

Characterization of $V_{H\epsilon}$ gene expressed in PBL from children with atopic diseases: detection of homologous V_{H1-69} derived transcripts from three unrelated patients

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Abstract

To elucidate the molecular background of IgE production in early infancy, we analyzed the nucleotide sequences of 36 $V_{H-C\epsilon}$ transcripts expressed in PBL from three infants with allergic diseases. We detected transcripts derived from V_{H1} , V_{H3} , V_{H4} gene family members, and no bias was observed in the usage of particular V_{H} gene family. However, some V_{H} members, V_{H1} ; 1–46, 1–69, V_{H3} ; 3–11, 3–21, V_{H4} ; 4–39, 4–59 were frequently seen and thus notable. V_{H4} gene was dominant in one patient with severe atopic dermatitis and food allergy, suggesting the involvement of this gene in pathogenesis of the disease. Even a limited number of clones were analyzed, we also found highly homologous V_{H1-69} derived sequences from all the three patients, which share the same somatic mutations or polymorphic variations in complementarity-determining region (CDR) 1, and 2 with the same CDR3 (D– J_{H}) sequences including the junctions. These findings might suggest that a rather limited V_{H} gene might be rearranged for specific IgE in early life.

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1. Introduction

In the last decade, allergic syndromes affected a significant population of the youngest generation worldwide. Specific sensitization is under strong genetic influence, although environmental factors may enhance the development of Ag specific IgE Ab in later life [1–3].

More recently, studies on the cellular basis of IgE regulation have provided important insights into the disease process. While the importance of cytokines, IL-4, IL-13 pro-

duced by antigen specific CD4⁺ Th2 type cells which control IgE class switch has been emphasized [4], little information are available about the molecular features of the IgE producing B cells. The study for IgE epitope using conventional immunochemistry [5,6] and recombinant system [7–9] has shown that allergen conformation is important for IgE eliciting properties. Such advanced molecular technology made it possible to realize the formation of Ag–IgE Ab complex, which initiates the activation of mast cells or basophils. In contrast to the refined molecular analysis of antigens, study of the variable region structure, which is involved in antigen recognition of IgE, remains unsuccessful.

Recently, the entire structure and physical map for the gene locus of heavy (H)-chain V-region (V_H) of human Ig have been elucidated [10,11]. These findings provide the tools for analysis of the molecular rearrangements in B cells which might be responsible for susceptibility to immune disorders.

Abbreviations: V_H , heavy chain variable region gene; CDR, complementarity-determining region; FR, framework region; D, diversity; J, joining; HDM, house dust mite; CM, cow's milk; RAST, radioallergosorbent test

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With a new approach using V_H family specific primers, the IgE V_H repertoire of patients with allergic disease have been analyzed [12–15]. The dominance of V_H5 genes from asthma patients was observed [12,13], on the other hand, no bias for the usage of IgE V_H was detected in AD patients [14]. It is generally known that adult individuals have more chance for sensitization, which makes studies for identification of specific V_H genes difficult. It is, therefore, of interest to inspect IgE V_H repertoire from young infants who are exposed to limited environmental antigens. Here we describe the nature and mutational patterns of V_H genes expressed in PBL from atopic infants.

2. Materials and methods

2.1. Patients

Under the informed consent, three children with clinical histories of allergic diseases were studied. Patient NK was born as low birth weight baby (Birth body weight 2200 g) and developed cow's milk (CM) allergy on day 14. Radioallergosorbent test (RAST) scores were positive only for CM components (Casein, β -lactoglobulin, α -lactalbumin) at the time of analysis. Patient SS, a 2-year-old boy, was diagnosed of egg hypersensitivity at 6 months of age and developed occasional asthma attacks and mild atopic dermatitis 1 year later. He had positive RAST score for Egg white and HDM. KY has been suffering from severe atopic dermatitis and bronchial asthma from 6 months of age. He also had repeated episodes of anaphylactic shock after cow's milk ingestion. Serum IgE level is high and specific IgE Abs against multiple allergens, including CM components, were detected. All patient profiles are summarized in Table 1.

2.2. Preparation of cDNA, and PCR amplification of V_H genes

Peripheral mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Total RNA was

Table 2

Oligonucleotide primers for the amplification of IgE V_H genes

Pst 1 ^a	
5'VH 1P	5'-CAG GTG CAG <u>CTG CAG</u> CAG TCT GG-3'
5'VH 2P	5'-CAG GTC AAC <u>CTG CAG</u> GAG TCT GG-3'
5'VH 3P	5'-GAG GTG CAG <u>CTG CAG</u> GAG TCT GG-3'
5'VH 4P	5'-CAG GTG CAG <u>CTG CAG</u> GAG TCG GG-3'
5'VH 5P	5'-GAG GTG CAG <u>CTG CAG</u> CAG TCT GG-3'
5'VH 6P	5'-CAG GTA CAG <u>CTG CAG</u> CAG TCA GG-3'
Sal 1 ^a	
3'C- ϵ S	5'-GG <u>GTC GAC</u> AGT CAC GGA GGT GGC ATT-3'

^a Restriction sites are underlined.

isolated using STAT-60 (Tel-Test, Friendswood, TX) and cDNA was prepared with oligo dT primer and AMV reverse transcriptase using Superscript Preamplification System (GIBCO BRL, Gaithersburg, MD). V_H family specific primers for human V_H1, V_H2, V_H3, V_H4, V_H5 and V_H6 containing a Pst 1 restriction site were generated (Table 2) according to the previous reports [16] with minor modification. For amplification of IgE V_H genes, a 5' primer for V_H in combination with 3' primer specific for the first exon of the ϵ constant region containing Sal 1 site were prepared. PCR amplification procedure consisted of an initial step of 5 min at 95 °C followed by 40 cycles of 95 °C 0.45 min, 64 °C 1 min, 72 °C 1 min with final extension of 10 min at 72 °C.

2.3. Cloning and sequencing of the PCR products

All PCR products were purified using QIA quick PCR Purification Kit (Quiagen Inc., Chatsworth, CA), and digested with two restriction enzymes (Sal 1, and Pst 1, New England Biolabs Inc., Beverly, MA) and ligated into pBlue-Script M13 (Stratagene, La Jolla, CA). JM 109 competent cells were transformed with the ligation mixture under heat shock. The transformed cells were then selected on the basis of blue/white color selection in the presence of X-gal. Plasmid DNA was purified from 4 ml overnight culture in LB medium containing antibiotics, using QIA Spin Miniprep

Table 1
Patient profiles

	Case 1 NK	Case 2 SS	Case 3 KY
Age	8 month	2 year	5 year
M/F	Male	Male	Male
Diagnosis	Food allergy	Bronchial asthma Atopic dermatitis Food allergy	Bronchial asthma Atopic dermatitis Food Allergy
Total IgE (U/ μ l)	180	882	12,410
RAST score			
House dust mite	(–)	139.5 (5+)	135.8 (5+)
Cedar pollen	(–)	ND ^a	96.9 (4+)
Egg white	(–)	3.4 (3+)	29.9 (3+)
Cow milk	1.86 (+)	(–)	142.0 (5+)

^a Not done.

Kit (Quiagen). Purified plasmids were digested with the two enzymes to confirm the presence of insert of the correct size. DNA sequencing was carried out on an automated DNA sequencer (310, Applied BioSystems Inc., Foster, CA), using dideoxy termination cycle sequencing kit (Applied Biosystem Inc.). Sequence analysis was conducted on totally 50 colonies from three patients (15 colonies from NK; 20 from SS; 15 from KY).

2.4. Sequence analysis and assignment of mutations

Sequence alignment analysis was performed by searching current database of EMBL (V base directory, MRC Center for Protein Engineering, Cambridge) and Matsuda, et al. [11], using GENETYX-MAC ver. 10.0 sequence analysis software (Software Development Co. Ltd., Tokyo, Japan).

3. Results

3.1. Characterization of IgE V_H transcripts

From three patients, a total of 50 IgE V_H transcripts (15 from NK; 20 from SS; 15 from KY) were analyzed. Interestingly, two each (total four) of completely identical sequences were repeatedly (four to five times among 15–20 sequences) obtained from two patients, (designated as NK-2, NK-8, SS-15, SS-10 sequences, respectively) suggesting that ϵ transcripts having these V_H sequences were rather dominant in these patients. Thus, we obtained 36 independent $V_H\epsilon$ sequences (eight from NK; 13 from SS; 15 from KY). Among these 36 sequences, NK-1, 4, 5, 6 and SS-4, 7, 9, 10 are highly related respectively, suggesting they arose from common sequences. Thus, in the patient NK, three sequences are dominant (four clones each in 15 sequences clones), and in the patient SS, two sequences are dominant (four and eight clones in 20 sequences clones). It is interesting younger two patients have such apparently dominant sequences suggesting a limited $V_H\epsilon$ complexity. Profiles of V_H transcripts of IgE from three patients are shown in Table 3.

3.2. V_H family usage

For all of the three cases, V_H primers, V_{H1} , V_{H3} , and V_{H4} , yielded the amplified PCR products of the expected size (~450 bp). There was a trace band seen in the product of V_{H5} specific primer at the same size, however no bands were seen with V_{H2} and V_{H6} primers (data not shown). These results indicate that $V_{H-C\epsilon}$ transcripts encoded by V_{H1} , 3 and 4 gene family members were present in all three patients. The V_{H2} , 5 and 6 families were composed of rather small numbers of segments in genome, therefore it is likely that the low numbers of these genes in the rearranged repertoire resulted from molecular processes. All detected transcripts were from functional germline genes with no stop codon and we did not find any unproductive sequences derived from pseudogenes or having out-of-frame junctions. Finally,

no apparent preferential usage of a certain V_H family was detected in all patients.

3.3. V_H member usage

Although the distribution of IgE V_H families was ordered in accordance with germline complexity, a bias toward some of the V_H members was found individually. The dominance of specific V_H members suggest that younger patients have a limited $V_H\epsilon$ transcript and those transcripts might come from a limited B cell clones, which expanded through antigen selective process.

The frequently found germline segments were V_{H1} , 1–46, 1–69; V_{H3} , 3–11, 3–21; V_{H4} , 4–39, and 4–59 and all of these segments were also detected in previous studies [14,15]. Interestingly, V_{H1} –69, and V_{H3} –11 derived transcripts (V_{H1} –69; NK-2, SS-3, KY-21, V_{H3} –11; NK-1, 3, 4, 5, 6, SS-1, 2, KY-14, 20) were identified in all the patients, which had similar DJ sequences but carried different point mutations at CDR (complementarity-determining region) 1, 2 and FR (framework region).

3.4. V_H deduced amino acid sequences and somatic mutations

Each was compared with the closest germline gene in database, but not with germline counterpart of the patient per se. This means some apparent somatic mutations could be germline polymorphism. Homology to the closest germline gene was distributed from minimum 85 to maximum 99%. The positions of mutations were not accumulated in CDR1, 2 but also seen in FR3. SS-15 and KY-3 transcripts were nearly identical to the germ-line sequences.

The deduced amino acid sequences of all $V_H\epsilon$ transcripts identified are shown in Fig. 1. The V_{H3} –21 derived transcripts SS-10, 9, 7, 4 found in SS have the same VDJ junctions and also share the same replacement mutations at the codons of 50, 57, 69 (50; Y $\rightarrow$ S, 57; T $\rightarrow$ Y, 69; A $\rightarrow$ T). Similar patterns are seen in V_{H3} –11 derived sequences of NK, K-1, 4, 5, 6, at the position of 57 (57; T $\rightarrow$ Y). These transcripts were thought to be from the common IgE producing B cell clone, which is expanded by the antigen stimulation. The KY-14 and KY-20 transcripts found in KY seem to have a unique relationship, because they share the same mutation at the codons 57, 58, 59 and 62 (57: T $\rightarrow$ Y, 58; I $\rightarrow$ T, 59; Y $\rightarrow$ N, 62; D $\rightarrow$ N) although they used different DJ segments. These coincidences might be resulted from selection by particular antigen and those mutations might increase the affinity to such antigen but the possibility that this patient have polymorphic V_H segments carrying these mutations in the germline could not be excluded.

3.5. D and J_H gene usage

The D gene segments were defined as the nucleotide sequences between the V_H and J_H genes, and include putative

	FR1	CDR1	FR2	CDR2	FR3	D (CDR3)	J (FR4)	Cε
<NC>								
1-69	QVQLVQSGAEVKKPGSSVKYKSCASGGTFS	SYAIT	WVRQAPGQGLEWMG	GIIPFGTANYAQKFGQ	RVITADKSTSTAYMELSSLRSEDTAVYYCAR			ASTQ
NK-2	G-----T	-G-R	-----	R--VL--SDS--EL-D	-----P-N-----T-----	EVERKRFYR	WFDPWGGGLTVTVSS	---
3-11	QVQLVESGGGLVKPGGSLRLSCAASGFTFS	DYMS	WIRQAPGKGLEWVS	YISSSGSTIYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR			
NK-1	-----E-----V-----	-H-----	-----	--K-YTN--R-	-----D-M-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
NK-4	-----E-----V-----	-Q-----	-----	--KS-YTN--R-	-----M-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
NK-5	-----PVC-----	-Q-----	-A-----	--KR-YTN--R-	-----K-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
NK-6	-----E-----V-P-----	-H-----	-----	--K-YTN-N-R-	-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
NK-3	-----R-----WTA-----L-----	-----	-----L-----	--S-YTN-----	-----D-----	EIVVYND	FDIWGGQTMITVSS	---
4-28	QVQLQESGPGLVKPSDTLSLTCAVSGYSIS	SSNWVG	WIRQPPGKMEWIG	YIYSGSTIYNPSLKS	RVIMSDTSKNQFSLKLSSTAVDTAVYYCAR			
NK-8	-----G-P-T-G-----	-N-T-----	-V-----L-----	E-H--N-Y-----	-----R-----	EPYNWVDVG	FDIWGGQTMVTVSS	---
4-61	QVQLQESGPGLVKPSSETLSLTCTVSGGSYS	SGGYWS	WIRQPPGKMEWIG	YIYSGSTIYNPSLKS	RVTISVDTSKNQFSLKLSSTAAEDTAVYYCAR			
NK-9	-----Q-----I-----	--S-----	-A-----	RT-T-----	-----F-----	AAYSSSWCWYIL	YWGQGNLTJTVSS	---
<SS>								
1-46	QVQLVQSGAEVKKPGASVKYKSCASGYTFT	SYMH	WVRQAPGQGLEWMG	IINPSGGSTSYAQKFGQ	RVMTIRDTSTSTVYMELSSLRSEDTAVYYCAR			
SS-15	-----L-----	-----	-----	-M-----	-----	AGSSWYGYL	QHWGGGLTVTVSS	---
1-69	QVQLVQSGAEVKKPGSSVKYKSCASGGTFS	SYAIT	WVRQAPGQGLEWMG	GIIPFGTANYAQKFGQ	RVITADKSTSTAYMELSSLRSEDTAVYYCAR			
SS-3	-----P-----	R-----	-----	R--VL--S-S--D	-----P-N-----	EVERKRFYR	WFDPWGGGLTVTVSS	---
3-11	QVQLVESGGGLVKPGGSLRLSCAASGFTFS	DYMS	WIRQAPGKGLEWVS	YISSSGSTIYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR			
SS-2	-----E-----V-----	-Q-----	-A-----	--K-YTK--R-	-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
SS-1	-----R-E-----VRR-----	-H-----	-----	--K-YTK--R-	-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
3-21	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	SYSMN	WVRQAPGKGLEWVS	YISSSSSTIYADSVKG	RFAISRDNAKNSLYLQMNSLRAEDTAVYYCAR			
SS-6	-----V-----	-----	-----	S--R-Y--A-----	-----V-----	EGYDWGMR	WYFDLWGGGLTVTVSS	---
SS-10	-----	-----	-----	S--Y-----	-T-----	DASELGYCSGGSCYVG	WFDPWGGGLTVTVSS	---
SS-9	-----L-----	-C-K-----	-----	S--Y-----	-T-----	DASELGYCSGGSCYVG	WFDPWGGGLTVTVSS	---
SS-4	-----WV--W-----	-H-----	-V-----	S--Y-----	-T-----	DASELGYCSGGSCYVG	WFDPWGGGLTVTVSS	---
SS-7	-----R-PW--IW-----	-----	-I-D-R-----	S--Y-----	-T--T-E-----	DASELGYCSGGSCYVG	WFDPWGGGLTVTVSS	---
3-53	EVQLVESGGGLIQPGGSLRLSCAASGFTVS	SNYMS	WVRQAPGKGLEWVS	VIYSGSTIYADSVKG	RFTISRDNKNTLYLQMNSLRAEDTAVYYCAR			
SS-5	-T-----C-N	NK-N	-T-----	-----N-----	-----M-----	VHSGSYHDSG	PHWGGGLTVTVSS	---
4-4	QVQLQESGPGLVKPSSETLSLTCTVSGGSIS	SSNWWS	WVRQPPGKLEWIG	EIYHSGSPNYNSLKS	RVTISVDKSKNQFSLKLSSTAAEDTAVYYCAR			
SS-16	-----	-N-T-----	-----	-T-P-----	-----R-----	DIAADTGS	YFDYWGQGLTVTVSS	---
4-59	QVQLQESGPGLVKPSSETLSLTCTVSGGSIS	SYWWS	WIRQPPGKLEWIG	YIYSGSTIYNPSLKS	RVTISVDTSKNQFSLKLSSTAAEDTAVYYCAR			
SS-17	-V-----G	T-----	-----	S-H--I-----	-----I--R-N-G--L-----	PLGVRGIVSY	WFDPWGGGLTVTVSS	---
SS-18	-----	-----	-----	-----Y-----	-----R-----	PYNWVDVG	FDIWGGQGMVTVSS	---
<KY>								
1-46	QVQLVQSGAEVKKPGASVKYKSCASGYTFT	SYMH	WVRQAPGQGLEWMG	IINPSGGSTSYAQKFGQ	RVMTIRDTSTSTVYMELSSLRSEDTAVYYCAR			
KY-19	-----L-A-----	-----	-R-----	-M-----	-----	AGSSWYGYL	QHWGGGLTVTVTVSS	---
1-69	QVQLVQSGAEVKKPGSSVKYKSCASGGTFS	SYAIT	WVRQAPGQGLEWMG	GIIPFGTANYAQKFGQ	RVITADKSTSTAYMELSSLRSEDTAVYYCAR			
KY-21	-----A-----Y-----	-G-S-----	-----	R--VL--S-S--E-D	-----P-N-----T-----	EVERKRFYR	WFDPWGGGLTVTVSS	---
3-11	QVQLVESGGGLVKPGGSLRLSCAASGFTFS	DYMS	WIRQAPGKGLEWVS	YISSSGSTIYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR			
KY-14	-----K-----	-----	-----L-----	--S-YTN--N-	-----D-----	EIVVYND	FDIWGGQTMVTVSS	---
KY-20	-----E-----V-----	-H-----	-----	-T-TK--YTN--N-R-	-----R-A-----	EGYDWGMR	WYFDLWGRGTPVTVSS	---
3-48	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	SYSMN	WVRQAPGKGLEWVS	YISSSSSTIYADSVKG	RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR			
KY-6	-----HQ-----V-----	R-Y-----	-----	-S-G-----Y-----	-----E--M-S-V--I-----	LTGSY	FDYWGQGCITVSS	---
3-53	EVQLVESGGGLIQPGGSLRLSCAASGFTVS	SNYMS	WVRQAPGKGLEWVS	VIYSGSTIYADSVKG	RFTISRDNKNTLYLQMNSLRAEDTAVYYCAR			
KY-22	-T-----K-----N	N-N-----	-----	A-----N-----	-----K--M-----K-----	VHSGSYHDSG	PHWGGGLTVTVSS	---
4-4	QVQLQESGPGLVKPSSETLSLTCTVSGGSIS	SSNWWS	WVRQPPGKLEWIG	EIYHSGSPNYNSLKS	RVTISVDKSKNQFSLKLSSTAAEDTAVYYCAR			
KY-15	T-V--G-----AL-----	-N-T-----	-----	-----CT--P-----	-----R-----	DIAADTGS	YFDYWGQGLTVTVSS	---
4-28	QVQLQESGPGLVKPSDTLSLTCAVSGYSIS	SSNWVG	WIRQPPGKMEWIG	YIYSGSTIYNPSLKS	RVIMSDTSKNQFSLKLSSTAVDTAVYYCAR			
KY-10	-----GG--VT-----	-----T-----	-----	-----K-----	-----N--A-----	PLGVRGIVSY	WFDPWGGGLTVTVSS	---
4-39	QLQLQESGPGLVKPSSETLSLTCTVSGGSIS	SSSYWG	WIRQPPGKLEWIG	SIYSGSTIYNPSLKS	RVTISVDTSKNQFSLKLSSTAAEDTAVYYCAR			
KY-3	-----	-----	-----	-----T-----	-----N-----	PLGVRGIVSY	WFDPWGGGLTVTVSS	---
KY-5	-----GG-T-----F-----	-----	-----	E-SH--T-----	-----N-----	EDVTTAG	FDYWGQGLTVTVSS	---
4-59	QVQLQESGPGLVKPSSETLSLTCTVSGGSIS	SYWWS	WIRQPPGKLEWIG	YIYSGSTIYNPSLKS	RVTISVDTSKNQFSLKLSSTAAEDTAVYYCAR			
KY-1	-----	-----	-----	-----N-----	-----T-----T-----	GFYFDS	WGQGLTVTVSP	---
KY-7	-----A--S-----	-----	-----	-----N-K-----	-----	EDVTTAG	FDYWGQGLTVTVSS	---
KY-11	-----	-----	-----	-----	-----N--N-----	GQYFDWLSL	DIWGQGLTVTVSS	---
KY-2	-----T-----F-----	-----	-----	-F--V-----	-S--P--L--R--R-----F-----	GPGYDITGGYYS	DFWGQGTPTVTVSS	---
KY-9	-V-----G	T--R-----	-----	S-H--I-----	-----I--R-N-G-----	PLGVRGIVSY	WFDPWGGGLTVTVSS	---

Fig. 1. Deduced amino acid sequences of V_H-Cε transcripts. Single-letter amino acid code is used. Dashes, identity of amino acid residue. The first eight amino acids were omitted.

Table 3
Characterization of V_H–C ϵ transcripts^a

V _H –C ϵ						(D)	(J _H)	Incidence ^b
	Family	Member	Homology (%) ^d	Mutations (R/S) ^c in				
				CDR	FR			
[NK]								
NK-2	1	1–69/DP-10	87 ^d	11/13	5/3	D 3–3	J5	4/15
NK-1	3	3–11/DP-35	94	6/0	4/2	D 7–27	J2	1/15
NK-4	3	3–11/DP-35	94	7/0	3/2	D 7–27	J2	1/15
NK-5	3	3–11/DP-35	92	7/2	5/6	D 7–27	J2	1/15
NK-6	3	3–11/DP-35	94	7/1	3/3	D 7–27	J2	1/15
NK-3	3	3–11/DP-35	94	4/0	7/5	D 5–12	J3	1/15
NK-7	4	4–39/DP-79	92	6/4	7/4	D 1–1	J3	1/15
NK-8	4	4–61/DP-66	94	4/1	4/4	D 6–13	J4	4/15
[SS]								
SS-15	1	1–46/DP-7	99	1/0	1/0	D 6–13	J1	4/20
SS-3	1	1–69/DP-10	92	8/2	3/1	D 3–3	J5	1/20
SS-2	3	3–11/DP-35	94	6/0	3/1	D 7–27	J2	1/20
SS-1	3	3–11/DP-35	93	6/1	5/1	D 7–27	J2	1/20
SS-6	3	3–21/DP-77	96	4/0	2/0	D 7–27	J2	1/20
SS-10	3	3–21/DP-77	97	2/1	1/2	D 2–15	J5	5/20
SS-9	3	3–21/DP-77	96	4/0	2/0	D 2–15	J5	1/20
SS-4	3	3–21/DP-77	95	3/2	5/0	D 2–15	J5	1/20
SS-7	3	3–21/DP-77	90	2/0	12/6	D 2–15	J5	1/20
SS-5	3	3–53/DP-42	91	4/1	5/6	D 3–22	J1	1/20
SS-16	4	4–4/DP-70	95	4/2	1/5	D 6–13	J4	1/20
SS-17	4	4–59/DP-71	95	4/1	7/0	D 3–10	J5	1/20
SS-18	4	4–59/DP-71	98	1/2	1/3	D 1–1	J5	1/20
[KY]								
KY-19	1	1–46/DP-7	97	1/2	3/3	D 6–13	J1	1/15
KY-21	1	1–69/DP-10	89	9/2	5/8	D 3–3	J5	1/15
KY-14	3	3–11/DP-35	94	5/0	3/5	D 5–12	J3	1/15
KY-20	3	3–11/DP-35	92	8/1	3/7	D 7–27	J2	1/15
KY-6	3	3–48/DP-51	92	5/0	8/3	D 7–27	J4	1/15
KY-22	3	3–53/DP-42	95	4/1	4/3	D 3–22	J1	1/15
KY-15	4	4–4/DP-70	92	5/1	7/3	D 6–13	J4	1/15
KY-10	4	4–28/DP-68	94	2/0	6/0	D 3–10	J5	1/15
KY-3	4	4–39/DP-79	98	1/1	1/1	D 3–10	J5	1/15
KY-5	4	4–39/DP-79	93	4/1	5/2	D 6–13	J4	1/15
KY-1	4	4–59/DP-71	97	1/1	2/3	D 4–17	N ^e	1/15
KY-7	4	4–59/DP-71	96	2/1	2/2	D 6–13	J4	1/15
KY-11	4	4–59/DP-71	98	0/1	2/2	D 3–9	J4	1/15
KY-2	4	4–59/DP-71	93	3/2	7/5	D 3–22	J2	1/15
KY-9	4	4–59/DP-71	95	5/0	6/0	D 3–10	J5	1/15

^a Columns show name of V_H–C ϵ transcript from three patients, Donor germline genes according to the nomenclature of V-base and Ref. [16].

^b Number of clones having identical sequence/total number of sequenced clones.

^c Homology to the candidate germline gene at the nucleotide level.

^d Total number of replacement (R) and silent (S) mutations in CDR and FR.

^e Could not be uniquely identified because of a large deletion at the junction.

N insertions (Fig. 2). D genes were aligned to the segments published so far [17] according to the criteria by Kabat et al. [18]. Among 30 distinct clones, we detected 10 species of D segments, and D6–13, D7–27 were prominent (8/30, 5/30). Other three D3 family segments (3–3, 3–10, 3–22) were used in three clones each. The entire CDR 3 length was varied among the IgE V_H transcripts.

Analyses of J_H region genes indicated that all of the J_H members except J_H6 were used at almost the same frequency. In one of these transcripts, J_H gene could not be uniquely identified because it has a shorter J_H se-

quence indistinguishable between two candidate germline genes.

3.6. Analysis of CDR 3/FR 4 region of the VH1–69, VH3–11 derived VH genes from three patients

The two groups of the V_H1–69, V_H3–11 derived transcripts (V_H1–69; NK-2, SS-3, KY-21, V_H3–11; NK-5, SS-2, KY-20) are unique, that is, they utilize the same D and J_H gene segments and are indistinguishable at the V_H–D, and D–J_H junctions (Figs. 3 and 4). To exclude the

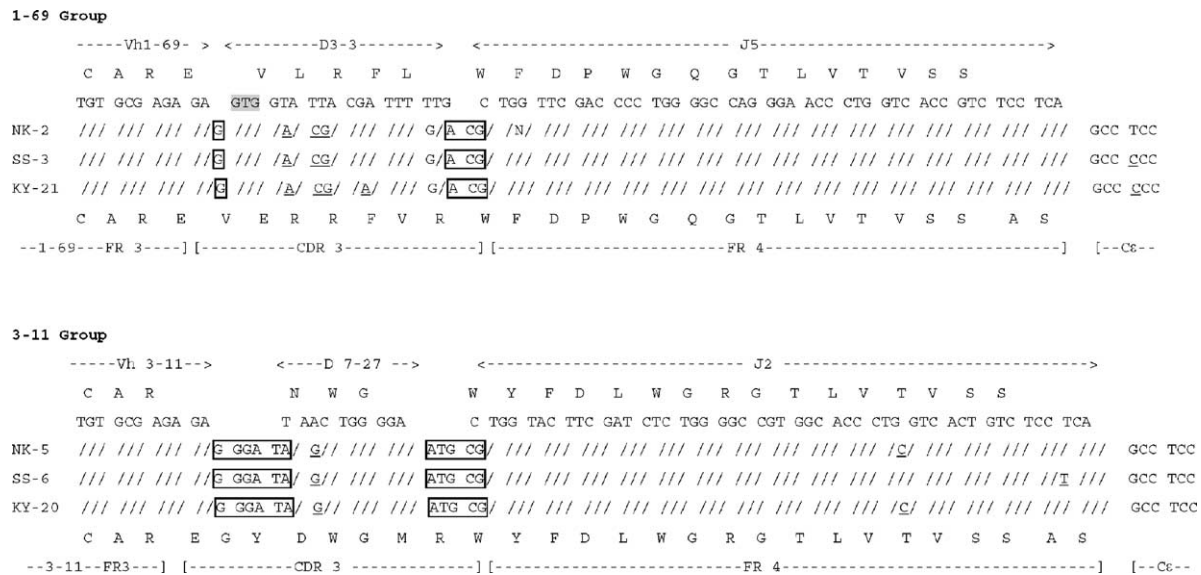


Fig. 4. Nucleotide sequences of the CDR3/FR4 region of the two groups of 1-69 and 3-11 derived transcripts from three patients. Nucleotide sequences are compared to the closest germline D and J_H genes. /, nucleotide identity. ■, RSS (recombination signal sequences). (□), putative N insertions.

at the same V_H-D-J_H junctions (V_H1-69/D3-3/J_H5 and V_H3-11/D7-27/J_H2) found in transcripts from different sources the common features are that putative N insertions around D are solely A/T and those attached to V_H or J_H are solely G/C (Fig. 4). Furthermore, the same D-J_H junction (D7-27/J_H2) was also found in another transcript having a different V_H (Fig. 1 and Table 3). These findings might suggest that a rather limit variety of D-J_H or V_H-D-J_H junctions are created in neonate or infant stages. It is therefore reasonable to assume that repeatedly found CDR3/FR4 sequences in our study might be critical in determining the selected V_H gene members to generate the functional antibody against limited environmental antigens.

These V_H1-69 derived V_H-Cε transcripts, even when taking allelic polymorphism into account, share the same point mutation in CDR or FR among individuals, and may encode the antigen-binding site. Of interest, the V_H sequence of a human monoclonal IgE (data not shown) provided us new evidence that V_H1-69 is the candidate gene encoding an IgE variable region to recognize an allergen.

In the human genome V_H repertoire, two types of polymorphisms (insertion and deletion) are found which affect the different alleles of the same segment [10,11]. According to this, one occurs around the position of the segment V_H3-30 to V_H4-31, and a second is in the telomeric region of the locus near the V_H1-69. Snow et al. [13] discussed that an asymmetrical use of V_H5 gene might reflect activation by B cell superantigen. Because V_H1-69 alleles and V_H5-51 share high homology in CDR 1 and 2, both V_H1-69 and V_H5-51 are considered to be candidate genes for the IgE V_H. Importantly, the evidence that V_H1 member 51p1, newly named V_H1-69, are preferentially expressed in fetal B cells or CLL, or the source of rheumatoid factors [26,27] gives us a clue to reconsider IgE production in infancy.

V_H3 family members, V_H3-11 and V_H3-21, were found at higher frequency in our study. V_H3-11 was reported to be rather negatively selected in PBL [20], however, we found V_H3-11 derived transcripts in three patients. The V_H sequence of a mouse monoclonal anti-Dp 1 Ab, which shows similar epitope specificity as human IgE, showed high homology with the human V_H3 member V_H3-11, V_H3-74, V_H3-7 [28]. This result suggests that independent B cell clones developed from V_H1, 3 and 5 family genes recognized different IgE epitopes of HDM.

There still remains an important fundamental question concerning the genetic control by selected V_H for switching to IgE Ab. Targeting detection of V_H1-69 in IgE V_H for high-risk infants and normal individuals, as well as searching for polymorphisms in the locus of V_H1-69 would give us useful hints, toward which our current research is aimed. Moreover, further study for light chain transcripts may be needed to accomplish the whole molecular structure of variable region of HDM specific IgE, which can be done with the human IgE-producing hybridomas.

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