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Towards a Pan-Anti-Allergy Vaccine

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ABSTRACT

Allergic manifestations affect 20% - 30% of the population in industrialized countries. The global market for asthma and allergy medications has been estimated to exceed USD 6 billion, since 40% of the human population has some form of IgE sensitization to diverse proteins. Most therapeutic intervention strategies cope with the symptoms of allergy without eliminating the underlying cause and many are associated with undesirable and often long-term debilitating side effects. We designed a peptide immunogen encompassing sequences of the human Cε2-3 linker region to prime rat (Rattus norvegicus) immune systems, we then designed a chimeric human-dog-human IgE antibody and used it to boost the immune system and produce high-affinity antibodies that targets native IgE. The investigation showed that this peptide immunogen elicit the formation of antibodies recognizing the native IgE of human, canine and equine origin. The current investigation describes novel approaches aimed at the development of safe anti-allergy vaccines based on active immunization with IgE-derived peptides that are involved in the complementary interaction with the high affinity receptor. The immunization strategy was successful but did not fully work as predicted, thus we propose that peptides described in the current study may lead to the development of a pan-anti-allergy vaccine with applications for the treatment of all IgE-mediated allergic response independent of the nature of the offending allergen.

Keywords: Anti-Allergy Vaccine; Hypersensitivity Responses; IgE; Immunotherapy; Original Antigenic Sin

1. Introduction

IgE antibodies are best known for their role as mediators of the allergic response, which in its most serious manifestations causes asthma and anaphylactic shock, reviewed in [1]. The current consensus is that allergic diseases become manifest as a result of an imbalance between Th_1 and Th_2 responses to environmental antigens/pollutants capable of creating a cytokine environment favoring Th_2 immune response. IgE mediates allergic responses by sensitizing cells expressing high- and low-affinity receptors for allergen-induced release of pharmacologically potent chemicals causing the symptoms associated with the diverse manifestations of the disease. Severity of symptoms ranges from mild to high and can be life threatening.

The high socio-economic cost of management of allergic disorders initiated the quest for effective therapeutic intervention strategies which started with the demonstration that a proteolytic fragment derived from human IgE inhibited the sensitization of mast cells with allergen-specific IgE [2], reviewed in [3]. Subsequently, progressively smaller IgE, and receptor (FcεRIα), derived peptides that can competitively inhibit ligand/receptor interaction, were developed [4], but the low affinity of these peptidomimetics proved a major drawback underlying this strategy. Furthermore, peptides based on receptor-derived sequences carry a potential danger of stimulating the synthesis of antibodies that might cross link the receptor and induce an anaphylactic response [4]. Also, most anti-IgE antibodies are also anaphylactogenic and can cross link receptor bound IgE leading to the onset of allergic responses. On the other hand, the observation by [5], who described an anti-human-IgE monoclonal antibody (mAb) that did not induce histamine release, indicated the presence of IgE epitopes which are obscured while engaged to the receptor and thus constitute

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valid anti-IgE targets only when free in solution. Several non-anaphylactogenic mAb have since been described that block the binding of IgE to its receptor [6,7].

Passive immunotherapy with non-anaphylactic antibodies has demonstrated that it is possible to treat type I hypersensitivity responses with mouse mAbs of which Omalizumab [6] is the best characterized. The humanized antibody has been approved by the Food and Drug Administration (FDA) and its efficacy has been demonstrated in numerous clinical trials since 2000. The high-affinity anti-IgE mAb, mAb12, can dissociate IgE from its receptor by competition for binding sites [7,8]. Passive immunotherapy with administered anti-IgE antibodies has, however, shown poor effectiveness in obese patients and in patients with IgE levels above 700 IU ml^{-1}. Furthermore, treatment only reduces symptoms temporarily, ~14 days, with 7% of individuals undertaking Omalizumab therapy reporting adverse reactions. In addition, logistics and annual cost exceeds USD 50,000 per patient.

The demonstration that some IgE-antibodies do not evoke anaphylactic responses suggested that it might be possible to devise a vaccine based on sequences in IgE that elicit the synthesis of non-anaphylactic antibodies. Such peptide immunogens can be expected to provide long term protection against all IgE mediated allergies, irrespective of the nature of the allergen evoking the IgE response [4]. The determination of the co-crystal structure of the human IgE/FcεRIα complex [9], which identified epitopes in IgE-Fc region that become masked following receptor engagement, identified a region that could be exploited to develop IgE-derived peptide vaccines that inhibit receptor sensitization and affect the dissociation of receptor bound IgE [10-17].

Humans (hu), dogs (d) and horses (ho) are known to suffer the clinical symptoms of IgE-mediated type I hypersensitivity responses. But no effective therapeutic intervention strategies are currently available. Based on an *in vivo* canine model system, several publications [13,16,18] described the generation of an apparently effective therapeutic anti-IgE antibody response based on the immunization with a chimeric IgE construct where the canine Cε3 domain is flanked by sequences of Cε2 and Cε4 from opossum (Opossum-Dog-Opossum = ODO Protein fragment). Following vaccination with this construct, the authors report a reduction in blood IgE levels of about 65%. But no information is available on the binding affinity of the resulting anti-sera for canine IgE. Furthermore, no assessment was made of the overall immune response to canine Cε3 determinants, several of which are potentially available after receptor docking and could give rise to anaphylactogenic antibodies with potentially lethal consequences.

Our current study assessed the immune response against IgE-derived peptide epitope based on sequences involved in the complementary interaction between human IgE and human FcεRIα and we obtained monoclonal and polyclonal antibodies recognizing the inter Cε2-3 linker region, the AB helix and the FG loop. We subsequently focused on immune responses to peptides encompassing the inter Cε2-3 linker region since this epitope is highly conserved between primates, horses and dogs and may therefore have potential applications as a universal vaccine to combat allergic responses in all these species. Since anti-peptide antibody responses commonly give rise to antibodies of low affinity, we decided to test the potential applicability of the "Original Antigenic Sin" [19,20] hypothesis to enhance the secondary immune response. Rats were primed with the disulphide linked Cε2-3 linker region sequence ($Fc\varepsilon_{2\text{-}3}$ dimer, **Figure 1**) and subsequently challenged with the same peptide or a human Cε2-dog Cε3-human Cε4 chimeric IgE (HDH) antibody construct (**Figure 2**).

To underpin these investigations we also developed cellular assay systems to assess and test the safety of anti-IgE immune responses directed against human, canine and equine IgE. Rat Basophil Leukemia (RBL-2H3.1) cells which were transfected with the gene encoding human [21], canine [22] or equine [23,24] FcεRIα and expressed the functional receptor complex on their surface, could be sensitized with species specific IgE for the assessment of both antigen and antibody induced *β*-hexosaminidase release.

2. Materials and Methods

2.1. Design of the Disulphide Linked $Fc\varepsilon_{2\text{-}3}$ Dimer and the FG Loop Dimer

Peptides corresponding to the Cε2-Cε3 linker region, and FG loop of the heavy chain of human and canine IgE were designed and employed as immunogens, aiming to provide the targets against which a specific anti-IgE antibody response would be raised. The amino acid sequence of these mentioned peptides are shown on **Table 1**.

As the peptide comprising the FG loop had to simulate the native form as faithfully as possible, cysteine residues were introduced at both ends in order to establish a

Table 1. Peptide immunogens. End cysteine residues were introduced in the FG loop peptide and were connected by a disulphide bridge, resulting in formation of loop peptide structure. Conserved residues are shown in bold.

IgE Cε2-Cε3 Linker Region Peptide	Human	MW = 1005	DSTKKCADSNPRGVS
	Canine	MW = 1050	DEARKCSESDPRGVT
IgE FG Loop Peptide	Human	MW = 1134	CTHPHLPRAC
	Canine	MW = 1150	CTHPHLPKDC

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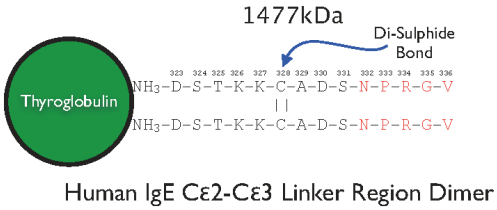

Figure 1. Structure of the Fcε$_{2\text{-}3}$ dimer showing the sequence residue 323 - 336 of the human IgE molecule (which includes the Cε2-3 323 - 326 linker region and the 332 - 336 PRGV sequence found in human, canine and equine IgEs highlighted in red) with a disulphide bridge at the cys 328 position, the dimer was then linked to the adjuvant thyroglobulin.

disulphide bridge constricting the peptides' shape into a loop form. Furthermore, a sample of each peptide was covalently linked to KLH (Synthesis and Sequencing Facility, the University of Sheffield), while another sample was mixed with MPL®/Tyrosine adjuvant (Allergy Therapeutics Ltd) before they were used in the respective mouse immunization protocols.

2.2. Generation of a Disulphide Linked Fcε$_{2\text{-}3}$ Dimer

The Cε2-3 linker region peptide (Fcε$_{2\text{-}3}$ dimer) encompassing residue 323 - 326 of the human IgE molecule (**Table 1**) was chemically synthesized prior to dimerization on a Millipore 9050 Peptide Synthesizer using Fmoc chemistry. The linear peptide was purified by High-Performance Liquid Chromatography (HPLC) and subjected to oxidation in 200 mM NH_4HCO_3 at 4˚C for 72 hours at a concentration of 5 mg·ml^{-1}. The NH_4HCO_3 was removed by freeze-drying and remaining traces of the bicarbonate were removed by freeze drying from water. Correct assembly and oxidation (at cys 328 residue) of the peptide was established by mass spectrometry. The oxidized peptide was coupled to either keyhole Limpet Haemocyanin or thyroglobulin via NH_3 groups using glutaraldehyde and mixed with MPL®/Tyrosine adjuvant (Allergy Therapeutics Ltd) to give the final Fcε$_{2\text{-}3}$ dimer (**Figure 1**).

2.3. Generation of the HDH Anti NIP-HSA Chimeric IgE Antibody Gene

The primers in (**Figure 2**) were used to amplify the respective canine and human IgE Fc heavy chain domains from canine IgE Fc heavy chain genomic DNA cloned into the pCRII-TOPO plasmid [22] and human IgE Fc heavy chain genomic DNA cloned into the pSV-V$_{NP}$ plasmid by [25]. The required domains (the human Cε1, Cε2 and Cε4 and the canine Cε3) were amplified by PCR, and sub cloned into the pUC18 plasmid for sequence verification by DNA sequencing. The domains were constructed to make the final HDH Fc heavy chain IgE gene, which was then sub cloned into the pSV-V$_{NP}$ plasmid, to make pSV-V$_{NP}$HDH, where the HDH IgE Fc heavy chain gene was inserted downstream of a mouse λ chain with NIP specificity (**Figure 2**).

2.4. Generating the HDH Anti NIP-HSA Chimeric IgE

The pSV-V$_{NP}$HDH plasmid was transfected into J558L cells (mouse B myeloma cells derived from BALB/c strain [26]) by electroporation at 250v 960 μF (Bio-Rad) and cultured using established procedures [27]. The cloned cells were selected by Surface Plasmon Resonance (SPR) and expanded for IgE expression.

2.5. Generation of Mouse Monoclonal Antibodies

Immunizations were performed under animal license PPL 50/01317. Balb/c mice were immunized on days 1, 14, and 35 with 50 μg peptide in the presence of adjuvant. Complete Freud's adjuvant was used for the primary injection, which was administered subcutaneously, followed by intraperitoneal boost with incomplete Freud's adjuvant. Mice showing optimal titers were boosted with 100 μg of peptide in the absence of adjuvant. Spleens were removed three days later to harvest spleen B cells for the generation of hybridomas using established procedures [28].

2.6. Production of Anti-IgE Peptide Antibodies in Rabbits

Rabbit polyclonal anti-IgE antibodies were produced by GeneScript Corporation (New Jersey, USA) employing a polyclonal express protocol and the proprietary T-Max adjuvant. Prior to vaccination, blood samples were removed from each rabbit as negative reference control. Subsequently, three rounds of conjugated peptide vaccinations were made via intradermal and subcutaneous routes. Serum was harvested from the rabbits displaying the highest titers by exsanguination and the Ig fraction was purified by Protein A column chromatography.

2.7. Rat Immunization Protocol

Immunizations were performed under animal license PPL/40/3371. Male rats (Rattus norvegicus) aged ~10 weeks old were primed by subcutaneously injection with 100 μl of 1 mg·ml^{-1} of Fcε$_{2\text{-}3}$ dimer (**Figure 1**) mixed with an equal volume of complete Freund's adjuvant, followed by a subsequent injection with the dimer mixed with incomplete Freund's adjuvant 14 days later. 10 days

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after the second injection ~200 µl of blood was taken from the rat's tail vein. After each bleed a minimum of ~20 days recovery was allowed before the rats were boosted.

Test rats were subsequently boosted with 100 µl of 1 $mg \cdot ml^{-1}$ of HDH chimeric IgE antibody construct (**Figure 2**) mixed with an equal volume of incomplete Freund's adjuvant, while control rats where boosted with the $Fc\varepsilon_{2\text{-}3}$ dimer. 10 days passed before a bleed was collected (**Table 2**).

After each bleed, blood was incubated at room temperature for ~1 hour followed by storage at 4˚C for ~24 hours before centrifugation at high speed (10,000 g for 1 minute). The resultant serum fraction was assessed for anti-IgE titers.

2.8. Assay Protocol (ELISA)

The mouse and rat anti-sera were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA), using immobilised peptide targets or native Fc IgE in order to assess specificity and strength of the immune response. The The non-thyroglobulin conjugated $Fc\varepsilon_{2\text{-}3}$ dimer was used as a ligand to measure anti-serum antibody titers at dilutions (1:200 - 1:102400). NIP-HSA was used to capture (human, canine or equine) IgE and to test binding of the anti-sera to native IgEs, identical anti-serum dilutions were used.

2.9. Cell Lines Expressing Human, Canine and Equine FcεRIα

Cell lines transfected with genes encoding either human [21], canine [22] or equine FcεRIα [23,24] were employed to assess the consequences of cell sensitization and challenge with immune serum raised against the IgE derived inter Cε2-3 dimeric peptide and HDH chimeric IgE.

2.10. *β*-Hexosaminidase Release Assays

Mediator degranulation assessed as *β*-hexosaminidase release [21] was assessed using RBL-2H3.1 cells expressing a functional human, canine and equine FcεRIα chain as described previously [2,3,22-24].

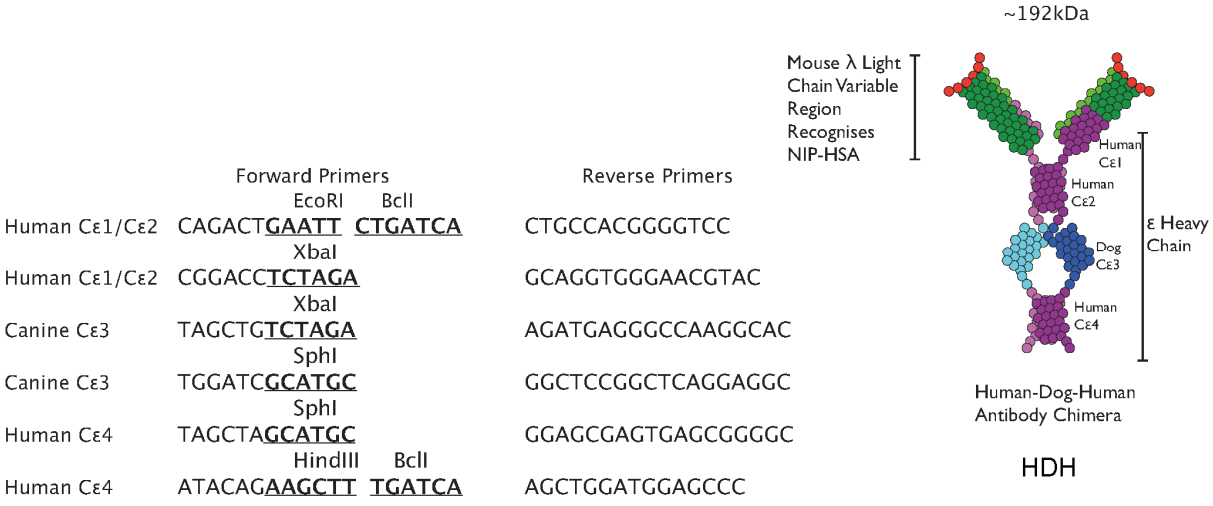

Figure 2. PCR primers used to amplify and assemble the different domains of the human and canine IgE to construct the final HDH IgE heavy chain gene (structure shown) having the human Cε1, Cε2 and Cε4 and the canine Cε3. The gene was then inserted downstream of a mouse *λ* chain variable region with NIP-HSA specificity which resulted in a full HDH IgE antibody with NIP-HSA specificity.

Table 2. The rat immunization schedule showing the pre-immunization bleed, the first and second bleed timings. Due to the results of this investigation the third bleed was not preformed.

Pre Immunization Bleed		Bleed 1		Bleed 2		Bleed 3	
		Injected on Day 0 & 14	Bled on Day 24	Injected on Day 42	Bled on Day 52	Injected on Day 92	Bled on Day 102
Rat 1	**No immunization**	Immunized with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 1 with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 2 with the $Fc\varepsilon_{2\text{-}3}$ dimer	
Rat 2		Immunized with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 1 with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 2 with the $Fc\varepsilon_{2\text{-}3}$ dimer	
Rat 3		Immunized with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 1 with HDH IgE		Boosted 2 with HDH IgE	
Rat 4		Immunized with the $Fc\varepsilon_{2\text{-}3}$ dimer		Boosted 1 with HDH IgE		Boosted 2 with HDH IgE	

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3. Results and Discussion

3.1. Binding of Anti-IgE Anti-Sera Directed Against Human IgE Derived Peptides

The anti-peptide antibody responses were assessed by SPR using the BIAcore 2000 system (General Electric and results are summarized in **Table 3**).

Antibodies generated against the $Fc\varepsilon_{2\text{-}3}$ dimer (human Cε2-3 linker region) recognized both the human and canine IgE-Fc with an affinity in the μM range.

When RBL-2H3.1 cells, transfected with human or canine FcεRIα, were sensitized with cognate IgE and challenged with $Fc\varepsilon_{2\text{-}3}$ dimer antiserum up to a concentration of 50 $\mu g \cdot ml^{-1}$ no evidence was obtained for receptor cross linking. In contrast, IgE-mediated cell degranulation was observed in response to challenge with the commercial anti-human IgE reference control, under identical conditions, which supported *β*-hexosaminidase release peaking at ~50% [27].

These observations clearly indicated that the immune response to the $Fc\varepsilon_{2\text{-}3}$ dimer recognized human, canine and equine IgEs and gives rise to non-anaphylactogenic antibodies. Since the affinity of anti-peptide antibodies is in the micromolar range, we assessed the potential of the $Fc\varepsilon_{2\text{-}3}$ dimer antibodies/sera to prevent receptor sensitization with human or canine IgE by investigating inhibition of *β*-hexoseaminidase release following incubation of human or canine IgE (1 $\mu g \cdot ml^{-1}$) with serial dilutions of pre and post vaccination mAb or anti-serum for 1 hour prior to sensitization of the respective transfected cell lines, followed by a challenge with NIP-HSA antigen (100 $\mu g \cdot ml^{-1}$) (data not shown). No inhibition of mediator release was observed at any concentration of anti serum (4.5 $mg \cdot ml^{-1}$, serum dilution range 1:10 - 10,000).

Since several distinct binding sites are known to contribute to the docking of IgE to FcεRI, it is not surprising that antibodies directed against a single peptide determinant are unlikely to induce an antibody response of sufficiently high affinity/avidity capable of inhibiting IgE/receptor interaction or effect displacement of receptor bound IgE. This is an important consideration in view of the fact that most IgE is found complexed to cognate receptors. We therefore decided to re-assess the observation by others [13,16,18], the results of which suggested that immunization of dogs with a chimera encompassing the complete canine Cε3 surrounded by opossum Cε2 and Cε4 domains (ODO) generated an immune response capable of inhibiting allergic responses in dogs. We designed a human Cε2-canine Cε3-human Cε4 chimeric IgE antibody (HDH) to test this concept, although we were aware that a complete Cε3 domain in context of surrounding domains could generate an immune response against Cε3 determinants which are not obscured when the molecule is in contact with its receptor and may therefore give rise to generation of anaphylactogenic antibodies.

Table 3. Binding of mAbs and rabbit antisera raised in response to immunization of mice and rabbits with IgE-derived peptides. Procedures leading to the generation of mouse monoclonal and rabbit polyclonal anti-IgE derived peptide antibodies are described in section 2.5 and 2.6. Canine and human anti NIP-HSA IgE were bound indirectly to a CM51 chip via immobilized NIP-HSA and followed by subsequent injections of each antibody, or serum, by passing over the chip.

Antibody	Target	K_A (M)
Mouse IgG3	Human FG Loop	1.3×10^9
Mouse IgG3	Human AB Helix	2.4×10^5
Mouse IgG3	Human Cε2-3 Linker Region	4.7×10^4
Mouse IgG2A	Canine FG Loop	9.01×10^4
Mouse IgG1	Canine Cε2-3 Linker Region	2.88×10^6
Rabbit Antiserum	Human Cε2-3 Linker Region	4.2×10^7 (Human IgE)
Rabbit Antiserum	Human Cε2-3 Linker Region	7.1×10^6 (Canine IgE)

3.2. Generation of HDH Anti NIP-HSA Chimeric IgE

The protocol outlined in Sections 2.3 and 2.4 lead to the development of a J558L cell line that expressed HDH anti NIP-HSA chimeric IgE. After purification the chimeric IgE was analyzed on a 12% SDS-PAGE (**Figure 3**) in the presence and absence of the reducing agent *β*-mercaptoethanol.

3.3. Binding of Rat Immune Serum to $Fc\varepsilon_{2\text{-}3}$ Dimer

The anti-$Fc\varepsilon_{2\text{-}3}$ dimer serum was raised in rats as described in section 2.7 and tested by ELISA as in section 2.8 for the assessment of the immune response to the $Fc\varepsilon_{2\text{-}3}$ dimer. The results showed that sera from all four rats recognized the $Fc\varepsilon_{2\text{-}3}$ dimer (**Figure 4**). Bleed 2 antibody titers were higher than bleed 1. Variations in antibody titer between rats were observed as expected.

3.4. Assessment of Rat Immune Serum Reactivity to HDH Anti NIP-HSA Chimeric IgE

Since the vaccine strategy employed the targeted recognition of the Cε2-3 linker region of the native IgE, binding to native canine IgE was assessed. The outcome, shown in **Figure 5**, indicated recognition of native canine IgE with sera from rats 3 and 4 responding with higher titer than rats 1 and 2, which were only boosted with the

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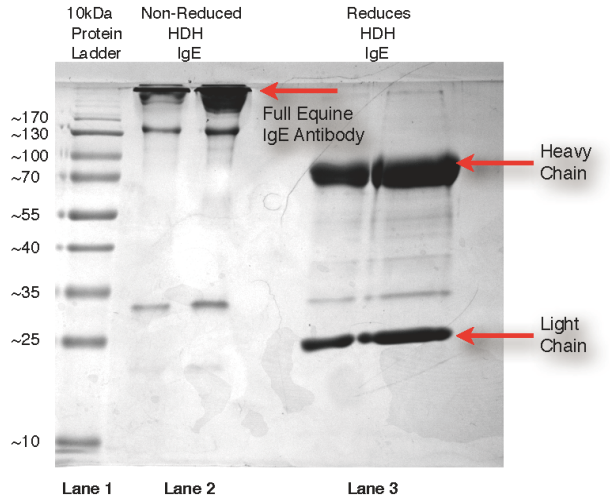

Figure 3. SDS-PAGE of purified HDH anti NIP-HSA chimeric IgE in the absence (lane 2) and presence (lane 3) of *β*-mercaptoethanol reducing agent. Lane 2 result shows the predicted size of the full chimeric IgE (~192 kDa), while lane 3 shows the predicted sizes of the heavy (~70 kDa) and lights chains (~25 kDa), the result also shows in lane 2 almost no bovine albumin impurity (~70 kDa) with minimal protein degradation (bands at ~35 kDa and ~130 kDa).

$Fc\varepsilon_{2\text{-}3}$ dimer. Since the $Fc\varepsilon_{2\text{-}3}$ dimer comprises the PRGV sequence, which is found in human, canine and equine IgEs, responses to native human and equine IgE were also assessed. The positive control was a rabbit anti human IgE Cε3 anti-serum while the negative controls were pre immunization sera.

Our results indicate that the immunization strategy induced higher antibody titers for binding to native IgE when rats were boosted with the chimeric HDH IgE compared to animals that were boosted with the $Fc\varepsilon_{2\text{-}3}$ dimer.

3.5. Assessment of Potential Anaphylactogenic Immune Response

Antibodies in the rat sera, isolated from rats primed with the $Fc\varepsilon_{2\text{-}3}$ dimer and boosted with the chimeric HDH IgE, recognized native IgE of human, canine and equine origin indicating that this peptide may form the basis of a common anti-allergy vaccine.

We further wished to establish whether the immune response adheres to the principal of the "original antigenic sin", a phenomenon commonly observed in response to viral infections [19,20] where an *in vivo* immunization showed there is usually only one antibody raised against one dominant epitope with traces of one or two more. Upon re-infection, there is a tendency to make antibodies against the first dominant epitope(s) encountered during the original exposure in spite of the presence of new epitopes during subsequent encounters. Should the second boost with the HDH chimera followed this principle, a high titer of antibodies against the Cε2-3 dimer should result, and additional Cε3 epitopes, which in principal, could be expected to stimulate the synthesis of anaphylactogenic antibodies, and would not, in fact, act as strong immunogens. The confirmation of such a response could then form the basis for the development of a safe anti-IgE vaccination schedule.

The sera, from bleed 2, of rats 3 and 4 were therefore assessed for their capacity to induce *β*-hexosaminidase release from cells sensitized with IgE as described in Sections 2.9 and 2.10. The outcome, shown in **Figure 6**, showed that antibodies in the sera of the rats immunized and boosted with the $Fc\varepsilon_{2\text{-}3}$ dimer did not induce mediator release. In contrast, sera from rats boosted with the HDH chimera did cross link receptor bound IgE and therefore activated downstream signaling from the FcεRI receptor, resulting in mediator degranulation. Results showed that this particular immunization protocol might ultimately lead to the development of anaphylactogenic antibodies.

The outcome of this experiment clearly shows that the immunization protocol has resulted in the development of antibodies that recognized receptor bound IgE and cross linked it, thus aggregating the receptors on the cell surface initiating downstream signaling resulting in degranulation and mediator release.

Our observations showed that immunization with a peptide encompassing the Cε2-3 linker region known to be involved in IgE/receptor [9] interaction gave rise to antibodies capable of recognition of human, canine and equine IgE. This suggests that further development of this strategy could provide the basis for the development of a pan-anti-allergic vaccine, provided that a safe and effective high-affinity antibody response can be induced. If successful, such an active immunisation strategy would circumvent the problems associated with passive antibody transfer, which are well reviewed in the literature. Even when the framework regions have been humanised, the resulting antibodies, when used for passive immunisation, may evoke immune responses to idiotypic determinants. The advantage of the approach outlined in this work is that it avoids this problem because it activates directly the species own immune system. The maximal affinity observed in our study shows that the immune response to the peptide is in the μM range, and these antibodies, as we have shown, are incapable of inhibiting receptor sensitization, since the affinity of the IgE for FcεRIα is in the nM range, reflecting the involvement of multiple binding sites in the interaction and the need to further improve the design of peptide targets with respect to their similarity with their counterparts in the native antibody. With respect to immunization with

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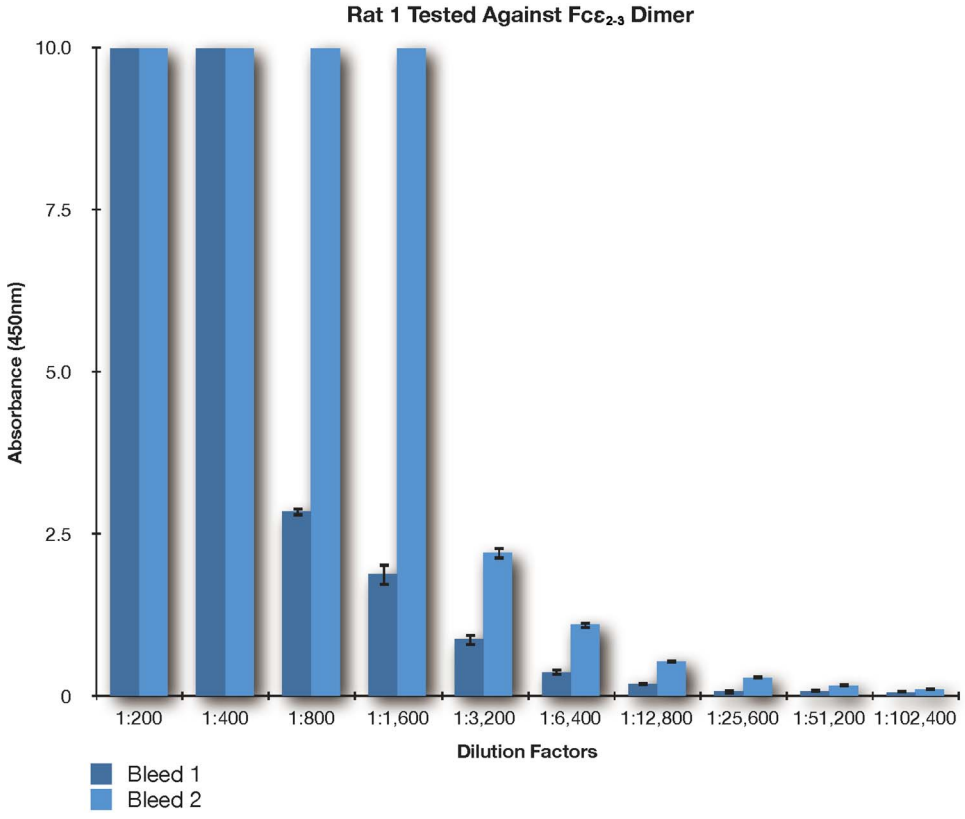

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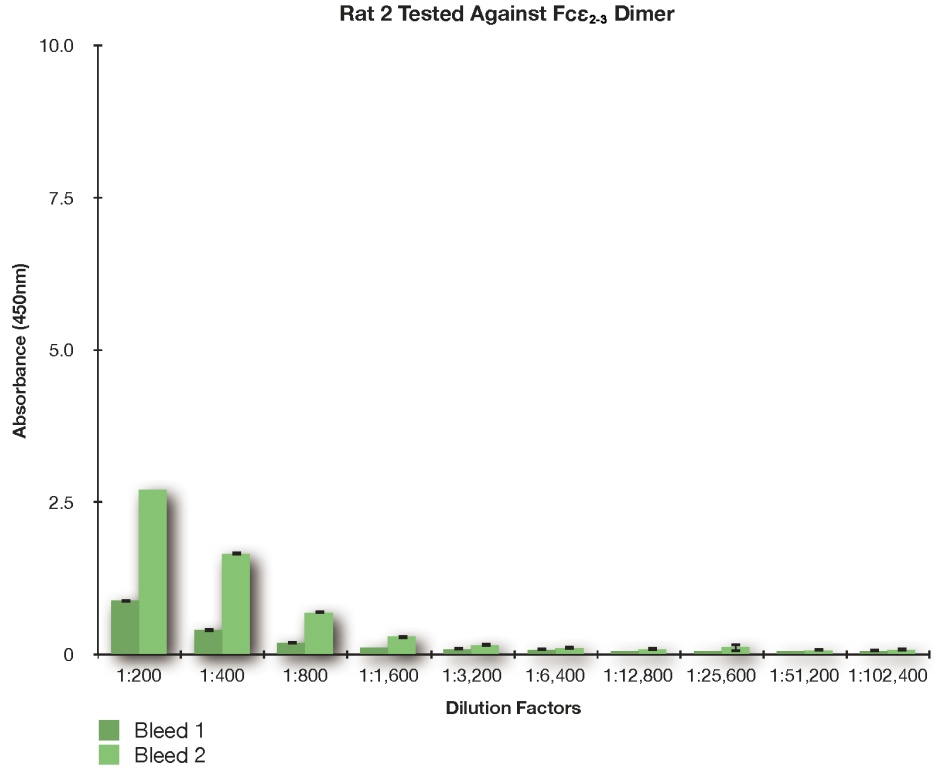

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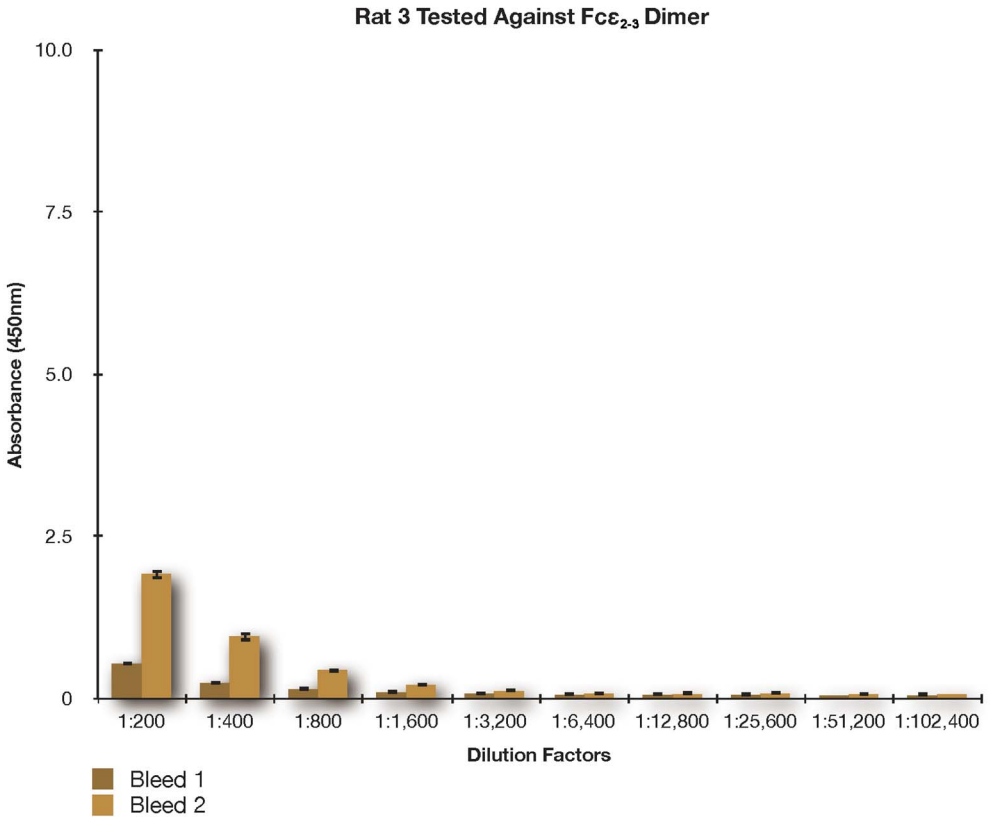

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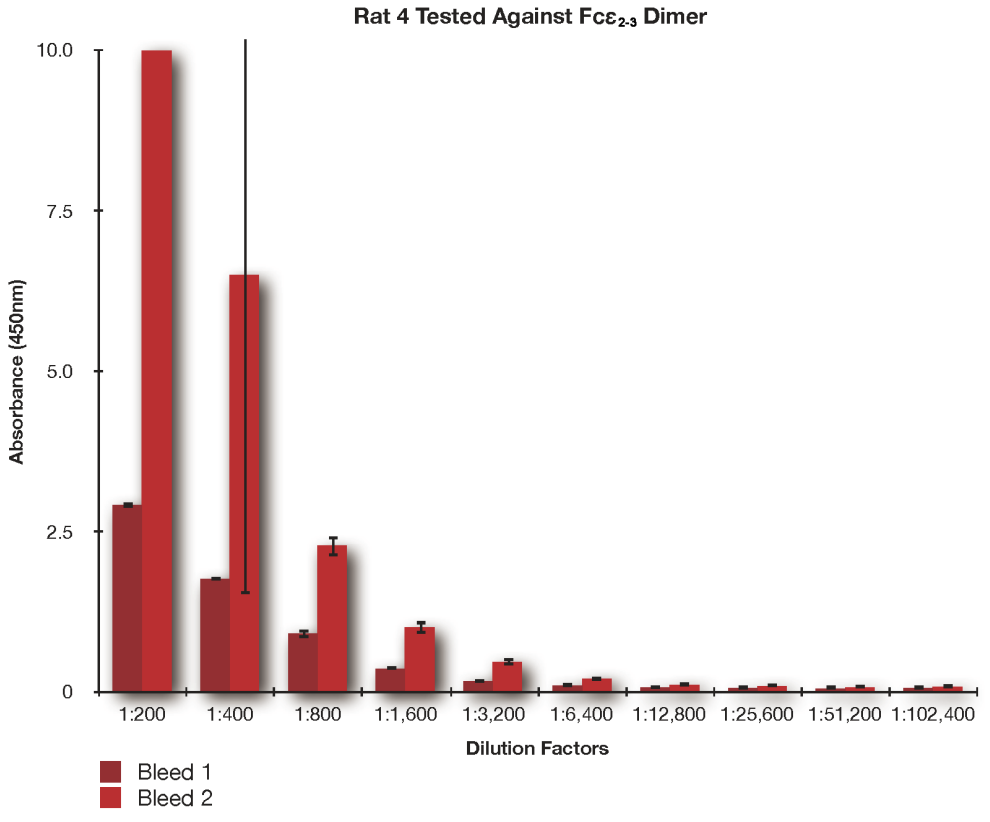

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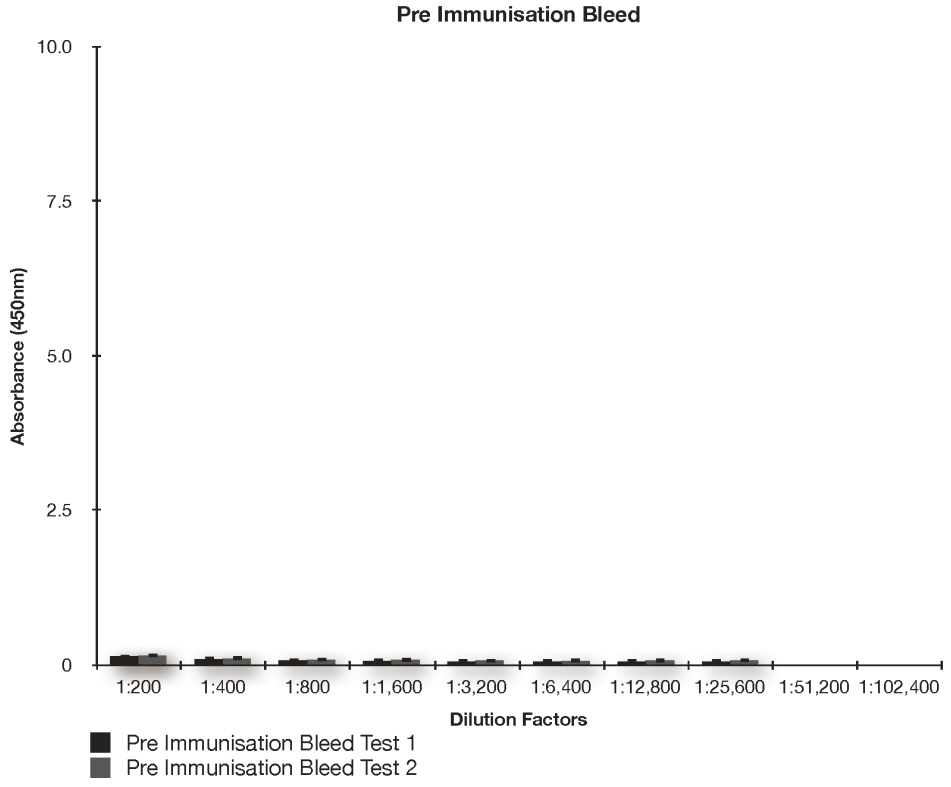

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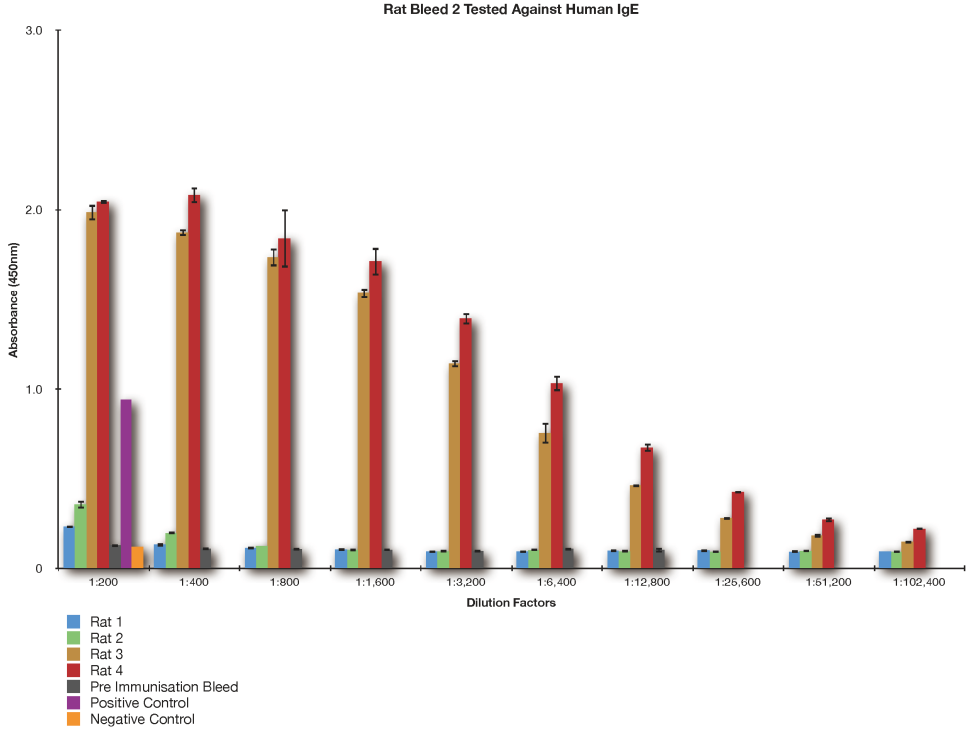
Figure 4. ELISA tests of both the first and second boost from each rat. All sera from immunized rats showed presence of antibodies that bound to the Fc$\varepsilon_{2\text{-}3}$ dimer as predicted. Rats 1 and 2 were immunized with the Fc$\varepsilon_{2\text{-}3}$ dimer and this was used in the subsequent boosts, while rats 3 and 4 were immunized, originally, with the same peptide but boosted with the HDH anti NIP-HSA chimeric IgE. The pre immunization serum showed no binding to the Fc$\varepsilon_{2\text{-}3}$ dimer.



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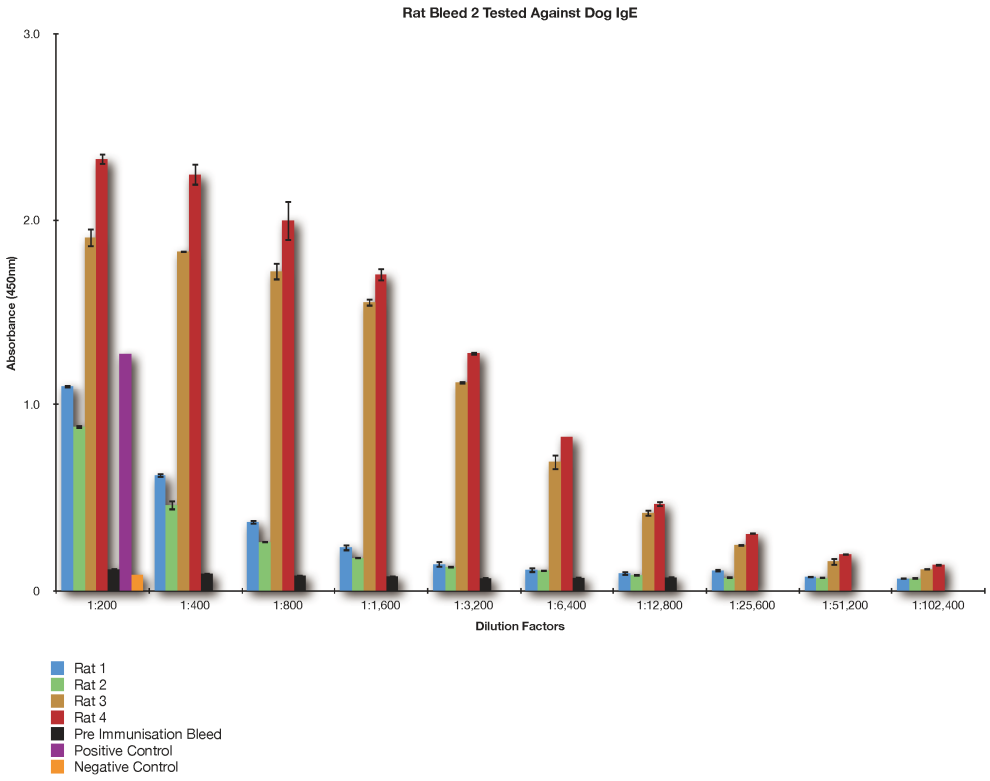

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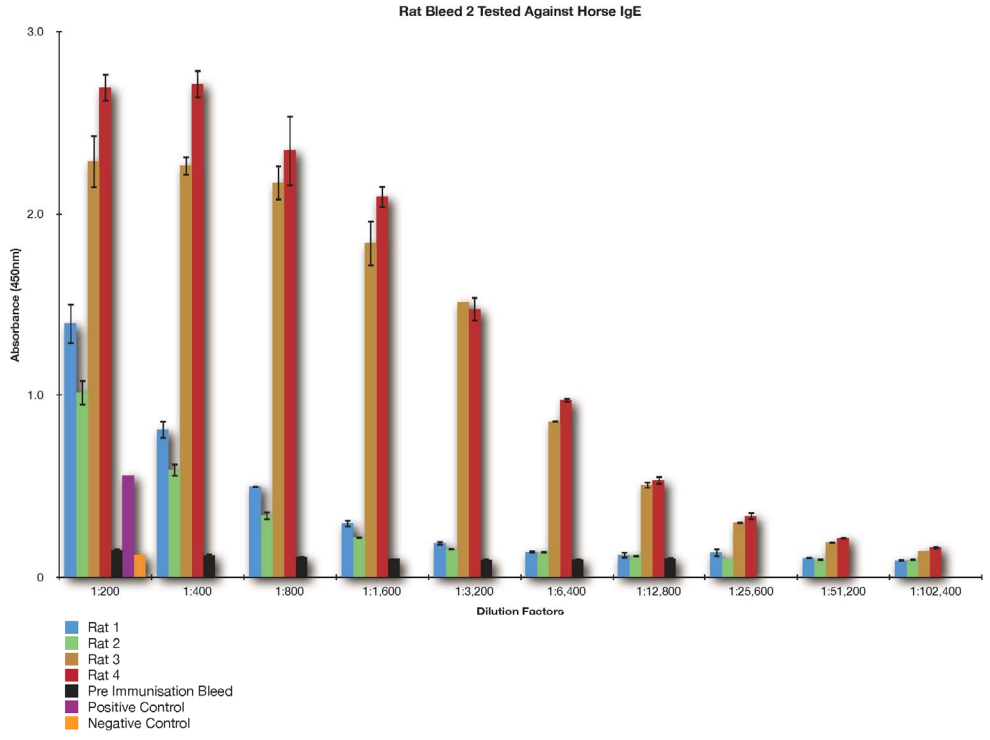

(c)

Figure 5. ELISA results of second boosts from each rat. Sera were tested for binding to native human, canine and equine IgE. Rats 1 and 2, which were boosted with the Fc$\varepsilon_{2\text{-}3}$ dimer, showed lower antibody titers than rats 3 and 4, which were boosted with the HDH anti NIP-HSA chimeric IgE. Rabbit anti human IgE Cε3 anti-serum was employed as a positive control and pre immunization sera were used as negative control.

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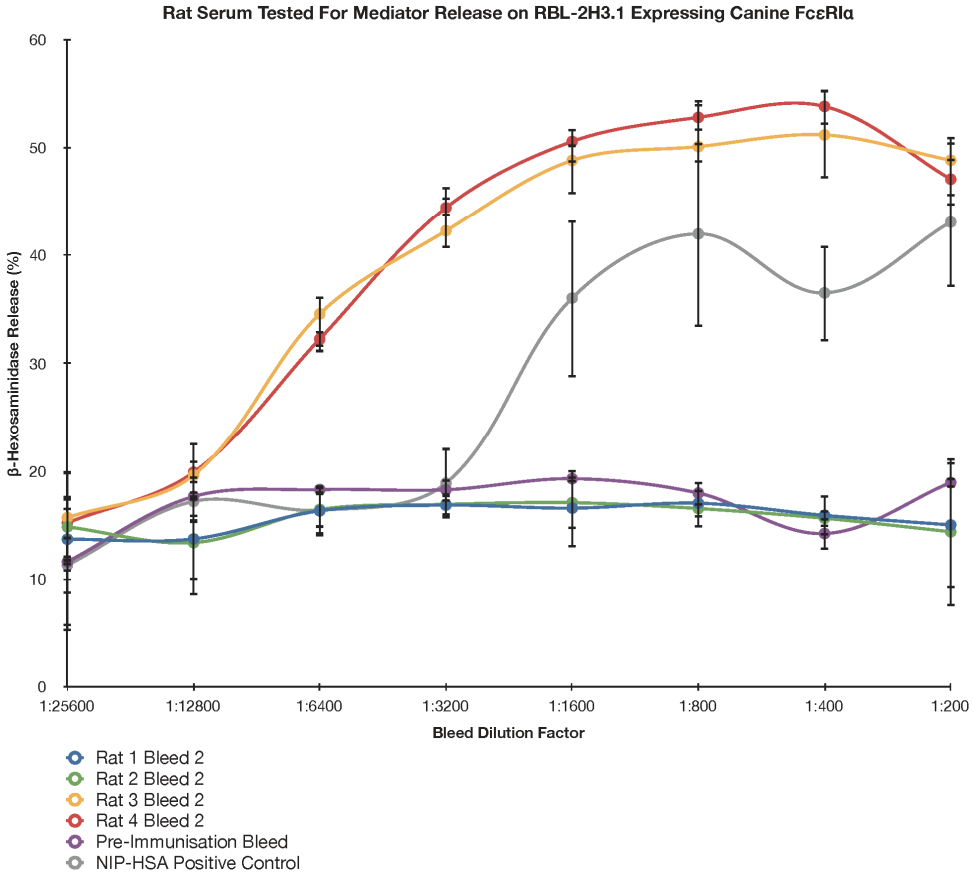

Figure 6. *β*-hexosaminidase release from RBL-2H3.1 cells expressing canine FcεRIα after sensitization with ca- nine IgE and challenged with antisera obtained after the second boosts of rats 1, 2, 3 and 4. The result shows that the immunization protocol has resulted in antibodies that recognized and cross linked receptor bound IgE, thus aggregating the receptors on the cell surface resulting in degranulation and mediator release.

the HDH IgE chimera encompassing the original immunogen, although it was shown to induce a strong immune response to IgE in all three species, it is evident that the generation of anaphylactogenic antibodies prohibits this methodology from providing the basis of a potential therapeutic strategy. It is interesting to note, however, that in spite of the anaphylactic antibody response elicited by the immunization schedule described in this study, the immunized rats suffered no obvious side effects.

Similarly, there was no indication that the dogs immunized with the chimeric ODO IgE described by [13,16,18] suffering from adverse effects, although it is highly probable that the immunization schedule described by these investigators would have induced the formation of potentially anaphylactic anti-IgE antibodies. Authors of these publications fail to indicate whether this possibility was investigated or even considered [13,16,18].

In order to improve the affinity/avidity of the immune response, a cocktail of peptides representing sequences of all the interactive sites between IgE and FcεRIα could be administered together with novel adjuvants capable of stimulating the synthesis of antibodies inhibiting and reversing receptor sensitization.

The reagents described in the current study form the basis for the initial *in-situ* safety testing of the immune response to such immunogens, which in time, may have an application in the prevention and treatment of all IgE-mediated allergies by active immunization, irrespective of the nature of the allergen.

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