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Tracing antigen signatures in the human IgE repertoire

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ABSTRACT

Allergen recognition by IgE antibodies is a key event in allergic inflammation.

In this study, the IgE IGHV repertoires of individuals with allergy to the major birch pollen allergen, Bet v 1, were analyzed over a four years period of allergen exposure by RT-PCR and sequencing of cDNA.

Approximately half of the IgE transcripts represented non-redundant sequences, which belonged to seventeen different IGHV genes. Most variable regions contained somatic mutations but also non-mutated sequences were identified. There was no evidence for relevant increases of somatic mutations over time of allergen exposure. Highly similar IgE variable regions were found after four years of allergen exposure in the same and in genetically non-related individuals.

Our results indicate that allergens select and shape a limited number of similar IgE variable regions in the human IgE repertoire.

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1. Introduction

Immunoglobulin E occurs in extremely low concentrations in serum and other body fluids but can induce severe inflammatory reactions via interaction with a variety of immune cells (Geha et al., 2003; Ishizaka et al., 1966). IgE-mediated cross-linking of FcεRI receptors on mast cells and basophils induces the immediate release of inflammatory mediators, enzymes and pro-inflammatory cytokines and represents a key mechanism in acute allergic inflammation (Kraft and Kinet, 2007). Furthermore, IgE antibodies can present minute amounts of antigens to T cells and thus contribute to efficient T cell activation (van Neerven et al., 2006). Besides its key role in allergic inflammation, IgE may play a part in defense against parasitic infestations via activation of eosinophils (Gounni et al., 1994). The class switch to IgE production in the course of the primary immune response is induced by Th2 cytokines secreted by Th2 helper cells upon allergen encounter (Geha et al., 2003; Romagnani, 2001). Most of the allergens inducing IgE responses in man are protein antigens of which many have been charac-

terized down to their three-dimensional structure and biological functions (Valenta, 2002). For some of these allergens evidence exists that they may promote the development of IgE responses through their intrinsic biological properties. Other studies suggest that non-allergenic components present in allergen sources, certain allergen carriers, Toll-receptor agonists/antagonists or environmental factors may induce or hamper the development of Th2 and IgE responses (Blumer et al., 2005; Traidl-Hoffmann et al., 2005).

Due to the extremely low concentrations of IgE in serum only few studies have investigated the molecular basis of allergen-specific IgE responses and the repertoire of IgE antibodies. In fact, respiratory allergen contact has been shown to up-regulate strongly IgE production in allergic subjects (Niederberger et al., 2007). Several studies have analyzed the IgE repertoire in allergic subjects but all the analyzed individuals mounted IgE responses against numerous and unknown allergens. Hence it has been impossible to draw conclusions regarding the repertoire of IgE antibodies specific for defined allergens (Bando et al., 2004; Coker et al., 2005; Davies and O'Hehir, 2004; Janezic et al., 1998; Lim et al., 2007; Snow et al., 1995; Tilgner et al., 1997; van der Stoep et al., 1993). Those studies reporting the molecular characterization of allergen-specific IgE antibodies have analyzed only few allergen-specific IgE antibodies (Andreasson et al., 2006; Edwards et al., 2002; Flicker et al., 2002, 2006; Niemi et al., 2007; Steinberger et al., 1996). To gain insight into the allergen-specific IgE IGHV repertoire and its development over several years of repeated allergen contact (i.e.,

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secondary IgE response) we have established a model for allergen-specific IgE responses in man. We identified allergic subjects with IgE reactivity against one defined protein allergen, the major allergen of birch pollen, Bet v 1 (Breiteneder et al., 1989). The fact that birch pollen allergic subjects are exposed to antigen during defined periods of the year allowed us to study the IgE IGHV repertoire over several years and episodes of antigen contact. Our finding that highly similar IgE IGHV transcripts were used in different allergic subjects after four years of allergen exposure strongly suggests that allergens directly select and stimulate allergen-specific IgE production (i.e., secondary IgE responses) via activation of a limited and pre-defined set of IgE memory cells.

2. Methods

2.1. Pollen counts, characterization of allergic subjects

Five hundred allergic subjects were screened to identify six individuals with exclusive allergic sensitization to birch pollen using a multi-allergen test system (MAST CLA allergen-specific IgE assay, Hitachi Chemical Diagnostics) containing 46 allergen sources (Alder pollen, Almond, Alternaria, Apple, Aspergillus, Birch pollen, Carrot, Casein, Cat dander, Celery, Cladosporium, Cockroach, Codfish, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, Dog dander, Grass pollen mix, Guinea pig dander, Hamster dander, Hazel pollen, Hazelnut, Horse dander, Juniper, Latex, Milk protein, Mugwort pollen, Olive tree pollen, Parietaria pollen, Peach, Peanut, Penicillium, Pine mix, Plantain pollen, Plume mix, Potato, Rabbit, Ragweed pollen, Rye pollen, Rye flour, Sesame, Shrimp, Soy bean, Tomato, Walnut, Wheat flour, whole Egg). Blood samples from the six allergic subjects were obtained in spring and summer 2002 and 2005. At each appointment, Bet v 1-specific IgE levels were quantified in plasma by CAP-RAST measurements (Phadia, Uppsala, Sweden) and allergic symptoms and anti-allergic medication were recorded. None of the selected subjects had received any kind of allergen-specific immunotherapy. Birch pollen exposure in the individuals living area was recorded as described (Drachenberg et al., 2001).

2.2. HLA typing of the allergic subjects

HLA typing of allergic subjects was performed using a commercially available typing HLA kit based on HLA-sequence specific primers (PCR-SSP) according to the manuals provided by the manufacturer (Dynal AllSet™ SSP DR “low resolution”; Invitrogen Corporation, Carlsbad, CA 92008, U.S.A.). Ambiguous HLA typing results were further clarified by direct DNA sequencing after HLA group-specific amplification with an automated DNA sequencer (ABI Prism 310 Genetic Analyzer; PE Applied Biosystems, Foster City, CA 94404, U.S.A.) as described elsewhere (McGinnis et al., 1997).

2.3. Allergen-specific IgE antibodies

To confirm that all IgE in the allergic subjects is directed exclusively against the major birch pollen allergen, Bet v 1, IgE inhibition experiments with recombinant Bet v 1 were performed. Recombinant Bet v 1, (Biomay, Vienna, Austria), was coupled to CNBr-activated sepharose 4B (GE Healthcare Bio-Sciences AB) in a concentration of five mg protein per ml medium according to the manufacturer's instructions. Samples of 1500 µl of plasma from each of the six allergic persons were incubated with 500 µl of allergen-coupled gel by end-over-end rotation overnight at 4 °C. Serum was recovered by centrifugation (4 °C, 5 min, 5000 × g). IgE levels against food allergen mix (egg white, milk protein, codfish, wheat flour, peanut and soy bean) and respiratory mix (mugwort,

birch pollen, parietaria, timothy grass and ribwort) as well as IgE levels against birch pollen extract and rBet v 1 were determined before and after the depletion by CAP-RAST measurements (Phadia). In the IgE inhibition experiments three subjects (B, D, H) were identified who reacted exclusively with Bet v 1 in birch pollen.

2.4. PBMC isolation and RT-PCR amplification of IgE transcripts

Peripheral mononuclear cells were isolated by Ficoll density-gradient centrifugation at the time of serum collection. Total cellular RNA was isolated using the guanidine isothiocyanate method and CsCl gradient centrifugation (Eibenstein et al., 2000). IgE transcripts were generated by the SuperScript™ One-Step RT-PCR with Platinum® Taq (Invitrogen) using VH1–VH6 family specific primers together with a primer specific for the first constant region of the IgE heavy chain (Eibenstein et al., 2000) (Table 2). Primers used for the experiments in 2002 had accessory Eco RI restriction sites (Table 2 in parenthesis). The PCR amplification procedure consisted of an initial step reverse transcription of 30 min at 47 °C and 5 min at 94 °C followed by 40 cycles of 20 s 94 °C, 30 s 59 °C and 1 min 72 °C with final extension of 5 min at 72 °C. All PCR products were agarose gel purified using the Wizard® SV Gel and PCR Clean-Up System (Promega) according to the manufacturer's instructions. Subsequently cDNA was cloned into the Acceptor™ Vector (Novagen) and transformed into *Escherichia coli* XL1-blue. Plasmid DNA was purified from 3 ml overnight cultures containing 100 µg/ml ampicillin using Wizard® Plus SV Miniprep DNA Purification System (Promega) and digested with the restriction enzymes KpnI and SacI (Roche). Plasmids with inserts of the correct size were sequenced (Microsynth AG, Buchs, Switzerland). Non-redundant sequences are defined as non-identical sequences from one PCR reaction.

2.5. Sequence analysis of IgE transcripts

Analyses of immunoglobulin rearrangement, N-diversity and somatic mutations were performed using SoDA (Volpe et al., 2006) and IMGT/V-QUEST (Giudicelli et al., 2004) (Version 3.0.0). For VDJ germline fitting the consensus of IMGT/V-QUEST and SoDA analyses was used. Mutation frequencies are listed based on the IMGT/V-QUEST results, taking into account consistency with SoDA mutation rates. Identification of sequences with more than 98% identity to each other (Table 4) has been performed using Cd-hit (Li and Godzik, 2006).

3. Results

3.1. Allergic patients with a defined IgE response against a protein allergen

Most of the allergic subjects exhibit IgE reactivity against a plethora of different allergenic proteins. In order to identify allergic subjects with IgE reactivity against one defined allergen we screened allergic patients for IgE antibody responses against 46 allergen sources covering the allergen repertoire in Europe and identified subjects who were monosensitized to birch pollen. Using recombinant Bet v 1 we finally identified three genetically unrelated subjects B, D, H (B: HLA-A *02, *32; HLA-B *39, *51; HLA-Cw *07, *14; HLA-DR *03, *08; HLA-DQ *02, *04; D: HLA-A *01, *02; HLA-B *08, *51; HLA-Cw *07, *15; HLA-DR *03, *07; HLA-DQ *02; H: HLA-A *01, *30; HLA-B *13, *44; HLA-Cw *06, *07; HLA-DR *11, *13; HLA-DQ *03, *06) with exclusive IgE reactivity to Bet v 1. The three subjects suffered from respiratory allergy to birch pollen in spring. They had never received any form of allergen-specific immunotherapy. Their Bet v 1-specific immune response therefore must have been induced by natural allergen exposure. Pre-adsorption of their

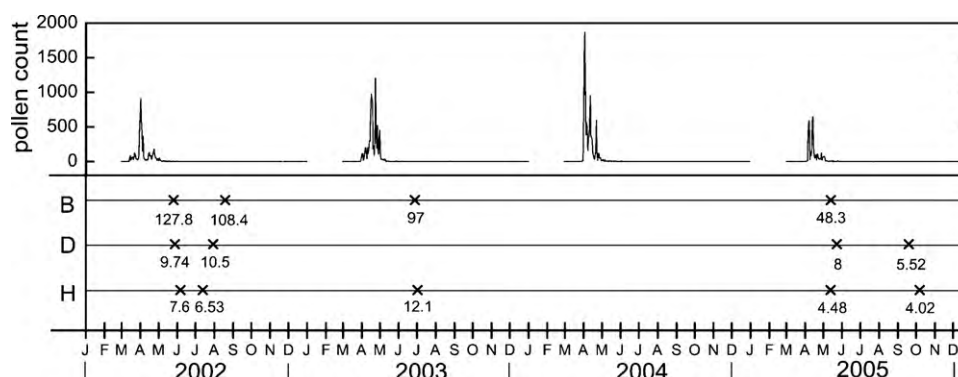


Fig. 1. Allergen exposure and allergen-specific IgE levels in the four years study period. Birch pollen exposure expressed as pollen counts per m³ of air (y-axis: pollen count) in the years 2002–2005 (x-axis: months are abbreviated). The time points when PBMCs were obtained from allergic subjects (B, D, H) for the characterization of IgE IGHV regions are indicated (x) and the IgE levels specific for the major birch pollen allergen, Bet v 1 are displayed in kUA/L.

Table 1

IgE antibodies in the allergic subjects B, D and H are mainly directed to Bet v 1. Quantitative measurements of IgE levels (kUA/L) against inhalant allergens (sx1), food allergens (fx5), birch pollen, rBet v 1 and total IgE before (pre) and after (post) pre-adsorption of sera with rBet v 1 in the allergic subjects (B, D, H). The percentage inhibition of IgE achieved by pre-adsorption with rBet v 1 is displayed. The cut-off level for the detection of specific IgE is 0.35 kUA/L.

	B	D	H
sx1-pre	41.1	5.79	4.53
sx1-post	0.36	0.37	0
Inhibition	99.12%	93.61%	100%
fx5 pre	neg	neg	neg
fx5 post	neg	neg	neg
Inhibition	–	–	–
Birch pollen pre	74.7	10.1	5.6
Birch pollen post	0	0	0
Inhibition	100%	100%	100%
rBet v 1 pre	65.7	9.78	4.92
rBet v 1 post	0	0	0
Inhibition	100%	100%	100%

sera with rBet v 1 depleted completely IgE antibodies against Bet v 1 and birch pollen extracts and either completely depleted IgE antibodies specific for a respiratory allergen mix (subject H) or reduced IgE levels close to the cut off detection level (0.35 kUA/L) for subjects B and D (Table 1). No IgE antibodies were detected against a food allergen mix in any of the three individuals (B, D, H; Table 1). The subjects were studied over a period of four years (2002–2005) with seasonal pollen exposure. The registration of pollen counts during the full study in the living area of the subjects demonstrates that they have been exposed to birch pollen in the spring of each year with the pollen exposure being lowest in spring 2005 (Fig. 1). Bet v 1-specific IgE antibodies were detected in serum of the three individuals and symptoms of respiratory birch pollen allergy were recorded in the first two years and at the end of the study (2005).

Table 2

Oligonucleotides used for RT-PCR amplification of cDNAs coding for IgE variable regions. Oligonucleotides used for RT-PCR amplification of cDNAs coding for IgE variable regions. Names, specificities and sequences of the PCR primers are displayed. Eco RI restriction sites are underlined.

Name	Specificity	Sequence 5'–3'
VH1	hu VH1 gene family	(GGA ATT) CAC TCC CAG GTG CAG CTG CTC GAG TCT GG
VH2	hu VH2 gene family	(GGA ATT C)GT CCT GTC CCA GGT CAA CTT ACT CGA GTC TGG
VH3	hu VH3 gene family	(GGA ATT C)GT CCA GGT GGA GGT GCA GCT GCT CGA GTC TGG
VH4	hu VH4 gene family	(GGA ATT C)GT CCT GTC CCA GGT GCA GCT GCT CGA GTC GGG
VH5	hu VH5 gene family	(GGA ATT C)GT CTG TGC CGA GGT GCA GCT GCT CGA GCT CGG
VH6	hu VH6 gene family	(GGA ATT C)GT CCT GTC ACA GGT ACA GCT GCT CGA GTC AGG
IgEC1	hu ε-chain first constant region	(GAG AGG AAT TC)G CTA CTA GTT TTG TTG TCG ACC CAG TCT GTG

3.2. Bet v 1-allergic subjects use a relatively limited and similar IgE-IGHV gene repertoire

From peripheral blood mononuclear cells of the three allergic subjects 296 IgE transcripts (2002: $n = 121$; 2003: $n = 2$; 2005: $n = 173$) were sequenced (B: $n = 130$; D: $n = 136$; H: $n = 30$). Among the 296 sequences, 152 non-redundant sequences (B: $n = 99$; D: $n = 27$; H: $n = 26$) were identified and compared with the germ line sequences deposited in the IMGT (Giudicelli et al., 2004) and SoDA (Volpe et al., 2006) database. Non-redundant sequences have been submitted to GenBank and accession numbers are displayed in the Supplemental list 1. Each of these sequences was derived from IgE-encoding transcripts due to the use of a 3'-primer located in the end of DNA coding for the first constant region of IgE. The presence of the IgE-encoding DNA in each of the sequences was verified by sequence comparison.

Fig. 2 displays the frequencies of the closest related V-genes for each subject and each point of time. The human germ line IGHV locus contains in principle 129 genes belonging to 7 subgroups of which 45 have been reported as functional genes (Pallares et al., 1999). We found that the three Bet v 1-allergic subjects used only 17 different IGHV genes belonging to three IGHV families (IGHV1, 3 and 4) (Fig. 2). From the 9 functional IGHV1 genes 3 were used. Eight of the 20 functional IGHV3 genes were utilized and 6 of the 10 IGHV4 genes. IGHV3-23 ($n = 55$) was the most frequently used IGHV gene among the sequenced clones followed by IGHV4-61 ($n = 15$), IGHV1-2 ($n = 11$) and IGHV4-31 ($n = 11$) (Fig. 2). Several IGHV genes were used by each of the three subjects. They included IGHV3-15, IGHV3-23, IGHV4-4, IGHV4-31, IGHV4-39 and IGHV4-61. The findings are therefore consistent with previous observations according to which IGHV3-23 is the most commonly used human V-gene (Brezinschek et al., 1997).

The human germ line IGHD locus comprises 27 (Lefranc, 2001) genes on chromosome 14 of which 19 were used by the three subjects. More than two-thirds of all D genes could be identified. IGHD

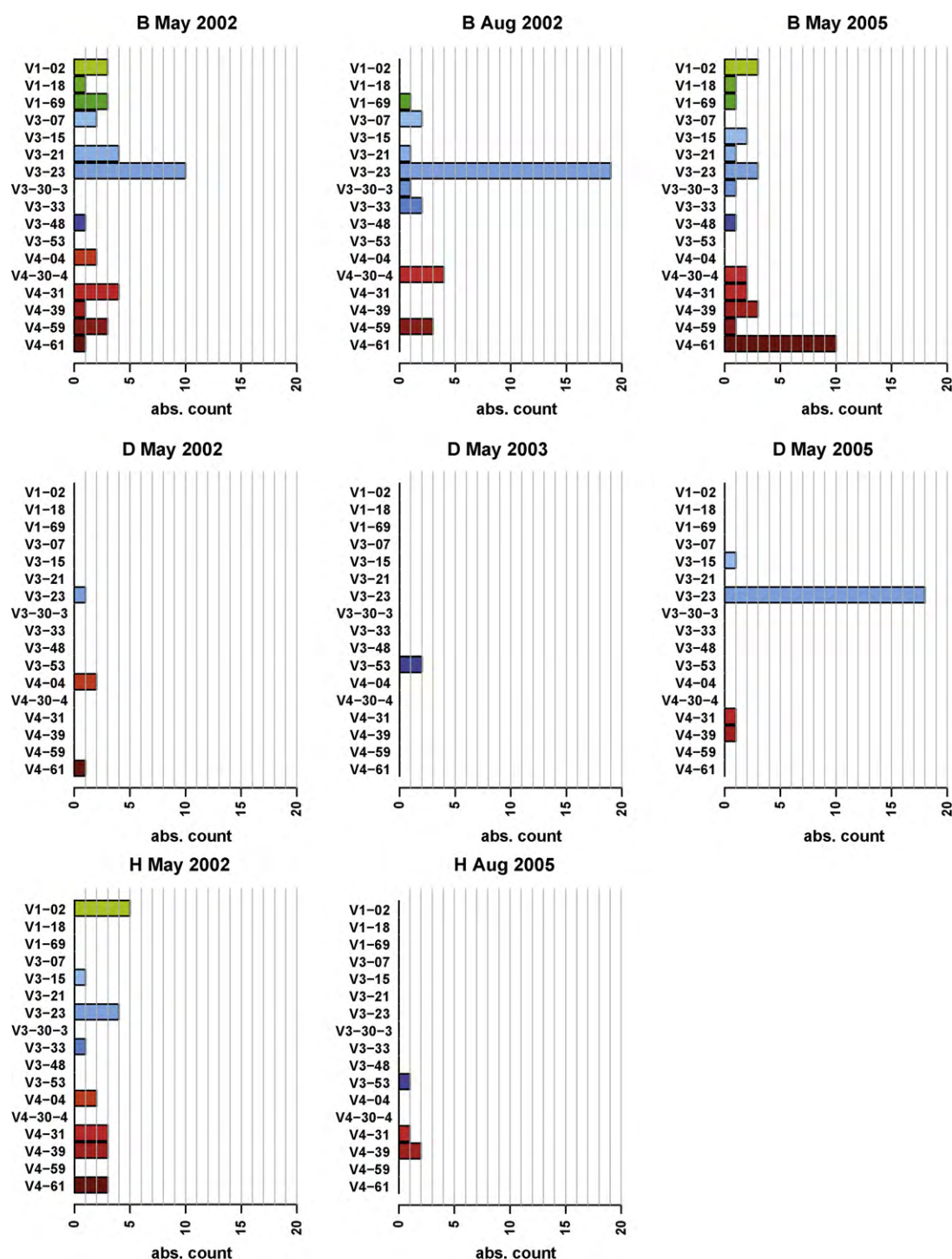


Fig. 2. Frequency of the usage of V genes in the IgE repertoires of the allergic subjects over a four years period. Frequencies (x-axes: absolute numbers of non-redundant sequences) of V genes are displayed for allergic subjects (B, D, H) at different dates (top of the figures).

3-3 was the most frequently used D gene and was found in 20% of the Bet v 1 specific IgE sequences (data not shown). Regarding the D genes it has to be noted that the annotation of the transcripts to the closest germ line sequences was hampered by the high mutation rates in this part of the transcripts and their short length. From the six J genes described for the human IGHJ locus (Lefranc, 2001), five were used by the Bet v 1-allergic subjects (data not shown). J4 was by far the most frequently J gene used ($n=88$) followed by J6 and J3. The results obtained for the usage of D and J genes were thus comparable to those obtained in a study analyzing the

heavy chain repertoire of human peripheral B cells by single cell PCR (Brezinschek et al., 1995).

3.3. Usage of similar IgE-VH genes in Bet v 1-allergic subjects during four years of allergen exposure

Fig. 2 shows that most of the identified V-genes are used in 2002 and after three additional seasons of pollen exposure in the year 2005. In the case of subject B PBMC were analyzed at three points of time and all but two of the V genes were used at least twice in the

Table 3

Number of sequences containing a certain number of somatic mutations displayed for the three IGHV families.

Number of somatic mutations	Number of sequences containing mutations as indicated			
	IGHV1	IGHV3	IGHV4	Sum
≤1	0	6	0	6
10	1	9	1	11
>10–30	12	57	47	116
>30	2	7	7	16
Sum	15	79	55	149

observation period. IGHV3-23 was the most frequently IGHV gene used by subject D and found in 2002 and 2005. Likewise, subject H used certain IGHV genes in 2002 and 2005 (e.g., IGHV4-31, IGHV4-39). The variation of the number of clones obtained for each IGHV gene at different time points might be due to a repertoire shift or to the fact that the primers used in 2002 and 2005 differed regarding the presence of an additional Eco RI site which might have affected PCR amplification.

3.4. Varying degree of somatic mutations in IgE variable regions: some IgE variable regions are highly mutated whereas others contain no somatic mutations

A comparison of the IgE variable DNA sequences with germ line sequences was performed in order to analyze the presence of somatic mutations in the FR1, FR2, FR3, CDR1 and CDR2. Since in the CDR3 somatic mutations cannot be unambiguously distinguished from junctional diversity, this part was not included in the analysis. The [Supplemental Table 1](#) shows the detailed frequency and distribution of somatic mutations in each of the 152 IgE variable regions. Replacement and silent mutations are also shown there. [Table 3](#) gives an overview how many variable regions of each IGHV family contained a certain number of somatic mutations in the FR1, FR2, FR3, CDR1 and CDR2 together. The majority of variable regions contained >10–30 somatic mutations (i.e., 116 out of 149 sequences). Variable regions with a number of somatic mutations between >10–40 were evenly distributed among the three IGHV families (IGHV1: 12/15; IGHV3: 57/77; IGHV4: 47/55). In each of the three IGHV families sequences containing more and less somatic mutations were detected. Interestingly, 6 sequences, which contained 1 or 0 mutations, were found in the IGHV3 family-derived sequences ([Table 3](#)). Silent as well as non-silent somatic mutations were evenly distributed over the CDR's as well as in the FR's in the analyzed sequences ([Supplemental Table 1](#)). Three sequences belonging to the IGHV1 family have been excluded from mutation analysis due to high differences in their length.

3.5. Highly similar IgE IGHV transcripts can be detected in genetically unrelated patients and in the same patient after several years

When we performed a multiple sequence alignment of all non-redundant IgE variable regions ([Supplemental Figure 1](#)) we found several variable region-encoding cDNAs with more than 98% identity in different patients and in the same patient after four years of allergen exposure. In patients D and H several highly similar IGHV sequences were retrieved, e.g. sequence D191218 IGHV3-15 that was isolated from patient D in May 2005 was highly similar to sequence H12125 (patient H) isolated in May 2002 ([Table 4](#)). Likewise, sequence H12115 (May 2002) and sequence D19111 (May

Table 4

Identification of IgE sequences in different patients and different years (cluster 1 and cluster 2) with an identity of more than 98% to each other. The sequences have been grouped according to IGHV subgroups (IGHV1: green; IGHV3: blue; IGHV4: red) and the sequence identities with the corresponding germline sequences are displayed. Designations of IgE variable regions and the colour codes correspond to those in the [Supplemental Figure 1](#). The first letter indicates the patient (B, D, H) and the time point of blood sampling is identified by the second number (1, 2, 3: May 2002; 4: August 2002; 8, 9: May 2005).

IGHV Gene	Cluster 1	Cluster 2
V1-02	B May 2002 B11229 (92%) B31214 (91%)	B May 2005 B383114 (92%) B59115 (91%)
V1-02	H May 2002 H12113 (62%) H42212 (61%)	B May 2005 B19113 (62%)
V1-18	B May 2002 B111126 (91%)	B May 2005 B383343 (91%)
V1-69	B May 2002 B112113 (93%)	B May 2005 B38419 (93%)
V3-15	H May 2002 H12125 (91%)	D May 2005 D191218 (91%)
V3-23	B May 2002 B311124 (88%) B311128 (88%) B311120 (88%)	B May 2005 B38336 (87%)
V3-23	B May 2002 B31211 (94%)	B May 2005 B383124 (95%)
V3-23	H May 2002 H12126 (94%) H12115 (94%)	D May 2005 members 18: a)
V3-48	B May 2002 B31118 (92%)	B May 2005 B38335 (92%)
V4-30-4	B Aug 2002 B441313 (91%)	B May 2005 B38414 (91%)
V4-30-4	B Aug 2002 B441216 (94%)	B May 2005 B59114 (93%)
V4-31	H May 2002 H42314 (96%) H42311 (96%)	D May 2005 D691213 (96%)
V4-31	B May 2002 B111213 (83%) B431111 (83%)	B May 2005 B491129 (84%) B383311 (83%)
V4-39	H May 2002 H42125 (92%) H12131 (92%) H12135 (93%)	D May 2005 D691110 (92%)

^aD591220 (95%), D191227 (94%), D691227 (94%), D49122 (94%), D59112 (93%), D591217 (95%), D591218 (94%), D59122 (94%), D691225 (94%), D191216 (94%), D591216 (94%), D691230 (94%), D191210 (94%), D59111 (94%), D59127 (94%), D19111 (94%), D191224 (94%), D49117 (93%).

2005), D691110 isolated from patient D in May 2005 and H12131 (patient H) from May 2002 ([Table 4](#)) showed more than 98% identity.

Interestingly, variable region transcripts with more than 98% identity were also found to occur in the very same patient but at different points of time. For example, transcript B111126 (IGHV1-18) detected in patient B in May 2002 was isolated as identical sequence (B383343) in May 2005. Additionally some other sequences, e.g. B11229-IGHV1-2 May 2002; B31214-IGHV1-2 May 2002; B11216-IGHV4-31 May 2002; B441313-IGHV4-30-4

August 2002, which had been found to reach more than 98% identity (Table 4 and Supplemental Figure 1).

4. Discussion

We have analyzed the IgE variable sequences in allergic subjects who were sensitized against one defined allergen, the major allergen of birch pollen Bet v 1 (Breiteneder et al., 1989). Similar as for most other important respiratory allergens, IgE antibodies of allergic patients recognize exclusively conformational epitopes on the Bet v 1 allergens which require the intact three-dimensional structure of the protein for IgE recognition (Valenta and Kraft, 2001). The IgE response to Bet v 1 thus resembles the features of typical IgE responses to other important respiratory allergens from pollens, house dust mites, animal dander and moulds. Since allergic subjects mostly exhibit IgE antibody reactivity against several independent allergen molecules allergic patients were screened for “IgE mono-reactivity” to Bet v 1 and its cross-reactive allergens in order to establish a human system of allergic sensitization to one defined antigen. It can thus be assumed that most of the IgE variable sequences in these patients are specific for Bet v 1 but outliers cannot be excluded. The advantage of this human system over experimental animal systems is that it is based on natural sensitization to physiological doses (i.e., ng) of inhaled allergen, whereas most animal systems require injection of adsorbed artificial antigens or inhalation/contact with non-physiologically high doses of antigen in mostly inbred strains (Epstein, 2004). Although Bet v 1-monosensitized patients are rare in middle Europe, we do not think that the patients studied by us represented a unique subgroup of allergic patients. Similar as poly-sensitized patients our patients did not change their molecular reactivity profile over time. Their clinical symptoms to birch pollen were not different from those of poly-sensitized patients and each of them had a different genetic background. Interestingly, we found highly similar IgE variable regions in the three different patients. Moreover, these sequences reappeared unchanged in the same patients again after several years of pollen exposure. We interpret this finding as a molecular imprint of the antigenic signature of the Bet v 1 allergen in the human IgE repertoire. Interestingly, we found a rather limited usage of IGHV sequences in the allergic subjects which has been suggested also for grass pollen allergic patients (Andreasson et al., 2006). In eight blood samples taken over a period of four years only 152 different non-redundant VH sequences were identified among more than 296 sequenced PCR products. The non-redundant sequences belonged to three IGHV families (i.e., IGHV1, IGHV3, IGHV4) indicating that there is no bias towards the use of one certain IGHV family in the human IgE response (Bando et al., 2004; Davies and O’Hehir, 2004; Edwards et al., 2002; Eibensteiner et al., 2000; Janezic et al., 1998; Lim et al., 2007; Tilgner et al., 1997).

There is evidence that IgE antibodies bind much tighter to allergen than IgG antibodies and that they recognize epitopes which are either different from IgE epitopes or overlap only partially (Niemi et al., 2007). The latter would explain why allergen-IgE immune complexes could form even in the presence of higher amounts of allergen-specific IgG antibodies in allergic patients. It was therefore quite surprising to discover also a considerable number of IgE variable sequences containing few or almost no somatic mutations, which are thought to contribute to high affinity binding.

There is evidence that the secondary IgE response is strongly triggered by allergen contact but this response seems to be already independent of T cell help (Linhart et al., 2007; Niederberger et al., 2007).

The identification of identical and highly similar IgE variable sequences at different points of time (i.e., after several periods of allergen contact) may be interpreted in at least two ways. Either

allergen contact reactivates a pool of IgE memory cells that then undergo development to IgE-secreting plasma cells. Consistent with this assumption it has been observed that allergen-contact strongly boosts allergen-specific IgE production in allergic subjects (Niederberger et al., 2007). It is also possible that an established pool of IgE-secreting plasma cells is the main source for continuous IgE production (Radbruch et al., 2006) but this hypothesis would not explain the strong boosts of IgE production which occur after allergen contact because plasma cells do not respond to antigen contact (Niederberger et al., 2007).

The fact that the allergen-specific IgE repertoire is rather limited opens interesting possibilities for therapeutic approaches targeting the allergen-specific IgE production. It is well established that the number of important disease-eliciting allergens is limited and most of the relevant allergens have been isolated and characterized down to their three-dimensional structure (Valenta, 2002; Valenta and Kraft, 2001). It should thus be possible to characterize the corresponding IgE variable sequences and to develop allergen mimicks, which target as anti-idiotypic reagents specifically IgE producing cells. Such a therapeutic approach would allow eliminating selectively those IgE producing cells that are responsible for allergic symptoms in patients and leave IgE specificities with potentially physiological roles untouched.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the data presented in the paper. R.V. is a scientific consultant of Phadia, Uppsala, Sweden and Biomay, Vienna, Austria.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2010.05.285.

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Supplemental Table I. Distribution of somatic mutations in the non-redundant IgE variable sequences. IgE variable sequences are named and grouped according to IGHV subgroups. The first letter identifies the patients (B, D, H) and the second number identifies the time point of blood sampling (1, 2, 3: May 2002; 4: August 2002; 8, 9: May 2005). IGHV genes are displayed, total number of replacement (R) and silent (S) mutations as well as for the FR's and CDR's are shown. The total number of R+S mutations and the ratio of R/S mutations is also indicated. Sequences containing a certain number of somatic mutations are indicated in a colour code (dark green: ≤ 1 ; light green: 2-10; white: >10-30; blue >30). Grey highlighted sequences have been excluded from mutation analysis.

name	IGHV gene	VH-R	FR1-R	CDR1-R	FR2-R	CDR2-R	FR3-R	VH-S	FR1-S	CDR1-S	FR2-S	CDR2-S	FR3-S	R+S	R/S
B111126	IGHV1-18	12	1	0	2	3	6	10	1	2	1	0	6	22	1.20
B383343	IGHV1-18	12	1	0	2	3	6	10	1	2	1	0	6	22	1.20
B111114	IGHV1-2	6	0	1	0	3	2	3	0	0	0	0	3	9	2.00
B11229	IGHV1-2	17	4	1	3	2	7	4	1	0	1	0	2	21	4.25
B19113	IGHV1-2														
B31214	IGHV1-2	19	4	2	3	2	8	3	1	0	1	0	1	22	6.33
B383114	IGHV1-2	17	4	1	3	2	7	4	1	0	1	0	2	21	4.25
B59115	IGHV1-2	19	4	2	3	2	8	3	1	0	1	0	1	22	6.33
H12113	IGHV1-2														
H121318	IGHV1-2	25	2	4	4	4	10	7	2	0	0	0	4	30	3.57
H12311	IGHV1-2	21	1	3	5	4	8	8	0	1	0	3	4	29	2.63
H12313	IGHV1-2	22	1	3	5	4	9	8	0	1	0	3	4	30	2.75
H42212	IGHV1-2														
B112113	IGHV1-69	9	0	2	0	3	4	9	3	0	0	1	5	18	1.00
B11222	IGHV1-69	25	3	6	3	5	8	8	1	0	3	2	2	33	3.13
B11227	IGHV1-69	30	3	3	5	1	18	11	2	1	1	1	6	41	2.73
B15345	IGHV1-69	13	1	3	1	2	6	8	2	1	2	2	1	21	1.63
B38419	IGHV1-69	9	0	2	0	3	4	9	3	0	0	1	5	18	1.00
B383316	IGHV3-15	5	0	2	1	1	0	3	0	1	0	0	2	7	1.67
B59112	IGHV3-15	8	1	0	0	0	7	5	0	0	0	0	5	13	1.60
D191218	IGHV3-15	15	0	2	0	3	8	9	3	0	0	1	5	22	1.67
H12125	IGHV3-15	15	0	2	0	3	8	9	3	0	0	1	5	22	1.67
B11111	IGHV3-21	0	0	0	0	0	0	0	0	0	0	0	0	0	-
B31113	IGHV3-21	10	0	0	2	2	5	9	2	1	0	1	5	18	1.11
B311134	IGHV3-21	1	0	0	1	0	0	0	0	0	0	0	0	1	-
B312110	IGHV3-21	2	0	0	1	0	1	0	0	0	0	0	0	2	-
B353413	IGHV3-21	17	3	4	3	2	5	5	2	1	0	0	2	22	3.40
B38333	IGHV3-21	0	0	0	0	0	0	0	0	0	0	0	0	0	-
B11214	IGHV3-23	12	1	4	0	0	5	3	0	0	0	0	3	13	4.00
B151213	IGHV3-23	8	0	0	3	2	3	3	0	1	0	0	2	11	2.67
B15321	IGHV3-23	14	1	2	0	4	7	12	2	0	1	2	7	26	1.17
B153410	IGHV3-23	8	0	0	2	3	3	10	1	0	1	3	5	18	0.80
B15412	IGHV3-23	6	0	1	0	3	2	2	1	0	1	0	0	8	3.00
B311120	IGHV3-23	21	2	1	3	7	7	11	2	1	0	2	6	31	1.91
B311122	IGHV3-23	10	3	2	1	1	3	6	1	0	1	2	2	16	1.67

B311124	IGHV3-23	22	3	1	3	7	7	10	1	1	0	2	6	31	2.20
B311128	IGHV3-23	21	2	1	3	7	7	11	2	1	0	2	6	31	1.91
B311131	IGHV3-23	12	1	4	0	0	5	3	0	0	0	0	3	13	4.00
B311137	IGHV3-23	0	0	0	0	0	0	0	0	0	0	0	0	0	-
B31114	IGHV3-23	8	1	1	0	4	2	4	0	1	1	0	2	12	2.00
B31211	IGHV3-23	10	3	2	1	1	3	5	0	0	1	2	2	15	2.00
B31218	IGHV3-23	27	3	3	5	8	7	9	3	1	1	1	3	35	3.00
B351116	IGHV3-23	17	4	1	4	1	7	6	2	0	0	1	3	23	2.83
B352220	IGHV3-23	13	1	2	0	4	6	12	2	0	1	2	7	25	1.08
B352228	IGHV3-23	10	0	1	1	6	2	6	1	0	2	0	2	15	1.67
B35228	IGHV3-23	21	6	2	6	0	7	7	1	1	1	1	3	28	3.00
B35229	IGHV3-23	8	0	0	3	2	3	2	0	0	0	0	2	10	4.00
B352311	IGHV3-23	10	1	1	2	3	3	12	1	1	2	3	5	22	0.83
B35233	IGHV3-23	10	0	0	1	6	3	6	1	0	2	0	2	15	1.67
B353112	IGHV3-23	5	0	1	1	1	2	2	1	0	0	1	0	7	2.50
B353120	IGHV3-23	17	4	1	4	1	7	6	2	0	0	1	3	23	2.83
B35324	IGHV3-23	10	0	0	1	6	3	6	1	0	2	0	2	15	1.67
B35326	IGHV3-23	10	0	0	1	5	4	3	0	0	0	0	2	12	3.33
B35331	IGHV3-23	7	0	2	2	1	2	7	1	0	1	1	3	13	1.00
B35334	IGHV3-23	8	1	3	1	2	1	2	1	0	0	0	1	10	4.00
B35335	IGHV3-23	6	0	1	2	1	2	1	0	0	0	1	0	7	6.00
B353411	IGHV3-23	23	4	3	2	4	10	10	1	1	0	1	7	33	2.30
B383124	IGHV3-23	11	3	2	2	1	3	2	0	0	1	0	1	13	5.50
B38336	IGHV3-23	22	2	1	3	7	8	13	2	3	0	2	6	34	1.69
B491121	IGHV3-23	17	0	3	3	5	6	5	1	0	1	2	1	22	3.40
D19111	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D191210	IGHV3-23	11	2	0	3	1	5	4	2	1	0	0	1	15	2.75
D191216	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D191224	IGHV3-23	10	2	0	3	1	4	5	2	1	0	0	2	15	2.00
D191227	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D333110	IGHV3-23	10	1	2	1	1	5	6	0	2	1	1	2	16	1.67
D49117	IGHV3-23	12	2	0	5	1	4	4	2	1	0	0	1	16	3.00
D49122	IGHV3-23	11	2	0	4	1	4	4	2	1	0	0	1	15	2.75
D59111	IGHV3-23	11	3	0	3	1	4	4	2	1	0	0	1	15	2.75
D59112	IGHV3-23	11	3	0	3	1	4	5	3	1	0	0	1	16	2.20
D591216	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D591217	IGHV3-23	8	0	0	3	1	4	3	1	1	0	0	1	11	2.67
D591218	IGHV3-23	9	1	0	3	1	4	4	2	1	0	0	1	13	2.25
D59122	IGHV3-23	8	0	0	3	1	4	3	1	1	0	0	1	11	2.67
D591220	IGHV3-23	8	0	0	3	1	4	3	1	1	0	0	1	11	2.67
D59127	IGHV3-23	11	3	0	3	1	4	4	2	1	0	0	1	15	2.75
D691225	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D691227	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
D691230	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
H12115	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
H12117	IGHV3-23	22	4	4	0	5	9	9	0	1	1	3	4	31	2.44
H12126	IGHV3-23	10	2	0	3	1	4	4	2	1	0	0	1	14	2.50
H12127	IGHV3-23	22	4	4	0	5	9	8	0	1	1	2	4	30	2.75
B352313	IGHV3-30-3	14	4	1	3	3	3	7	1	3	2	0	1	21	2.00
B38126	IGHV3-30-3	5	0	1	1	1	2	5	0	0	1	1	3	10	1.00
B15221	IGHV3-33	8	0	1	0	3	4	2	0	0	0	0	2	10	4.00
B15325	IGHV3-33	7	1	2	0	1	3	2	0	0	0	0	2	9	3.50
H12111	IGHV3-33	19	3	4	1	2	9	8	2	0	0	2	4	27	2.38
B31118	IGHV3-48	16	2	3	1	6	4	6	1	0	2	1	1	21	2.67
B38335	IGHV3-48	14	1	3	0	6	4	6	1	0	2	1	1	19	2.33
D36116	IGHV3-53	4	0	1	0	1	2	5	1	1	1	1	1	9	0.80
D36313	IGHV3-53	8	0	1	1	3	3	7	1	0	1	2	3	15	1.14
H310117	IGHV3-53	8	0	2	1	4	1	5	1	0	0	1	3	13	1.60
B11123	IGHV3-7	4	0	1	0	1	1	0	0	0	0	0	0	3	-
B31115	IGHV3-7	2	0	0	0	1	1	0	0	0	0	0	0	2	-
B35313	IGHV3-7	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00
B353412	IGHV3-7	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00
B352227	IGHV4-30-4	18	3	3	1	4	7	7	0	1	2	1	3	25	2.57

B38414	IGHV4-30-4	16	0	3	2	1	10	7	1	0	1	0	5	23	2.29
B441216	IGHV4-30-4	11	1	0	1	3	6	3	0	0	1	1	1	14	3.67
B441313	IGHV4-30-4	16	0	3	2	1	10	7	1	0	1	0	5	23	2.29
B441520	IGHV4-30-4	16	3	1	3	2	7	6	2	2	0	0	2	22	2.67
B59114	IGHV4-30-4	13	1	1	2	3	6	4	0	0	1	1	2	17	3.25
B111121	IGHV4-31	22	2	3	6	3	7	7	0	0	1	0	6	28	3.14
B111213	IGHV4-31	29	2	3	5	3	16	14	1	5	2	1	5	43	2.07
B383311	IGHV4-31	29	2	3	5	3	16	14	1	5	2	1	5	43	2.07
B431110	IGHV4-31	18	1	3	3	2	9	2	0	1	0	0	1	20	9.00
B431111	IGHV4-31	29	2	3	5	3	16	14	1	5	2	1	5	43	2.07
B491129	IGHV4-31	29	2	3	5	3	16	13	1	5	1	1	5	42	2.23
D691213	IGHV4-31	6	0	3	1	0	2	4	0	0	1	0	3	10	1.50
H12137	IGHV4-31	6	0	2	1	1	2	3	0	0	1	0	2	9	2.00
H410119	IGHV4-31	16	2	3	1	3	7	7	1	1	2	0	3	23	2.29
H42311	IGHV4-31	6	0	3	1	0	2	4	0	0	1	0	3	10	1.50
H42314	IGHV4-31	6	0	3	1	0	2	4	0	0	1	0	3	10	1.50
B391113	IGHV4-39	13	2	2	3	1	5	8	0	3	3	1	1	21	1.63
B431314	IGHV4-39	19	4	2	3	4	6	9	1	4	3	0	1	28	2.11
B491115	IGHV4-39	16	2	5	3	1	5	8	0	2	3	1	2	24	2.00
B491126	IGHV4-39	34	3	12	5	4	10	15	4	0	3	0	8	49	2.27
D691110	IGHV4-39	14	1	5	1	3	4	6	0	0	1	1	4	20	2.33
H12131	IGHV4-39	14	1	5	1	3	4	6	0	0	1	1	4	20	2.33
H12135	IGHV4-39	13	1	5	1	2	4	5	0	0	1	1	3	18	2.60
H410111	IGHV4-39	17	0	5	3	4	5	12	0	4	2	1	5	29	1.42
H4101122	IGHV4-39	17	0	5	3	4	5	12	0	4	2	1	5	29	1.42
H42125	IGHV4-39	13	1	4	1	3	4	7	0	1	1	1	4	20	1.86
B111112	IGHV4-4	8	1	1	1	2	3	8	0	1	3	1	3	16	1.00
B43135	IGHV4-4	9	0	4	1	1	3	2	1	0	0	0	1	11	4.50
D331124	IGHV4-4	11	2	1	1	3	4	2	0	0	1	0	1	13	5.50
D43112	IGHV4-4	12	1	1	2	3	5	2	0	0	0	1	1	14	6.00
H422112	IGHV4-4	13	0	2	3	1	7	3	0	0	0	0	3	16	4.33
H423110	IGHV4-4	14	0	2	3	1	8	3	0	0	0	0	3	17	4.67
B11125	IGHV4-59	14	2	3	1	3	5	4	0	1	1	1	1	18	3.50
B15235	IGHV4-59	16	0	7	2	4	3	13	2	0	4	3	4	29	1.23
B15344	IGHV4-59	10	0	0	1	4	5	3	0	0	1	0	2	13	3.33
B31212	IGHV4-59	10	0	3	1	3	3	2	0	0	0	0	2	12	5.00
B432119	IGHV4-59	14	2	3	1	3	5	4	0	1	1	1	1	18	3.50
B441229	IGHV4-59	11	1	2	0	3	5	5	1	0	2	0	2	16	2.20
B491130	IGHV4-59	16	2	4	1	2	7	6	0	2	1	2	1	22	2.67
B43119	IGHV4-61	21	2	5	3	3	8	6	1	0	1	0	4	27	3.50
B49111	IGHV4-61	21	1	3	5	4	8	7	0	2	1	1	2	27	3.00
B491114	IGHV4-61	19	2	8	0	2	7	8	0	1	1	1	4	26	2.38
B491118	IGHV4-61	21	2	8	0	2	9	7	0	1	1	1	3	27	3.00
B491119	IGHV4-61	22	2	8	0	2	10	7	0	1	1	1	3	28	3.14
B49112	IGHV4-61	21	2	5	0	1	13	11	1	1	2	0	7	32	1.91
B491122	IGHV4-61	21	2	5	0	1	13	11	1	1	2	0	7	32	1.91
B49113	IGHV4-61	21	2	8	0	2	9	7	0	1	1	1	3	27	3.00
B49116	IGHV4-61	21	1	3	5	4	8	8	0	2	1	1	3	28	2.63
B49118	IGHV4-61	22	2	3	5	4	8	7	0	2	1	1	2	28	3.14
B69111	IGHV4-61	23	2	9	0	2	10	8	1	1	1	1	3	30	2.88
D43116	IGHV4-61	11	0	4	0	4	3	10	0	0	3	1	6	21	1.10
H12136	IGHV4-61	14	1	3	3	3	3	6	1	2	1	0	2	19	2.33
H422119	IGHV4-61	15	1	3	3	3	4	6	1	2	1	0	2	20	2.50
H42316	IGHV4-61	14	1	3	3	3	3	6	1	2	1	0	2	19	2.33

Supplemental list 1:

Sequence identification number	Accession number
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B15235	GQ504853
D59122	GQ504854
B311134	GQ504855
D331124	GQ504856
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B491114	GQ504858
B31118	GQ504859
B69111	GQ504860
B111114	GQ504861
H422119	GQ504862
B31212	GQ504863
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B31214-B59115	GQ504867
B35334	GQ504868
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B111126-B383343	GQ504871
B31218	GQ504872
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B43135	GQ504874
B353112	GQ504875
B11125-B432119	GQ504876
D43112	GQ504877
D191227	GQ504878
B383124	GQ504879
D43116	GQ504880
B311131	GQ504881
B111112	GQ504882
D591217	GQ504883
B31211	GQ504884
B31113	GQ504885
B311124	GQ504886
B38336	GQ504887
D191224	GQ504888
D191210	GQ504889
B31114	GQ504890
H12135	GQ504891
B112113	GQ504892
B11123	GQ504893

D59112	GQ504894
B35326	GQ504895
B311128	GQ504896
H12117	GQ504897
H12311	GQ504898
B15344	GQ504899
B11229-B383114	GQ504900
D59127	GQ504901
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B351116	GQ504904
B49118	GQ504905
D191218-H12125	GQ504906
H12136	GQ504907
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B352227	GQ504921
B491122	GQ504922
B35229	GQ504923
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B38126	GQ504925
B491115	GQ504926
B38419	GQ504927
B49112	GQ504928
B491130	GQ504929
H42314	GQ504930
H42316	GQ504931
D691213	GQ504932
D49122	GQ504933
B441520	GQ504934
H410111	GQ504935
B153410	GQ504936
B35335	GQ504937
B35233	GQ504938
B11111	GQ504939
H12137	GQ504940

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B59114	<u>GQ504943</u>
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H121318	<u>GQ504945</u>
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B111121	<u>GQ504952</u>
B311120	<u>GQ504953</u>
B49111	<u>GQ504954</u>
H422112	<u>GQ504955</u>
B441216	<u>GQ504956</u>
D36116	<u>GQ504957</u>
B49113	<u>GQ504958</u>
D691110-H12131	<u>GQ504959</u>
B38335	<u>GQ504960</u>
B391113	<u>GQ504961</u>
B15321	<u>GQ504962</u>
B353412	<u>GQ504963</u>
D36313	<u>GQ504964</u>
B441229	<u>GQ504965</u>
B35228	<u>GQ504966</u>
B312110	<u>GQ504967</u>
D591218	<u>GQ504968</u>
B43119	<u>GQ504969</u>
H12111	<u>GQ504970</u>
H423110	<u>GQ504971</u>
D59111	<u>GQ504972</u>
B491119	<u>GQ504973</u>
D591216	<u>GQ504974</u>
B35313	<u>GQ504975</u>
H12126	<u>GQ504976</u>
H42125	<u>GQ504977</u>
D691225	<u>GQ504978</u>
B35324	<u>GQ504979</u>
B59112	<u>GQ504980</u>
B353413	<u>GQ504981</u>
D333110	<u>GQ504982</u>
D691227	<u>GQ504983</u>
B353120	<u>GQ504984</u>
B311137	<u>GQ504985</u>
B431110	<u>GQ504986</u>
D191216	<u>GQ504987</u>

D691230	<u>GQ504988</u>
B491129	<u>GQ504989</u>
H42212	<u>GQ504990</u>
B19113-H12113	<u>GQ504991</u>