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## COMMUNICATION

**Mimicry of Human IgE Epitopes by Anti-idiotypic Antibodies****Monique Vogel<sup>1\*</sup>, Sylvia Miescher<sup>1</sup>, Sonja Kuhn<sup>1</sup>, Adrian W. Zürcher<sup>1</sup>  
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According to Jerne's network hypothesis, the binding site of an anti-idiotypic antibody also represents the internal image of an epitope present on a foreign, or even a self antigen. In recent years, antigen mimicry has been defined at the molecular level for some xeno-antigens. However, until now there has been no demonstration of structural mimicry between a human anti-idiotypic antibody and a self structure. To address this question, we used human IgE as the self structure and a well-defined anti-human IgE mAb (BSW17). We describe the isolation of two anti-idiotypic antibodies specific for the anti-IgE antibody BSW17 from a non-immune human Fab phage display library. Interestingly, these two anti-idiotypic antibodies mimic the same molecular surface region as a previously described IgE peptide mimotope isolated by panning on BSW17, but they cover a much larger epitope on the IgE molecule. Accordingly, immunisation of rabbits with the two anti-idiotypic antibodies induced high-affinity antibodies with the same characteristics as BSW17. Thus, our data demonstrate that it is possible to isolate anti-idiotypic antibodies derived from the human genome without the need for hyperimmunisation, and confirm Jerne's hypothesis that both foreign antigens and self structures can be mimicked by our own immunoglobulins.

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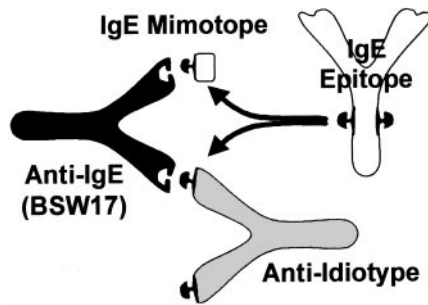
The immune network theory proposes the idea that anti-idiotypic antibodies are produced during the immune response to a given antigen (Jerne, 1974). A subset of these anti-idiotypic antibodies, termed internal image antibodies, or Ab2 $\beta$ , are able to mimic the molecular structure of the nominal antigen (Jerne *et al.*, 1982; Pan *et al.*, 1995). Such anti-idiotypic antibodies have been successfully isolated and characterised from animals immunised with external antigens (Goldbaum *et al.*, 1998; Bona, 1996; Pan *et al.*, 1995). In humans, natu-

rally occurring auto-anti-idiotypic antibodies have been detected serologically in patients with autoimmune diseases, but also in normal donors (Jayne *et al.*, 1993; Bronshtein *et al.*, 1992; Zouali & Eyquem, 1983). However, the isolation and characterisation of such antibodies has not been achieved on the monoclonal level using conventional hybridoma technology (Köhler & Milstein, 1975). We have previously shown that human anti-idiotypic antibodies against human IgE can be isolated from a non-immune repertoire using phage display technology (Vogel *et al.*, 1994). Here, we used the same technology to isolate human anti-idiotypic Fab fragments able to mimic an epitope from the constant part of human IgE. To define such an epitope, we used a monoclonal anti-human (mAb) IgE antibody, termed BSW17 (Knutti-Mueller *et al.*, 1986). With this mAb we had screened phage-displayed random peptide libraries and isolated peptides mimicking an epitope located in the third and

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Abbreviations used: PMBC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; CHO, Chinese hamster ovarian cells; mAb, monoclonal antibody; CDR, complementarity-determining region; FR, framework.

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**Figure 1.** IgE mimotope or anti-idiotypic antibody as the mimicry of an IgE epitope.

fourth constant domain of IgE (Rudolf *et al.*, 1998, 2000). Here, we address the question of whether it would be possible to isolate an anti-idiotypic antibody that mimics the same epitope (Figure 1).

To find the anti-idiotypic antibodies, we searched in combinatorial Fab libraries generated from the peripheral blood mononuclear cells (PBMC) of allergic and non-allergic donors (Vogel *et al.*, 1994), and of purified B-cells from children's tonsils. RNA was extracted from each source and used independently to prepare PCR-amplified cDNA corresponding to the variable heavy chain immunoglobulin (Ig) region and a part of the constant region of IgG1 (Fd) and the Ig light chains  $\kappa$  and  $\lambda$ . The cDNAs were cloned into the pComb3 vector system, and Fab libraries from the three different sources were expressed separately on the surface of filamentous phage and then pooled for further use. To select anti-Id antibodies specific for BSW17, the pooled libraries were subjected to four rounds of biopanning on solid-phase BSW17 (Marks *et al.*, 1991). After the last round of panning, eight positive clones were selected out of 50

clones, and all of them recognised the antigen-binding site of BSW17. This was demonstrated in an inhibition assay where the binding of the Fab clones to BSW17 was inhibited by the two previously isolated IgE mimotopes corresponding to the third and fourth domain of IgE (data not shown).

To delineate the regions on anti-Id Fabs responsible for mimicking IgE structures, the nucleotide sequences of the heavy and light chain variable domains from the selected clones were determined (Vogel *et al.*, 1996). Sequence analysis revealed that only two types of Fab clones were isolated. Figure 2 shows the deduced amino acid sequences of the two anti-Id Fab clones compared with the most homologous germline genes. The two Fab clones (anti-Id-BSW17.52 and anti-Id-BSW17.43) showed the highest level of homology to two genes derived from different VH families, 22-2 B from VH3 and DP-65 from VH4. The VH CDR3 regions of both clones were also markedly different in length and sequence, suggesting that they originated from two different B-cell clones. Further, the light chains of these Fab clones were homologous to two different germline genes belonging to different families, V $\kappa$ 1 and V $\lambda$ 1 (Figure 2). The V-genes of both clones showed several differences from the germline V-gene segments, suggesting that they are derived from somatically mutated B-cells. As the source of our immunoglobulin genes was a pool of three donors, we cannot conclude whether the anti-idiotypic specificities were derived from one individual or whether both specificities may actually be present in all three donors.

To further characterise the epitope specificity of the anti-Id-BSW17 Fab clones, soluble Fab fragments were produced and analysed in a competitive binding assay against the mAb BSW17. The mAb BSW17 has been shown to recognise IgE on the surface of CHO cells expressing the  $\alpha$  chain of

#### A. Heavy Chains

Clones	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
22-2B	QVQLVDSGGGLVLPKGGSLRLSCAASGFTFS	DYYS	WIRQAPFGKLEWVS	YISSSGSTIYYADSVKG	RFTISRDNAKNSLYLQMNLSRAEDTAVYYCAR		
$\alpha$ -Id-B52	--K-L-----V-----	N-N-N	-V-----	S--RN-Y-----	---EST---DN-GV---F-TS	GRLFDY	WGQGLTVTVSS
DP-65	QVQLQESGPGLVKPSQTLTCTVSGGSIS	SGGYWS	WIRQPPGKLEWIG	YIYSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSVVTAADTAVYYCAR		
$\alpha$ -Id-B43	--K-L-----E-----	-----T	---R-----	-----S-----	---N-----R-T-----	ERGETGLYPPYIDV	WGTGTTTVVSS

#### B. Light chains

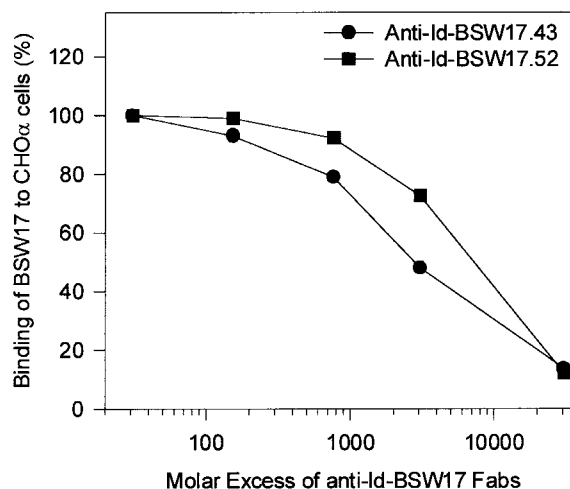
Clones	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
<i>Kappa Chain</i>							
O2	QMIQSPSSLSASVGDRTIITC	RASQSISSYLM	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFRSGSGSIDFTLTISLQPEDFATYYC	QQSYSTP	
$\alpha$ -Id-B52	V-T-----	---CG-NN--A	-F-----S---	S--I---	---K-----T-----N-----	--YNY-LT	FGGKTKVEIK
<i>Lambda Chain</i>							
D11	QSALTQFASVSGSPGQSITISIC	TGTSSDVGCYNYVS	WYQQHFGKAPKLMIIY	EVSNRPS	GVSNRFRSGSKSGNTASLTISGLQAEDEADYYC	SSYSSSTL	
$\alpha$ -Id-B43	ELVV-----	---R-----	-----	D-----	-----	-----GV	FGGKTKVEIK

**Figure 2.** VH and VL-deduced residue sequences of the two anti-Id-BSW17 Fab clones. The sequences  $\alpha$ -Id-B52:anti-Id-BSW17.52 and  $\alpha$ -Id-B43:anti-Id-BSW17.43 are segregated into CDR (complementarity determining regions) and FR (framework regions) and compared to the most homologous germline protein sequences. The sequence data are available from EMBL Genbank under the numbers AJ249736 and AJ249791 for the heavy chains of clones anti-Id-BSW17.52 and anti-Id-BSW17.43, respectively, and AJ249737 and AJ249792 for the light chains of clones anti-Id-BSW17.52 and anti-Id-BSW17.43, respectively.

the FcεRI receptor (CHOα) (Rudolf *et al.*, 1996). A flow cytometric assay was used to determine whether the anti-Id-BSW17 Fab clones were able to inhibit the binding of BSW17 to IgE-primed CHOα cells. The CHOα cells were first incubated with a monoclonal human IgE (Zürcher *et al.*, 1995), followed by incubation with preformed complexes of BSW17 and soluble anti-Id-BSW17 Fab fragments. As shown in Figure 3, the binding of BSW17 to CHOα cells decreased with increasing concentrations of anti-Id-BSW17 Fab fragments, indicating that the two anti-Id-BSW17 Fab clones were able to inhibit the binding of BSW17 to IgE. These results imply that the two anti-Id-BSW17 Fab clones mimic epitope regions on IgE recognised by the mAb BSW17.

To determine the epitopes on IgE mimicked by the anti-Id-BSW17 Fabs, their amino acid sequences were compared with the constant ε chain amino acid sequence using the homology search function algorithm developed by Lipman & Pearson (1985). As shown in Figure 4(a), the heavy chains of both anti-Id Fab clones showed homology with the third heavy chain constant domain of the IgE molecule (Cε3). Sequence comparison revealed a 46% identity (dark green) and 73% similarity (light green) between the residues 370 and 383 of Cε3 and a region of anti-Id-BSW17.52 comprising the first complementarity-determining region (CDR1) and a part of the adjacent framework (FR2). For anti-Id-BSW17.43, values of 31% identity (dark blue) and 67% similarity (light blue) were obtained between residues 355 and 383 of Cε3 and a sequence of 29 amino acid residues encompassing the CDR1 and a part of the adjacent frameworks (FR1 and FR2). Remarkably, the previously isolated Cε3 peptide mimotope specific to BSW17 revealed homology with a similar region of the Cε3 domain corresponding to residues Val370 to Ser375 (Figure 4(a); Rudolf *et al.*, 2000).

To visualise the epitopes responsible for the observed mimicry on IgE, the sequences were aligned with a homology-based three-dimensional computer model of the IgE molecule developed for human Fcε on the basis of sequence homology with Fcγ (Padlan & Davies, 1986; Helm *et al.*, 1991). Visual and computational analysis revealed that the VH of both Fab clones, as well as a part of the Cε3 mimotope, share sequence homology and mimic a common IgE epitope covering the region from Thr373 to Asn383 (Figure 4(a)). This common epitope emerges on the surface of the IgE molecule and has considerable surface solvent accessibility. These data, and the fact that structural analysis of the common epitope revealed a β-turn, support the prediction that this region is antigenic (Chou *et al.*, 1978) and is recognised by BSW17 as described by Knutti-Mueller *et al.* (1986). Furthermore, this region has been reported to overlap with the possible IgE-binding site of the high-affinity IgE receptor. Using alanine scanning mutagenesis, two amino acid residues (Arg376, Ser378) present in this region were found to be directly involved in



**Figure 3.** Inhibition of BSW17 binding to sensitised FcεRIα-transfectoma cells by soluble anti-Id-BSW17 Fabs. To produce soluble anti-Id BSW17.52 and anti-Id BSW17.43 Fabs, the gIII sequence was replaced by six histidine residues at the carboxy-terminal end of the Fab. After transformation in XL1-Blue, single clones were chosen for Fab large-scale production. Purification of Fab was performed using a nickel-nitriloacetate column (Qiagen) according to the manufacturer's instructions. A series of test samples containing  $5 \times 10^4$  CHOα cells were incubated in FACS buffer (PBS, 0.3% bovine serum albumin, 0.02% NaN<sub>3</sub>) with 48 ng of a monoclonal human IgE (Zürcher *et al.*, 1995) for 20 minutes at room temperature. After washing once with FACS buffer, each sample was incubated for 20 minutes at room temperature with preformed complexes of fluoresceine isothiocyanate conjugated BSW17 (BSW17-FITC) and anti-Id-BSW17 Fabs. The complexes were prepared as follows: 50 μl of BSW17-FITC at a concentration of 1.3 nM were incubated with different concentrations of anti-Id-BSW17 Fabs (40 nM, 200 nM, 1 μM, 4 μM and 40 μM) for 30 minutes at room temperature. A control sample containing only the CHOα cells was incubated for 20 minutes at room temperature with BSW17-FITC for determination of non-specific binding. The CHOα cells were washed once with FACS buffer and after addition of 150 μl of FACS buffer the cells were analysed in FACSCalibur (Becton Dickinson) flow cytometer equipped with an argon laser tuned to 488 nm. Gates in the forward scatter/side scatter dot blot were set around monomeric cells, and fluorescence was quantified and expressed as mean channel fluorescence (mcf). The percentage of positive cells was calculated as the percentage of BSW17 binding to CHOα cells.

high-affinity receptor binding (Presta *et al.*, 1994; Helm *et al.*, 1996).

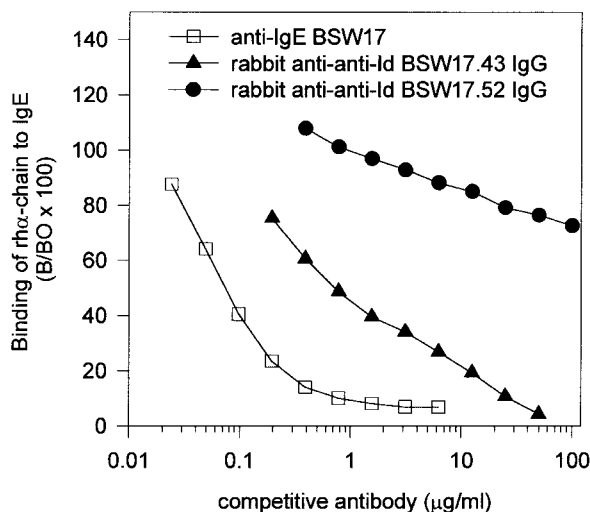
The heavy chains and the light chains of both anti-Id-BSWS17 Fab clones showed homology with additional regions in the third domain of IgE (Figure 4(b)). For anti-Id-BSW17.52, alignment showed 38% identity (dark purple) and 75% similarity (light purple) between the residues 343-358 of Cε3 and a region mapping the first framework (FR1). Similarly, the anti-Id-BSW17.43 revealed values of 36% identity (dark red) and 65% simi-



**Figure 4.** Sequence homology between IgE and anti-Id-BSW17 Fabs. (a) Computer-simulated model of IgE C $\epsilon$ 2-C $\epsilon$ 4 domains depicting C $\epsilon$ 3 regions homologous to anti-Id-BSW17 VH regions and C $\epsilon$ 3 mimotope amino acid residues. (b) Computer-simulated model of IgE C $\epsilon$ 2-C $\epsilon$ 4 domains depicting C $\epsilon$ 3 regions homologous to anti-Id-BSW17 VH and VL regions. For each comparison with the C $\epsilon$ 3 domain, different colour schemes were chosen. Colour intensities are defined according to the homology: dark or light colours for identical or similar residues, respectively. The homology was calculated according to the algorithm developed by Lipman *et al.* (1985).

larity (light red) between the region of Cε3 covering the residues from 413 to 438 and a region encompassing CDR1 and the adjacent framework (FR2). The homologous region of this anti-Id-BSW17.43 light chain appears to span over the common epitope (Figure 4(b)) mimicked by the heavy chains of both anti-Id Fabs, suggesting that for this second anti-Id the light chain might be involved in the antigen mimicry. Furthermore, two residues, Arg427 and Met430, present in this homologous region have been shown by Presta *et al.* (1994) to lie within the hinge-proximal-bend of IgE and to be critical for the binding of IgE to FcεRI. Using the IAsys system (Horn *et al.*, 1999) the  $K_d$  of anti-Id-BSW17.52 and anti-Id-BSW17.43 for BSW17 was determined as  $3.5 \times 10^8 \text{ M}^{-1}$  and  $2.4 \times 10^8 \text{ M}^{-1}$ , respectively, which compares well with values for antibodies obtained by other techniques, such as hybridoma technology (Köhler & Milstein, 1975).

The observed structural mimicry implies that our two anti-Id BSW17 Fab clones might serve as immunogens by providing a "simulated" epitope of IgE and inducing a "BSW17-like anti-IgE" antibody response. To assess this functional mimicry, two rabbits were immunised with soluble anti-Id-BSW17.52 and anti-Id-BSW17.43 Fab preparations four times at two-week intervals; after seven weeks, the sera were tested for anti-IgE activity. Because the Cε3 epitope region, mimicked by the heavy and light chains of both anti-Id Fab clones, has been shown to be involved in high-affinity receptor binding, the antibodies raised against the two anti-Id Fab clones were expected to inhibit binding of IgE to its high-affinity receptor by blocking the IgE-binding domain. To assess this inhibitory activity, the rabbit antisera were first affinity-purified on human IgE and then tested in an inhibition ELISA. Figure 5 shows that the two purified rabbit IgG preparations inhibit the binding of IgE to the α-chain of FcεRI. However, the affinity-purified IgG of the rabbit immunised with the second anti-Id BSW17.43 Fab showed a stronger inhibition of IgE binding to the α-chain than the IgG purified from the rabbit immunised with the first anti-Id BSW17.52 Fab. Using surface plasmon resonance in the BIAcore system (Malborg *et al.*, 1996), the affinity of both purified IgG preparations to human IgE was determined. The polyclonal anti-anti-idiotypic IgG preparation purified from the rabbits immunised with anti-Id BSW17.52 and anti-Id BSW17.43 bound to human IgE with  $K_d$  of  $6 \times 10^9 \text{ M}^{-1}$  and of  $4 \times 10^{10} \text{ M}^{-1}$ , respectively. This result suggests that both anti-Id can act as a functional image of the IgE molecule. Accordingly, the anti-Id BSW17.43 Fab, which shows the closest molecular mimicry to the IgE molecule, induces antibodies *in vivo* with the greatest inhibitory effect and the highest affinity to the IgE molecule.



**Figure 5.** Inhibition of FcεRIα chain binding to IgE by BSW17 and by BSW17 like rabbit anti-anti-Id IgG preparations. Preparation of rabbit anti-anti-Id IgG. Two New Zealand white female rabbits were given a primary immunisation subcutaneously with 300 μg/ml of either soluble anti-Id BSW17.52 or anti-Id BSW17.43 Fabs emulsified 1:1 in Freund's complete adjuvant and then boosted three times with the same amount of Fab emulsified 1:1 in Freund's incomplete adjuvant every two weeks. Animals were bled seven days after the last injection. To purify rabbit serum, the IgG fraction was first isolated by precipitation in 45% saturated ammonium sulphate. After resuspension and dialysis against PBS at 4°C, Fab was purified on an affinity column (CH-Sepharose 4B, Pharmacia, LKB Biotechnology, Inc., Piscataway, NJ) coupled to human IgE. The dialysed fraction was filtered on a 0.2 μm MILLEX-GV filter unit (Millipore) and applied to the column equilibrated with PBS at a flow rate of 2 ml/minute. The flowthrough was pooled and, after washing with PBS, the IgG was eluted with 0.1 M glycine-HCl (pH 2.8) and neutralised immediately with 3.3 M Tris-HCl (pH 8.0). The fractions containing the IgG were pooled, concentrated and extensively dialysed against PBS. The quantity of purified IgG was determined using the Bradford assay, B1gG Standard (BioRad). Inhibition assay. Microtiter plate wells were coated with 1 μg/ml of a mouse anti-human IgE mAb Le27 (Knutti-Muller *et al.*, 1986) for 16 hours at 4°C. After five washes in PBS/0.05% Tween-20, wells were incubated for two hours at 37°C with complexes preformed for 16 hours at 4°C by a monoclonal human IgE (Zürcher *et al.*, 1995) at a concentration of 50 ng/ml and increasing concentrations of BSW17 (open squares), rabbit anti-anti-Id BSW17.52 IgG (filled circles) and rabbit anti-anti-Id BSW17.43 IgG (filled triangles). After washing, 100 ng/ml of peroxidase-labelled recombinant FcεRI (Nechansky *et al.*, 1997) was added for a second incubation of two hours at 37°C. The wells were washed, and the amount of bound FcεRIα chain was determined by colour reaction using chromogenic *o*-phenylene diamine dihydrochloride (OPD; Sigma) OPD substrate. Results were calculated as percentage binding of FcεRIα chain to IgE in the presence (B) or the absence of competitor (B0) multiplied by 100.

Although functional mimicry has been reported for some anti-idiotypic antibodies mimicking self antigens, there have been no studies to date that have demonstrated structural similarity between self antigens and human anti-idiotypic antibodies. Mouse monoclonal anti-idiotypic antibodies that mimic self structures have already been reported (Lubahn *et al.*, 1990; Taub *et al.*, 1992; Abrams *et al.*, 1992; Garcia *et al.*, 1992; Perosa *et al.*, 1994). Garcia *et al.* (1992) described a system using angiotensin which is a phylogenetically conserved octapeptide and thus can be considered as a self antigen. In this system, rabbit polyclonal anti-idiotypic antibodies (Ab2) specific to a monoclonal anti-angiotensin (Ab1) were used to obtain anti-anti-idiotypic antibodies (Ab3). The conformation of the angiotensin in the complex with Ab3 has suggested that a CDR of the Ab2 could have elicited the angiotensin-specific Ab3. However, due to the polyclonal nature of the anti-idiotypic antibodies, no structural study of Ab2 was performed to confirm this hypothesis. In our system we have isolated two human monoclonal human anti-idiotypic antibodies and demonstrated amino acid sequence-based structural homology by identifying the cross-reactive residues responsible for the mimicry. Additionally, we have shown that neither immunisation nor affinity maturation is a prerequisite for generating high-affinity anti-idiotypic antibodies from phage display libraries. Recently, the isolation of human recombinant anti-idiotypic scFv against coronavirus from a non-immune phage display library has been reported (Lamarre & Talbot, 1997). However, these scFv antibodies were not able to induce an antiviral immune response sufficiently strong to protect immunised animals. In our case, the fact that two human anti-idiotypic Fab fragments behaved as surrogate antigens and induced an anti-IgE response in rabbits may circumvent the problem of using non-human antibodies in humans. Preliminary results of monkey immunisations have shown that the anti-idiotypic antibodies can induce an anti-IgE response that is able to abolish partially reactivity in an *in vivo* passive cutaneous anaphylaxis test in the case of allergen challenge. Thus, the anti-idiotypic antibodies can be used as an active vaccine analogous to the IgE mimotope peptides that have been previously isolated and are currently in the preclinical testing phase. Clearly, the anti-idiotypic antibodies represent a much larger epitope of IgE than the mimotope derived from random phage peptide libraries. Moreover, in a vaccination procedure these anti-idiotypic antibodies may be better tolerated than peptides that have to be coupled to immunogenic carriers. Thus, it can be expected that in humans the anti-idiotypic antibodies will induce, as already seen in monkeys, an anti-IgE immune response that would prevent binding of IgE to the Fc $\epsilon$ RI, and consequently the sensitisation of the effector cells implicated in allergic disease.

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