

Clonally related IgE and IgG4 transcripts in blood lymphocytes of patients with asthma reveal differing patterns of somatic mutation

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Isotype switching to IgE contributes to atopic asthma, therefore strategies to divert this process to alternative isotypes could have therapeutic relevance. It is known that patients with allergic disease have serum IgE and IgG4 antibodies with similar specificities, and that cytokines such as IL-4 mediate switching to both of these isotypes. Availability of variable region gene analysis has allowed us to probe isotype variants at the single-cell level. An earlier report described identification in a single atopic patient of short transcripts with a common complementarity-determining region "clonal signature" in combination with C μ , C γ 4 and C ϵ . We have extended this analysis, and have identified V μ -C γ 4 transcripts with clear clonal relationship to IgE-derived sequences in blood lymphocytes from three of four patients with atopic asthma. No other IgG subclasses were detected, confirming the link between IgE and IgG4. Full sequences were obtained from each clonally related isotype in all patients, and showed extensive somatic mutation. As previously found for IgE, the IgG4 isotypes had evident intraclonal variation. There were shared mutations between isotypes, but also many differences, indicative of separate cell populations with divergent mutational histories. These findings indicate that, in atopic patients, an individual B cell commonly switches to either IgE or IgG4. Cells producing each isotype then co-exist in the recirculating pool, and the balance between them may influence the disease process.

Key words: IgE / IgG4 / Somatic mutation / V μ gene

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1 Introduction

IgE antibodies have a central role in allergic disease, with elevated levels of both total and allergen-specific IgE in serum of atopic patients [1, 2]. This is frequently paralleled by high levels of total and allergen-specific IgG4 [3]. The role of IgG4 Ab in allergic disease is still unclear, but some studies have found a significant negative correlation between symptom score and IgG4 Ab titer following immunotherapy [4, 5]. It is possible that non-reaginic Ag-specific IgG4 Ab may have a protective role as blocking Ab that neutralize allergens [6–8], as has been demonstrated for parasite-specific IgG4 in helminthic infections [9].

Isotype switching of B cells to both IgE and IgG4 is induced by two signals: the Th2-type cytokines IL-4 or IL-13, and CD40:CD40 ligand interaction between B and T cells. It is unknown if a choice is made during isotype switching between IgE and IgG4 or if their expression is co-regulated. Recently, IL-7 has been found to potentiate IL-4-induced class switching to both IgE and IgG4 [10]. The same group has demonstrated that IL-10 can decrease IL-4-induced switching to IgE and increase switching to IgG4, and may have an additional influence on secretion of these isotypes [10]. There are other levels of control that differentially regulate production, with high IFN- γ :IL-4 ratios enhancing IgG4 and suppressing IgE, while lower levels of IFN- γ and increased IL-4 or IL-13 enhance IgE [11, 12]. A recent study found that different T cell subsets from the peripheral blood of patients with atopic dermatitis could induce IgE or IgG4 production from purified B cells. The activated skin-homing T cells secreted high levels of IL-13 and induced IgE production, while, in contrast, resting T cells without the skin-

[18175]

Abbreviations: CDR: Complementarity determining region
FWR: Framework region

homing receptor secreted high levels of IFN- γ and induced IgG4 production [12].

One interesting question is whether different cohorts of B cells are producing the IgG4 and IgE or if B cells derived from the same cell of origin are involved. If they are produced by clonally related cells they may have been stimulated by a common Ag, and share Ag and possibly epitope specificity. This is difficult to analyze at the serological level, but can be investigated by probing the Ig V_H genes. The clonal relationship of B cells is evident from the CDR3 sequence that is unique for each B cell clone. It is encoded by D segment genes, N additions and part of J_H, forming the most diverse amino acid sequence of the V region. Gene sequences of V_H-C ϵ transcripts from allergic individuals have been analyzed by PCR and have revealed a preferential use of the V_H5 family perhaps indicative of stimulation by a superantigen [13, 14]. Repeated sequences with a common CDR3 and differing somatic mutations have also been detected in IgE [15]. Identification of intraclonal variants indicates that B cells switched to IgE are derived from only a few progenitors.

The complementarity-determining region (CDR)3 can also be used to investigate alternative isotypes. One study identified clonally related C μ , C γ 4 and C ϵ transcripts in a single individual by sequencing of the short CDR3 framework region (FWR)4 and part of the C region [16]. We have shown that existence of alternative isotypes may be a common phenomenon, with three out of four patients having clonally related IgE and IgG4 transcripts co-existing in the peripheral blood. We have extended the analysis by obtaining nucleotide sequences of the entire H chain V regions, and have been able to compare the patterns of somatic mutation in the two isotypes.

2 Results

2.1 Sequences of related V_H-C ϵ and V_H-C γ 4 transcripts

Using the approach outlined in Fig. 1, steps 1–3, we have found IgG4 transcripts clonally related to V_H5-C ϵ transcripts in three patients. It was not possible to identify clonally related transcripts for all V_H-C ϵ sequences. From a total of seven V_H5-C ϵ sequences detected from four patients (two sequences from patient 1, two from patient 2, one from patient 3 and two from patient 4), clonally related IgG4 transcripts were identified for three sequences. Clonal relationships were evident from the closely similar CDR3/FWR4 sequences of the IgG4 and IgE transcripts (Fig. 2) from the end of the 5'CDR3 primer onwards. The same J_H gene is used within each set of

clones, also indicating derivation from the same progenitor B cell: J_H3a in patient 1, J_H3b in patient 2 and J_H5b in patient 3, all of which are relatively uncommon in the expressed repertoire [17]. For patients 1 and 2 the IgG4 and IgE clones show common D segment gene alignments [18] of the same length and reading frame, which provides strong evidence for a clonal relationship (Fig. 2). In addition, there are common N additions in patient 1, at amino acids 110 (TGG) and 111 (TTT), and a shared mutation at position 112 (GCT→GGT). In patient 2 the last nucleotide of amino acid 110 (TAT) is common to both isotypes, either arising from a mutation of the D gene or an N addition. Although there is clear clonal relatedness, there are also some mutational differences between the V_H-C ϵ - and V_H-C γ 4-derived clones of patients 1 and 2 that result in different amino acids, for example in the D21-9 segment of patient 2, at positions 106 (serine) and 108 (glycine).

For patient 3, the CDR3 is quite short, therefore the 5' primer overlaps most of the D segment area, limiting the number of nucleotides available for comparison. The D segment in patient 3 is either too truncated or somatically mutated to confidently match it to a germ-line D segment using the strict criterion of ten consecutive nucleotides [18]. However, the close similarity of the sequences in the two isotypes, and the likelihood from the best matched D gene (Fig. 2) that the tryptophan at position 104 (TGG) is an N addition, confirm a common clonal origin. In addition, the same J_H5b gene is present in both transcripts. Further evidence of clonal relationship was found by the results of the PCR in the other direction (see below).

2.2 Absence of clonally related C μ , C γ and C α transcripts

No related transcripts of isotypes other than IgG4 were identified using the same approach (steps 1–3 in Fig. 1). The C region primers are routinely used with success in our laboratory [19] and the CDR3 probes amplified the appropriate V_H5-C ϵ sequence when tested. One explanation is that there was insufficient homology between the CDR3-specific primers and the CDR3 of related clones of other isotypes due to somatic mutation occurring in this region which may have prevented primer recognition and annealing. However, this is unlikely as the same primers were successful at annealing to IgG4 transcripts, and detection of only related V_H5-C ϵ and V_H5-C γ 4 clones was consistent in three different patients. Obviously, the IgM precursor B cells did exist at some earlier time but they may not have persisted in the circulating lymphocyte pool, or been present in the material sampled. Alternatively, they may have been present, but

at too low a frequency to be detected by our PCR approach.

2.3 Analysis of V_H gene sequences

In the next stage, the upstream region of the V_H -C γ 4 transcripts was sought, both to confirm the origin of the transcripts, and also to look for intraclonal variation (Fig. 1, step 4). Additional evidence was obtained for the common origin of the C ϵ and C γ 4 clones by finding identical N additions within each patient at the V_H -D junction (Fig. 2). This step was particularly important for the clones from patient 3, where enough common nucleotides were identified in the 5' end of the CDR3 to be confident of clonal relatedness, with the first two amino acids of the CDR3 probably arising from N additions. In patient 1 common replacement mutations were also

observed in both the IgE and IgG4 clones at the start of the D segment, at positions 101 (T→H) and 103 (Y→S) (Fig. 2). As expected, the same V_H5 genes were used by the related IgE and IgG4 transcripts, patients 1 and 2 using the V_{H5-51} gene and patient 3 using the V_{H32} gene. The clonal relationship of the V_H -C ϵ and V_H -C γ 4 transcripts is further underlined by the presence of common mutations in the V_H regions shown in Fig. 3 and discussed later.

2.4 Intraclonal variation in V_H5 -C γ 4 clones

In all three cases the sets of V_H5 -C γ 4 transcripts demonstrated intraclonal heterogeneity (Fig. 3). Similar intraclonal variation has been described previously for V_H5 -C ϵ transcripts [15]. In this study the limited V_H5 -C ϵ sequences identified did not show intraclonal heteroge-

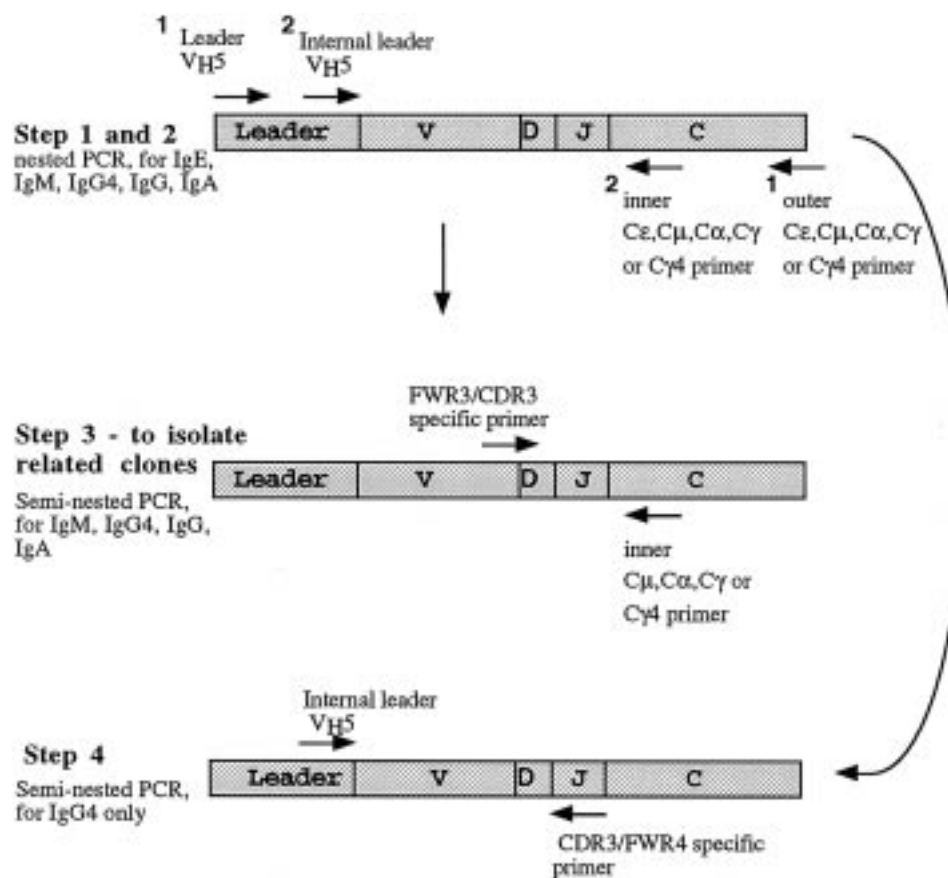


Figure 1. Outline of the position of primers used in each PCR step relative to the complete V_H -C γ 4 region. To obtain the complete IgE sequences only steps 1 and 2 were necessary. Steps 1, 2 and 3 were then used for IgM, IgG, IgG4 and IgA to look for related clones. As transcripts with the appropriate CDR3 sequence were only obtained for IgG4 in each case, step 4 was only carried out for this isotype. As indicated by the arrow, step 4 was carried out on the product from step 2. This was to obtain the V_H sequence and confirm clonal relatedness by the CDR3 sequence.

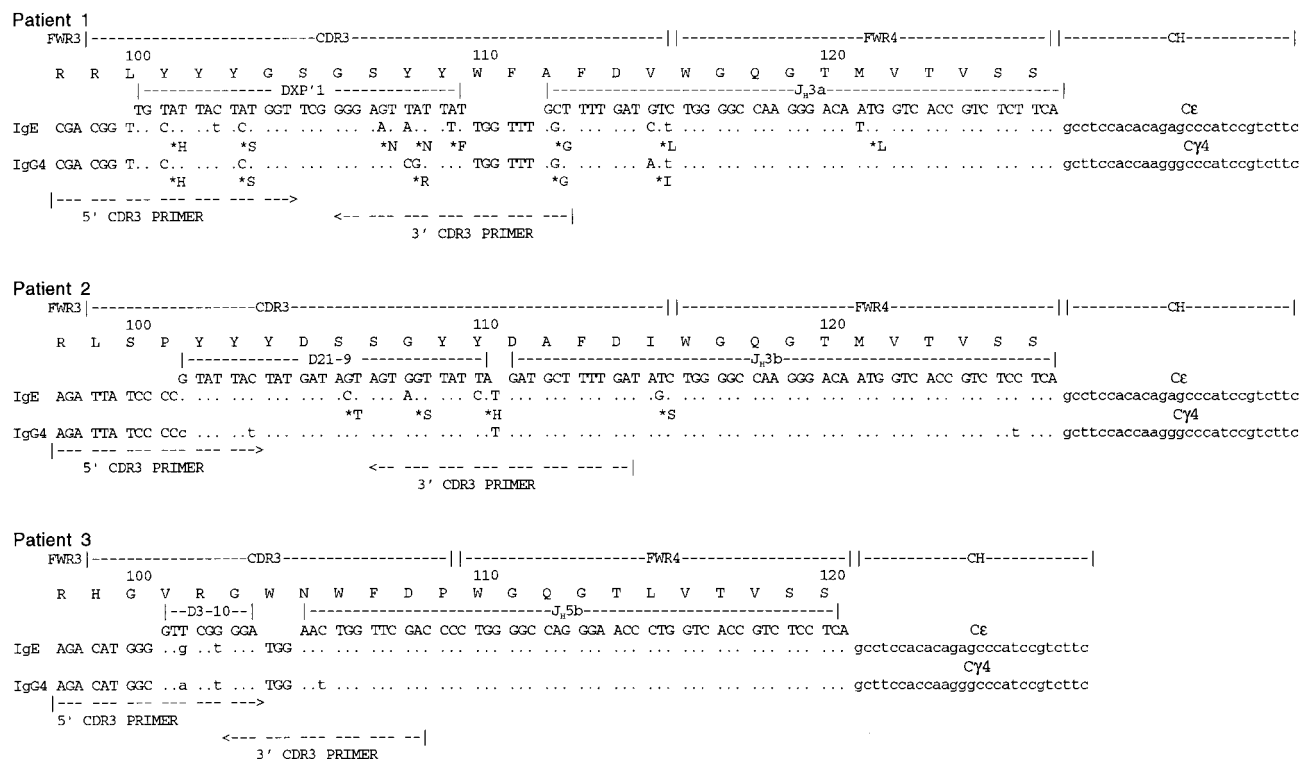


Figure 2. Nucleotide sequences of the CDR3/FWR4 regions of the IgE and IgG4 transcripts from each patient, compared with the germ line-encoded D and J_H segment genes. The closely similar CDR3 sequences of IgE and IgG4 for each patient are evidence for clonal relatedness. The sequences for IgG4 have been compiled from two different PCR reactions (CDR3-C_γ4 and V_H5L-CDR3, see Fig. 1), while the complete IgE sequences were from one PCR reaction (V_H5L-Cε). The positions of the 5' and 3' CDR3 primers used to obtain the IgG4 clones are indicated with dashed lines, with the 5' primers including the arginine from the V_H-encoded gene at the V_H-D junction. Uppercase: replacement mutation, lowercase: silent mutation, * followed by one-letter amino acid represents replacement substitutions when compared with the germ line-encoded amino acids. The amino acids are numbered from the first amino acid in V_H.

neity and therefore represent the predominant sequence, being found in 3/3, 4/4, and 5/5 clones from patients 1, 2 and 3, respectively (Fig. 3). Intracлонаl heterogeneity was more evident in IgG4 sequences (Fig. 3) in these patients. Some of the intracлонаl variants were very similar and closely related, for example sequences 1 to 4 in patient 3 (Fig. 3). Other sets differed more widely, as seen for sequence 1 in patient 1 when compared with sequences 2 and 3 (Fig. 3). Although the clones were clearly all related, there was no evidence for a step-wise accumulation of somatic mutation. There were no obvious parental clones that gave rise to daughter clones as one might expect to see within the confines of a germinal center; instead mutation appeared to accumulate randomly across the V region. It is likely that we have obtained only a limited view of mutational variability in the clones.

2.5 Analysis of mutational pattern of clonally related C_γ4 and Cε transcripts

The nucleotide sequences were compared with those of the related IgE clones. All the transcripts were aligned to the closest germ-line genes and, because the V_H5 family has little polymorphism, somatic mutations could be assessed with confidence [20]. There were common mutations found in each set of IgG4 and IgE clones, but overall the pattern of mutation was quite different for each isotype, with unique mutations in the FWR and CDR. Sets of IgG4 clones were more closely related to each other than to IgE.

Clustering of replacement amino acids in CDR1 and CDR2 is considered to be indicative of conventional Ag selection; however, a crude visual inspection of the sequences does not reveal this pattern. When the mutation patterns of the sequences were analyzed codon by

		CDR1										CDR2										CDR3													
		10	20	30	40	50	60	70	80	90																									
		↑	↑	↑	↑	↑	↑	↑	↑	↑																									
V5-51		EVQLVQSGAEVKKPGESLKISCKGSGYSFT	SYWIG	WVRQMPGKGLEWNG	IIPGDSITRYSPSPQG	QVTISADKSISTAYLQWSSLKASDTAMYYCAR																													
Patient 1																																			
IgE		-----pg-s-----FT--	-----g	V-----G--A-----f-----	-----T-----	-----r																RLHYSGSGNFWF										GFDL	WGQGTILTVSS	JH3a	
IgG4		1	--qVL-----p-----s--A--Ef	N-----	g--Pe--A	M-----T-----yG	q--F-----V-----l--N--R-----G-y--r																RLHYSGSGSRYWF										GFDI	WGQGTIMTVSS	JH3a
		2	-----p-e-----T-----	Ty-----	q-----	A-Nt-y-q-	--Ii-V-N--T--l--N--R-----y--r																RLHYSGSGSRYWF										GFDI	WGQGTIMTVSS	JH3a
		3	-----S--lS-----S	TD-----	q--g-----	A-Nt-y-q-	--Ii-V-N--T--l--N--R-----y--r																RLHYSGSGSRYWF										GFDI	WGQGTIMTVSS	JH3a
Patient 2																																			
IgE		-----F-----S	-----NM-----	d-----	H-----	S-----l-----R-----F-----																LSPYYDTSSYH										DAFDS	WGQGTIMTVSS	JH3b	
IgG4		1	-----	-----g-----	-----V-----	-----S-----l-----F-----																LSPYYDSSGY										DAFDI	WGQGTIMTVsS	JH3b	
		2	-----P--S--N--I-----	-----V-----	-----SN--l-----F-----																LSPYYDSSGY										DAFDI	WGQGTIMTVsS	JH3b		
		3	-----Q-F--s-----	-----Q-----	-----S-----	-----F-----																LSPYYDSSGY										DAFDI	WGQGTIMTVsS	JH3b	
		4	-----NH-----	-----Q-----	-----S--V--N--R-----	-----F-----																LSPYYDSSGY										DAFDI	WGQGTIMTVsS	JH3b	
Patient 3																																			
VH32		↑	↑	↑	↑	↑	↑	↑	↑	↑																									
		EVQLVQSGAEVKKPGESLRISCKGSGYSFT	SYWIS	WVRQMPGKGLEWNG	RIDPSDSYTYSPSPQG	HVTISADKSISTAYLQWSSLKASDTAMYYCAR																													
Patient 3																																			
IgE		--H-----k-----Q--yK-S	NF--T-----	r-----	QF-----	-----T-----LNt-l--s-----I-----																HGVRGW										NMFDP	WGQGTILTVSS	JH5b	
IgG4		1	---L-s-----QA--R--	N-----V-----	---G-s-Q-----f-----	-----T--l-----I-y--																HGVRGW										nMFDP	WGQGTILTVSS	JH5b	
		2	---L-s-----QA--R--	N-----V-----	---G-s-Q-----f-----	-----T--l-----GI-y--																HGVRGW										nMFDP	WGQGTILTVSS	JH5b	
		3	---L-s--M-----r-----QA--R--	N-----I-y--	---G-s-Q-----f-----	-----T--l-----I-y--																HGVRGW										nMFDP	WGQGTILTVSS	JH5b	
		4	e---L-s-a-----K--r--QA--R--	N-----I-y--	---G-s-Q-----f-----	-----T--l-----I-y--																HGVRGW										nMFDP	WGQGTILTVSS	JH5b	
		5	e---L-s-a-----K--r--QA--R--	N-----I-y--	---G-s-Q-----Q--a-----t-----LS--SGI-y--																HGVRGW										nMFDP	WGQGTILTVSS	JH5b		

Figure 3. Amino acid sequences of related IgE and IgG4 clones from patients 1–3 including the intraclonal variants for IgG4. The sequences are aligned to the closest germ-line V_H5 gene. Although there are some common mutations in both IgE and IgG4, the overall patterns are quite distinct. Upper-case: replacement mutation; lower-case: silent mutation; †: sites of previously observed hotspots of mutation [15]. The complete IgE sequences were obtained from one PCR, while the IgG4 sequences have been compiled from the products of two PCR reactions (CDR3-C_γ4 and V_H5L-CDR3, see Fig. 1). EMBL accession numbers AJ002767 – AJ002781.

codon for significant clustering by the method of Chang and Casali, using the binomial distribution method [21], none was found (data not shown). There is an ongoing accumulation of replacement mutations in the CDR of related IgG4 clones at a number of sites (Fig. 3). However, these additional R mutations are not confined to the CDR but are also seen in the FWR, therefore it is unlikely that they are Ag selected. Distinct replacement mutations are present in CDR1 and 2 of V_H5-C_ε transcripts when compared with the IgG4 clones (Fig. 3). Again, these differences are also found in FWR, reducing the likelihood of conventional Ag selection.

Some of the common mutations that occurred in both IgE and IgG4 clones arose at sites previously described as hotspots [15]. These are sites within V_H5 that are particularly prone to somatic mutation, and have been analyzed in detail for V_H5-C_μ, C_α, C_γ and C_ε clones and are indicated (†) in Fig. 3 [15]. Hotspots tend to occur at sites with certain nucleotide motifs, which appear to act as a target for the mutation machinery. They occur independently of Ag selection, although they may subsequently be selected by Ag [22, 23]. For example the triplet AGY, often within the motif CAGCT/A, is commonly mutated. This was found to be the case in patient 1 at Ser 28 (AGC→ACC) and Ser 77 (AGC→ACC), and patient 3 at Ser 31 (AGC→AAC). Another known consensus motif for hotspot sites is TAA [24], and a shared mutation within this motif is found in patient 3 (Lys 23, K→Q). These mutations may have arisen independently in the

different isotypes, given that these sites are known to be particularly susceptible to the hypermutation mechanism. Equally, they may have been present in the IgM precursor. More of the mutations (8/12) arose in both related IgE and IgG4 clones at non-hotspot sites and are likely to have been present in a common IgM precursor cell [15]. For example, two replacement mutations were found in patient 3 at Asp 59 (N→Q, AAC→CAA) in both the IgE and IgG4 clones (Fig. 3).

The overall pattern of mutation is different for IgE and IgG4 in each set, and is indicative of independent switching to either isotype from expanded IgM precursor clones, with the clones subsequently following divergent pathways of mutation.

3 Discussion

We have isolated related IgG4 and IgE transcripts from peripheral blood where the co-existence of related IgG4 and IgE clones in three of four atopic patients appeared to be a relatively common phenomenon. Previously, short CDR3/FWR4 sequences of related clones were obtained from a single patient by Efremov et al. [16] that showed mutational differences between IgG4 and IgE, with the IgM clone having a sequence similar to that of the IgE clone. However, the very short transcripts did not allow analysis of the V_H region.

By compiling complete V_H -C γ 4 transcripts from two PCR we have been able to demonstrate a distinct pattern of mutation in the IgG4 and IgE transcripts. The somatic mutation indicates that these are derived from clonally related but distinct B cells. Our similar sequences of the CDR3 confirm the clonal relatedness of the IgG4 and IgE transcripts in each patient. The full V region sequences demonstrate that the different isotypes have probably arisen from an IgM progenitor that already harbored some somatic mutation. Studies of switch fragments and switch circles from B cells in peripheral blood have revealed that the switch to IgE can either go directly from IgM, or, less frequently, via intermediate isotypes, including IgG4 [25]. The divergent mutational patterns seen in the IgE and IgG4 clones would indicate a direct switch from IgM to either IgE or IgG4, after which the cells had an independent history of somatic mutation. However, the possibility of sequential switching via IgG4 to IgE remains, with some of the clones remaining at the IgG4 stage and undergoing further somatic mutation. The common mutations in each patient may have been present in the original IgM precursor, although some may have arisen independently as they are at known hotspot sites. Intracloal variants have been described previously in IgE, but in these patients it was more evident for IgG4.

B cells from the same cell of origin switch to both IgG4 and IgE and co-exist together in the blood while other isotypes are not evident. We do not know if the clonally related B cells bind the same Ag or perhaps different epitopes of a common Ag. Two of the patients had specific IgG4 and IgE to *Der p* I in their serum. If the clonally related IgG4 and IgE do have common Ag specificities this may allow them to compete for allergen binding. The clones we identified had closely similar CDR3 for IgE and IgG4, which is central to Ag recognition. However, the most common pattern of mutation is for distinct replacement mutations in both the CDR and FWR of the V region of IgE and IgG4. It is difficult to know where specificity for antigen lies in IgE as there is a clear bias for usage of the V_H5 family, which may be due to a superantigen effect, with binding through the FWR [14]. The different patterns of mutation observed may lead to a divergence in specificity, with each isotype recognizing different epitopes of a common Ag. Distinct epitope specificity by IgG4 and IgE Ab has been described for certain allergens, *Fel d* I and *Bet v* I [3]. Blocking Ab of the IgG4 subclass may operate by binding to different epitopes of allergens also recognized by IgE. There are potential therapeutic benefits to increasing IgG4 production over IgE if IgG4 does indeed act in this way, but the mechanisms of regulation need to be better understood. As yet, the allergen specificity, if any, of the individual clones isolated also remains to be examined. Expressing them as

Fab molecules in phages is a potential way of addressing this question [26].

4 Material and methods

4.1 Patients

Peripheral blood was obtained from four patients with clinical histories of atopic asthma, elevated levels of total IgE ranging from 300–5200 IU/ml and radioimmunosorbent test (RAST) scores of 4 for *Der p* I-specific IgE (DPC, Gwynedd, GB). Patients 2–4 also had positive RAST scores for other aeroallergens such as cat, tree or grass allergens. In addition, patients 1 and 2 had a positive RAST for *Der p* I-specific IgG4, detected by a monoclonal mouse anti-human IgG4 (The Binding Site, Birmingham, GB) in place of anti-IgE.

4.2 Preparation of cDNA

RNA was extracted as previously described [14]. It was divided into five aliquots and reverse transcription was performed on each using a 3' outer C region primer for either IgM, IgG (a consensus primer for all IgG subclasses), IgG4, IgA or IgE [15, 16] with a first-strand cDNA synthesis kit (Pharmacia, Uppsala, Sweden). The exception to this was patient 3 where the cDNA was prepared with an oligo-dT primer.

4.3 Amplification and sequencing of V_H5 -C ϵ transcripts

We focused on the V_H5 family as we previously found it to be over-represented in the V_H genes used to encode IgE [14]. Therefore, V_H5 -C ϵ transcripts were amplified (Fig. 1, step 1 and 2), cloned and sequenced as previously described [15].

4.4 Investigation of related V_H -C ϵ clones

4.4.1 Identification of CDR3-FWR4-C H sequences

For seven V_H5 -C ϵ sequences from four patients, clonally related transcripts were sought by the use of CDR3-specific oligonucleotides. A three-step PCR was used, outlined in Fig. 1, using V_H5L and inner V_H5L (IV_H5L) primers in combination with C region primers as previously described for IgM, IgG and IgA [15] and IgG4 [16], followed by amplification with 5'CDR3-specific primers with C region primers. The 5'CDR3 primer sequences used for the clones obtained were as follows: patient 1 5'cgacgggtgcattattctggt3', patient 2 5'agattatccccgtattac3', patient 3 5'agacatggggtgcgtggatgg3' (Fig. 2). Products were purified, cloned and sequenced. The nucleotide sequence of the CDR3-C H transcripts obtained from step 3 were compared with that of the IgE clones to check for clonal relatedness.

4.4.2 Identification of V_H5-CDR3 sequences for IgG4

For clones with the appropriate CDR3-C_H sequence, shared with the V_H5-C_ε transcripts, step 4 was undertaken to obtain the upstream V_H-CDR3 sequence (Fig. 1). Of step 2 product, 1–3 μl was amplified with the IV_H5 leader primer together with a 3' CDR3/FWR4-specific primer. The CDR3/FWR4 primer sequences used for the clones obtained were as follows: patient 1 5'accaaaccacaaac/tg/tac/tccc3', patient 2 5'tcaaaagcatcatgataactact3', patient 3 5'tcgaaccaa/gttccatcc3' (Fig. 2). PCR products of predicted size were cloned and sequenced.

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5 References

- 1 Houri, M., Mayer, A. L., Houghton, L. E. and Jacobs, D., Correlation of skin, nasal and inhalation tests with the IgE in the serum, nasal fluid and sputum. *Clin. Allergy* 1972. **2**: 285–298.
- 2 Barbee, R. A., Halonen, M., Lebowitz, M. and Burrows, B., Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J. Allergy Clin. Immunol.* 1981. **68**: 106–111.
- 3 Aalberse, R. C., Van Milligen, F., Tan, K. Y. and Stapel, S. O., Allergen-specific IgG4 in atopic disease. *Allergy* 1993. **48**: 559–569.
- 4 Nakagawa, T., Kozeki, H., Katagiri, J., Fujita, Y., Yamashita, N., Miyamoto, T. and Skvaril, F., Changes of house dust mite-specific IgE, IgG and IgG subclass antibodies during immunotherapy in patients with perennial rhinitis. *Int. Arch. Allergy Appl. Immunol.* 1987. **82**: 95–99.
- 5 Ohashi, Y., Nakai, Y., Okamoto, H., Ohno, Y., Sakamoto, H., Tanaka, A. and Kakinoki, Y., Significant correlation between symptom score and IgG4 antibody titer following long-term immunotherapy for perennial allergic rhinitis. *Ann. Otol. Rhinol. Laryngol.* 1997. **106**: 483–489.
- 6 Devey, M. E., Wilson, D. V. and Wheeler, A. W., The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin. Allergy* 1976. **6**: 227–236.
- 7 Djurup, R., Malling, H. J., Sondergaard, I. and Weeke, B., The IgE and IgG subclass antibody response in patients allergic to yellow jacket venom undergoing different regimens of venom immunotherapy. *J. Allergy Clin. Immunol.* 1985. **76**: 46–55.
- 8 Nakagawa, T., Takaishi, T., Sakamoto, Y., Ito, K., Miyamoto, T. and Skvaril, F., IgG4 antibodies in patients with house-dust-mite-sensitive bronchial asthma: relationship with antigen-specific immunotherapy. *Int. Arch. Allergy Appl. Immunol.* 1983. **71**: 122–125.
- 9 Hussain, R., Poindexter, R. W. and Ottesen, E. A., Control of allergic reactivity in human filariasis. Predominant localization of blocking antibody to the IgG4 subclass. *J. Immunol.* 1992. **148**: 2731–2737.
- 10 Jeannin, P., Delneste, Y., Lecoanet-Henchoz, S., Gretenner, D. and Bonnefoy, J.-Y., Interleukin-7 (IL-7) enhances class switching to IgE and IgG4 in the presence of T cells via IL-9 and sCD23. *Blood* 1998. **91**: 1355–1361.
- 11 Carballido, J. M., Carballido-Perrig, N., Oberli-Schrammli, A., Heusser, C. H. and Blaser, K., Regulation of IgE and IgG4 responses by allergen specific T-cell clones to bee venom phospholipase A2 in vitro. *J. Allergy Clin. Immunol.* 1994. **93**: 758–767.
- 12 Akdis, M., Akdis, C. A., Weigl, L., Disch, R. and Blaser, K., Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA-memory T cells. *J. Immunol.* 1997. **159**: 4611–4619.
- 13 van der Stoep, N., van der Linden, J. and Logtenberg, T., Molecular evolution of the human immunoglobulin E response: high incidence of shared mutations and clonal relatedness among epsilon VH5 transcripts from three unrelated patients with atopic dermatitis. *J. Exp. Med.* 1993. **177**: 99–107.
- 14 Snow, R. E., Chapman, C. J., Frew, A. J., Holgate, S. T. and Stevenson, F. K., Analysis of Ig VH region genes encoding IgE antibodies in splenic B lymphocytes of a patient with asthma. *J. Immunol.* 1995. **154**: 5576–5581.
- 15 Snow, R. E., Chapman, C. J., Frew, A. J., Holgate, S. T. and Stevenson, F. K., Pattern of usage and somatic hypermutation in the V(H)5 gene segments of a patient with asthma: implications for IgE. *Eur. J. Immunol.* 1997. **27**: 162–170.
- 16 Efremov, D. G., Batista, F. D. and Burrone, O. R., Molecular analysis of IgE H-chain transcripts expressed in vivo by peripheral blood lymphocytes from normal and atopic individuals. *J. Immunol.* 1993. **151**: 2195–2207.
- 17 Brezinschek, H. P., Brezinschek, R. I. and Lipsky, P. E., Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. *J. Immunol.* 1995. **155**: 190–202.
- 18 Corbett, S. J., Tomlinson, I. M., Sonnhammer, E. L. L., Buck, D. and Winter, G., Sequence of the human immunoglobulin diversity (D) segment locus: a systematic analysis provides no evidence for the use of DIR segments, inverted D segments, minor D segments or D-D recombination. *J. Mol. Biol.* 1997. **270**: 587–597.

- 19 **Sahota, S. S., Leo, R., Hamblin, T. J. and Stevenson, F. K.**, Ig VH gene mutational patterns indicate different tumor cell status in human myeloma and monoclonal gammopathy of undetermined significance. *Blood* 1996. **87**: 746–755.
- 20 **Sanz, I., Kelly, P., Williams, C., Scholl, S., Tucker, P. and Capra, J. D.**, The smaller human VH gene families display remarkably little polymorphism. *EMBO J.* 1989. **8**: 3741–3748.
- 21 **Chang, B. and Casali, P.**, The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. *Immunol. Today* 1994. **15**: 367–373.
- 22 **Betz, A. G., Neuberger, M. S. and Milstein, C.**, Discriminating intrinsic and antigen-selected mutational hot-spots in immunoglobulin V genes. *Immunol. Today* 1993. **14**: 405–411.
- 23 **Dorner, T., Brezinschek, H. P., Brezinschek, R. I., Foster, S. J., Domiati-Saad, R. and Lipsky, P. E.**, Analysis of the frequency and pattern of somatic mutations within nonproductively rearranged human variable heavy chain genes. *J. Immunol.* 1997. **158**: 2779–2789.
- 24 **Rogozin, I. B. and Kolchanov, N. A.**, Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighbouring base sequences on mutagenesis. *Biochim. Biophys. Acta* 1992. **1171**: 11–18.
- 25 **Baskin, B., Islam, K. B., Evengard, B., Emtestam, L. and Smith, C. I.**, Direct and sequential switching from mu to epsilon in patients with *Schistosoma mansoni* infection and atopic dermatitis. *Eur. J. Immunol.* 1997. **27**: 130–135.
- 26 **Mockridge, C. I., Chapman, C. J., Spellerberg, M. B., Isenberg, D. A. and Stevenson, F. K.**, Use of phage surface expression to analyze regions of human V4-34 (VH4-21)-encoded IgG autoantibody required for recognition of DNA: no involvement of the 9G4 idiotope. *J. Immunol.* 1996. **157**: 2449–2454.

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