Therapeutic anti-IgE monoclonal antibody single chain variable fragment (scFv) safety and immunomodulatory effects after one time injection in four dogs

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Background – The therapeutic monoclonal antibody omalizumab that is specific for IgE has proven to be an effective addition to the treatment of allergic disease in humans.

Hypothesis/Objectives – The aims of this study were to demonstrate the safety and immunomodulating effects of a single injection of a monoclonal antibody single chain variable fragments (scFv) specific for canine IgE in normal dogs.

Animals – Three normal dogs were bled for EDTA whole blood samples for 112 days post-injection (dpi). A fourth dog was monitored for 28 days.

Methods – Anti-IgE scFv was pegylated to minimize scFv dimerization. Four normal dogs were injected once subcutaneously with anti-IgE scFv at 1 mg/kg. Flow cytometry was performed on whole blood. Plasma levels of IgE were measured by ELISA.

Results – None of the four dogs showed signs of anaphylaxis. All dogs demonstrated decreases in IgE(+) cells in lymphocyte-gated events by 14 dpi. Dogs C and D returned to pre-injection levels by 21 days, whereas dogs A and B remained below pre-injection levels until Day 112. Similar differences were seen in IgE-bearing granulocyte-gated cells. Free plasma IgE decreased below pre-injection levels by 47% in Dog A and by 52% in Dog B at 112 days. Dogs C and D did not change by more than 32% from preinjection levels.

Conclusion – A single injection of monomeric, pegylated scFv with high affinity for dog IgE was demonstrated to be safe. Marked reduction in IgE-bearing lymphocytes and granulocytes accompanied by reduced “free” plasma IgE level in two of four dogs is analogous to omalizumab in humans.

Introduction

Allergic disease pathogenesis is initiated primarily by allergen-specific immunoglobulin class IgE. IgE is strongly bound by the high affinity IgE epsilon receptor I (FcεRI) found on the surface of inflammatory cells such as mast cells and basophils, and on keratinocytes, monocytes and dendritic cells. When IgE that is bound to these cells is cross-linked by multivalent allergens, cytokines and inflammatory mediators are generated with results ranging from acute systemic anaphylaxis to dermal pruritic inflammation to immunomodulation.

The efficacy and safety of monoclonal antibodies with specificity for IgE epitopes located in the binding site that interacts with FcεRI have been the basis for the highly successful therapeutic humanized IgG monoclonal antibody omalizumab, marketed as Xolair. Because of this specificity, omalizumab does not cross-link IgE bound to cells bearing FcεRI and thus does not stimulate the release of inflammatory mediators such as histamine from mast cells or basophils.

Recent studies of the complex molecular conformational changes in IgE that are associated with the high affinity binding to FcεRI indicate that other sites outside the direct binding site contribute to IgE high affinity binding by FcεRI. Thus, potentially therapeutic antibody binding to IgE sites distant from the FcεRI binding site may diminish IgE affinity for mast cells and basophils. However, the consequences of bivalent antibody binding limit the use of intact immunoglobulin and require a monovalent form of the potentially therapeutic antibody.

The explanation of therapeutic efficacy by a simplistic mechanism of antibody binding to IgE at a site that blocks interaction with FcεRI is currently accepted for omalizumab. However, there are recent reports which suggest that binding of omalizumab to IgE expressed on the surface of B cells committed to production of IgE results in inhibition of the production of IgE in vitro. Production of IgE is regulated by cytokine-driven switching of B cells to IgE commitment and by IgE feedback through binding to low affinity receptor for IgE, CD23/...
The question of whether monovalent binding of IgE on B lymphocytes also reduces IgE production has not been addressed.

If it can be demonstrated that monovalent antibodies directed against epitopes on IgE other than those at the FcRRI binding site can be safely administered at therapeutic doses, then new antibodies with specificities for novel epitopes on IgE may be tested in vivo for empirical evidence for potential efficacy.

Single chain variable fragments (scFv) of monoclonal antibodies can be generated from the sequences of the heavy and light chain variable regions linked by a short bridge of four glycine and one serine sequence repeats. The scFv are monovalent but often show a low level of aggregation at high concentrations. Polyethylene glycol (PEG) has been used for many years to reduce the aggregation and antigenicity of therapeutic peptides.

The aim of this study was to determine if monoclonal forms of antibodies against IgE epitopes distant from epitopes that directly bind FcRRI could be developed to empirically test for immunomodulatory effects. In the approach used here, short chain PEG was covalently blocked by the FcRI alpha chain to measure “free” IgE (not complexed with omalizumab or FcRI alpha chain), and the latter being referred as “total” IgE based on scFv binding at a site not blocked by the FcRRI alpha chain. Absolute values of IgE µg/ml are reported based on standard curves using affinity purified monoclonal canine IgE; however, the use of low pH elution of the IgE standard from affinity matrix reduces the signal values from biotinylated FcRRI alpha chain detection, which precludes direct comparison of the plasma values generated by the two different protocols.

Materials and methods

Study dogs

The protocol of this study was approved by the North Carolina State University (NCSU), Institutional Animal Care and Use Committee. Mature, mixed breed dogs were randomly sourced by the Laboratory Animal Resources at North Carolina State University from animal shelters as healthy dogs and maintained with standard vaccination protocol of this study for the measurement of plasma IgE levels to minimize possible confounding effects of various plasma proteins that bind IgE, such as secreted FcRRI alpha chain, CD23 and autologous autoimmune IgG anti-IgE. Thus, biotinylated human recombinant FcRRI alpha chain (R&D Systems; Minneapolis, MN, USA) and biotinylated scFv were used to detect plasma IgE, with the former being conventionally referred to in human studies with omalizumab as detecting “free” IgE (not complexed with omalizumab or FcRRI alpha chain), and the latter being referred to as “total” IgE based on scFv binding at a site not blocked by the FcRRI alpha chain. Absolute values of IgE µg/ml are reported based on standard curves using affinity purified monoclonal canine IgE; however, the use of low pH elution of the IgE standard from affinity matrix reduces the signal values from biotinylated FcRRI alpha chain detection, which precludes direct comparison of the plasma values generated by the two different protocols.

Generation of scFv anti-IgE

The heavy and light chain variable regions of a mouse monoclonal antibody (mAb 5.91) with high affinity binding to an epitope in the C2 domain of the epsilon chain of canine IgE were sequenced (Creative Biolabs; Shirley, NY, USA) and a scFv DNA sequence created that contained a linkage between the carboxy terminal of the heavy chain and the amino terminal of the light chain using three repeats of glycine 4 serine 1 (GenScript; Piscataway, NJ, USA). This sequence was incorporated into the vector pcDNA3.4TOPO (Thermo Fisher Scientific; Rockford, IL, USA) for transfection of Expi293F cells by the ExpiFectamine 293 transfection kit (Thermo Fisher Scientific). The scFv secreted by Expi293F cells in culture for 7 days was purified by affinity chromatography with HiTrap protein L agarose beads (GE Healthcare; Pittsburgh, PA, USA). Affinity pure scFv was pegylated with SAT(PEG)4 (Thermo Fisher Scientific) at lysine primary amine moieties, and concentrated in phosphate buffered saline, pH 8.0 (PBS) to 2 mg/ml using 9K molecular weight cut-off filtration (Thermo Fisher Scientific). All procedures were conducted with endotoxin-free water and buffers with the resulting concentrated pegylated scFv containing less than 0.25 EU/ml of endotoxin.

scFv binding to IgE in vitro

ELISA was used to compare scFv with the original mAb 5.91 binding to IgE. Microtitre plates (Thermo Fisher Scientific) were coated with a canine monoclonal IgE, generated from mouse x dog heterohybridoma cell line 2.39, overnight at pH 9.0 in 0.05M sodium carbonate buffer, washed in PBS with 0.05% tween 20 (PBST) and blocked with 1% bovine serum albumin (BSA). After 2 h, plates were washed and dilutions of biotinylated scFv or mAb 5.91 were added and incubated for a further 2 h. Streptavidin-HRP was added after washing followed by a final wash and addition of ABTS. Absorbance at 450 nm was read after 1 h.

Measurement of plasma IgE

ELISA was used to measure IgE in plasma by two different methods to determine total and “free” IgE levels. Both measurements used the same coating of microtitre plates with 10 µg/ml of rabbit IgG anti-IgE that was affinity-purified by canine IgE linked to agarose beads. Blocking with 4% heat inactivated fetal bovine serum (FBS), and washing was as described above. Detection of IgE after incubation with appropriate dilutions of plasma on coated plates was with biotinylated human recombinant FcRRI alpha chain to measure “free” canine IgE, and with biotinylated scFv to measure total IgE. Standard curves for concentration were generated for each plate by using serial dilutions of canine monoclonal IgE in place of dog plasma samples.

SPOTS ELISA

The CDNA-derived amino acid sequence for canine IgE heavy chain constant region, or epsilon chain, with accession number AAB72882 was used to produce a matrix sequence of 13 amino acid long peptides offset by three amino acids representing the entire epsilon chain as 139 spots on a cellulose membrane (LPT Peptide Technologie GmbH; Berlin, Germany). Biotinylated mAb 5.91 and scFv were tested for binding to the membrane spots as described by the manufacturer.

Flow cytometry

Five millilitres of whole blood from each dog was collected into EDTA and centrifuged at 400 g for 20 min. Plasma was harvested for analysis of total and free IgE. The packed cells were washed with HBSS-0.5 mM EDTA and cells were suspended back to the original volume. One hundred microlitres of washed cells were added to each polystyrene tube for flow cytometry analysis and incubated for 3 min with 3 ml of 4.1 mM lactic acid, pH 3.9 for IgE stripping or with HBSS-0.5 mM EDTA, respectively. Cells were centrifuged and washed once with FBS staining buffer containing 0.1% NaN3 and suspended in 100 µl of staining buffer for labelling with

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allophycocyanin (APC) conjugated anti-canine IgE antibodies (scFv, mAb 5.91) and anti-CD21 (AbD Serotec, Raleigh, NC, USA) to detect bound and expressed IgE, and anti-PEG (GenScript; Piscataway, NJ, USA) to detect pegylated scFv. Cells were incubated with labeled antibodies for 1 h at 4°C with gentle shaking. After incubation, red blood cells were lysed using 1-Step Fix/Lyse Solution (eBioscience, San Diego, CA, USA). Samples were then analysed on a Becton Dickinson LSRII system using FCS Express 4 Flow (Denovo Analysis software; Glendale, CA, USA). Cell populations (granulocytes, monocytes and lymphocytes) were identified by gating on forward (FSC-A) and side angle (SSC-A) light scatter. The total numbers of labelled granulocytes, monocytes and lymphocytes in specific gated regions were recorded and those labelled with anti-IgE were expressed as a percentage of the total gated population.

Results

scFv characterization

scFv isolated from Expi293F cell culture supernatants after 7 days by protein L affinity chromatography and concentrated to 2 mg/ml showed slight opacity that clarified in buffers above pH 9.0. On non-denaturing, native PAGE of scFv, a strong band was visible at the expected molecular weight of the monomer, 27 kDa, as well as a weak band at 54 kDa (Figure 1). In order to eliminate aggregation and minimize dimerization affinity, purified scFv was pegylated at multiple primary amine groups with SAT (PEG)4 (Thermo Fisher Scientific) creating scFv-PEG4-S-acetyl. The pegylated scFv showed minimal dimeric form on native PAGE (Figure 1).

Binding of scFv to canine IgE was compared to the intact IgG2b mAb 5.91 from which it was derived. The end-point molar concentration for signal on ELISA plates coated with 10 µg/ml of IgE was 2.0 × 10^{-12} M for biotinylated mAb 5.91 and 2.6 × 10^{-9} M for biotinylated scFv.

Biotinylated mAb 5.91 and scFv bound the same IgE epsilon 13mer peptide sequences as demonstrated on SPOTS ELISA (data not shown). Two adjacent spots were strongly positive, representing a shared amino acid sequence of QKATNIFPYTAPG which is located near the amino terminus of the C2 domain of the IgE epsilon chain (Figure 2).

Clinical response to scFv injection

Subcutaneous injection of pegylated scFv at 2 mg/ml in volumes required to deliver doses of 1 mg/kg to dogs weighing 10–12 kg showed no change in behaviour or vital signs in any of the four dogs as continuous observation for 1 h, 60 min intervals for 8 h and at 24 h after injection. Observation measurements included respiratory rate, heart rate, mucous membrane reperfusion and dermal hyperaemia. No defaecation was observed within 1 h of injection, nor was vomiting observed during the 24 h after injection. No reaction was observed at the injection site of any of the dogs.

Plasma IgE levels

Figure 3 shows a sustained, long-term reduction in plasma IgE over 112 days after a single injection of pegylated scFv in three (dogs A–C) of the four dogs. The most notable differences in IgE values seen in comparing the two different detection protocols for each dog were during the first 28 days, after which the sustained reduction pattern for each dog was similar for both protocols. Dog D showed no reduction during the 28 days post-injection (dpi) period it was available for sampling.

Whole blood leucocyte surface IgE

Whole blood leucocytes were gated into granulocyte, monocyte and lymphocyte populations based on FSC-A and SSC-A scatter, as shown in Figure 4. The numbers of cells in these populations were within normal values for all dogs and the populations in each dog fluctuated very little over the course of the study. Responses to injection of pegylated scFv did not include changes in gated population numbers. This allowed comparison of percentages of APC-scFv staining cells within gated populations to be reported.

Detection of IgE on blood cells by flow cytometry was carried out with APC-labelled scFv instead of mAb.
5.91, because it was shown that APC-scFv positive staining cell populations were more distinctly separated from negative populations (Figure 5) for each dog. No pegylated scFv could be detected with anti-PEG antibodies by flow cytometry of blood cells 24 h after injection of pegylated scFv (results not shown) which eliminated the possibility of pegylated scFv blocking APC-scFv binding to cell surface IgE in flow cytometry measurements.

Because APC-scFv binds IgE that is bound by cell surface FccRI, as would be expected for monocytes and leucocytes, as well as IgE expressed by B lymphocytes committed to IgE production, lactic acid treatment of blood samples, as previously reported, was attempted to distinguish bound and expressed IgE. Comparison of nontreated and lactic acid treated samples showed no consistent difference in APC-scFv positive cell numbers in any of the gated populations (Figures 6, 8 and 9), nor any loss of total cell numbers.

The most consistent changes in IgE(+) cell numbers were in the lymphocyte gate where all dogs showed reductions by 14 dpi (Figure 6). Notably, dogs A and B maintained low numbers of IgE(+) lymphocytes, whereas dogs C and D IgE(+) lymphocytes returned quickly to pre-injection levels. This decrease in IgE(+) lymphocytes was not associated with any decrease in CD21(+) cells. Indeed, there appeared to be an increase in CD21(+)...
lymphocytes following injection of scFv in all four dogs (Figure 7).

The more dramatic responses of dogs A and B in reduction of IgE(+) lymphocytes compared to dogs C and D was also reflected in changes in IgE(+) granulocytes. Dogs A and B showed rapid and sustained loss of APC-scFv staining of cells in the granulocyte gate; however, dog C showed varying changes with both increased and decreased IgE(+) cell numbers, and dog D showed a consistently higher level of IgE(+) granulocytes (Figure 8). APC-scFv staining of cells in the monocyte gate did not show any consistent change following scFv injection (Figure 9).

**Discussion**

This preliminary report of safety and immune modulation in four dogs injected with potentially therapeutic levels of a scFv specific for IgE indicates that the development of anti-IgE therapies need not be constrained to targeting IgE epitopes directly interacting with FcεRI. The scFv generated from the sequence of mAb 5.91 retained the original mAb specificity for an epitope in the C2 domain of IgE that was accessible whether or not IgE was bound to FcεRI.

The major barrier to targeting epitopes on IgE that are not in the FcεRI binding site is the risk of anaphylactic response resulting from crosslinking of IgE bound to basophils and mast cells. Testing mAb 5.91 by whole blood in vitro release of histamine and by intradermal...
injection showed no detectable histamine release; however, a single subcutaneous injection trial at a potentially therapeutic level (1 mg/kg) showed clinical signs of anaphylaxis which were reversible with diphenhydramine (unpublished observation: Bruce Hammerberg BH). This barrier was circumvented by creating the monovalent

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scFv of mAb 5.91 and pegylating scFv to minimize the dimerization that is often reported for scFv. The scFv reported here retains the murine framework sequences for heavy and light chain variable regions. The SAT(PEG)₄ reagent contains an N-hydroxysuccinimide ester that reacts with primary amine groups on lysine. There are 14

Figure 8. Changes in IgE(+) granulocytes following injection of pegylated single chain variable fragments (scFv). The percentage of granulocyte gated cells staining with APC (allophycocyanin)-scFv demonstrate highly variable responses between dogs with dogs A and B showing rapid and marked declines in APC-scFv staining.

Figure 9. Changes in IgE(+) monocytes following injection of pegylated single chain variable fragments (scFv). The percentage of monocyte gated cells staining with APC (allophycocyanin)-scFv demonstrate no consistent pattern of response to pegylated scFv.
lysine amino acids in the scFv sequence, all but three of which are within the framework regions, and this may contribute to reduced antigenicity for scFv, which will be evaluated in future studies of repeated injections. It may be necessary for safety in prolonged repeated therapeutic injections to prevent anti-mouse IgG antibody development in treated subjects by caninizing the framework regions of the scFv.

Measurement of circulating IgE has been used as a marker for anti-IgE therapy, other than improvement in clinical signs, for the development and therapeutic use ofomalizumab in humans. A mechanism for the rapid reduction in circulating IgE seen in humans injected withomalizumab is proposed to be complex formation by the humanized complement-fixing IgG1 anti-IgE with IgE and its subsequent clearance by phagocytic cells. This could not be the mechanism for the more gradual reduction in circulating IgE shown after pegylated scFv injection in dogs due to the lack of crosslinking or complement-fixing ability by pegylated scFv. A more likely mechanism may be associated with the decrease in IgE expressing circulating lymphocytes following the pegylated scFv injection shown in this report. It has been reported recently thatomalizumab reduces IgE production by human tonsil-derived B lymphocytes in vitro. The precise mechanism whereby monovalent or bivalent antibodies against IgE expressed on B lymphocytes cause a reduction in IgE production remains to be discovered. A limited number of cells in the granulocyte gate, likely to be eosinophils and basophils, showed APC-scFv staining; however, only dogs A and B demonstrated a consistent, long-term reduction in IgE(+) cells following pegylated scFv injection. Eosinophil counts were not done on the serial samples.

The attempt to differentiate detection of IgE expressed on cells from receptor bound IgE – the former expected to be in granulocyte and monocyte gates, the latter expected to be in the lymphocyte gate – was not successful using lactic acid stripping. The failure of lactic acid stripping to remove IgE from granulocytes and monocytes suggests that conformational changes in IgE and/or FcεRI induced by low pH have less effect on their binding in the dog than in humans or mice.

The evidence presented here for the safety of monovalent anti-IgE forms specific for IgE epitope sites distant from the FcεRI binding site, that are modified to assure the maintenance of monovalent form at concentrations used for therapeutic doses, should encourage future studies with these types of antibodies to verify safety and to test for immunomodulatory efficacy of antibodies specific for novel IgE epitopes. Because of the complexity of possible mechanisms for immunomodulation that is clinically relevant, empirical studies testing for clinical efficacy in allergic dogs may be more effective in identifying therapeutic antibodies than pre-screening candidate antibodies based solely upon reduction of circulating IgE levels.

References
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**Sujetos** – Tres chisnes normales ont été prélevés en sang total sur EDTA pendant 112 jours post-injection (dpi). Un quatrième chien a été suivi pendant 28 jours.

**Métodos** – Les scFv anti-IgE ont été pégylés afin de minimiser la dimérisation. Quatre chiens sains ont reçu une injection sous-cutanée avec scFv anti-IgE à 1 mg/kg. Une cytométrie de flux a été réalisée sur sans total. Les taux de plasma d’IgE ont été mesurés par ELISA.

**Résultats** – Aucun des quatre chiens n’a montré de signes d’anaphylaxie. Tous les chiens ont montré une diminution des cellules IgE (+) à 14 dpi. Les chiens C et D sont restés sous les niveaux de pré-injection à jour 21 tandis que les chiens A et B sont restés sous les niveaux de pré-injection jusqu’au jour 112. Des différences identiques étaient observées dans les cellules granulocytes portant des IgE. Les IgE plasmiques libres diminuaient sous les niveaux de pré-injection de 47% chez le chien A et de 52% chez le chien B à jour 112. Les chiens C et D n’évoluaient pas de plus de 32% des niveaux de pré-injection.

**Conclusion** – Une seule injection de scFv pégylés, monomérique à forte affinité pour les IgE de chien s’est montré sûre. Une diminution marquée de lymphocytes portant les IgE et les granulocytes était accompagnée par une diminution des niveaux d’IgE plasmiques libres pour deux des quatre chiens de façon analogue à l’omalizumab chez l’homme.

**Resumen**

**Introducción** – El anticuerpo monoclonal terapéutico omalizumab específico para IgE ha demostrado ser una adición eficaz para el tratamiento de las enfermedades alérgicas en los seres humanos.

**Hipothesis/Objetivos** – Los objetivos de este estudio fueron demostrar la seguridad y los efectos inmunomoduladores de una única inyección única de un anticuerpo monoclonal de cadena sencilla de fragmentos variables (scFv) específicos para la IgE canina en perros normales.

**Animales** – A tres perros normales se les extrajo sangre en EDTA durante 112 días después de la inyección (dpi). Un cuarto perro se controló durante 28 días.

**Métodos** – El anti-IgE scFv fue pegilado para minimizar la dimerización de scFv. Cuatro perros normales se inyectaron una vez por vía subcutánea con anti-IgE scFv a 1 mg/kg. Se usó citometría de flujo en sangre entera. Los niveles plasmáticos de IgE se midieron por ELISA.

**Resultados** – Ninguno de los cuatro perros mostraron signos de anafilaxia. Todos los perros demostraron disminuciones de células IgE (+) en el área de linfocitos para el día 14 dpi. Los perros C y D volvieron a los niveles pre-inyección para el día 21, mientras que los perros A y B permanecieron por debajo de los niveles previos a la inyección hasta el día 112. Se observaron diferencias similares en las células con IgE de la zona de granulocitos. La IgE libre en plasma disminuyó por debajo de los niveles previos a la inyección en un 47% en el perro A y un 52% en el perro B a los 112 días. Los perros C y D cambiaron en un 32% respecto a los niveles preinyección.

**Conclusion** – Una sola inyección de monómero, scFv pegilado con una alta afinidad para la IgE canina demostró ser segura. Hubo una marcada reducción en los linfocitos y granulocitos con IgE acompañado de reducción del nivel IgE libre plasmática en dos de los cuatro perros, análoga al efecto de omalizumab en seres humanos.

**Zusammenfassung**

**Hintergrund** – Der therapeutisch eingesezte monoklonale Antikörper Omalizumab, der speziell auf IgE ausgerichtet ist, hat sich als effektive Zusatztherapie bei allergischen Krankheiten des Menschen erwiesen.

**Hypothesen/Ziele** – Die Ziele dieser Studie waren es, die Sicherheit und die immunmodulierende Wirkung einer einzigen Injektion eines monoklonalen Antikörpers [Einzelkette variabler Fragment (scFv)] spezifisch für canines IgE bei normalen Hunden zu erfassen.

**Tiere** – Drei normalen Hunden wurde EDTA Blut bis zu 112 Tage nach der Injektion (dpi) entnommen. Ein viertter Hund wurde 28 Tage lang überwacht.

**Methode** – Anti-IgE scFv wurde pegyliert, um die scFv Dimerisierung zu minimieren. Vier normalen Hunden wurde einmal eine subkutane Injektion mit anti-IgE scFv von 1 mg/kg verabreicht. Flowzytometrie wurde an Ganzblut durchgeführt. Die Plasmaelevations von IgE wurden mittels ELISA bestimmt.


要約
背景 - IgEに特異的で、治療用モノクローナル抗体であるオマリズマブはヒトにおけるアレルギー性疾患の治療として効果があると証明された。
仮説/目的 - この研究の目的は、正常犬においてヒスイIgE特異的単筋(scFv)モノクローナル抗体単回注射の安全性および免疫調整効果を立証することである。
供与動物 - 注射後112日間(dpi)にわたるEDTA全血サンプルを得るため、3頭の正常犬を採血した。4頭目のイスを28日間観察した。
方法 - 抗IgE scFvはscFv二量化を最小限にするためにペプチド化した。4頭の正常犬に抗IgE scFvを1mg/kgの量で単回皮下投与し、フローサイトメトリーを全血で実施した。IgEの血清レベルをELISAで測定した。
結果 - 1日目に1頭の反応を示す約60%の減少を示した。根拠は14dpiまでにリンパ球を介した事象におけるIgE-陽性細胞の減少を示し、すべてのイスで14dpiまでにIgE水平は正常レベルにまで戻った。IgE産生細胞間性細胞においても同様の差が認められた。過敏反応
IgEは112日目にIgEの37%、IgEの52%の減少を示した。イスおよびDでは注射前の値の32%以上に大きな変化は生じなかった。
結論 - ヒスイIgE高親和性単量体・ペプチドscFvの単回注射は安全であることが示された。IgE産生性リンパ球および顆粒球の明らかな減少は1頭中2頭における“過敏”血清IgE値の減少と関係しており、ヒトにおけるオマリズマブと類似していた。

Resumo
Contexto - O anticorpo monoclonal terapeutico omalizumab, que é específico para IgE, tem se provado como um acrématico eficaz no tratamento de doenças alérgicas em humanos.
Hipóteses/Objetivos - Este estudo teve como objetivo demonstrar a segurança e os efeitos imunomodulatórios de aplicação, em dose única, de um anticorpo monoclonal em cadênia única com fragmentos variados(scFv), específicos para IgE canina, em cachorros híbridos.
Animais - Três cães saudáveis foram submetidos à coletas de sangue total durante 112 dias após injeção (dpi). Um quarto cão foi monitorado por 28 dias.
Métodos - Anti-IgE scFv foi pegulado para minimizar a dimerização de scFv. Injeções subcutâneas de anti-IgE scFv em uma dose de 1mg/kg, foram aplicadas em quatro cães normais. Citometria de fluxo foi realizado na sangue total. Os níveis plasmáticos de IgE foram mensurados por ELISA.
Resultados - Nenhum dos cães demonstrou sinais de anafilaxia. Todos demonstraram redução dos níveis de células IgE - positivas (+) nos eventos de gate linfocitário, por volta de 14 dpi. Os cães C e D retornaram ao nível pré-injeção com 21 dias, enquanto que os cães A e B continuaram com os níveis abaixo da pré-injeção até o dia 112. Diferenças semelhantes foram vistas em gales de granulócitos ligados a IgE. O nível de IgE livre no plasma sofreu redução em 47% no căo A e 52% no căo B, ficando abaixo dos níveis pré-injeção. Os cães C e D não apresentaram alteração de mais de 32% em relação aos valores pré-injeção.
Conclusão - Uma aplicação única de scFv monomérico, pegulado, com alta afinidade para IgE canina demonstrou-se segura. Redução acentuada de linfócitos e granulócitos carreadores de IgE, acompanhada de redução no IgE plasmático livre em dois dos quatro cães é análogo ao omalizumab em humanos.