium-111 [*See e.g.*, Gordon *et al.*, *International Rev. Neurobiol.* 67:385-440 (2005)].

Cross-Blocking Antibodies

Furthermore, an anti-canine IL-4R$_\alpha$ antibody or antigen-binding fragment thereof of the present invention includes any antibody or antigen-binding fragment thereof that binds to the same epitope in canine IL-4R$_\alpha$ to which the antibodies and fragments discussed herein bind and any antibody or antigen-binding fragment that cross-blocks (partially or fully) or is cross-blocked (partially or fully) by an antibody or fragment discussed herein for canine IL-4R$_\alpha$ binding; as well as any variant thereof.

The cross-blocking antibodies and antigen-binding fragments thereof discussed herein can be identified based on their ability to cross-compete with the antibodies disclosed herein (on the basis of the CDRs as provided below in Example 5), *i.e.*, 1A3, 1A9, 1B12, 10C12, 10F2, 10E10, 10G8, and/or 11D3; or more particularly, 11B6 and/or 6C12; and even more particularly 4D8,

4H3, 2E2, and/or 11H2, in standard binding assays (*e.g.*, BIACore®, ELISA, as exemplified below, or flow cytometry). For example, standard ELISA assays can be used in which a recombinant canine IL-4R$_\alpha$ protein is immobilized on the plate, one of the antibodies is fluorescently labeled and the ability of non-labeled antibodies to compete off the binding of the labeled antibody is evaluated. Additionally or alternatively, BIAcore® analysis can be used to assess the ability of the antibodies to cross-compete. The ability of a test antibody to inhibit the binding of, for example, 1A3, 1A9, 1B12, 10C12, 10F2, 10E10, 10G8, and/or 11D3; or more particularly, 11B6 and/or 6C12; and even more particularly 4D8, 4H3, 2E2, and/or 11H2, to canine IL-4R$_\alpha$ demonstrates that the test antibody can compete with 1A3, 1A9, 1B12, 10C12, 10F2, 10E10, 10G8, 11D3, 11B6, 6C12, 4D8, 4H3, 2E2, and/or 11H2 for binding to canine IL-4R$_\alpha$ and thus, may, in some cases, bind to the same epitope on canine IL-4R$_\alpha$ as 1A3, 1A9, 1B12, 10C12, 10F2, 10E10, 10G8, 11D3, 11B6, 6C12, 4D8, 4H3, 2E2, and/or 11H2. As stated above, antibodies and fragments that bind to the same epitope as any of the anti-canine IL-4R$_\alpha$ antibodies or fragments of the present invention also form part of the present invention.

Pharmaceutical Compositions and Administration

To prepare pharmaceutical or sterile compositions of a caninized murine anti-canine IL-4R$_\alpha$ antibody or antigen binding fragment thereof it can be admixed with a pharmaceutically acceptable carrier or excipient. [See, *e.g.*, *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984)].

Formulations of therapeutic and diagnostic agents may be prepared by mixing with acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions or suspensions [see, *e.g.*, Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY]. In one embodiment, anti- IL-4R$_\alpha$ antibodies of the present

invention are diluted to an appropriate concentration in a sodium acetate solution pH 5-6, and NaCl or sucrose is added for tonicity. Additional agents, such as polysorbate 20 or polysorbate 80, may be added to enhance stability.

Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with another agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD_{50}/ ED_{50}). In particular aspects, antibodies exhibiting high therapeutic indices are desirable. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in canines. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The mode of administration can vary. Suitable routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial. In particular embodiments, the murine anti-canine IL-4R$_\alpha$ antibody or antigen binding fragment thereof can be administered by an invasive route such as by injection. In further embodiments of the invention, a murine anti-canine IL-4R$_\alpha$ antibody or antigen binding fragment thereof, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, or by inhalation, aerosol delivery. Administration by non-invasive routes (*e.g.*, orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle, including, *e.g.*, a prefilled syringe or autoinjector. The pharmaceutical compositions disclosed herein may also be administered with a needleless hypodermic injection device; such as

the devices disclosed in U.S. Patent Nos.: 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

The pharmaceutical compositions disclosed herein may also be administered by infusion. Examples of well-known implants and modules form administering pharmaceutical compositions include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

Alternately, one may administer a murine anti-canine or a caninized murine anti-canine IL-4R$_\alpha$ antibody in a local rather than systemic manner, for example, via injection of the antibody directly into an arthritic joint or pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, arthritic joint or pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibody, the level of symptoms, the immunogenicity of the therapeutic antibody, and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibody to effect improvement in the target disease state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibody and the severity of the condition being treated. Guidance in selecting appropriate doses of therapeutic antibodies is available [*see, e.g.*, Wawrzynczak *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK (1996); Kresina (ed.) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New

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York, NY (1991); Bach (ed.) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY (1993); Baert, *et al. New Engl. J. Med.* 348:601-608 (2003); Milgrom *et al. New Engl. J. Med.* 341:1966-1973 (1999); Slamon *et al. New Engl. J. Med.* 344:783-792 (2001); Beniaminovitz *et al. New Engl. J. Med.* 342:613-619 (2000); Ghosh *et al. New Engl. J. Med.* 348:24-32 (2003); Lipsky *et al. New Engl. J. Med.* 343:1594-1602 (2000)].

Determination of the appropriate dose is made by the veterinarian, *e.g.*, using parameters or factors known or suspected in the art to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

Antibodies or antigen binding fragments thereof disclosed herein may be provided by continuous infusion, or by doses administered, *e.g.*, daily, 1-7 times per week, weekly, bi-weekly, monthly, bimonthly, quarterly, semiannually, annually etc. Doses may be provided, e.g., intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A total weekly dose is generally at least 0.05 μg/kg body weight, more generally at least 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/ml, 10 mg/kg, 25 mg/kg, 50 mg/kg or more [*see, e.g.*, Yang, *et al. New Engl. J. Med.* 349:427-434 (2003); Herold, *et al. New Engl. J. Med.* 346:1692-1698 (2002); Liu, *et al. J. Neurol. Neurosurg. Psych.* 67:451-456 (1999); Portielji, *et al. Cancer Immunol. Immunother.* 52:133-144 (2003)]. Doses may also be provided to achieve a pre-determined target concentration of a caninized murine anti-canine IL-4R$_\alpha$ antibody in the subject's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 μg/ml or more. In other embodiments, a caninized murine anti-canine IL-4R$_\alpha$ antibody of the present invention is administered subcutaneously or intravenously, on a weekly, biweekly, "every 4 weeks," monthly, bimonthly, or quarterly basis at 10, 20, 50, 80, 100, 200, 500, 1000 or 2500 mg/subject.

The antigenic peptides recognized by anti-canine IL-4R$_\alpha$ mAbs also may be used as vaccines to elicit antibodies that block the binding of canine IL-4 and canine IL-13 to the type-I and type II

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IL-4 receptors. Such vaccines may be useful as therapeutic vaccines for diseases such as atopic dematitis. In order to use these antigenic peptides as vaccines, one or more of these peptides may be coupled chemically or through the techniques of recombinant DNA technology to another carrier protein in order to enhance the immunogenicity of these peptides and elicit peptide-specific antibodies. Techniques for coupling peptides to carrier proteins are known to those skilled in the art. Peptide vaccines may be used to vaccinate animals by IM, S/C, oral, spray or *in ovo* routes. Peptide vaccines may be used as subunit proteins expressed from bacterial, viral, yeast or baculovirus virus systems. Alternatively such peptide vaccines may be delivered following administration of a variety of viral or bacterial vectors that express such peptide vaccines as can be practiced by methods known to those skilled in the art. The peptide vaccines may be administered in doses from 1-1000 μg and may optionally contain an adjuvant and an acceptable pharmaceutical carrier.

As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with a disorder and/or a reduction in the severity of the symptoms of such disorder. The terms further include ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with a disorder, disease or symptom, or with the potential to develop such a disorder, disease or symptom.

As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of a caninized murine anti-canine IL-4R_α antibody or antigen binding fragment thereof of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, is effective to cause a measurable improvement in one or more symptoms of a disease or condition or the progression of such disease or condition. A therapeutically effective dose further refers to that amount of the binding compound sufficient to result in at least partial amelioration of symptoms, *e.g.,* treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient

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alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. An effective amount of a therapeutic will result in an improvement of a diagnostic measure or parameter by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. An effective amount can also result in an improvement in a subjective measure in cases where subjective measures are used to assess disease severity.

Other Combination Therapies

As previously described, a caninized murine anti-canine IL-4R$_\alpha$ antibody or antigen binding fragment thereof and/or an antigenic peptide of the present invention may be coadministered with one or other more therapeutic agents (such as an inhibitor as discussed in the next paragraph) and/or a murine (or caninized murine) anti-canine TSLP antibody [*see*, U.S. 8,791,242]. The antibod(ies) may be linked to the agent (as an immunocomplex) and/or can be administered separately from the agent or other antibody. In the latter case (separate administration), the antibodies can be administered before, after or concurrently with the agent or can be co-administered with other known therapies.

Kits

Further provided are kits comprising one or more components that include, but are not limited to, an antibody or antigen binding fragment, as discussed herein, which specifically binds IL-4R$_\alpha$ (*e.g.*, a caninized murine anti-canine IL-4R$_\alpha$ antibody or antigen binding fragment thereof) in association with one or more additional components including, but not limited to a pharmaceutically acceptable carrier and/or an inhibitor such as a Janus kinase (JAK) inhibitor, *e.g.*, oclacitinib [*see*, WO 2013/040241], a spleen tyrosine kinase (SYK) inhibitor [*see e.g.*, U.S. 8,759,366], or an antagonist to a chemoattractant receptor-homologous molecule expressed on TH2 cells [*see e.g.*, WO 2010/099039; WO 2010/031183; and U.S. 8,546,422]. The binding composition and/or an inhibitor, as described directly above, can be formulated as a pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

In one embodiment, the kit includes a binding composition of the present invention (*e.g.*, a caninized murine anti-canine IL-4R$_\alpha$ or a pharmaceutical composition thereof in one container (*e.g.*, in a sterile glass or plastic vial) and a pharmaceutical composition thereof and/or an inhibitor as described above in another container (*e.g.*, in a sterile glass or plastic vial).

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can also include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above. The kit can also include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids pet owners and veterinarians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

As a matter of convenience, an antibody or specific binding agent disclosed herein can be provided in a kit, *i.e.*, a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic or detection assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (*e.g.*, a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

EXAMPLES

EXAMPLE 1

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IDENTIFICATION AND CLONING OF CANINE IL-4 RECEPTOR α CHAIN RECEPTOR

The cDNA encoding a predicted full length canine IL-4 receptor *alpha* chain (SEQ ID NO: 1) was identified through a search of the Genbank database (accession # XM_547077.4; *see also*, US 7,208,579 B2). This predicted cDNA encodes an 823 amino acids (SEQ ID NO: 2) including a 25 amino acid leader sequence and is identified as accession # XP_547077.3. The mature predicted canine IL-4 receptor α chain protein (SEQ ID NO: 4) shares 65% identity with human IL-4 receptor α chain (accession # NP_000409.1) and 70 % identity with swine IL-4 receptor α chain (accession # NP_999505.1). The mature predicted canine IL-4 receptor α chain protein is encoded by the nucleotide sequence identified as SEQ ID NO: 3. Comparison of the predicted mature IL-4 receptor α chain with the known sequences of human IL-4 receptor α chain identified the extracellular domain (ECD) of the mature canine IL-4 receptor α chain protein and is designated as SEQ ID NO: 6. The DNA sequence encoding the ECD of the mature canine IL-4 receptor α chain is identified as SEQ ID NO: 5.

Canine IL-4 receptor α chain full length DNA with signal sequence (SEQ ID NO:1):

atgggcagactgtgcagcggcctgaccttccccgtgagctgcctggtgctggtgtgggtggccagcagcggcagcgtg
aaggtgctgcacgagcccagctgcttcagcgactacatcagcaccagcgtgtgccagtggaagatggaccaccccacc
aactgcagcgccgagctgagactgagctaccagctggacttcatgggcagcgagaaccacacctgcgtgcccgagaac
agagaggacagcgtgtgcgtgtgcagcatgcccatcgacgacgccgtggaggccgacgtgtaccagctggacctgtgg
gccggccagcagctgctgtggagcggcagcttccagcccagcaagcacgtgaagcccagaaccccccggcaacctgacc
gtgcaccccaacatcagccacacctggctgctgatgtggaccaaccctacccccaccgagaaccacctgcacagcgag
ctgacctacatggtgaacgtgagcaacgacaacgaccccgaggacttcaaggtgtacaacgtgacctacatgggcccc
accctgagactggccgccagcaccctgaagagcggcgccagctacagcgccagagtgagagcctgggcccagacctac
aacagcacctggagcgactggagccccagcaccacctggctgaactactacgagccctgggagcagcacctgccccctg
ggcgtgagcatcagctgcctggtgatcctggccatctgcctgagctgctacttcagcatcatcaagatcaagaagggc
tggtgggaccagatccccaaccccgcccacagccccctggtggccatcgtgatccaggacagccaggtgagcctgtgg
ggcaagagaagcagaggccaggagcccgccaagtgcccccactggaagacctgcctgaccaagctgctgccctgcctg
ctggagcacggcctgggcagagaggaggagagccccaagaccgccaagaacggccccctgcagggccccggcaagccc
gcctggtgccccgtggaggtgagcaagaccatcctgtggcccgagagcatcagcgtggtgcagtgcgtggagctgagc
gaggcccccgtggacaacgaggaggaggaggaggtggaggaggacaagagaagcctgtgccccagcctggagggcagc
ggcggcagcttccaggagggcagagagggcatcgtggccagactgaccgagagcctgttcctggacctgctgggcggc
gagaacggcggcttctgccccccagggcctggaggagagctgcctgcccccccccagcggcagcgtgggcgcccagatg
ccctgggcccagttccccagagccggccccagagccgcccccgagggccccgagcagcccagaagacccgagagcgcc
ctgcaggccagccccacccagagcgccggcagcagcgccttccccgagccccccccccgtggtgaccgacaaccccgcc
tacagaagcttcggcagcttcctgggccagagcagcgaccccggcgacggcgacagcgaccccgagctggccgacaga
cccggcgaggccgaccccggcatccccagcgccccccagccccccgagccccccgccgccctgcagcccgagcccgag
agctgggagcagatcctgagacagagcgtgctgcagcacagagccgccccccgccccccggccccggccccggcagcggc
tacagagagttcacctgcgccgtgaagcagggcagcgcccccgacgccggcggccccggcttcggccccagcggcgag
gccggctacaaggccttctgcagcctgctgcccggcggcgccacctgccccggcaccagcggcggcgaggccggcagc
ggcgagggcggctacaagcccttccagagcctgaccccggctgccccggcgcccccacccccgtgcccgtgccccctg
ttcaccttcggcctggacaccgagccccccggcagcccccaggacagcctgggcgccggcagcagccccgagcacctg
ggcgtggagcccgccggcaaggaggaggacagcagaaagaccctgctggcccccgagcaggccaccgacccccctgaga
gacgacctggccagcagcatcgtgtacagcgccctgacctgccacctgtgcggccacctgaagcagtggcacgaccag

gaggagagaggcaaggcccacatcgtgcccagcccctgctgcggctgctgctgcggcgacagaagcagcctgctgctg
agccccctgagagcccccaacgtgctgcccggcggcgtgctgctggaggccagcctgagccccgccagcctggtgccc
agcggcgtgagcaaggagggcaagagcagccccttcagccagcccgccagcagcagcgcccagagcagcagccagacc
cccaagaagctggccgtgctgagcaccgagcccacctgcatgagcgccagc

Canine IL-4 receptor α full length protein with signal sequence in bold font (SEQ ID NO: 2).
MGRLCSGLTFPVSCLVLVWVASSGSVKVLHEPSCFSDYISTSVCQWKMDHPTNCSAELRLSYQLDFMGSENHTCVPEN
REDSVCVCSMPIDDAVEADVYQLDLWAGQQLLWSGSFQPSKHVKPRTPGNLTVHPNISHTWLLMWTNPYPTENHLHSE
LTYMVNVSNDNDPEDFKVYNVTYMGPTLRLAASTLKSGASYSARVRAWAQTYNSTWSDWSPSTTWLNYYEPWEQHLPL
GVSISCLVILAICLSCYFSIIKIKKGWWDQIPNPAHSPLVAIVIQDSQVSLWGKRSRGQEPAKCPHWKTCLTKLLPCL
LEHGLGREEESPKTAKNGPLQGPGKPAWCPVEVSKTILWPESISVVQCVELSEAPVDNEEEEEVEEDKRSLCPSLEGS
GGSFQEGREGIVARLTESLFLDLLGGENGGFCPQGLEESCLPPPSGSVGAQMPWAQFPRAGPRAAPEGPEQPRRPESA
LQASPTQSAGSSAFPEPPPVVTDNPAYRSFGSFLGQSSDPGDGDSDPELADRPGEADPGIPSAPQPPEPPAALQPEPE
SWEQILRQSVLQHRAAPAPGPGPGSGYREFTCAVKQGSAPDAGGPGFGPSGEAGYKAFCSLLPGGATCPGTSGGEAGS
GEGGYKPFQSLTPGCPGAPTPVPVPLFTFGLDTEPPGSPQDSLGAGSSPEHLGVEPAGKEEDSRKTLLAPEQATDPLR
DDLASSIVYSALTCHLCGHLKQWHDQEERGKAHIVPSPCCGCCCGDRSSLLLSPLRAPNVLPGGVLLEASLSPASLVP
SGVSKEGKSSPFSQPASSSAQSSSQTPKKLAVLSTEPTCMSAS

Canine IL-4 receptor mature full length protein without signal sequence (SEQ ID NO: 4)
VKVLHEPSCFSDYISTSVCQWKMDHPTNCSAELRLSYQLDFMGSENHTCVPENREDSVCVCSMPIDDAVEADVYQLDL
WAGQQLLWSGSFQPSKHVKPRTPGNLTVHPNISHTWLLMWTNPYPTENHLHSELTYMVNVSNDNDPEDFKVYNVTYMG
PTLRLAASTLKSGASYSARVRAWAQTYNSTWSDWSPSTTWLNYYEPWEQHLPLGVSISCLVILAICLSCYFSIIKIKK
GWWDQIPNPAHSPLVAIVIQDSQVSLWGKRSRGQEPAKCPHWKTCLTKLLPCLLEHGLGREEESPKTAKNGPLQGPGK
PAWCPVEVSKTILWPESISVVQCVELSEAPVDNEEEEEVEEDKRSLCPSLEGSGGSFQEGREGIVARLTESLFLDLLG
GENGGFCPQGLEESCLPPPSGSVGAQMPWAQFPRAGPRAAPEGPEQPRRPESALQASPTQSAGSSAFPEPPPVVTDNP
AYRSFGSFLGQSSDPGDGDSDPELADRPGEADPGIPSAPQPPEPPAALQPEPESWEQILRQSVLQHRAAPAPGPGPGS
GYREFTCAVKQGSAPDAGGPGFGPSGEAGYKAFCSLLPGGATCPGTSGGEAGSGEGGYKPFQSLTPGCPGAPTPVPVP
LFTFGLDTEPPGSPQDSLGAGSSPEHLGVEPAGKEEDSRKTLLAPEQATDPLRDDLASSIVYSALTCHLCGHLKQWHD
QEERGKAHIVPSPCCGCCCGDRSSLLLSPLRAPNVLPGGVLLEASLSPASLVPSGVSKEGKSSPFSQPASSSAQSSSQ
TPKKLAVLSTEPTCMSAS

Canine IL-4 receptor mature full length DNA without signal sequence (SEQ ID NO: 3)
gtgaaggtgctgcacgagcccagctgcttcagcgactacatcagcaccagcgtgtgccagtggaagatggaccacccc
accaactgcagcgccgagctgagactgagctaccagctggacttcatgggcagcgagaaccacacctgcgtgcccgag
aacagagaggacagcgtgtgcgtgtgcagcatgcccatcgacgacgccgtggaggccgacgtgtaccagctggacctg
tgggccggccagcagctgctgtggagcggcagcttccagcccagcaagcacgtgaagcccagaaccccccggcaacctg
accgtgcaccccaacatcagccacacctggctgctgatgtggaccaaccctacccaccgagaaccacctgcacagc
gagctgacctacatggtgaacgtgagcaacgacaacgaccccgaggacttcaaggtgtacaacgtgacctacatgggc
cccaccctgagactggccgccagcaccctgaagagcggcgccagctacagcgccagagtgagagcctgggcccagacc
tacaacagcacctggagcgactggagccccagcaccacctggctgaactactacgagccctgggagcagcacctgccc
ctgggcgtgagcatcagctgcctggtgatcctggccatctgcctgagctgctacttcagcatcatcaagatcaagaag
ggctggtgggaccagatccccaaccccgcccacagccccctggtggccatcgtgatccaggacagccaggtgagcctg
tggggcaagagaagcagaggccaggagcccgccaagtgcccccactggaagacctgcctgaccaagctgctgccctgc
ctgctggagcacggcctgggcagagaggaggagagccccaagaccgccaagaacggccccctgcagggccccggcaag
cccgcctggtgccccgtggaggtgagcaagaccatcctgtggcccgagagcatcagcgtggtgcagtgcgtggagctg
agcgaggcccccgtggacaacgaggaggaggaggaggtggaggaggacaagagaagcctgtgccccagcctggagggc
agcggcggcagcttccaggagggcagagagggcatcgtggccagactgaccgagagcctgttcctggacctgctgggc
ggcgagaacggcggcttctgcccccagggcctggaggagagctgcctgccccccccagcggcagcgtgggcgcccag
atgccctgggcccagttccccagagccggccccagagccgcccccgagggccccgagcagcccagaagacccgagagc
gccctgcaggccagccccacccagagcgccggcagcagcgccttccccgagccccccccgtggtgaccgacaacccc
gcctacagaagcttcggcagcttcctgggccagagcagcgaccccggcgacggcgacagcgaccccgagctggccgac
agacccggcgaggccgaccccggcatccccagcgccccccagcccccgagcccccgccgccctgcagcccgagccc
gagagctgggagcagatcctgagacagagcgtgctgcagcacagagccgccccccgccccccggccccggccccggcagc
ggctacagagagttcacctgcgccgtgaagcagggcagcgcccccgacgccggcggccccggcttcggccccagcggc

gaggccggctacaaggccttctgcagcctgctgcccggcggcgccacctgccccggcaccagcggcggcgaggccggc
agcggcgagggcggctacaagcccttccagagcctgacccccggctgccccggcgcccccaccccgtgcccgtgccc
ctgttcaccttcggcctggacaccgagccccccggcagcccccaggacagcctgggcgccggcagcagccccgagcac
ctgggcgtggagcccgccggcaaggaggaggacagcagaaagaccctgctggcccccgagcaggccaccgacccccctg
agagacgacctggccagcagcatcgtgtacagcgccctgacctgccacctgtgcggccacctgaagcagtggcacgac
caggaggagagaggcaaggcccacatcgtgcccagcccctgctgcggctgctgctgcggcgacagaagcagcctgctg
ctgagcccccctgagagcccccaacgtgctgcccggcggcgtgctgctggaggccagcctgagccccgccagcctggtg
cccagcggcgtgagcaaggagggcaagagcagccccttcagccagcccgccagcagcagcgcccagagcagcagccag
acccccaagaagctggccgtgctgagcaccgagcccacctgcatgagcgccagc

Canine IL-4 receptor α chain extracellular protein domain without the signal sequence (SEQ ID NO: 6):

VKVLHEPSCFSDYISTSVCQWKMDHPTNCSAELRLSYQLDFMGSENHTCVPENREDSVCVCSMPIDDAVEADVYQLDL
WAGQQLLWSGSFQPSKHVKPRTPGNLTVHPNISHTWLLMWTNPYPTENHLHSELTYMVNVSNDNDPEDFKVYNVTYMG
PTLRLAASTLKSGASYSARVRAWAQTYNSTWSDWSPSTTWLNYYEPWEQHLP

Canine IL-4 receptor α chain extracellular DNA domain without the signal sequence (SEQ ID NO: 5):

gtgaaggtgctgcacgagcccagctgcttcagcgactacatcagcaccagcgtgtgccagtggaagatggaccacccc
accaactgcagcgccgagctgagactgagctaccagctggacttcatgggcagcgagaaccacacctgcgtgcccgag
aacagagaggacagcgtgtgcgtgtgcagcatgcccatcgacgacgccgtggaggccgacgtgtaccagctggacctg
tgggccggccagcagctgctgtggagcggcagcttccagcccagcaagcacgtgaagcccagaaccccggcaacctg
accgtgcaccccaacatcagccacacctggctgctgatgtggaccaacccctaccccaccgagaaccacctgcacagc
gagctgacctacatggtgaacgtgagcaacgacaacgaccccgaggacttcaaggtgtacaacgtgacctacatgggc
cccaccctgagactggccgccagcaccctgaagagcggcgccagctacagcgccagagtgagagcctgggcccagacc
tacaacagcacctggagcgactggagccccagcaccacctggctgaactactacgagccctgggagcagcacctgccc

Canine IL-4 receptor α chain extracellular domain with a c-terminal 8 HIS Tag (SEQ ID NO: 8):

VKVLHEPSCFSDYISTSVCQWKMDHPTNCSAELRLSYQLDFMGSENHTCVPENREDSVCVCSMPIDDAVEADVYQLDL
WAGQQLLWSGSFQPSKHVKPRTPGNLTVHPNISHTWLLMWTNPYPTENHLHSELTYMVNVSNDNDPEDFKVYNVTYMG
PTLRLAASTLKSGASYSARVRAWAQTYNSTWSDWSPSTTWLNYYEPWEQHLP**HHHHHHHH**

Canine IL-4 receptor α chain extracellular DNA domain with a c-terminal 8 HIS Tag (SEQ ID NO: 7):

gtgaaggtgctgcacgagcccagctgcttcagcgactacatcagcaccagcgtgtgccagtggaagatggaccacccc
accaactgcagcgccgagctgagactgagctaccagctggacttcatgggcagcgagaaccacacctgcgtgcccgag
aacagagaggacagcgtgtgcgtgtgcagcatgcccatcgacgacgccgtggaggccgacgtgtaccagctggacctg
tgggccggccagcagctgctgtggagcggcagcttccagcccagcaagcacgtgaagcccagaaccccggcaacctg
accgtgcaccccaacatcagccacacctggctgctgatgtggaccaacccctaccccaccgagaaccacctgcacagc
gagctgacctacatggtgaacgtgagcaacgacaacgaccccgaggacttcaaggtgtacaacgtgacctacatgggc
cccaccctgagactggccgccagcaccctgaagagcggcgccagctacagcgccagagtgagagcctgggcccagacc
tacaacagcacctggagcgactggagccccagcaccacctggctgaactactacgagccctgggagcagcacctgccc
caccaccaccaccaccaccac

Canine IL-4 receptor α chain extracellular domain *plus* human IgG1 Fc (SEQ ID NO: 10):

VKVLHEPSCFSDYISTSVCQWKMDHPTNCSAELRLSYQLDFMGSENHTCVPENREDSVCVCSMPIDDAVEADVYQLDL
WAGQQLLWSGSFQPSKHVKPRTPGNLTVHPNISHTWLLMWTNPYPTENHLHSELTYMVNVSNDNDPEDFKVYNVTYMG
PTLRLAASTLKSGASYSARVRAWAQTYNSTWSDWSPSTTWLNYYEPWEQHLEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Canine IL-4 receptor α chain extracellular DNA domain *plus* human IgG1 Fc (SEQ ID NO: 9):

gtgaaggtgctgcacgagcccagctgcttcagcgactacatcagcaccagcgtgtgccagtggaagatggaccacccc
accaactgcagcgccgagctgagactgagctaccagctggacttcatgggcagcgagaaccacacctgcgtgcccgag
aacagagaggacagcgtgtgcgtgtgcagcatgcccatcgacgacgccgtggaggccgacgtgtaccagctggacctg
tgggccggccagcagctgctgtggagcggcagcttccagcccagcaagcacgtgaagcccagaaccccggcaacctg
accgtgcaccccaacatcagccacacctggctgctgatgtggaccaaccccctaccccaccgagaaccacctgcacagc
gagctgacctacatggtgaacgtgagcaacgacaacgaccccgaggacttcaaggtgtacaacgtgacctacatgggc
cccaccctgagactggccgccagcaccctgaagagcggcgccagctacagcgccagagtgagagcctgggcccagacc
tacaacagcacctggagcgactggagccccagcaccacctggctgaactactacgagccctgggagcagcacctggag
cccaagagctgcgacaagacccacacctgccccccctgccccgccccgagctgctgggcggccccagcgtgttcctg
ttcccccccaagcccaaggacaccctgatgatcagcagaacccccgaggtgacctgcgtggtggtggacgtgagccac
gaggaccccgaggtgaagttcaactggtacgtggacggcgtggaggtgcacaacgccaagaccaagcccagagaggag
cagtacaacagcacctacagagtggtgagcgtgctgaccgtgctgcaccaggactggctgaacggcaaggagtacaag
tgcaaggtgagcaacaaggccctgcccgcccccatcgagaagaccatcagcaaggccaagggccagcccagagagccc
caggtgtacaccctgcccccccagcagagacgagctgaccaagaaccaggtgagcctgacctgcctggtgaagggcttc
taccccagcgacatcgccgtggagtgggagagcaacggccagcccgagaacaactacaagaccacccccccccgtgctg
gacagcgacggcagcttcttcctgtacagcaagctgaccgtggacaagagcagatggcagcagggcaacgtgttcagc
tgcagcgtgatgcacgaggccctgcacaaccactacacccagaagagcctgagcctgagccccggcaag

EXAMPLE 2

MURINE ANTI-CANINE IL-4 RECEPTOR *ALPHA* CHAIN ANTIBODIES

Generation of anti-Canine Il-4 receptor α chain monoclonal antibodies:
A total of three Balb/c mice were immunized multiple times (with 10 μg each time) over a 17 day period. The immunizing antigen was the canine IL-4 R alpha chain extracellular domain (ECD)-human Fc fusion protein. Following immunization, serum was collected from each mouse and tested for reactivity with canine IL-4 receptor alpha chain ECD HIS-tagged protein. The spleen cells of the mouse with the highest serum anti- IL-4 receptor alpha chain ECD titer were fused to the myeloma P3X63Ag8.653 cell line. Approximately 2 weeks following fusion, supernatant from putative hybridoma cells were tested by ELISA for their reactivity to the IL-4 receptor alpha chain ECD HIS- tagged protein. Hybridomas producing strong positive signals in the ELISA were subcloned by limiting dilution and tested again for reactivity to canine IL-4 receptor alpha chain ECD HIS-tagged protein.

Confirmation of monoclonal antibodies reactivity against canine IL-4 receptor α chain:
The reactivity of antibodies secreted by hybridomas to ECD of canine IL-4 receptor alpha chain was confirmed by ELISA. Hybridoma cells were cultured using CELLine bioreactors (Integra-biosciences) for 10-30 days. Cells were initially maintained in DMEM supplemented with 4 mM L-glutamine and 10% Ultra Low IgG fetal bovine serum (FBS) from Gibco. Hybridoma cells were seeded in CELLine bioreactor cell chambers at a cell density of approximately $2x10^6$ cells/mL in 15 mL of the same medium with the FBS concentration increased to 20%. The outer

chamber was filled with 1 L of nutrient medium (DMEM with 4mM L-glutamine and 2% standard FBS). Hybridoma cells in the cell chamber were expanded to approximately $2.5x10^7$ cells/mL over 3-7 days. Then, 10 mL of cell suspension was harvested from the cell chamber and replaced with fresh media to allow for re-expansion of cells and subsequent harvests. This procedure was repeated as necessary to obtain adequate amounts of mAb from each hybridoma clone. Harvested cell suspensions were centrifuged and the supernatants were filtered through 0.2 micron filter membranes. For antibody purification, each clone's supernatant was purified using a Protein G Sepharose™ 4 Fast flow 5 mL column (GE Healthcare) by gravity flow. After washing with Tris-EDTA (TE) buffer pH 8.0, bound antibodies were eluted using 0.1 M glycine buffer, pH 2.7, followed by pH neutralization using 1 M Tris, pH 8.0. Antibodies were concentrated and buffer exchanged into phosphate-buffered saline (PBS) using Centriprep YM-10 kDa NMWL centrifugal filter units (Millipore). Antibody concentrations were quantified using spectrophotometry. Purified anti-canine IL-4 receptor α chain mAbs were tested for reactivity with the HIS-tagged ECD domain of canine IL-4 receptor alpha chain by ELISA as follows: HIS-tagged canine IL-4 receptor alpha chain protein is diluted to 10μg/mL in coating buffer (Carbonate/Bicarbonate pH 9.0) and dispensed at 100 μl/well in 96-well flat bottomed ELISA plates (NUNC). The plates are incubated at 4°C overnight. The plates are then washed three times with phosphate buffered saline containing 0.05% Tween™-20 (PBST). Next, 200 μl of blocking buffer (5% skim milk in PBST) is added to each well and the plates are incubated at 37°C for 60 minutes. The plates are then washed three times with PBST. Next, 100 μl of test mAbs diluted in blocking buffer is added to the first wells of the appropriate columns. Test mAbs are then diluted three-fold to the appropriate plate position. Following incubation of the plates at 37°C for 60 minutes, the plates are washed three times with PBST. Next, 100 μl per well of a 1:2,000 dilution of a horseradish peroxidase conjugated goat anti-mouse IgG (KPL) is added to the plates, which are then incubated at 37°C for 60 minutes. Then the plates are washed three times with PBST, and 100 μl/well of 3,3',5,5' tetramethyl benzidine, (TMB) substrate (from KPL) is added to the plates. The color reaction is allowed to develop for 5-20 minutes at 37°C prior to measuring absorbance at 650nm.

Various mouse anti-canine IL-4R$_\alpha$ monoclonal antibodies (mAbs) were assayed by ELISA for their ability to bind the extracellular domain of canine IL-4R$_\alpha$. As depicted in Figure 1, a majority of these mAbs exhibit positive dosage-dependent binding.

EXAMPLE 3

IDENTIFICATION OF THE DNA AND PREDICTED PROTEIN SEQUENCES OF THE HEAVY AND LIGHT CHAINS VARIABLE DOMAINS OF ANTI-CANINE IL-4 RECEPTOR *ALPHA* CHAIN MONOCLONAL ANTIBODIES

The DNA sequence of mouse VH and VL chains are identified following isolation of mRNA from each hybridoma using standard molecular biology methods. The SEQ ID NOs. of the DNA and predicted amino acid sequences of the VH and VL from these hybridomas are listed below. The DNA encoding the signal sequence and the amino acids corresponding to predicted signal sequence are underlined, those corresponding to the CDRs are in bold, and the FRs are neither underlined nor in bold (*i.e.*, signal sequence-FR1-**CDR1**-FR2-**CDR2**-FR3-**CDR3**-FR4).

mAb 1A3

Heavy chain: DNA sequence (SEQ ID NO: 11):

ATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCGGTGTGAGGTGCAGCTGGTGGAGTCT
GGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGT**GACTTTGGA**
ATGCACTGGGTTCGTCAGGCTCCAGAGAAGGGGCTGGGGTGGGTTGCA**TACATTAGTAGTGGCAGTGGTACCATCTAC**
TATGCAGACACAGTGAGGGGCCGATTCACCATCTCCAGAGACAATGTCAAGAACACCCTGTTCCTGCAAATGACCAGT
CTGAGGTCTGAGGACACGGCCATGTATTACTGTGTAAGG**GGGGACCTTTACTACGGTAGTAGTTTCGATGCTTAT**TGG
GGCCGAGGGACTCTGGTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 12):

MDSRLNLVFLVLILKGVRCEVQLVESGGDLVKPGGSLKLSCAASGFTFS**DFGMH**WVRQAPEKGLGWVA**YISSGSGTIY**
YADTVRGRFTISRDNVKNTLFLQMTSLRSEDTAMYYCVR**GDLYYGSSFDAY**WGRGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 13):

ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCCAGAGGACAAATTGTTCTC
TCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGC**AGGGCCAGCTCAAGTGTAAGT**
TTCATGTTCTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTAT**GACACATCCAACCTGGCTTCT**GGA
GTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGATGCT
GCCACTTATTACTGC**CAGCAGTGGAGTAGTAACCCACTCACG**TTCGGTGCTGGGACCAAGCTGGAGCTGAAA

Light chain: Amino acid sequence (SEQ ID NO: 14):

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTMTC**RASSSVSFMF**WYQQKPGSSPKPWIY**DTSNLAS**G
VPARFSGSGSGTSYSLTISRVEAEDAATYYC**QQWSSNPLT**FGAGTKLELK

mAb 1A9

Heavy chain: DNA sequence (SEQ ID NO: 15):

ATGGAATGGCCTTGTATCTTTCTCTTCCTCCTGTCAGTAACTGAAGGTGTCCACTCCCAGGTTCCGCTGCAGCAGTCT
GGACCTGAGCTGGTGAAGCCTGGGGCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTACGCATTCAGT**AGCTCCTGG**
ATGAACTGGGTGAAGCAGAGGCCTGGAAAGGGTCTTGAGTGGATTGGA**CGGATTTATCCTGGAGATGGAGATACTAAG**

TACAATGGGAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTCAGCAGC
CTGACATCGGAGGACTCTGCGGTTTACTTCTGTGCAAGA**GATGATTACGACGAGGCTTCC**TGGGGCCAAGGGACTCTG
GTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 16):
MEWPCIFLFLLSVTEGVHSQVPLQQSGPELVKPGASVKISCKASGYAFS**SSWMN**WVKQRPGKGLEWIG**RIYPGDGDTK**
YNGKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAR**DDYDEAS**WGQGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 17):
ATGGGCATCAAGATGGAGTTTCAGACCCAGGTCTTTGTATTCGTGTTGCTCTGGTTGTCTGGTGTTGATGGAGACATT
GTGATGACCCAGTCTCAAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCATCACCTGC**AAGGCCAGTCAGAAT**
GTTCGTTCTGCTGTAGCCTGGTATCAACAGAAACCAGGGCAGTCTCCTAAATCACTGATTTAC**TTGGCATCCAACCGG**
CACACTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATTAGCAATGTGCAATCT
GAAGACCTGGCAGATTATTTCTGT**CTGCAACATTGGAATTATCCATTCACG**TTCGGCTCGGGGACAAAGTTGGAAATA
AAA

Light chain: Amino acid sequence (SEQ ID NO: 18):
MGIKMEFQTQVFVFVLLWLSGVDGDIVMTQSQKFMSTSVGDRVSITC**KASQNVRSAVA**WYQQKPGQSPKSLIY**LASNR**
HTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFC**LQHWNYPFT**FGSGTKLEIK

mAb 1B12

Heavy chain: DNA sequence (SEQ ID NO: 19):
ATGGGATGGAGCTGGATCTTTCTCTTTCTCCTGTCAGGAACTGCAGGTGTCCTCTCTGAGGTCCAGCTGCAACAATCT
GGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCTTCTGGATACACGTTCACT**GACTATTAC**
ATGAACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGA**GACATTATTCCTAGCAATGGTGGTACTAGC**
TACAACCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGACAAGTCCTCCAGCGCAGCCTACATGGAGCTCCGCAGC
CTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGA**GGGATCAGCTACTATGGTAACCGATATTACTTTACTATG**
GACTATTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 20):
MGWSWIFLFLLSGTAGVLSEVQLQQSGPELVKPGASVKISCKASGYTFT**DYYMN**WVKQSHGKSLEWIG**DIIPSNGGTS**
YNQKFKGKATLTVDKSSSAAYMELRSLTSEDSAVYYCAR**GISYYGNRYYFTMDY**WGQGTSVTVSS

Light chain: DNA sequence (SEQ ID NO: 21):
ATGAGGTGCCTAGCTGAGTTCCTGGGGCTGCTTGTGCTCTGGATCCCTGGAGCCATTGGGGATATTGTGATGACTCAG
GCTGCACCCTCTGTACCTGTCACTCCTGGAGAGTCAGTATCCATCTCCTGC**AGGTCTAGTAAGAGTCTCCTGCATAGT**
AATGGCAACACTTACTTGTTTTGGTTCGTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGATATAT**CGGATGTCCAAC**
CTTGCCTCAGGAGTCCCAGACAGGTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGGAG
GCTGAGGATGTGGGTGTTTATTACTGT**ATGCAACATCTAGAATATCCATTCACG**TTCGGCTCGGGGACAAAGTTGGAC
ATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 22):
MRCLAEFLGLLVLWIPGAIGDIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLF**WFVQRPGQSPQLLIY**RMSN**
LASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYPFT**FGSGTKLDIK

mAb 10C12

Heavy chain: DNA sequence (SEQ ID NO: 23):
ATGGAATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGTAACTGCAGGTGTCCAATCCCAGGTTCAACTGCAGCAGTCT
GGGGCTGAGCTGGTGAGGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCGGGCTACACATTTACT**GACTATGAA**
ATGCACTGTGTGAAGCAGACACCTGTGCACGGCCTGGAATGGATTGGA**GCTATTGATCCTGAAACTTGTGGTACTGCC**
TACAATCAGAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATGGAGCTCCGCAGC
CTGACATCTGAGGACTCTGCCGTCTATTACTGTACAAGA**TCGAAACTGGGACGAGGGTGGTACTTCGATGTC**TGGGGC
ACAGGGACCACGGTCACCGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 24):
MEWSWIFLFLLSVTAGVQSQVQLQQSGAELVRPGASVKLSCKASGYTFT**DYEMH**CVKQTPVHGLEWIG**AIDPETCGTA**
YNQKFKGKATLTADKSSSTAYMELRSLTSEDSAVYYCTR**SKLGRGWYFDV**WGTGTTVTVSS

Light chain: DNA sequence (SEQ ID NO: 25):
ATGGAATCACAGACCCAGGTCCTCATGTTTCTTCTGCTCTGGGTATCTGGTGCCTGTGCAGACATTGTGATGACACAG
TCTCCATCCTCCCTGGCTATGTCAGTAGGACAGAAGGTCACTATGAGCTGC**AAGTCCAGTCAGAGCCTTTTAAATAGT**
AGCAATCAAAAGAACTATTTGGCCTGGTACCAGCAGAAACCAGGACAGTCTCCTAAACTTCTGGTATAC**TTTGCATCC**
ACTAGGGAATCTGGGGTCCCTGATCGCTTCATAGGCAGTGGATCTGGGACAGATTTCACTCTTACCATCAGCAGTGTG
CAGGCTGAAGACCTGGCAGATTACTTCTGT**CAGCAACATTATAGCACTCCGTACACG**TTCGGAGGGGGGACCAAGCTG
GAAATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 26):
MESQTQVLMFLLLWVSGACADIVMTQSPSSLAMSVGQKVTMSC**KSSQSLLNSSNQKNYLA**WYQQKPGQSPKLLVY**FAS**
TRESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFC**QQHYSTPYT**FGGGTKLEIK

mAb 10F2

Heavy chain: DNA sequence (SEQ ID NO: 27):
ATGGCTGTCCTGGCACTGCTCCTCTGCCTGGTGACATTCCCAAACTGTGTCCTGTCCCAGGTGCACCTGAAGGAGTCA
GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACTGTCTCAGGGTTCTCTTTAACC**AGCTATGGT**
GTAAGCTGGGTTCGCCAGCCTCCAGGAGAGGGTCTGGAGTGGCTGGGA**GTAATATGGGGTGACGGGAGCACATATTTT**
CATTCAGCTCTCATATCCAGACTGAGCATCAGCAAGGATGACTCCAAGAGCCAAGTTTTCTTAAAATTGAACAGTCTA
CAAACTGATGACACAGCCACGTACTACTGTGCCAAA**CAAGGGACGATCTATGATGGTTACTACAACTATGCTATGGAC**
TACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 28):
MAVLALLLCLVTFPNCVLSQVHLKESGPGLVAPSQSLSITCTVSGFSLT**SYGVS**WVRQPPGEGLEWLG**VIWGDGSTYF**
HSALISRLSISKDDSKSQVFLKLNSLQTDDTATYYCAK**QGTIYDGYYNYAMDY**WGQGTSVTVSS

Light chain: DNA sequence (SEQ ID NO: 29):
ATGGATTCACAGGCCCAGGTTCTTATGTTACTGCTGCTATGGGTATCTGGTACCTGTGGGGACATTGTGATGTCACAG
TCTCCATCCTCCCTAACTGTGTCAGTTGGAGAGAAGGTTACTATGAGCTGC**AAGTCCAGTCAGAACCTTTTATATGGT**
GGCAATCAAAAGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGATTTAC**TGGGCATCC**
ACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTG
AGGGCTGAAGACCTGGCAGTTTATTACTGT**CAGCAATATTATGACTATCCGTACACG**TTCGGAGGGGGGACCAAGCTG
GAAATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 30):
MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLTVSVGEKVTMSC**KSSQNLLYGGNQKNYLA**WYQQKPGQSPKLLIY**WAS**
TRESGVPDRFTGSGSGTDFTLTISSVRAEDLAVYYC**QQYYDYPYT**FGGGTKLEIK

mAb 10E10

Heavy chain: DNA sequence (SEQ ID NO: 31):
ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCACTCCCAGGTTCAGCTGCAGCAGTCT
GGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGCTACACCTTCACA**ACCTACGAT**
ATACACTGGGTGAAGCAGAGGCCTGGGCAGGGCCTTGAGTGGATTGGA**TGGATTTATCCTAGAGATGGTCGTACTACT**
TACAATGAGAAGTTCAAGGCCAAGGCCACATTGACTGTAGACACATCCTCCACCACAGCGTACATGGAGCTCCACAGC
CTGACATCTGAGGACTCTGCGGTCTATTTCTGTGCGAGA**AGTAGCCCCTTTGGCTAC**TGGGGCCAAGGCACCACTCTC
ACAGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 32):

MGWSWIFLFLLSGTAGVHSQVQLQQSGPELVKPGASVKLSCKASGYTFT**TYDIH**WVKQRPGQGLEWIG**WIYPRDGRTT**
YNEKFKAKATLTVDTSSTTAYMELHSLTSEDSAVYFCAR**SSPFGY**WGQGTTLTVSS

Light chain: DNA sequence (SEQ ID NO: 33):
ATGAAGTTTCCTTCTCAACTTCTGCTCTTCCTGCTGTTCAGAATCACAGGCATAATATGTGACATCCAGATGACACAA
TCTTCATCCTACTTGTCTGTATCTCTAGGAGGCAGAGTCACCATTACTTGC**AAGGCAAGTGACCACATTAATAATTGG**
TTAGCCTGGTATCAGCAGAAACCAGGAAATGCTCCTAGGCTCTTAATATCT**GGTGCAACCAGTTTGGAAACT**GGGGTT
CCTTCAAGATTCAGTGGCAGTGGATCTGGAAAGGATTACACTCTCAGCATTACCAGTCTTCAGACTGAAGATGCTGCT
ACTTATCACTGT**CACCAGTATTGGAGTATTCCGTACACG**TTCGGAGGGGGGACCAAGGTGGAAATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 34):
MKFPSQLLLFLLFRITGIICDIQMTQSSSYLSVSLGGRVTITC**KASDHINNWLA**WYQQKPGNAPRLLIS**GATSLET**GV
PSRFSGSGSGKDYTLSITSLQTEDAATYHC**HQYWSIPYT**FGGGTKVEIK

mAb 10G8

Heavy chain: DNA sequence (SEQ ID NO: 35):
ATGGAATGGAGCTGGGTCTTTCTCTTCCTCCTGTCAGTAATTGCAGGTGTCCAATCCCAGGTTCAACTGCAGCAGTCT
GGGGCTGAGCTGGTGGGGCCTGGGGCTTCAGTGACGCTGTCCTGCAAGGCTTCGGGCTACACATTTACT**GACTATGAA**
ATGCACTGGGTGAAGCAGACACCTGTGCATGGCCTGGAATGCATTGGA**GCTATTGATCCTGAAACTGGTGGTACTGCC**
TACAATCAGAAGTTCAAGGGCAAGGCCATACTGACTGCAGACAAATCCTCTAGCACAGCCTACATGGAGCTCCGCAGC
CTGACATCTGAGGACTCTGCCGTCTATTACTGTCTAACT**GGGTTTGACTAC**TGGGGCCAAGGCACCACTCTCACAGTC
TCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 36):
MEWSWVFLFLLSVIAGVQSQVQLQQSGAELVGPGASVTLSCKASGYTFT**DYEMH**WVKQTPVHGLECIG**AIDPETGGTA**
YNQKFKGKAILTADKSSSTAYMELRSLTSEDSAVYYCLT**GFDY**WGQGTTLTVSS

Light chain: DNA sequence (SEQ ID NO: 37):
ATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGTCTCAGTCATAATGTCCAGAGGACAAATTGTTCTC
ACCCAGTCTCCAGCAATCATGTCTGCATCTCCTGGGGAGAAGGTCACCTTGACCTGC**AGTGCCAGCTCAAGTGTGAAT**
TCCAGCTACTTGTACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACTCTGGATTTAT**AGCACATCCAACCTGGCT**
TCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAA
GATGCTGCCTCTTATTTCTGCC**ATCAGTGGAGTAGTTACCCGTACACG**TTCGGAGGGGGGACCAAGCTGGAAATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 38):
MDFQVQIFSFLLISVSVIMSRGQIVLTQSPAIMSASPGEKVTLTC**SASSSVNSSYLY**WYQQKPGSSPKLWIY**STSNLA**
SGVPARFSGSGSGTSYSLTISSMEAEDAASYFC**HQWSSYPYT**FGGGTKLEIK

mAb 11B6

Heavy chain: DNA sequence (SEQ ID NO: 39):
ATGATGGTGTTAAGTCTTCTGTACCTGTTGACAGCCCTTCCGGGTATCCTGTCAGAGGTGCAGCTTCAGGAGTCAGGA
CCTGGCCTGGCAAAACCTTCTCAGACTCTGTCCCTCACCTGTTCTGTCACTGGCTACTCCATCACC**AGTGATTACTGG**
AACTGGATCCGGAAATTCCCAGGGAATAAACTTGAATACATGGGG**TACATAAACTACAGTGGTAACACTTACTACAAT**
CCATCTCTCAAAAGTCGAATCTCCATAACTCGAGACACATCCAAGAACCAGTATTACCTGCAATTGAATTCTGTGACT
ACTGAGGACACAGCCACGTATTACTGTGCAAGA**TATGGGGGATTACGACAGGGTTCCTGGCACTTCGATGTC**TGGGGC
CCAGGGACCACGGTCACCGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 40):
MMVLSLLYLLTALPGILSEVQLQESGPGLAKPSQTLSLTCSVTGYSIT**SDYWN**WIRKFPGNKLEYMG**YINYSGNTYYN**
PSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCAR**YGGLRQGSWHFDV**WGPGTTVTVSS

Light chain: DNA sequence (SEQ ID NO: 41):

ATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATAATGTCCAGAGGACAAATTGTTCTC
ACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACCATATCCTGC**AGTGCCAGCTCAAGTGTAAGT**
TACATGTACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTAT**CGCACATCCAACCTGGCTTCT**GGA
GTCCCTGCGCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCT
GCCACTTATTACTGC**CAGCAGTATCATAGTTACCCAGCGACG**TTCGGTGGAGGCACCAAGCTGGAAATCAAA

Light chain: Amino acid sequence (SEQ ID NO: 42):
MDFQVQIFSFLLISASVIMSRGQIVLTQSPAIMSASPGEKVTISC**SASSSVSYMY**WYQQKPGSSPKPWIY**RTSNLAS**G
VPARFSGSGSGTSYSLTISSMEAEDAATYYC**QQYHSYPAT**FGGGTKLEIK

mAb 11D3

Heavy chain: DNA sequence (SEQ ID NO: 43):
ATGGGTTGGCTGTGGAACTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCCAAGCACAGATCCAGTTGGTACAGTCT
GGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGGTATATCTTCACA**ACCTATGGA**
ATGTACTGGGTGAAACAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGC**TGGATAAACACCTACTCTGGAGTGCCAACA**
TATGTTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACATCTGCCAGCACTGCCTATTTGCAGATCAACAAC
CTCAAAAATGAGGACACGGCTACATATTTCTGTGTAGTT**GCCGGGTGGTTTGCTTAC**TGGGGCCAAGGGACTCTGGTC
ACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 44):
MGWLWNLLFLMAAAQSAQAQIQLVQSGPELKKPGETVKISCKASGYIFT**TYGMY**WVKQAPGKGLKWMG**WINTYSGVPT**
YVDDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCVV**AGWFAY**WGQGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 45):
ATGGACATGAGGACCCCTGCTCAGTTTCTTGGAATCTTGTTGCTCTGGTTTCCAGGTATCAAATGTGACATCAAGATG
ACCCAGTCTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTCACTATCACTTGC**AAGGCGAGTCAGGACATTAAG**
AGCTATTTAAGCTGGTTCCAGCAGAAACCAGGGAAATCTCCTAAGACCCTGATCTAT**CGTGCAAATATATTGATAGAT**
GGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGCAAGATTATTCTCTCACCATCAGCAGCCTGGAGTATGAAGAT
ATGGGAATTTATTATTGT**CTACAATATGATGAGTTTCCGTACACG**TTCGGAGGGGGGACCAAGCTGGAAATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 46):
MDMRTPAQFLGILLLWFPGIKCDIKMTQSPSSMYASLGERVTITC**KASQDIKSYLS**WFQQKPGKSPKTLIY**RANILID**
GVPSRFSGSGSGQDYSLTISSLEYEDMGIYYC**LQYDEFPYT**FGGGTKLEIK

mAb 11H2

Heavy chain: DNA sequence (SEQ ID NO: 105)
ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTGACGTGAAGCTGGTGGAGTCT
GGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGG
ATTCACTTTCAGT**GACTATTACATGTAT**TGGGTTCGCCAGACTCCAGAGAAGAGACTGGAGTGGGTCGCA**TATGTTAG**
TAGTGGTGGTGGTAGTATCTATTATCCAGACACTGTAAAGGGCCGATTCACCATCT
CCAGAGACAATGCCAAGAACACCCTGTATTTGCAAATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTG
CAAGG**CATGGGTCCCCCTTCGGTAGTAGCCGAGGGGCCTGGTTTGCTTAC**TGGGGC
CAGGGGACTCTGGTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 106)
MNLGLSLIFLVLVLKGVQCDVKLVESGGGLVQPGGSLKLSCAASGFTFS**DYYMY**WVRQTPEKRLEWVA**YVSSGGGSIY**
YPDTVKGRFTISRDNAKNTLYLQMSRLKSEDTAMYYCAR**HGSPFGSSRGAWFAY**WG
QGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 107)
ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGGTGCCAGATGTGACATCCAGATGACTCAG
TCTCCAGCCTCCCTGTCTGCATCTGTGGGAGAAACTGTCACCATCACATGT**CGAGC**

AAGTGAGAATATTTACAGTTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTAT**AATGC**
AAAAACCTTAGCAGAGGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCACAC
AGTTTTCTCTGAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAATTATTACTGT**CAACATTATGATGGTTTTCCGT**
TCACGTTCGGTGGTGGGACCAAGCTGGAGCTGAAA

Light chain: Amino acid sequence (SEQ ID NO: 108)
MSVPTQVLGLLLLWLTGARCDIQMTQSPASLSASVGETVTITC**RASENIYSYLA**WYQQKQGKSPQLLVY**NAKTLAE**GV
PSRFSGSGSGTQFSLKINSLQPEDFGNYYC**QHYDGFPFT**FGGGTKLELK

mAb 6C12

Heavy chain: DNA sequence (SEQ ID NO: 109)
ATGGGTTGGCTGTGGAACTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCCAAGCACAGATCCAGTTGATACAGTCT
GGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGGTATACCTTCACA**ACCTTTGGA**
ATGAGCTGGGTGAAACAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGC**TGGATAAGCACCTACTCTGGAGTGCCAACA**
TATGCTGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCTATTTGCAGATCAACAAC
CTCAAAAATGAGGACACGGCTTCATATTTCTGTGCAAGA**CACACCTTCCAAAGTCGCGGGTTGGCTTA**CTGGGGCCAA
GGGACTCTGGTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 110)
MGWLWNLLFLMAAAQSAQAQIQLIQSGPELKKPGETVKISCKASGYTFT**TFGMS**WVKQAPGKGLKWMG**WISTYSGVPT**
YADDFKGRFAFSLETSASTAYLQINNLKNEDTASYFCAR**HTFQSRGLAY**WGQGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 111)
ATGGGCATCAAAATGGAGTCACAGATTCAGGTCTTTGTATTCGTGTTTCTCTGGTTGTCTGGTGTTGACGGAGACATT
GTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCATCACCTGC**AAGGCCAGTCAGGAT**
GTGATTACTACTGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACTACTGATTTAC**TCGGCATCCTACCGG**
TACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATCACCAGTGTGCAGACT
GAAGACCTGGCAGTTTATTACTGT**CAGCAACATTATAGTACTCCGTGGACG**TTCGGTGGAGGCACCAAGCTGGAAATC
AAA

Light chain: Amino acid sequence (SEQ ID NO: 112)
MGIKMESQIQVFVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSITC**KASQDVITTVA**WYQQKPGQS
PKLLIY**SASYRYT**GVPDRFTGSGSGTDFTFTITSVQTEDLAVYYC**QQHYSTPWT**FGGGTKLEIK

mAb 4H3

Heavy chain: DNA sequence (SEQ ID NO: 113)
ATGGGATGGAGCTGTATCATGCTCTTCTTGGCAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGCAGCCT
GGGGCTGAGCTTGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCACC**AACTACTGG**
ATACACTGGATGAAGCAGAGGCCTGGACGAGGCCTTGAGTGGATTGGA**AGGATTGATCCTAATAGTGGTGGTACTAAG**
TACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTCGACAAACCCTCCATCACAGCCTACATGCAGCTCAGCAGC
CTGACATCTGAGGACTCTGCGGTCTATTATTGTGCAGCA**TTCGGTAGTACCTACGGGTTTGCTTAC**TGGGGCCAAGGG
ACTCTGGTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 114)
MGWSCIMLFLAATATGVHSQVQLQQPGAELVKPGASVKLSCKASGYTFT**NYWIH**WMKQRPGRGLEWIG**RIDPNSGGTK**
YNEKFKSKATLTVDKPSITAYMQLSSLTSEDSAVYYCAA**FGSTYGFAY**WGQGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 115)
ATGGATTCACAGGCCCAGGTTCTTATATTGCTGCTGCTATGGGTATCTGGTACCTGTGGGGACATTGTGATGTCACAG
TCTCCATCCTCCCTGGCTGTGTCAGCAGGAGAGAAGGTCACTATGAGTTGC**AAATCCAGTCAGAGTCTGCTCAACAGT**
AGAACCCGAAAGAACTACTTGGCTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGATCTAC**TGGGCATCC**
ACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTG

CAGGCTGAAGACCTGGCAGTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTCGGAGGGGGGACCAAGCTGGAA
ATAAAA

Light chain: Amino acid sequence (SEQ ID NO: 116)
MDSQAQVLILLLLWVSGTCGDIVMSQSPSSLAVSAGEKVTMSC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WAS**
TRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC**KQSYNLYT**FGGGTKLEIK

mAb 4D8

Heavy chain: DNA sequence (SEQ ID NO: 117)
ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTGAAGTGACGCTGGTGGAGTCT
GGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGT**GACTATTAC**
ATGTATTGGGTTCGCCAGACTCCAGAGAAGAGGCTGGAGTGGGTCGCA**TACATTAGTCCTGGTGGTGGTAGCACCTAT**
TATCCGGACACTATAAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGAGCCGT
CTGAAGTCTGAGGACACAGCCATGTATTACTGTACAAGA**CATGGGTCCCCCTACGGTAGTAGTCGAGGGGCCTGGTTT**
GCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

Heavy chain: Amino acid sequence (SEQ ID NO: 118)
MNLGLSLIFLVLVLKGVQCEVTLVESGGGLVQPGGSLKLSCAASGFTFS**DYYMY**WVRQTPEKRLEWVA**YISPGGGSTY**
YPDTIKGRFTISRDNAKNTLYLQMSRLKSEDTAMYYCTR**HGSPYGSSRGAWFAY**WGQGTLVTVSA

Light chain: DNA sequence (SEQ ID NO: 119)
ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGGTGCCAGATGTGACATCCAGATGACTCAG
TCTCCAGCCTCCCTATCTGCATCTGTGGGAGAAACTGTCACCATCACATGT**CGAGCAAGTGAGAATATTTACAGTTAT**
TTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTAT**AATGGAAAAACCTTAGCAGAA**GGTGTG
CCAGCAAGGTTCAGTGGCAGTGGATCAGGCACACAGTTTTCTCTGAAGATCAACAGCCTACAGCCTGAAGATTTTGGG
AGTTATTACTGT**CAACATCATGATGGTATTCCGGTCACG**TTCGGTGCTGGGACCAAGCTGGAGCTGAAA

Light chain: Amino acid sequence (SEQ ID NO: 120)
MSVPTQVLGLLLLWLTGARCDIQMTQSPASLSASVGETVTITC**RASENIYSYLA**WYQQKQGKSPQLLVY**NGKTLAE**GV
PARFSGSGSGTQFSLKINSLQPEDFGSYYC**QHHDGIPVT**FGAGTKLELK

mAb 2E2

Heavy chain: DNA sequence (SEQ ID NO: 121)
ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTGAAGTGAAGC
TGGTGGAGTCGGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGTAGCCTCTGG
ATTCACTTTCAGT**GACTATCACATGCAT**TGGGTTCGCCAGACTCCAGAGAAGAGGCTGGAGTGGGTC
GCA**TACATTAGTAAAGGTGGTGGTAGCACCTATTATCCAGACACTGAAAAGGG**CCGATTCACCATCT
CCAGAGACAATGCCAAGAATACCCTGTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACACAGCCAT
GTATTACTGTGCAAGA**TCCCCCGGCCCTAGTAGCTTCTACTGGTACTTCGATGTC**TGGGGCACAGGG
ACCACGGTCACCGTCTCCTCA

Heavy chain: Amino acid sequence (SEQ ID NO: 122)
MNLGLSLIFLVLVLKGVQCEVKLVESGGGLVQPGGSLKLSCVASGFTFS**DYHMH**WVRQTPEKRLEWV
A**YISKGGGSTYYPDTEKG**RFTISRDNAKNTLYLQMSRLKSEDTAMYYCAR**SPGPSSFYWYFDV**WGTG
TTVTVSS

Light chain: DNA sequence (SEQ ID NO: 123)
ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGGTGCCAGATGTGACATCC
AGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAAACTGTCACCATCACATGT**CGAGC**
AAGTGAGAATATTTACAGTTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTG
GTCTAT**AATGCAAAAACCTTAGCAGAA**GGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCACAC
AGTTTTCTCTGAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGT**CAACATCATTA**
TGGTATTCCGGTCACGGTCGGTGTAGGGACCAAGCTGGAGCTGAAA

Light chain: Amino acid sequence (SEQ ID NO: 124)
MSVPTQVLGLLLLWLTGARCDIQMTQSPASLSASVGETVTITC**RASENIYSYLA**WYQQKQGKSPQLL
VY**NAKTLAE**GVPSRFSGSGSGTQFSLKINSLQPEDFGSYYC**QHHYGIPVT**VGVGTKLELK

EXAMPLE 4
CONSTRUCTION OF CHO CELL LINE EXPRESSING CANINE IL-4 RECEPTOR *ALPHA* CHAIN AND USE IN LIGAND BLOCKADE ASSAYS

The gene encoding full length canine IL-4 receptor *alpha* chain (cIL-4R$_\alpha$; SEQ ID NO: 4) was synthesized and sub-cloned into a mammalian expression vectors. The resulting plasmid was transfected into CHO DG44 cells. At 48 hours post-transfection, the cells were diluted into 96-well plates to generate single cell clones. About 130 clones were obtained after a 4-week incubation. All of the clones were screened for expression of cIL-4Rα by FACS using the anti-cIL-4Rα monoclonal antibody 6B2. Three clones were selected for stability evaluation. Stability was monitored for 20 passages by FACS.

In order to assess the ability of monoclonal antibodies specific to canine IL-4 receptor *alpha* to block the binding of canine IL-4 to canine IL-4 R *alpha* expressed on the surface of CHO cells, a ligand blockade assay was set as follows:

Reagent and equipments:

- Cell growth medium: CD OptiCHO medium + 8mM L-Glutamine + 0.018% F-68
- FACS Buffer: BD Pharmingen Stain Buffer (BD cat#: 554657)
- R-phycoerythin conjugated Streptavidin (Life Technologies: SB66)
- Canine IL-4 (R&D system, cat #754-CL/CF)
- Lightning-Link Biotin Conjugation Kit Type A (Novus: 704-0010) used to biotinylate canine IL-4 as per manufacturer's recommendation
- Flow cytometer: BD Accuri-C6

Procedure:

1. CHO-DH44-canIL-4Rα cell grown to 2 - 4 x10^6 cells/mL with more than 96% viability.
2. The cells were spun down, the supernatant discarded, and the cells were suspended in FACS buffer to 2 x 10^7 cells /mL.

3. The cells were distributed into a U-shape 96-well plate, 50 µl each well.
4. The anti-canine IL-4Rα mAbs in FACS buffer was diluted three-fold on a 96 - well plate from top down to bottom well, starting at 50 µg/mL.
5. 50 µl of each diluted Ab was transfered into the cell plate and then incubated on ice for 30 min.
6. The cells were washed twice with FACS buffer.
7. The cells were resupended into 100 µl of biotinylated canine IL-4 at 0.32 µg/mL in FACS buffer and incubated on ice for 30 min.
8. The cells were washed twice with FACS buffer.
9. The cells were responded into 100 µl of R-phycoerythin conjugated Streptavidin (1:1000 dilution) in FACS buffer and incubated on ice for 30 min.
10. The cells were washed twice with FACS buffer.
11. The cells were brought up to 300 µl in FACS buffer.
12. 10,000 cells were read for each sample by BD Accuri-C6.
13. The resulting readout were analyzed by FlowJo to get the mean fluorescent intensity (MFI).

A dose response curve for the binding of canine IL-4 to canine IL-4R_α expressed on the surface of CHO cells was obtained using the cell-based CHO-cIL-4R_α binding assay (*see*, Figure 2A). A half maximal effective concentration (EC50) of 25 nM was determined from this curve. Next, dose response curves for the binding of CHO-cIL-4R_α by the mouse anti-canine IL-4R_α monoclonal antibodies (mAbs): 11B6, 4D8, 4H3, 2E2, 11H2, and 6C12 were obtained (*see*, Figure 2B). The half maximal effective concentrations (EC50) for each of the antibodies is provided in Table 2 below.

TABLE 2
Binding/Blocking of Various mABs

mABs	EC50 (nM)	IC50 (nM)
11B6	7.5	53.2
4D8	1.1	4.2
4H3	1.6	3.9
2E2	1.2	2.1
11H2	1.2	1.7 / 1.0*
6C12	8.6	19.3

*Determinations from two separate studies

The mouse anti-canine IL-4R$_\alpha$ monoclonal antibodies (mAbs) were then assayed for their ability to block the binding of canine IL-4 to the cell-based CHO-cIL-4R$_\alpha$. As depicted in Figure 3A the five mAbs, 11B6, 4D8, 4H3, 2E2, and 11H2 displayed significant blocking ability. In a complementary study a sixth mAbs was tested (6C12), and compared with one of the five mAbs tested (11H2) in Figure 3A. As is apparent from Figure 3B and Table 2, 6C12 mAbs has a significantly higher half maximal inhibitory concentration (IC50) than the 11H2 mAbs. Four of anti- cIL-4Rα monoclonal antibodies, 4D8, 2E2, 4D8, and 11H2 showed superior blocking ability, as can be seen in Figures 3A and 3B, as well as in Table 2.

EXAMPLE 5
AMINO ACID SEQUNCES OF THE MOUSE CDRS

CDRs from mouse anti-canine IL-4 receptor α chain monoclonal antibodies:

VL CDR-1		SEQ ID NO:
1A3	Arg Ala Ser Ser Ser Val Ser Phe Met Phe	47
1A9	Lys Ala Ser Gln Asn Val Arg Ser Ala Val Ala	48
1B12	Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Phe	49
10C12	Lys Ser Ser Gln Ser Leu Leu Asn Ser Ser Asn Gln Lys Asn Tyr Leu Ala	50
10F2	Lys Ser Ser Gln Asn Leu Leu Tyr Gly Gly Asn Gln Lys Asn Tyr Leu Ala	51
10E10	Lys Ala Ser Asp His Ile Asn Asn Trp Leu Ala	52
10G8	Ser Ala Ser Ser Ser Val Asn Ser Ser Tyr Leu Tyr	53
11B6	Ser Ala Ser Ser Ser Val Ser Tyr Met Tyr	54
11D3	Lys Ala Ser Gln Asp Ile Lys Ser Tyr Leu Ser	55
11H2	Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Leu Ala	129
6C12	Lys Ala Ser Gln Asp Val Ile Thr Thr Val Ala	130
4D8	Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Leu Ala	129
4H3	Lys Ser Ser Gln Ser Leu Leu Asn Ser Arg Thr Arg Lys Asn Tyr Leu Ala	131
2E2	Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Leu Ala	129
VL CDR-2		SEQ ID NO:

1A3	Asp Thr Ser Asn Leu Ala Ser	56
1A9	Leu Ala Ser Asn Arg His Thr	57
1B12	Arg Met Ser Asn Leu Ala Ser	58
10C12	Phe Ala Ser Thr Arg Glu Ser	59
10F2	Trp Ala Ser Thr Arg Glu Ser	60
10E10	Gly Ala Thr Ser Leu Glu Thr	61
10G8	Ser Thr Ser Asn Leu Ala Ser	62
11B6	Arg Thr Ser Asn Leu Ala Ser	63
11D3	Arg Ala Asn Ile Leu Ile Asp	64
11H2	Asn Ala Lys Thr Leu Ala Glu	132
6C12	Ser Ala Ser Tyr Arg Tyr Thr	133
4D8	Asn Gly Lys Thr Leu Ala Glu	134
4H3	Trp Ala Ser Thr Arg Glu Ser	60
2E2	Asn Ala Lys Thr Leu Ala Glu	132

VL CDR-3		SEQ ID NO:
1A3	Gln Gln Trp Ser Ser Asn Pro Leu Thr	65
1A9	Leu Gln His Trp Asn Tyr Pro Phe Thr	66
1B12	Met Gln His Leu Glu Tyr Pro Phe Thr	67
10C12	Gln Gln His Tyr Ser Thr Pro Tyr Thr	68
10F2	Gln Gln Tyr Tyr Asp Tyr Pro Tyr Thr	69
10E10	His Gln Tyr Trp Ser Ile Pro Tyr Thr	70
10G8	His Gln Trp Ser Ser Tyr Pro Tyr Thr	71
11B6	Gln Gln Tyr His Ser Tyr Pro Ala Thr	72
11D3	Leu Gln Tyr Asp Glu Phe Pro Tyr Thr	73
11H2	Gln His Tyr Asp Gly Phe Pro Phe Thr	135
6C12	Gln Gln His Tyr Ser Thr Pro Trp Thr	136
4D8	Gln His His Asp Gly Ile Pro Val Thr	137
4H3	Lys Gln Ser Tyr Asn Leu Tyr Thr	138
2E2	Gln His His Tyr Gly Ile Pro Val Thr	139

VH CDR-1		SEQ ID NO:
1A3	Asp Phe Gly Met His	74
1A9	Ser Ser Trp Met Asn	75
1B12	Asp Tyr Tyr Met Asn	76
10C12	Asp Tyr Glu Met His	77
10F2	Ser Tyr Gly Val Ser	78
10E10	Thr Tyr Asp Ile His	79
10G8	Asp Tyr Glu Met His	80
11B6	Ser Asp Tyr Trp Asn	81
11D3	Thr Tyr Gly Met Tyr	82
11H2	Asp Tyr Tyr Met Tyr	140
6C12	Thr Phe Gly Met Ser	141
4D8	Asp Tyr Tyr Met Tyr	140
4H3	Asn Tyr Trp Ile His	142
2E2	Asp Tyr His Met His	143

VH CDR-2																SEQ ID NO:
1A3	Tyr	Ile	Ser	Ser	Gly	Ser	Gly	Thr	Ile	Tyr	Tyr	Ala	Asp	Thr	Val	Arg Gly 83
1A9	Arg	Ile	Tyr	Pro	Gly	Asp	Gly	Asp	Thr	Lys	Tyr	Asn	Gly	Lys	Phe	Lys Gly 84
1B12	Asp	Ile	Ile	Pro	Ser	Asn	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys Gly 85
10C12	Ala	Ile	Asp	Pro	Glu	Thr	Cys	Gly	Thr	Ala	Tyr	Asn	Gln	Lys	Phe	Lys Gly 86
10F2	Val	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Tyr	Phe	His	Ser	Ala	Leu	Ile	Ser 87
10E10	Trp	Ile	Tyr	Pro	Arg	Asp	Gly	Arg	Thr	Thr	Tyr	Asn	Glu	Lys	Phe	Lys Ala 88
10G8	Ala	Ile	Asp	Pro	Glu	Thr	Gly	Gly	Thr	Ala	Tyr	Asn	Gln	Lys	Phe	Lys Gly 89
11B6	Tyr	Ile	Asn	Tyr	Ser	Gly	Asn	Thr	Tyr	Tyr	Asn	Pro	Ser	Leu	Lys	Ser 90
11D3	Trp	Ile	Asn	Thr	Tyr	Ser	Gly	Val	Pro	Thr	Tyr	Val	Asp	Asp	Phe	Lys Gly 91
11H2	Tyr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ile	Tyr	Tyr	Pro	Asp	Thr	Val	Lys Gly 144
6C12	Trp	Ile	Ser	Thr	Tyr	Ser	Gly	Val	Pro	Thr	Tyr	Ala	Asp	Asp	Phe	Lys Gly 145
4D8	Tyr	Ile	Ser	Pro	Gly	Gly	Gly	Ser	Thr	Tyr	Tyr	Pro	Asp	Thr	Ile	Lys Gly 146
4H3	Arg	Ile	Asp	Pro	Asn	Ser	Gly	Gly	Thr	Lys	Tyr	Asn	Glu	Lys	Phe	Lys Ser 147
2E2	Tyr	Ile	Ser	Lys	Gly	Gly	Gly	Ser	Thr	Tyr	Tyr	Pro	Asp	Thr	Glu	Lys Gly 148

VH CDR-3																SEQ ID NO:
1A3	Gly	Asp	Leu	Tyr	Tyr	Gly	Ser	Ser	Phe	Asp	Ala	Tyr				92
1A9	Asp	Asp	Tyr	Asp	Trp	Ala	Ser									93
1B12	Gly	Ile	Ser	Tyr	Tyr	Gly	Asn	Arg	Tyr	Tyr	Phe	Thr	Met	Asp	Tyr	94
10C12	Ser	Lys	Leu	Gly	Arg	Gly	Trp	Tyr	Phe	Asp	Val					95
10F2	Gln	Gly	Thr	Ile	Tyr	Asp	Gly	Tyr	Tyr	Asn	Tyr	Ala	Met	Asp	Tyr	96
10E10	Ser	Ser	Pro	Phe	Gly	Tyr										97
10G8	Gly	Phe	Asp	Tyr												98
11B6	Tyr	Gly	Gly	Leu	Arg	Gln	Gly	Ser	Trp	His	Phe	Asp	Val			99
11D3	Ala	Gly	Trp	Phe	Ala	Tyr										100
11H2	His	Gly	Ser	Pro	Phe	Gly	Ser	Ser	Arg	Gly	Ala	Trp	Phe	Ala	Tyr	149
6C12	His	Thr	Phe	Gln	Ser	Arg	Gly	Leu	Ala	Tyr						150
4D8	His	Gly	Ser	Pro	Tyr	Gly	Ser	Ser	Arg	Gly	Ala	Trp	Phe	Ala	Tyr	151
4H3	Phe	Gly	Ser	Thr	Tyr	Gly	Phe	Ala	Tyr							152
2E2	Ser	Pro	Gly	Pro	Ser	Ser	Phe	Tyr	Trp	Tyr	Phe	Asp	Val			153

TABLE 3
CANONICAL STRUCTURES

	L1	**L2**	**L3**	**H1**	**H2**	**H3**
1A3	L1-1	L2-1	L3-1	H1-1	H2-3A	H3-12
1A9	L1-2A	L2-1	L3-1	H1-1	H2-2A	H3-7
1B12	L1-4	L2-1	L3-1	H1-1	H2-2B	H3-15
10C12	L1-3	L2-1	L3-1	H1-1	*	H3-11
10F2	L1-3	L2-1	L3-1	H1-1	H2-1	H3-15
10E10	L1-2A	L2-1	L3-1	H1-1	H2-2B	H3-6
10G8	L1-6	L2-1	L3-1	H1-1	H2-2B	H3-4
11B6	L1-1	L2-1	L3-1	H1-1	H2-1	H3-13
11D3	L1-2A	L2-1	L3-1	H1-1	H2-2A**	H3-6

Date Reçue/Date Received 2024-03-19

11H2	L1-6	L2-1	L3-1	H1-1	H2-3A	H3-15
6C12	L1-6	L2-1	L3-1	H1-1	H2-2A	H3-10
4D8	L1-6	L2-1	L3-1	H1-1	H2-3A	H3-15
4H3	L1-3	L2-1	L3-3	H1-1	H2-3A	H3-9
2E2	L1-6	L2-1	L3-1	H1-1	H2-3A	H3-13

* Cysteine in the CDR
** The best assignment that could be made in view of the particular pattern.

EXAMPLE 6

EPITOPE MAPPING OF MURINE ANTI-CANINE IL-4 RECEPTOR *ALPHA* ANTIBODIES

The interaction of antibodies with their cognate protein antigens is mediated through the binding of specific amino acids of the antibodies (paratopes) with specific amino acids (epitopes) of target antigens. An epitope is an antigenic determinant that causes a specific reaction by an immunoglobulin. An epitope consists of a group of amino acids on the surface of the antigen. A protein of interest may contain several epitopes that are recognized by different antibodies. The epitopes recognized by antibodies are classified as linear or conformational epitopes. Linear epitopes are formed by a stretch of a continuous sequence of amino acids in a protein, while conformational epitopes are composed of amino acids that are discontinuous (*e.g.*, far apart) in the primary amino acid sequence, but are brought together upon three-dimensional protein folding.

Epitope mapping refers to the process of identifying the amino acid sequences (*i.e.,* epitopes) that are recognized by antibodies on their target antigens. Identification of epitopes recognized by monoclonal antibodies (mAbs) on target antigens has important applications. For example, it can aid in the development of new therapeutics, diagnostics, and vaccines. Epitope mapping can also aid in the selection of optimized therapeutic mAbs and help elucidate their mechanisms of action. Epitope information on IL-4 receptor *alpha* can also elucidate unique epitopes, and define the protective or pathogenic effects of vaccines. Epitope identification also can lead to development of subunit vaccines based on chemical or genetic

coupling of the identified peptide epitope to a carrier protein or other immunostimulating agents.

Epitope mapping can be carried out using polyclonal or monoclonal antibodies and several methods are employed for epitope identification depending on the suspected nature of the epitope (*i.e.*, linear *versus* conformational). Mapping linear epitopes is more straightforward and relatively, easier to perform. For this purpose, commercial services for linear epitope mapping often employ peptide scanning. In this case, an overlapping set of short peptide sequences of the target protein are chemically synthesized and tested for their ability to bind antibodies of interest. The strategy is rapid, high-throughput, and relatively inexpensive to perform. On the other hand, mapping of a discontinuous epitope is more technically challenging and requires more specialized techniques such as x-ray co-crystallography of a monoclonal antibody together with its target protein, Hydrogen-Deuterium (H/D) exchange, Mass Spectrometry coupled with enzymatic digestion as well as several other methods known to those skilled in the art.

Mapping of canine IL-4 receptor alpha epitopes using Mass Spectroscopy:

A method based on chemical crosslinking and mass spectrometry detection was employed to identify epitopes recognized by anti-canine IL-4 receptor *alpha* mAbs [CovalX Instrument Incorporated]. The application of this technology to epitope mapping of canine IL-4 receptor *alpha* chain resulted in identification of epitopes recognized by the mAbs listed in Table 4.

The results from the epitope mapping of canine IL-4 receptor *alpha* with the six antibodies included in Table 4, indicates that the mAbs recognize specific peptide epitopes that are present within the extracellular domain of canine IL-4 receptor *alpha*. Notably, two to three epitopes were identified for each of the six monoclonal antibodies (mAbs) tested. Interestingly, one of the epitopes identified for mAbs 2E2 was found to have the exact same amino acid sequence as that for mAbs 11B6 (*i.e.*, SEQ ID NO: 158). As depicted in Table 4 below, mAbs: 4D8, 11H2, and 11B6 all recognize an epitope, labeled with a "[1]" that is a portion of the same linear amino acid sequence; mAbs: 11H2, 4H3, and 2E2 all recognize an

epitope labeled with a "[2]" that is a portion of another linear amino acid sequence; and mAbs 4H3 and 2H2 recognize an epitope labeled with a "[3]" that is a portion of a third linear amino acid sequence. This relative consistency in the identification of the relevant epitopes indicates that these six monoclonal antibodies recognize a limited number of portions of canine IL-4 receptor *alpha*, within its extracellular domain.

TABLE 4
IL-4 RECEPTOR *ALPHA* EPITOPES RECOGNIZED BY ANTI-CANINE IL-4 RECEPTOR *ALPHA* MONOCLONAL ANTIBODIES

ANTIBODY	SEQ ID NO:	EPITOPE SEQUENCE
4D8	125	SAELRLSYQLD
	126	**FQPSKHVKPRT[1]**
11H2	127	AGQQLL**WSGSFQPSKHVKPRT[1]**
	128	**TLKSGASYS[2]**
4H3	154	**EDSVCVCSMPI[3]**
	155	MWTNPYPTENHL
	156	AS**TLKSG[2]**
11B6	157	**WSGSFQPSKHVKPR[1]**
	158	**VYNVTYMGPTLR**
2E2	159	VLHEPSCFSDYISTSVCQ
	160	ENR**EDSVCVCSMPI[3]**
	161	**KSGASYS**ARVRAW[2]
6C12	158	**VYNVTYMGPTLR**
	162	YYEPWEQHLP

[1, 2, 3] identify three individual groups of epitopes arising from three portions of the antigen.

Together with the CDRs provided in Example 5 for the six antibodies listed in Table 4 above, a one to one relationship is defined between each set of CDRs and their corresponidng epitopes in Table 4. This relationship allows a defined linkage between the set of 6 CDRs in Example 5 for each of the six antibodies in Table 4 and the corresponding epitopes that they bind. Accordingly, antibodies (*e.g.*, caninized antibodies) with the defined set of 6 CDRs provided in Example 5 that bind corresponding epitopes in Table 4 are also part of the present invention.

EXAMPLE 7

CONSTRUCTION OF CANINIZED ANTI-CANINE IL-4 RECEPTOR *alpha* MONOCLONAL ANTIBODIES

In order to execute the process of caninization, the DNA sequence that encodes the heavy and light chains of canine IgG were determined. The DNA and protein sequence of the canine heavy and light chains are known in the art and can be obtained by searching of the NCBI gene and protein databases. As indicated above, for canine antibodies there are four known IgG subtypes: IgG-A, IgG-B, IgG-C, and IgG-D, and two types of light chains, *i.e.*, *kappa* and *lambda*. Without being bound by any specific approach, the overall process of producing caninized heavy and light chains that can be mixed in different combinations to produce caninized anti-canine IL-4 receptor alpha mAbs involves the following scheme:

i) Identify the DNA sequence of VH and VL domains comprising the CDRs of desired anti-IL-4 receptor alpha mAbs

ii) Identify the H and L chain CDRs of desired anti-IL-4 receptor mAbs

iii) Identify a suitable sequence for H and L chain of canine IgG

iv) Identify the DNA sequence encoding the endogenous CDRs of canine IgG H and L chains of the above sequence.

v) Replace the DNA sequence encoding endogenous canine H and L chain CDRs with DNA sequences encoding the desired anti-IL-4 receptor alpha CDRs. In addition, optionally replace some canine framework residues with selected residues from the desired anti-IL-4 receptor mAb framework regions.

vi) Synthesize the DNA from step (v), clone it into a suitable expression plasmid, and transfect the plasmids containing desired caninized H and L chains into HEK 293 cells.

vii) Purify expressed caninized antibody from HEK 293 supernatant.

viii) Test purified caninized antibody for binding to canine IL-4 receptor alpha chain.

The application of the above outlined steps resulted in a set of caninized H and L chain sequences for which the SEQ ID NOs. are listed in Table 5 below.

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TABLE 5
CANINIZED FULL-LENGTH HEAVY AND LIGHT CHAIN SEQUENCES

H chain or L chain	Nucleic Acid	Amino Acid
vH1	SEQ ID NO: 163	SEQ ID NO: 164
vH2	SEQ ID NO: 165	SEQ ID NO: 166
vH3	SEQ ID NO: 167	SEQ ID NO: 168
vL1	SEQ ID NO: 169	SEQ ID NO: 170
vL2	SEQ ID NO: 171	SEQ ID NO: 172
vL3	SEQ ID NO: 173	SEQ ID NO: 174

The present invention provides caninized antibodies formed by the combination of various caninized heavy and light chains listed in the Table 5 above; such antibodies have particularly tight binding with canine IL-4 receptor *alpha*. In a particular embodiment the heavy chain comprises the amino acid sequence of SEQ ID NO: 164 and the light chain comprises the amino acid sequence of SEQ ID NO: 170. In a more particular embodiment of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 163 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 169. In another embodiment the heavy chain comprises the amino acid sequence of SEQ ID NO: 166 and the light chain comprises the amino acid sequence of SEQ ID NO: 172. In a more particular embodiment of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 165 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 171. In still another embodiment the heavy chain comprises the amino acid sequence of SEQ ID NO: 168 and the light chain comprises the amino acid sequence of SEQ ID NO: 174. In a more particular embodiment of this type, the heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 167 and the light chain is encoded by the nucleotide sequence of SEQ ID NO: 173. Binding studies to IL-4 receptor *alpha* by these caninized antibodies are depicted in Figure 4, as described in Example 8, below.

As indicated above, the Fc portion of the caninized antibodies is based on modified sequences of canine IgG-B in order to remove ADCC and CDC effector functions. The Fc regions of these antibodies may be replaced with a modified Fc from other canine IgG isotypes and/or

can be combined with substitute hinge regions as discussed above, and exemplified and disclosed in U.S. provisional application 62/030,812 filed July 30, 2014; U.S. provisional application 62/057,541 filed September 30, 2014; U.S. provisional application 62/092,496 filed December 16, 2014; U.S. provisional application 62/172,511, filed June 8, 2015; and WO 2015/091910.

CANINZED 4H3 (VH1)

SEQ ID NO: 163

GAGGTGCAGCTGGTGGAGAGCGGAGGCGACCTGGTGAAACCCGGAGGCAGCCTGAGACTGAGCTGTGTGGCCAGCGGCT
ACACCTTCACCAACTACTGGATTCATTGGGTGAGGCAGGCTCCCGGCAAAGGACTGCAGTGGGTGGCCAGGATTGATCC
CAACAGCGGCGGCACCAAGTACAACGAGAAGTTCAAGAGCAGGTTCACCATCAGCAGGGACAACGCCAAGAACACCCTC
TACCTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGTTCGGCAGCACCTACGGCTTCG
CCTACTGGGGCCAAGGCACCCTGGTGACCGTGAGCAGCGCTTCCACAACCGCGCCATCAGTCTTTCCGTTGGCCCCATC
ATGCGGGTCGACGAGCGGATCGACTGTGGCCCTGGCGTGCTTGGTGTCGGGATACTTTCCCGAACCCGTCACGGTCAGC
TGGAACTCCGGATCGCTTACGAGCGGTGTGCATACGTTCCCCTCGGTCTTGCAATCATCAGGGCTCTACTCGCTGTCGA
GCATGGTAACGGTGCCCTCATCGAGGTGGCCCTCCGAAACGTTCACATGTAACGTAGCACATCCAGCCTCCAAAACCAA
GGTGGATAAACCCGTGCCGAAAAGAGAGAATGGGCGGGTGCCTCGACCCCCTGATTGCCCCAAGTGTCCGGCTCCGGAA
ATGCTCGGTGGACCCTCAGTGTTTATCTTCCCTCCGAAGCCCAAGGACACTCTGCTGATCGCGCGCACTCCAGAAGTAA
CATGTGTAGTGGTGGCACTTGATCCCGAGGACCCCGAAGTCCAGATCTCCTGGTTTGTAGATGGGAAACAGATGCAGAC
CGCAAAAACTCAACCCAGAGAGGAGCAGTTCGCAGGAACATACCGAGTGGTATCCGTCCTTCCGATTGGCCACCAGGAC
TGGTTGAAAGGGAAGCAGTTTACGTGTAAAGTCAACAATAAGGCGTTGCCTAGCCCTATTGAGCGGACGATTTCGAAAG
CTAGGGGACAGGCCCACCAGCCATCGGTCTATGTCCTTCCGCCTTCCCGCGAGGAGCTCTCGAAGAATACAGTGAGCCT
TACATGCCTCATTAAGGATTTCTTCCCGCCTGATATCGACGTAGAGTGGCAATCAAACGGTCAACAGGAGCCGGAATCC
AAGTATAGAACCACTCCGCCCCAGCTTGACGAGGACGGATCATACTTTTTGTATTCAAAACTGTCGGTGGATAAGAGCC
GGTGGCAGAGAGGTGACACCTTCATCTGTGCGGTGATGCACGAAGCACTCCATAATCACTACACCCAAGAGAGCCTCTC
GCATTCCCCCGGAAAG

SEQ ID NO: 164

EVQLVESGGDLVKPGGSLRLSCVASGYTFTNYWIHWVRQAPGKGLQWVARIDPNSGGTKYNEKFKSRFTISRDNAKNTL
YLQMNSLRAEDTAVYYCTRFGSTYGFAYWGQGTLVTVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVS
WNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPE
MLGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQD
WLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPES
KYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

CANINZED 4H3 (VH2)

SEQ ID NO: 165

GAGGTGCAGCTGGTGGAGAGCGGCGGAGATCTGGTGAAGCCCGGCGGAAGCCTGAGACTGAGCTGTGTGGCCAGCGGCT
ACACCTTCACCAACTACTGGATTCATTGGGTGAGACAGGCCCCTGGCAAGGGCCTGCAGTGGATCGGCAGGATCGACCC
CAACAGCGGCGGCACCAAGTACAACGAGAAGTTCAAGAGCAAGGCCACCCTGAGCGTGGACAAGGCCAAGAACACCCTG
TACCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCGCCTTTGGCAGCACCTACGGCTTCG
CCTACTGGGGCCAGGGAACCCTGGTGACCGTGAGCAGCGCTTCCACAACCGCGCCATCAGTCTTTCCGTTGGCCCCATC

ATGCGGGTCGACGAGCGGATCGACTGTGGCCCTGGCGTGCTTGGTGTCGGGATACTTTCCCGAACCCGTCACGGTCAGC
TGGAACTCCGGATCGCTTACGAGCGGTGTGCATACGTTCCCCTCGGTCTTGCAATCATCAGGGCTCTACTCGCTGTCGA
GCATGGTAACGGTGCCCTCATCGAGGTGGCCCTCCGAAACGTTCACATGTAACGTAGCACATCCAGCCTCCAAAACCAA
GGTGGATAAACCCGTGCCGAAAAGAGAGAATGGGCGGGTGCCTCGACCCCCTGATTGCCCCAAGTGTCCGGCTCCGGAA
ATGCTCGGTGGACCCTCAGTGTTTATCTTCCCTCCGAAGCCCAAGGACACTCTGCTGATCGCGCGCACTCCAGAAGTAA
CATGTGTAGTGGTGGCACTTGATCCCGAGGACCCCGAAGTCCAGATCTCCTGGTTTGTAGATGGGAAACAGATGCAGAC
CGCAAAAACTCAACCCAGAGAGGAGCAGTTCGCAGGAACATACCGAGTGGTATCCGTCCTTCCGATTGGCCACCAGGAC
TGGTTGAAAGGGAAGCAGTTTACGTGTAAAGTCAACAATAAGGCGTTGCCTAGCCCTATTGAGCGGACGATTTCGAAAG
CTAGGGGACAGGCCCACCAGCCATCGGTCTATGTCCTTCCGCCTTCCCGCGAGGAGCTCTCGAAGAATACAGTGAGCCT
TACATGCCTCATTAAGGATTTCTTCCCGCCTGATATCGACGTAGAGTGGCAATCAAACGGTCAACAGGAGCCGGAATCC
AAGTATAGAACCACTCCGCCCCAGCTTGACGAGGACGGATCATACTTTTTGTATTCAAAACTGTCGGTGGATAAGAGCC
GGTGGCAGAGAGGTGACACCTTCATCTGTGCGGTGATGCACGAAGCACTCCATAATCACTACACCCAAGAGAGCCTCTC
GCATTCCCCCGGAAAG

SEQ ID NO: 166

EVQLVESGGDLVKPGGSLRLSCVASGYTFTNYWIHWVRQAPGKGLQWIGRIDPNSGGTKYNEKFKSKATLSVDKAKNTL
YLQMNSLRAEDTAVYYCAAFGSTYGFAYWGQGTLVTVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVS
WNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPE
MLGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQD
WLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPES
KYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

CANINZED 4H3 (VH3):

SEQ ID NO: 167

GAGGTGCAGCTGGTGGAGAGCGGCGGCGATCTGGTGAAGCCTGGCGGAAGCCTGAGACTGAGCTGCGTGGCCAGCGGCT
ACACCTTCACCAACTACTGGATTCATTGGATGAGGCAGGCCCCTGGCAAGGGACTGCAGTGGATCGGCAGAATCGACCC
CAACAGCGGCGGCACCAAGTACAACGAGAAGTTCAAGAGCAAGGCCACCCTGAGCGTGGACAAGGCCAAGAACACCGCC
TACATGCAGCTGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCGCCTTTGGCAGCACCTACGGCTTCG
CCTATTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTTCCACAACCGCGCCATCAGTCTTTCCGTTGGCCCCATC
ATGCGGGTCGACGAGCGGATCGACTGTGGCCCTGGCGTGCTTGGTGTCGGGATACTTTCCCGAACCCGTCACGGTCAGC
TGGAACTCCGGATCGCTTACGAGCGGTGTGCATACGTTCCCCTCGGTCTTGCAATCATCAGGGCTCTACTCGCTGTCGA
GCATGGTAACGGTGCCCTCATCGAGGTGGCCCTCCGAAACGTTCACATGTAACGTAGCACATCCAGCCTCCAAAACCAA
GGTGGATAAACCCGTGCCGAAAAGAGAGAATGGGCGGGTGCCTCGACCCCCTGATTGCCCCAAGTGTCCGGCTCCGGAA
ATGCTCGGTGGACCCTCAGTGTTTATCTTCCCTCCGAAGCCCAAGGACACTCTGCTGATCGCGCGCACTCCAGAAGTAA
CATGTGTAGTGGTGGCACTTGATCCCGAGGACCCCGAAGTCCAGATCTCCTGGTTTGTAGATGGGAAACAGATGCAGAC
CGCAAAAACTCAACCCAGAGAGGAGCAGTTCGCAGGAACATACCGAGTGGTATCCGTCCTTCCGATTGGCCACCAGGAC
TGGTTGAAAGGGAAGCAGTTTACGTGTAAAGTCAACAATAAGGCGTTGCCTAGCCCTATTGAGCGGACGATTTCGAAAG
CTAGGGGACAGGCCCACCAGCCATCGGTCTATGTCCTTCCGCCTTCCCGCGAGGAGCTCTCGAAGAATACAGTGAGCCT
TACATGCCTCATTAAGGATTTCTTCCCGCCTGATATCGACGTAGAGTGGCAATCAAACGGTCAACAGGAGCCGGAATCC
AAGTATAGAACCACTCCGCCCCAGCTTGACGAGGACGGATCATACTTTTTGTATTCAAAACTGTCGGTGGATAAGAGCC
GGTGGCAGAGAGGTGACACCTTCATCTGTGCGGTGATGCACGAAGCACTCCATAATCACTACACCCAAGAGAGCCTCTC
GCATTCCCCCGGAAAG

SEQ ID NO: 168

EVQLVESGGDLVKPGGSLRLSCVASGYTFTNYWIHWMRQAPGKGLQWIGRIDPNSGGTKYNEKFKSKATLSVDKAKNTA
YMQLNSLRAEDTAVYYCAAFGSTYGFAYWGQGTLVTVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVS
WNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPE

MLGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQD
WLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPES
KYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

CANINZED 4H3 (VL1)

SEQ ID NO: 169

GACATCGTGATGACCCAGACCCCTCTGAGCCTGTCCGTGAGCCCTGGCGAACCTGCCAGCATCAGCTGCAAGAGCAGCC
AGAGCCTGCTGAACAGCAGGACCAGGAAGAACTACCTGGCCTGGTTCAGACAGAAGCCCGGCCAGAGCCCCCAGAGACT
GATCTACTGGGCCAGCACCAGAGAGAGCGGCGTGCCTGACAGATTTAGCGGCAGCGGCAGCGGCACAGACTTCACCCTG
AGGATCAGCAGAGTGGAGGCCGACGATGCCGGCGTGTACTACTGCAAGCAGAGCTACAACCTGTACACCTTCGGCCAGG
GCACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCATAC
GGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACGGGGTA
ATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTCAACCTTGA
CGATGTCAAGCACCGAGTATCTTAGCCATGAGCTGTATTCGTGCGAGATCACCCACAAGTCCCTCCCCTCCACTCTTAT
CAAATCCTTTCAGCGGTCGGAATGTCAGCGGGTCGAT

SEQ ID NO: 170

DIVMTQTPLSLSVSPGEPASISCKSSQSLLNSRTRKNYLAWFRQKPGQSPQRLIYWASTRESGVPDRFSGSGSGTDFTL
RISRVEADDAGVYYCKQSYNLYTFGQGTKVEIKRNDAQPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGV
IQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFQRSECQRVD

CANINZED 4H3 (VL2)

SEQ ID NO: 171

GACATCGTGATGACCCAGACCCCTCTGAGCCTGAGCGTGAGCCCTGGAGAGCCTGCCAGCATCAGCTGCAAGAGCAGCC
AGAGCCTGCTGAACAGCAGGACCAGGAAGAACTACCTGGCCTGGTACAGGCAGAAGCCTGGCCAGAGCCCCCAGCTGCT
GATCTACTGGGCCAGCACCAGAGAGAGCGGAGTGCCTGACAGGTTCAGCGGAAGCGGCAGCGGCACCGACTTCACCCTG
AGGATCAGCAGAGTGGAGGCCGATGACGCCGGCGTGTACTACTGCAAGCAGAGCTACAACCTGTACACCTTCGGCCAGG
GCACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCATAC
GGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACGGGGTA
ATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTCAACCTTGA
CGATGTCAAGCACCGAGTATCTTAGCCATGAGCTGTATTCGTGCGAGATCACCCACAAGTCCCTCCCCTCCACTCTTAT
CAAATCCTTTCAGCGGTCGGAATGTCAGCGGGTCGAT

SEQ ID NO: 172

DIVMTQTPLSLSVSPGEPASISCKSSQSLLNSRTRKNYLAWYRQKPGQSPQLLIYWASTRESGVPDRFSGSGSGTDFTL
RISRVEADDAGVYYCKQSYNLYTFGQGTKVEIKRNDAQPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGV
IQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFQRSECQRVD

CANINZED 4H3 (VL3)

SEQ ID NO: 173

GACATCGTGATGACCCAGACCCCTCTGAGCCTGAGCGTGAGCCCTGGAGAGCCTGCCAGCATCAGCTGCAAGAGCAGCC
AGAGCCTGCTGAACAGCAGGACCAGGAAGAACTACCTGGCCTGGTACCAGCAGAAGCCTGGCCAGAGCCCCCAGCTGCT
GATCTACTGGGCCAGCACCAGAGAGAGCGGAGTGCCTGACAGGTTCAGCGGAAGCGGCAGCGGCACCGACTTCACCCTG
AGGATCAGCAGAGTGGAGGCCGATGACGCCGGCGTGTACTACTGCAAGCAGAGCTACAACCTGTACACCTTCGGCCAGG
GCACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCATAC

GGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACGGGGTA
ATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTCAACCTTGA
CGATGTCAAGCACCGAGTATCTTAGCCATGAGCTGTATTCGTGCGAGATCACCCACAAGTCCCTCCCCTCCACTCTTAT
CAAATCCTTTCAGCGGTCGGAATGTCAGCGGGTCGAT

SEQ ID NO: 174
DIVMTQTPLSLSVSPGEPASISCKSSQSLLNSRTRKNYLAWYQQKPGQSPQLLIYWASTRESGVPDRFSGSGSGTDFTL
RISRVEADDAGVYYCKQSYNLYTFGQGTKVEIKRNDAQPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGV
IQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFQRSECQRVD

EXAMPLE 8

REACTIVITY OF CANINIZED ANTIBODIES AGAINST CANINE IL-4 RECEPTOR *alpha*

The caninized antibodies were tested for reactivity with canine IL-4 receptor *alpha* as follows:

1. Coat 200 ng/well of IL-4 receptor *alpha* on an immunoplate and incubate the plate at 4°C overnight.
2. Wash the plate 3 times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST).
3. Block the plate with 0.5% bovine serum albumin (BSA) in PBS for 45 – 60 min at room temperature.
4. Wash the plate 3 times with PBST.
5. Three – fold dilute the caninized antibody in each column or row of dilution plate starting at 0.3µg/mL.
6. Transfer the diluted caninized antibody into each column or row of the immunoplate, and incubate the plate for 45 – 60 min at room temperature.
7. Wash the plate 3 times with PBST.
8. Add 1:4000 diluted horseradish peroxidase labeled anti – canine IgG Fc into each well of the plate, and then incubate the plate for 45 – 60 min at room temperature.
9. Wash the plate 3 times with PBST.
10. Add 3,3',5,5'-tetramethylbenzidine (TMB) Substrate into each well of the plate, and incubate the plate for 10 to 15 min at room temperature to develop the color.

11. Add 100 µL 1.5 M phosphoric acid into each well to stop the reaction. Read plate at 450 nm with 540 nm reference wavelength.

As depicted in Figure 4, the binding of five (5) antibodies to the IL-4 receptor *alpha* was studied: 4H3 M-C, c4H3 H1-L1, c4H3 H2-L2, c4H3 H3-L3, and 2G9 M-C. 2G9 M-C was used as a negative control antibody. 4H3 M-C is a chimeric antibody consisting of the mouse variable heavy regions of the presently disclosed 4H3 antibody together with canine constant regions, and the light chain from the mouse 4H3 antibody. c4H3 H1-L1, c4H3 H2-L2, c4H3 H3-L3 are three caninized variants of the mouse 4H3 antibody, and include specific heavy chains and light chains as depicted in Table 5 above. 2G9 M-C is a chimeric antibody consisting of the mouse variable heavy regions of a mouse antibody to an antigen that is completely unrelated to the IL-4 receptor *alpha* together with canine constant regions, and the light chain from the mouse antibody to that unrelated antigen. Consistently, 2G9 M-C did not bind to the IL-4 receptor *alpha*, whereas the remaining four antibodies studied, *i.e.*, 4H3 M-C, c4H3 H1-L1, c4H3 H2-L2, and c4H3 H3-L3, all bound relatively tightly (*see,* Figure 4).

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CLAIMS

1. An isolated mammalian antibody or antigen binding fragment thereof that binds canine interleukin-4 receptor α (IL-4$R_α$) with specificity, comprising a light chain and heavy chain, wherein the light chain comprises three light chain complementary determining regions (CDRs): a CDR light 1 (CDRL1), a CDR light 2 (CDRL2), and a CDR light 3 (CDRL3); and wherein the heavy chain comprises three heavy chain CDRs: a CDR heavy 1 (CDRH1), a CDR heavy 2 (CDRH2), and a CDR heavy 3 (CDRH3), wherein:

a) the CDRL1 comprises the amino acid sequence of SEQ ID NO: 129;
b) the CDRL2 comprises the amino acid sequence of SEQ ID NO: 134;
c) the CDRL3 comprises the amino acid sequence of SEQ ID NO: 137;
d) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 140;
e) the CDRH2 comprises the amino acid sequence of SEQ ID NO: 146;
f) the CDRH3 comprises the amino acid sequence of SEQ ID NO: 151;

wherein the antibody and antigen binding fragment thereof bind canine IL-4$R_α$ and block the binding of canine IL-4$R_α$ to canine interleukin-4.

2. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1, that is a caninized antibody or a caninized antigen binding fragment thereof.

3. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1 or 2, that comprises a hinge region that comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, and SEQ ID NO: 104.

4. The isolated mammalian antibody or antigen binding fragment thereof of any one of the previous claims, wherein said antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 118 and a light chain comprising the amino acid sequence of SEQ ID NO: 120.

5. The isolated mammalian antibody or antigen binding fragment thereof of any one of the previous claims, wherein when bound to canine IL-4$R_α$ said antibody or antigen binding fragment thereof binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, or any combination thereof; wherein the antibody or antigen binding fragment thereof binds canine IL-4$R_α$ and blocks the binding of canine IL-4$R_α$ to canine interleukin-4.

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6. The isolated mammalian antibody or antigen binding fragment thereof of any one of the previous claims, wherein said antibody or antigen binding fragment thereof or said exhibits one, two, three, four, five, six, or all of the following properties:

i. binding to canine IL-4R$_\alpha$ with a dissociation constant (Kd) of 1 X 10^{-5} M to 1 X 10^{-12} M;
ii. binding to canine IL-4R$_\alpha$ with an on rate (k_{on}) of 1 X 10^{2} M^{-1} s^{-1} to 1 X 10^{7} M^{-1} s^{-1};
iii. binding to canine IL-4R$_\alpha$ with an off rate (k_{off}) of 1 X 10^{-3} s^{-1} to 1 X 10^{-8} s^{-1};
iv. blocking the binding of IL-4 to a type I IL-4 receptor;
v. blocking the binding of IL-13 to a type I IL-4 receptor;
vi. blocking the binding of IL-4 to a type II IL-4 receptor; and
vii. blocking the binding of IL-13 to a type II IL-4 receptor.

7. A mammalian monoclonal antibody or antigen binding fragment thereof that cross-competes for binding with canine IL-4Rα with the mammalian antibody or antigen binding fragment thereof of any one of the previous claims; wherein the caninized monoclonal antibody and antigen binding fragment thereof bind canine IL-4R$_\alpha$ and block the binding of canine IL-4R$_\alpha$ to canine IL-4.

8. A pair of isolated nucleic acids that encodes the light chain and heavy chain of the mammalian antibody or antigen binding fragment thereof of Claims 1 – 7.

9. The pair of isolated nucleic acids of Claim 8, that encodes one or more amino acid sequences selected from the group consisting of SEQ ID NOs: 118, 120, 129, 134, 137, 140, 146, 147 and 151.

10. An expression vector comprising the pair of isolated nucleic acid of Claims 8 or 9.

11. A host cell comprising the expression vector of Claim 10.

12. A pharmaceutical composition comprising the isolated mammalian antibody or antigen binding fragment thereof of any one of Claims 1 – 7, the pair of nucleic acids of Claim 8 or 9, the expression vectors of Claim 10, the host cell of claim 11or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

13. A pharmaceutical composition according to Claim 12 for use in the treatment of atopic dermatitis and/or the treatment of asthma, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition.

* * * *

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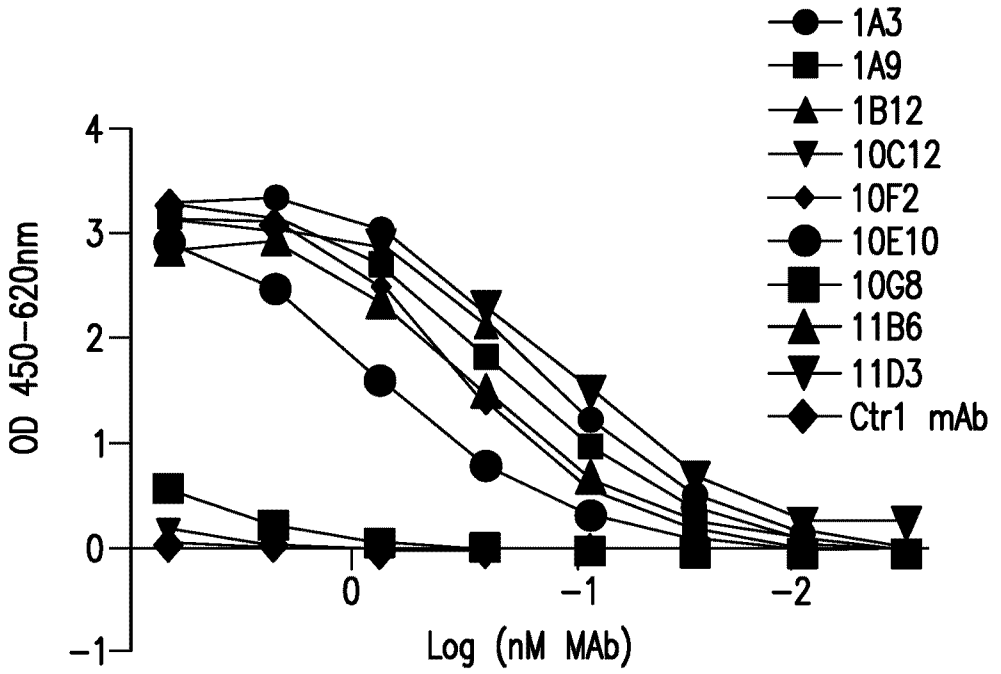

FIG.1

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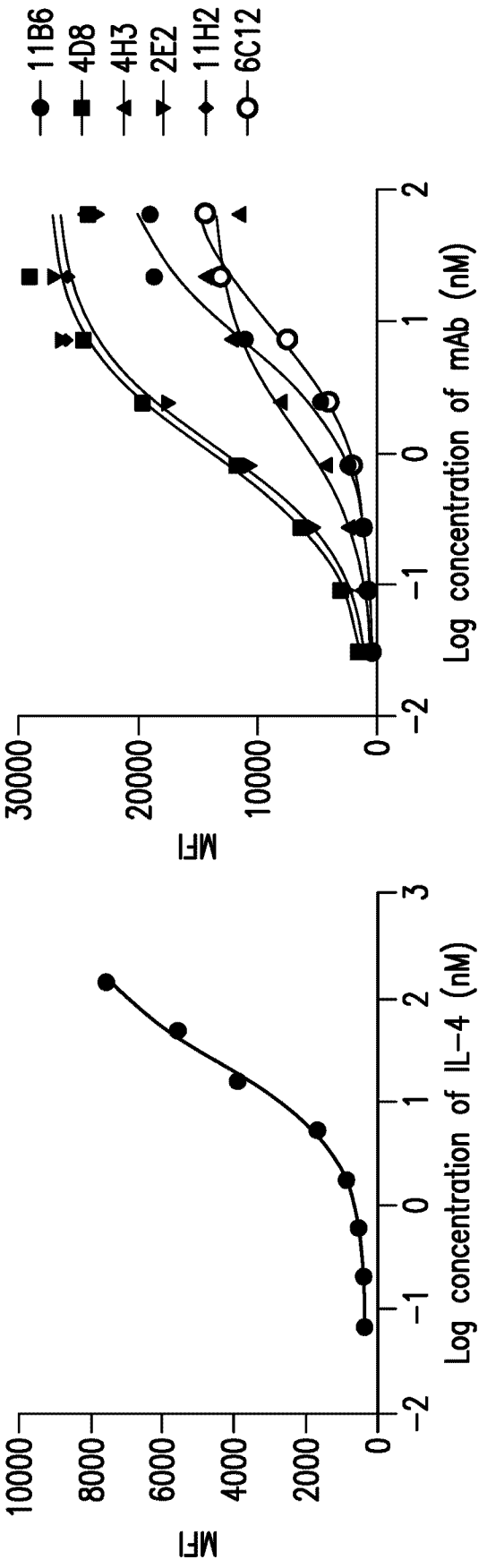
MFI
10000
8000
6000
4000
2000
0
−2
−1
0
1
2
3
Log concentration of IL−4 (nM)
MFI
30000
20000
10000
0
−2
−1
0
1
2
Log concentration of mAb (nM)
11B6
4D8
4H3
2E2
11H2
6C12

FIG.2A

FIG.2B

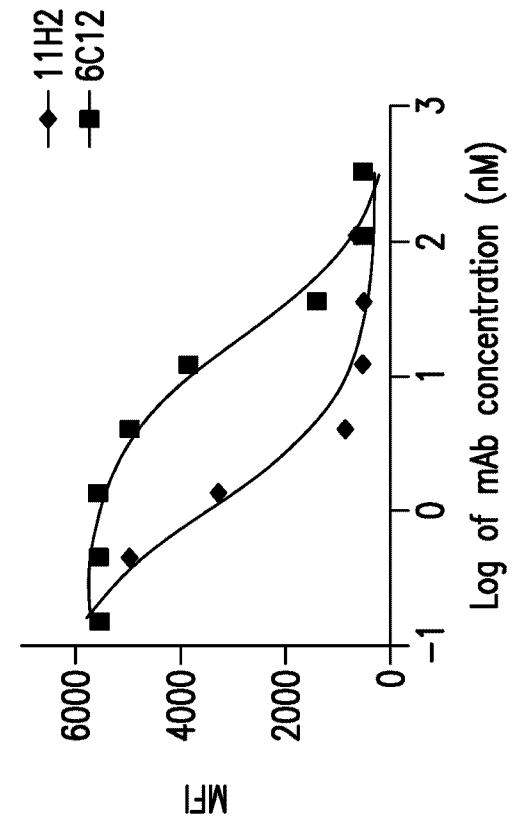
11H2
6C12
MFI
6000
4000
2000
0
−1
0
1
2
3
Log of mAb concentration (nM)

FIG.3B

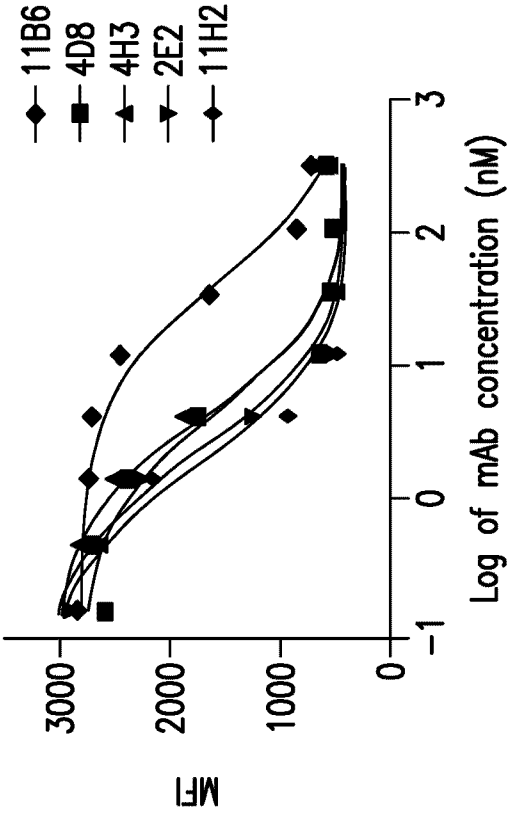
11B6
4D8
4H3
2E2
11H2
MFI
3000
2000
1000
0
−1
0
1
2
3
Log of mAb concentration (nM)

FIG.3A

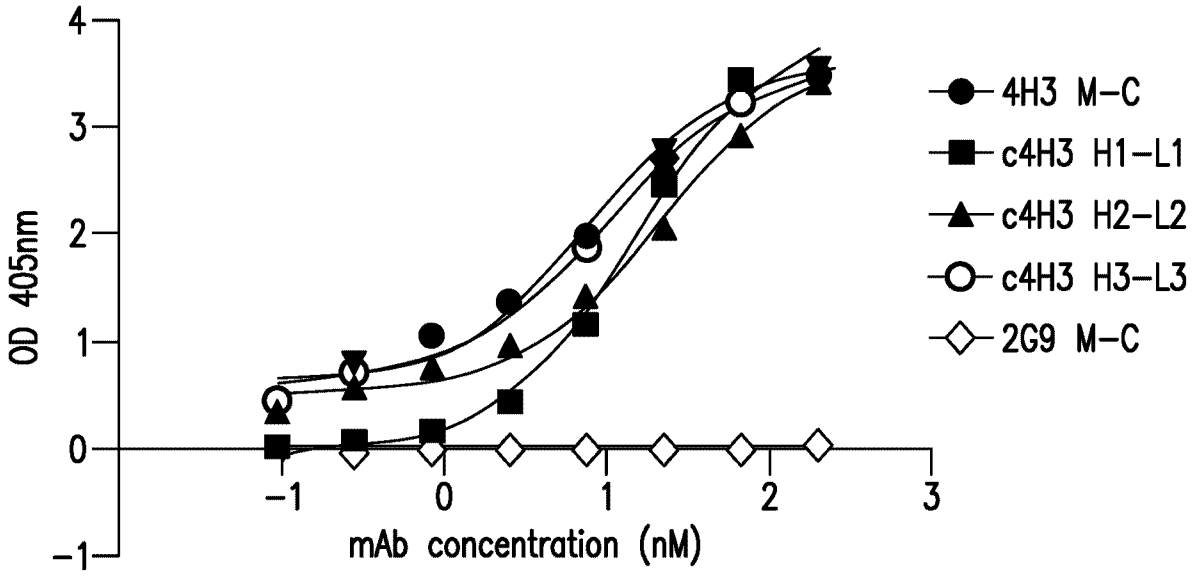

FIG.4

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