Molecular Evolution of the Human Immunoglobulin E Response: High Incidence of Shared Mutations and Clonal Relatedness among ε Vh5 Transcripts from Three Unrelated Patients with Atopic Dermatitis

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Summary

We have analyzed the nucleotide sequences of 19 ε Vh5 transcripts derived from in vivo isotype switched peripheral blood B cells of three patients with atopic dermatitis. Comparison with the patients' own germline Vh5 gene segments revealed that the ε transcripts were derived from both functional members of the human Vh5 gene family and harbored numerous somatic mutations (range 5–36 per Vh5 gene). In two patients, we detected clonally related but diverged transcripts, permitting the construction of a genealogical tree in one patient. We observed a high proportion of shared silent (S) and replacement (R) mutations among ε Vh5 sequences derived from all three individuals, even among transcripts descending from the two different germline Vh5 gene segments. A remarkably high number of these mutations is shared with previously reported Vh5 genes encoding antibodies with defined specificities. The shared S mutations, and likely a fraction of the R mutations, appear to mark preferential sites ("hot spots") of somatic hypermutations in human Vh5 genes. The distribution of R and S mutations over complementarity determining region and framework regions in the majority of Vh regions deviated from that characteristic of antigen-driven immune response. We hypothesize that the V regions of immunoglobulin E-bearing B cells have accumulated "selectively neutral" mutations over extended periods of clonal expansion, resulting in unusual R/S ratios. We propose that the molecular characteristics of the ε Vh regions in atopic dermatitis may be representative of antigens that recurrently or chronically stimulate the immune system.

Atopic dermatitis is an inflammatory skin disorder characterized by a chronically relapsing course with frequent exacerbations and a distinctive clinical morphology. Although the primary cause of atopic dermatitis is unknown, substantial evidence suggests that excessive production of IgE antibodies likely contributes to the pathogenesis of this disorder (1, 2). These antibodies are reactive with a wide variety of environmental allergens. The regulatory effects of allergen-reactive T lymphocytes and their lymphokine products appear to be a major cause of the high serum levels of IgE in patients with atopic dermatitis (3–5).

Although the role of allergen-reactive T cells in controlling IgE class switching is beginning to be elucidated, little is known about the B lymphocytes that are recruited into the IgE response. In particular, questions related to the Ig gene segments encoding the V regions of IgE antibodies and the role of somatic hypermutation in the clonal evolution of IgE responses have not been addressed. Similar studies on the molecular origin of human (auto)antibodies have largely depended on hybridoma technology and EBV transformation to generate monoclonal B cell lines secreting relevant specificities. Attempts to immortalize B lymphocytes switched to IgE in vivo have generally been unsuccessful.

In the present study we have employed a cDNA/PCR approach to analyze the molecular structure of V regions expressed in IgE antibodies of atopic dermatitis patients. This strategy permitted the selective amplification of ε Vh5 region transcripts from RNA extracted from PBMC. Our data show that members of the Vh3-Vh6 gene families may be utilized in IgE antibodies from patients with atopic dermatitis. The small human Vh5 gene family, which contains only two functional members, was abundantly expressed in ε transcripts. Comparison of the nucleotide sequences of 19 ε Vh5 transcripts with the germline Vh5 genes of these patients revealed the accumulation of high numbers of somatic mutations. A surprisingly high proportion of silent (S) and
replacement (R) mutations was shared among sequences derived from different members of the V_5 gene family. In two patients, we detected evidence for transcripts derived from clonally related but diverged IgE-bearing B cells which, in one patient, permitted the construction of a genealogical tree. The results of these experiments are discussed in the context of the molecular mechanisms contributing to a clinically relevant IgE-mediated chronic immune response.

Materials and Methods

 Patients. All patients analyzed suffered from severe atopic dermatitis according to the diagnostic criteria formulated by Hanifin et al. (6). Patient P1 is a female adult with a total serum IgE level of 18 x 10^3 KU/liter. Patient P2 is a 12-yr-old boy with a total serum IgE level of 5.3 x 10^3 KU/liter, and patient P3 is a 9-yr-old boy with an IgE serum level of 9.9 x 10^3 KU/liter. IgE in the serum of all three patients reacted with more than 15 different environmental allergens as detected in Radio Allergosorbent Test (RAST).

DNA and RNA Isolation. PBMC from atopic dermatitis patients were isolated by Ficoll-Hypaque density gradient centrifugation. Total cellular RNA was prepared from 2 x 10^6 PBMC using RNAzo® (Cinna/biotecx, Friendwood, TX) as described previously (7). EBV-transformed polyclonal B cell lines were generated from 5 x 10^6 PBMC, and genomic DNA was isolated from 10 EBV-transformed B cells as detailed elsewhere (8).

First-strand cDNA Synthesis and PCR. Total RNA was used as a template for first-strand cDNA with an oligo primer specific for the first exon of the Ce constant region (Ce, 5'-TGTCCCGTT-GAGGGAG CCTGT-3') and 10 U of avian myeloblastosis virus (AMV) reverse transcriptase (Pharmacia, Uppsala, Sweden) according to standard procedures (7). One tenth of the cDNA reaction mixture was amplified in a PCR employing a previously described set of 5' primers specific for the human V_3, V_4, V_5, and V_6 gene families (9), in combination with a second, nested 3' Ce-specific primer containing a Sall restriction site (5'-GGGT-CGACAGTCGGAGTTGCGAT-3'). Genomic DNA was amplified with the V_5 V_5-specific primer and a 3' primer 100% homologous to sequences in the 3' flanking region of both functional V_5 genes (5'-GGGT-CGACAGTCGGAGTTGCGAT-3'). All PCR reaction mixtures contained 200 ng of each primer and 2.5 U Taq polymerase (Promega Biotec, Madison, WI). Amplifications were performed in a Bioexcellence thermal cycler (Biores., Woerden, The Netherlands) and consisted of 35 cycles of 1 min denaturation at 94°C, 1.5 min primer annealing at 65°C, and 1.5 min extension at 72°C. After the last cycle, reaction mixtures were incubated for 10 min at 72°C to ensure complete extension of all products.

Cloning and Sequencing of PCR-amplified Material. PCR-amplified material was digested with restriction enzymes EcoRI and Sall (Pharmacia) and separated on a 0.8% low-melting agarose gel (Nusieve; FML Bioproducts, Rockland, ME). A fragment of ~450 bp was excised and ligated into Bluescript (Stratagene Inc., La Jolla, CA) using standard procedures (7). After transformation of competent DH5α cells, colonies were transferred to nitrocellulose and screened with a 32P-labeled V_5 probe (11). DNA sequence analysis was according to Sanger et al. (12) using the T7 sequencing kit (Pharmacia).

Results

V_5 Gene Family Utilization in V_5 Transcripts from Atopic Dermatitis Patients. Total RNA extracted from PBMC of three patients with atopic dermatitis was extended with a Ce-specific primer and AMV reverse transcriptase, and first-strand cDNA was amplified by PCR using a nested Ce-specific primer in combination with 5' primers specific for the human V_3-V_6 gene families. All four V_5 primers yielded amplified PCR products of the expected size. The identity of PCR products was confirmed by hybridizing Southern blots of amplified material with 32P-labeled V_5 gene family-specific probes, indicating the V_5 gene family specificity of all V_5 primers used. The results demonstrated that transcripts encoded by members of the human V_3-V_5 gene families were present in three atopic dermatitis patients, whereas V_6-encoded transcripts were found in patient P3 only (data not shown). We were unable to amplify V_6 transcripts from PBMC of four nonatopic, healthy donors.

Molecular Analysis of Germline V_5 Gene in Atopic Dermatitis Patients. In all three patients, we obtained abundant PCR products with the V_5 gene family-specific primer. The human V_5 gene family consists of only two functional members both of which are extremely well-conserved in the human population (10). We focused on this gene family to analyze the molecular structure of the Ce V_5 gene transcripts. To that end, we first determined the nucleotide sequences of V_5 genes present in the germline of these patients. Genomic DNA from polyclonal, EBV-transformed B lines was used as starting material in PCR reactions employing primers specific for the leader and 3' end flanking sequences of both functional V_5 genes. For each patient, a minimum of 10 bacterial colonies hybridizing to a V_5 probe were randomly picked and used for nucleotide sequencing. In the genome of each patient, the two functional members of the V_5 gene family were detected, and no other V_6 family members or fragments were detected in this sequence analysis. In patients P1 and P2, both members displayed 100% sequence identity to the published germline genes (represented by the sequences of the V_5-1R1 and V_5-2R1 rearrangements (11)). In patient P3, we detected a single nucleotide difference in one of the V_5-1R1 alleles (see legend to Fig. 1). Based on the large number of germline V_5 genes sequenced, we estimate the PCR-induced error frequency to be <1/1200 in 35 cycles.

Inter- and Intrachromosomal Somatic Mutations in Ce V_5 Transcripts. To investigate the molecular structure of Ce V_5 regions, first-strand cDNA was synthesized from total RNA of PBMC and subsequently amplified in the PCR using a Ce and V_5-specific primer pair. PCR-amplified material was cloned into Bluescript vector and after transformation, multiple V_5-hybridizing colonies were used for nucleotide sequence analysis. All 19 nucleotide sequences contained open reading frames and were derived from Ce V_5 transcripts, confirming the specificity of the Ce and V_5 primers. In Figs. 1 and 2, we have compared the nucleotide and deduced amino acid sequences of the expressed and germline V_5 genes of patients P1, P2, and P3. Table 1 summarizes the distribution of R and S mutations over framework and CDR regions. In this analysis, we excluded somatic mutations occurring in the junctional region, because the patient's germ-
line diversity (Dₜ) and joining (Jₜ) gene segments were not available for comparison.

cDNA clones VHP2-57 and VHP1-58 derive from the germline Vₛₕ-2R₁ gene, whereas the other 17 cDNA clones descend from the germline Vₛₕ-1R₁ gene. All ε Vₛₕ sequences contain somatic mutations, ranging from 6 to 37 in the Vₛ portion of individual transcripts. Close inspection of sequences from patient P2 reveals that nucleotide sequences P2-51, P2-52, P2-53, and P2-54 are not identical (between 95.7 and 99.2% sequence homology) but utilize the same Dₛ and Jₛ gene segments and are indistinguishable at the Vₛ/Dₛ and Dₛ/Jₛ junctions (Fig. 1). This strongly suggests that the corresponding B cell clones are the progeny of a common precursor (13). By the same criteria, the collection of eight sequences from patient P1 contains two clonally related IgE transcripts, P1-54 and P1-57, that differ by four nucleotides in the Vₛ portion (Fig. 1).

Comparison of the nucleotide sequences of all ε Vₛₕ transcripts revealed that numerous mutations are shared among clonally related and unrelated sequences within one individual and among individuals. These shared mutations are present in both CDR and framework (FR) regions and are silent or result in amino acid replacements. Among the most extreme examples are mutations that occur in multiple independent ε transcripts derived from all three donors (e.g., compare the C→T substitution at position 173, the G→A at position 230, and the C→T substitution at position 180). It is notable that these mutations involve descendants from both the Vₛₕ-1R₁ and Vₛₕ-2R₁ germline gene segments.

Based on the four clonally related ε Vₛₕ sequences from patient P2, we constructed a genealogical tree that shows the nature and reconstructs the likely order of somatic mutations that occurred during expansion of this B cell clone (Fig. 3).

The tree has been constructed to require the lowest number of parallel mutations. A total of 14 mutations (six R and seven S mutations) is shared by all four sequences. At the first branchpoint, four mutations are shared by clones P2-52 and P2-53 (three R and one S mutation). These clones differ from each other by four R and one S mutation. Similarly, clone P2-54 and P2-51 share four R and two S mutations and differ from each other by one S and one R mutation.

Dₛ and Jₛ Gene Utilization. All Jₛ gene segments except Jₛ₂ were present in the ε transcripts. The Dₛ gene segments were defined as the nucleotide sequences between the Vₛₕ- and Jₛ genes, and include putative N insertions. The Dₛ gene segments varied extensively in nucleotide sequence and length and displayed partial sequence homology with previously published germline or expressed Dₛ elements (Fig. 4).

Discussion

Antibody diversity is generated through a number of mechanisms including somatic assembly and imprecise joining of multiple gene segments, combinatorial assortment of different Ig H and L chains, and somatic hypermutation. Although the genetic mechanisms of antibody diversity are fairly well understood, little is known about their contribution to the human antibody response to clinically relevant exogenous antigens. We have analyzed Ig ε Vₛₕ gene transcripts from peripheral blood B cells of three patients with atopic dermatitis. The sera of these patients contained IgE antibodies reactive with at least 15 different environmental allergens. Our inability to amplify ε transcripts from peripheral blood B cells of nonatopic individuals strongly suggests that these ε Vₛₕ transcripts were relevant to the allergic immune response. The results demonstrate that members of all human Vₛ gene

| Sequence | Total no. mutations | Overall R/S | CDR R/S | FR R/S | Sequence | Total no. mutations | Overall R/S | CDR R/S | FR R/S |
|----------|---------------------|------------|---------|--------|----------|---------------------|------------|---------|--------|
| P1-51    | 36                  | 2.2        | 0.6     | 3.3    | P2-51    | 23                   | 1.1        | 1       | 1.1    |
| P1-52    | 18                  | 2.0        | 2.0     | 3.0    | P2-52    | 20                   | 1.2        | 3       | 1.0    |
| P1-53    | 9                   | 8.0        | 3.0     | 1.0    | P2-53    | 19                   | 0.9        | 3       | 0.7    |
| P1-54    | 7                   | 0.7        | 1.0     | 0.2    | P2-54    | 21                   | 1.0        | 1       | 1.1    |
| P1-55    | 23                  | 2.8        | 5.0     | 2.4    | P2-55    | 11                   | 0.8        | 1       | 0.8    |
| P1-56    | 14                  | 1.8        | 2.0     | 1.7    | P2-56    | 32                   | 1.6        | 2       | 1.5    |
| P1-57    | 8                   | 1.0        | 2.0     | 0.5    | P2-57    | 6                    | 1.0        | -       | 1.0    |
| P1-58    | 17                  | 2.4        | 1.5     | 3.0    | P3-9     | 6                    | 5.0        | 4.0     | 1.0    |
|          |                     |            |         |        | P3-60    | 14                   | 1.8        | 2.5     | 1.3    |
|          |                     |            |         |        | P3-63    | 36                   | 2.6        | 2.5     | 2.7    |
|          |                     |            |         |        | P3-69    | 8                    | 1.7        | 4.0     | 0.3    |

For each ε transcript, the total number of mutations (columns 2 and 7), the R/S ratio in the overall Vₛₕ region (columns 3 and 8), the CDR region (columns 4 and 9) and the FR regions (columns 5 and 10) are depicted.
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Figure 1. Nucleotide sequences of \( V_{\delta}5 \) transcripts from atopic dermatitis patients P1, P2, and P3. Nucleotide sequences are compared to the rearranged \( VH_{5-1R1} \) and \( VH_{5-1R2} \) genes that represent the two functional \( VN_{5} \) genes in germline configuration (T. Logtenberg, unpublished data). The nucleotide sequences of the corresponding germline \( V_{\delta}5 \) gene segments of patients P1, P2, and P3 were 100\% identical, except for a single difference at position 42 in one of the alleles of patient P3 (* and **). (Dashes) Nucleotide identity. The \( J_{\delta} \) gene segments are aligned with the germline \( JK_{3} \) gene (35). Upper and lower case letters refer to R and S mutations, respectively.

| \( VH_{5-1R1} \) | \( VH_{5-1R2} \) |
|-----------------|-----------------|
| JH3             | CDR3            |
| P1-51           | GCT GCC TAC TCG |
| P1-52           | GCT ATC TT     |
| P1-53           | TGT GCC TAC GGA|
| P1-55           | GCT TCG ATG GGC|
| P1-56           | TAG CAA TCC AC |
| P2-51           | ACT TAT TAT AGT|
| P2-52           | ACT TAT TAT ACT|
| P2-53           | ACT TAT TAT ACT|
| P2-54           | ACT TAT TAT ACT|
| P2-55           | ACC TCC TTC TCA|
| P2-56           | AGA TGC TCC GGC|
| P3-69           | TAT TAT GTG TCG|
| P3-60           | ACC AAC TAC    |
| P3-63           | CAC AGT TCC GCT|
| P2-57           | AAG TCC        |
| P1-58           | TGG GCT ACC TGC|

**CDR3**

| P1-51 | AAA GTC CAC GCC GAC |
|-------|---------------------|
| P1-52 | GCT ATC TT          |
| P1-53 | TGT GCC TAC GGA     |
| P1-55 | GCT TCG ATG GGC     |
| P1-56 | TAG CAA TCC AC      |

| P2-51 | ACT TAT TAT AGT GG  |
|-------|---------------------|
| P2-52 | ACT TAT TAT ACT GG  |
| P2-53 | ACT TAT TAT ACT GG  |
| P2-54 | ACT TAT TAT ACT GG  |
| P2-55 | ACC TCC TTC TCA AGC|
| P2-56 | AGA TGC TCC GGC     |

| P3-69 | TAT TAT GTG TCG GGG|
|-------|--------------------|
| P3-60 | ACC AAC TAC        |

**GCT**

| GCT ACC GTC TCT CAG GCC TCC |
|-----------------------------|

| P1-51 | GCT ACC GTC TCT CAG GCC TCC |
|-------|-----------------------------|
| P1-52 | GCT ACC GTC TCT CAG GCC TCC |
| P1-53 | GCT ACC GTC TCT CAG GCC TCC |
| P1-55 | GCT ACC GTC TCT CAG GCC TCC |
| P1-56 | GCT ACC GTC TCT CAG GCC TCC |

| P2-51 | GCT ACC GTC TCT CAG GCC TCC |
|-------|-----------------------------|
| P2-52 | GCT ACC GTC TCT CAG GCC TCC |
| P2-53 | GCT ACC GTC TCT CAG GCC TCC |
| P2-54 | GCT ACC GTC TCT CAG GCC TCC |
| P2-55 | GCT ACC GTC TCT CAG GCC TCC |

| P3-69 | GCT ACC GTC TCT CAG GCC TCC |
|-------|-----------------------------|
| P3-60 | GCT ACC GTC TCT CAG GCC TCC |
| P3-63 | GCT ACC GTC TCT CAG GCC TCC |

**GAT**

| GAT GTC TGG CAC AGC CTA |
|------------------------|

| GAT GTC TGG CAC AGC CTA |
|------------------------|

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families tested (V_n3-V_n6) may be utilized in the IgE response in atopic dermatitis patients. In PCR experiments, we noted that the small V_n5 gene family, consisting of two functional
members, was abundantly expressed in ε transcripts from all three patients, whereas for example V_n6, a family of comparable size, was not detectable in two patients, and generated a scarcely detectable PCR fragment in the third patient.

Nucleotide sequence analysis of the V_n5 genes present in the genome of the atopic dermatitis patients permitted the unequivocal assignment of somatic mutations in the ε V_n5 transcripts. In 19 transcripts analyzed, we detected a total of 328 nucleotide differences (range 5-36 per V_n segment) with the patient's own germline V_n5 genes, including both S and R mutations. Based on a PCR-related error frequency of <1/1,200, virtually all nucleotide differences represent somatic mutations acquired during in vivo growth and differentiation of the corresponding B cells. The distribution and types of mutations in the V regions of a B cell clone have been suggested to reflect the nature of the selective forces causing its expansion (14, 15). In the absence of selection and under the restraint of preserving the Ig molecule's structure, R/S ratios have been estimated to be 2.9 in the CDR and 1.5 in the FR regions (13). R/S ratios in the CDR regions that are substantially higher than 2.9 have been interpreted to reflect a process of positive selection by antigen. The R/S ratios in the CDR and FR regions of the ε V_n5 transcripts do not exhibit a consistent pattern (Table 1). In fact, all combinations of high and low R/S ratios in FR regions with high and low R/S ratios in CDR regions occur (Table 1).

Although this appears unusual for an antigen-driven selection process, several notions argue in favor of a role for antigen. Activation of the somatic hypermutation mechanism is associated with antigen stimulation and does not occur in polyclonally stimulated B cells (16, 17). Moreover, the allergen specificity of the IgE antibodies in the sera of these patients suggests an important role for allergen. Finally, the occurrence of clonally related B cells, as observed in two out of three patients, is characteristic for an antigen-driven selec-

Figure 2. Deduced amino acid sequences of ε V_n5 transcripts. Single-letter amino acid code is used. (Dashes) Identity of amino acid residue.

Figure 3. Genealogical tree of clonally related transcripts from patient P2. This tree was constructed to require the fewest independent parallel replacements. Single-letter amino acid code is used to denote R mutations.
tion process. Several explanations, not mutually exclusive, for the heterogeneous R/S patterns in e V.5 transcripts are conceivable. The FR regions, especially FR3, may contribute to antigen binding, as has been argued on the basis of experimental data and theoretical considerations (18-21). In this respect, it is important to realize that antigen determines the shape of the antigen-binding portion of V regions and that current concepts concerning R/S distributions have largely been derived from analysis involving immunization with haptenic groups. The antigen combining site of hapten-binding antibodies may differ profoundly from that of protein-binding antibodies. Examples of nonhaptenic immune responses generating high affinity antibodies with "aberrant" R/S mutations have been reported in the literature (22-24). An alternative interpretation is an extension of a model proposed by Manser (25). It may be envisaged that initially, somatic mutations are efficiently selected by antigen resulting in affinity maturation of the immune response. In case of recurrent or prolonged antigenic stimulation, the extent to which somatic mutations lead to higher binding affinity in an already selected B cell clone becomes limited and finally saturated. At this point, persistent antigenic stimulation may result in the accumulation of "neutral" R and S mutations, i.e., mutations that do not affect the affinity or the structural integrity of the Ig molecule. Such mutations are not selected for or against, and may shift the R/S ratio towards a pattern resembling random mutations. It may be envisaged that such a pattern of mutations of V regions is associated with antigens that chronically and/or recurrently stimulate the immune system, as is the case with allergens and perhaps particular autoantigens (22). In the above scenario, mutations continue to occur in secondary and higher order immune responses as a result of repeated or chronic exposure to the same antigen.

Four out of seven e V.5 sequences from patient P2 were clonally related as judged by their junctional regions. This set of sequences contained, in addition to many shared somatic mutations, at least three unique mutations. These numbers represent a minimum estimate because somatic mutations in the Dn and Jn segments were not included in our analysis. We constructed a genealogical tree based on the premise that the most shared mutations were acquired early, and the least shared mutations were acquired late in the evolution of the clone. Indeed, the general pattern of mutations appears to reflect the temporal order of somatic mutations acquired during expansion of the clone (17, 26). A small number of shared mutations in the clonally related set of e transcripts likely arose as a result of parallel events (i.e., the A→T at position 17 and the A→G at position 127; Fig. 1).

It has been previously reported that the V regions of murine hybridomas secreting monoclonal anti-hapten antibodies share somatic mutations, even among hybridomas from different mice and generated in different laboratories (27, 28). In addition, shared mutations have been noted in hybridomas carrying "passenger" transgenes that are not functionally expressed (29, 30). Shared R mutations have been interpreted to reflect the influence of antigen selection conferring proliferative advantage to the B cell clone, whereas shared S mutations may mark positions that are especially prone to somatic mutation, so-called hot spots (28, 31). In the collection of 19 e V.5 transcripts, we found a remarkably high incidence of shared mutations, even among transcripts derived from different germline members of the V.5 gene family. The shared mutations were distributed across CDR and FR regions and included both S and R mutations. In the current analysis, it can be excluded that these mutations represent artifacts introduced, for example, as a consequence of cell fusion or in vitro growth of hybridomas. A high number of mutations in the collection of e V.5 transcripts was also found in two V.5 expressed genes reported by Andris et al. (32). Thus, out of 17 mutations present in a V.5 gene encoding an anti-HIV antibody, 12 are also present in the same position in at least one but often multiple e V.5 transcripts, seven of which constitute identical substitutions (32). Similarly, the majority of mutations present in the V.5 genes encoding an antinsulin autoantibody and an antirabies virus antibodies also appear in multiple independent e V.5 transcripts (33). Thus, the seemingly improbable event of isolating multiple parallel somatic alterations in Vn transcripts from unrelated individuals and in antibodies with different specificities constitutes, in fact, a frequently occurring phenomenon.

The molecular basis for the high incidence of shared mutations remains to be elucidated. Based on their diversity, multitude, and distribution pattern in the collection of e V.5 transcripts, it seems unlikely that a somatic gene conversion mechanism, even when considering multiple segmental exchanges, is a major contributor (34). Rather, the accumulation of point mutations, perhaps in combination with intrinsic sequence specificity of the somatic hypermutation mechanism, constitutes the mechanism underlying shared mutations (30). In addition, selection by antigen probably contributes to the occurrence of shared R mutations. Although we have not addressed the antigen specificity of the IgE antibodies encoded by these transcripts, it seems unlikely that they all bind to the same epitope.

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References
1. Hanifin, J.M. 1984. Atopic dermatitis. J. Allergy Clin. Immunol. 73:211.
2. Sampson, H.A. 1989. Role of immediate hypersensitivity in the pathogenesis of atopic dermatitis. Allergy 44:52.
3. Pérez, J., F. Rousset, F. Brière, I. Chrétien, J.V. Bonnefoy, H. Spits, T. Yokota, N. Arai, K. Arai, J. Banchereau, and J.E. de Vries. 1988. IgD production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferon γ and α and prostaglandine E2. Proc. Natl. Acad. Sci. USA. 85:6880.
4. Parronchi, P., D. Macchia, M. Piccinni, P. Biswas, C. Simonelli, E. Maggi, M. Ricci, A.A. Ansari, and S. Romagnani. 1991. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc. Natl. Acad. Sci. USA. 88:4538.
5. Wierenga, E.A., M. Snoek, H.M. Jansen, J.D. Bos, R.A.W. van Lier, and M.L. Kapsenberg. 1991. Human atopic-specific types 1 and 2 T helper cell clones. J. Immunol. 147:2942.
6. Hanifiin, J.M., and G. Rajka. 1980. Diagnostic features of atopic dermatitis. Acta Dermato-Venereol. Suppl. 92:44.
7. Ausubel, F.M., R. Brent, R.E. Kingston, R.E. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1988. Current Protocols in Molecular Biology. Vol. 1 and 2. Greene Publishing Associates and Wiley-Interscience, New York.
8. Higuchi, R. 1989. Rapid, efficient DNA extraction for cells or blood. Amplifications. 2:1.
9. Van Es, J.H., F.H.J. Gmelig Meyling, W.R.M. van de Akker, H. Aanstoot, R.H.W. Derksen, and T. Logtenberg. 1991. Molecular cloning and sequencing with chain-terminating inhibitors. EMBO (Eur. Mol. Biol. Organ.) J. 10:2397.
10. Van Es, J.H., F.H.J. Gmelig Meyling, W.R.M. van de Akker, H. Aanstoot, R.H.W. Derksen, and T. Logtenberg. 1991. Structure and evolution of mammalian Vα families. Int. Immunol. 4:52.
11. Berman, J.E., S.J. Mellis, R. Pollock, C.L. Smith, H. Suh, B. Heinke, C. Kowal, U. Surti, L. Chess, C.R. Cantor, and F. Alt. 1988. Content and organization of the human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. J. Exp. Med. 173:461.
12. Sanger, F., S. Nicklen, and A.R. Coulsen. 1977. DNA sequencing. Proc. Natl. Acad. Sci. USA. 74:5463.
13. Stohlmacher, M.J., A.H. McMichael, A.B. Phipps, and M.G. Weigert. 1987. Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. Proc. Natl. Acad. Sci. USA. 84:9150.
14. Stohlmacher, M.J., D.A. Nemazee, J. van Snick, and M. Weigert. 1987. Variable region sequences of murine IgG anti-IgG monoclonal autoantibodies (rheumatoid factors). II. Comparison of hybridomas derived by lipopolysaccharide stimulation and secondary protein immunization. J. Exp. Med. 165:970.
15. Stohlmacher, M.J., A. Marshak-Rothstein, C.B. Woldowicz, T.L. Rothstein, and M.G. Weigert. 1987. The role of clonal selection and somatic mutation and autoimmunity. Nature (Lond.). 328:805.
16. Manser, T., L.J. Wysocky, M.N. Margolies, and M.L. Gefter. 1987. Evolution of antibody variable structure during the immune response. ImmunoL Rev. 96:141.
17. Rudikoff, S., M. Pawlita, J. Pumphrey, and M. Heller. 1984. Somatic diversification of immunoglobulins. Proc. Natl. Acad. Sci. USA. 81:2162.
18. Alzari, P.M., S. Spinelli, R.A. Mariuzza, G. Boulou, R.J. Poljak, J.M. Jarvis, and C. Milstein. 1990. Three-dimensional structure determination of an anti-2-phenyloxazolone antibody: the role of somatic mutation and light/light chain pairing in the maturation of an immune response. EMBO (Eur. Mol. Biol. Organ.) J. 9:3807.
19. Amit, A.G., R.A. Mariuzza, S.E.V. Phillips, and R.J. Poljak. 1986. Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. Science (Wash. DC). 233:747.
20. Schroeder, H.W., Jr, J.L. Illinson, and R.M. Perlmuter. 1989. Structure and evolution of mammalian Vα family. Int. Immunol. 2:41.
21. Rose, D.R., K.K. Strong, M.N. Margolies, L.M. Gefter, and G.A. Petsko. 1990. Crystal structure of the antigen-binding fragment of the murine anti-arsonate monoclonal antibody 36-71 at 2.9-Å resolution. Proc. Natl. Acad. Sci. USA. 87:338.
22. Randen, I., D. Brown, K.M. Thompson, N. Hughes-Jones, V. Pascual, K. Victor, J.D. Capra, O. Ferre, and J.B. Natvig. 1992. Clonally related IgM rheumatoid factors undergo affinity maturation in the rheumatoid synovial tissue. J. Immunol. 148:3296.
23. Adderson, E.E., P.G. Shackelford, A. Quinn, and W.L. Carroll. 1991. Restricted Ig H chain V region gene usage in the human antibody response to Haemophilus influenzae type b capsular polysaccharide. J. Immunol. 147:1667.
24. Clarke, S., R. Rickert, M.K. Wloch, L. Staudt, W. Gerhard, and M. Weigert. 1990. The BALB/c secondary response to the sib site of influenza virus hemagglutinin. J. Immunol. 145:2286.
25. Manser, T. 1989. Evolution of antibody structure during the immune response. J. Exp. Med. 170:1211.
26. Mckean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. Proc. Natl. Acad. Sci. USA. 81:3180.
27. Wysocky, L.J., M.L. Gefter, and M.N. Margolies. 1990. Parallel evolution of antibody variable regions by somatic processes: consecutive shared somatic alterations in the Vα genes expressed by independently generated hybridomas apparently acquired by point mutation and selection rather than by gene conversion. J. Exp. Med. 172:315.
28. Berek, C., and C. Milstein. 1987. Mutation drift and repertoire shift in the maturation of the immune response. ImmunoL Rev. 96:23.
29. Sharpe, M.J., C. Milstein, J.M. Jarvis, and M.S. Neuberger. 1991. Somatic hypermutation of immunoglobulin κ may de-
depend on sequences 3' of C, and occurs on passenger trans-
genomes. EMBO (Eur. Mol. Biol. Organ.) J. 10:2139.
30. Hackett, J., Jr., B.J. Rogerson, R.L. O'Brien, and U. Storb. 1990. Analysis of somatic mutations in \( \kappa \) transgenes. J. Exp. Med. 172:131.
31. Levy, S., E. Mendel, S. Kon, Z. Avnur, and R. Levy. 1988. Mutational hot spots in Ig V region genes of human follicular lymphomas. J. Exp. Med. 168:475.
32. Andris, J.S., S. Johnson, S. Zolla-Pazner, and J.D. Capra. 1991. Molecular characterization of five human anti-human immunodeficiency virus type 1 antibody heavy chains reveals extensive somatic mutation typical of an antigen-driven immune response. Proc. Natl. Acad. Sci. USA. 88:7783.
33. Sanz, I., P. Casali, J.W. Thomas, A.L. Notkins, and J.D. Capra. 1989. Nucleotide sequences of eight human natural autoantibody \( \kappa \) regions reveals apparent restricted use of \( \kappa \) families. J. Immunol. 142:4054.
34. Wysocki, L.J. 1989. Gene conversion and the generation of antibody diversity. Annu. Rev. Biochem. 58:509.
35. Kabat, E.A., T.T. Wu, M. Reid-Miller, H.M. Perry, and K.S. Gottesman. 1987. Sequences of Proteins of Immunological Interest. 4th edition. U.S. Government Printing Office, Bethesda, MD. 674 pp.