It is now evident that most if not all major intrastrain crossreactive idiotypes (CRI) result from the expression of unmutated germline genes or germline genes that have undergone a moderate degree of somatic mutation (1-11). The association with germline genes can account for the ubiquitous presence of such idiotypes within a strain (12), as contrasted with private idiotypes, which may be the products of highly mutated genes (13).

In general, there appears to be no restriction of expression of heavy chain isotype in CRIs. This includes the expression of IgE, which has been identified in CRI-bearing antibodies with specificities for GAT (14, 15), Ars (16), and PC (17).

The number of somatic mutations expressed in CRI + antibodies increases with time after immunization. For example, in the phenyloxazolone system, mAb prepared with spleens taken 1 wk after primary immunization reflect very few mutations, whereas mutations are frequent 1 wk later (18, 19). In the PC system, mutations are much more common in IgG or IgA than in IgM mAb bearing the major CRI (2). Whether this reflects time-dependence of mutations (18, 20), association of mutations with a class switch (2), or a combination of the two mechanisms is uncertain.

Although IgE antibodies bearing CRIs have been identified, there are no data available on their amino acid sequences. Such data are of general interest; also, the degree of somatic mutation may relate to the mechanism of the IgM to IgE switch. This paper presents data on the primary structures of two IgE mAb that express idiotypes associated with antibodies to the Ars hapten in A/J mice.

Materials and Methods

Mice. A/J and (BALB/c × A/J)F1 (CAF1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were 8-12 wk old at the start of an experiment.

Hybridomas. Hybridomas were prepared according to Köhler and Milstein (21), as modified by Gefter et al. (22). The hybridoma that produces protein R16.7 (anti-Ars, 1)

This work was supported by grants AI-22068 and AI-10947 from the National Institutes of Health, Bethesda, MD.

Abbreviations used in this paper: CDR, complementarity-determining region; CRI, crossreactive idiotype; CRIa, major intrastrain CRI of anti-Ars in A/J mice; CRIa, minor intrastrain CRI of anti-Ars in A/J mice.

J. Exp. Med. © The Rockefeller University Press - 0022-1007/86/7/0291/12 $1.00 291
Volume 164 July 1986 291-302
CRI\textsubscript{a} [major intrastrain CRI of anti-Ars in A/J mice], IgG1\textsubscript{a}) was described previously (23). A hybridoma producing mAb SE20.2 (anti-Ars, CRI\textsubscript{a}, IgE\textsubscript{a}) (24) was prepared using mesenteric lymph node cells from an A/J mouse immunized i.p. three times at 2-wk intervals, with 5 μg portions of KLH-Ars in alum. A fourth inoculation, in saline without alum, was given i.p. 2 wk later, 3 d before the fusion. The myeloma cell line used was the nonsecretor Sp2/0-Ag14 (25). Supernatants were screened for IgE anti-Ars production and for the presence of CRI\textsubscript{a} by the RIA described below. A hybridoma secreting mAb SE1.3 (anti-Ars, CRI\textsubscript{m} [minor intrastrain CRI of anti-Ars in A/J mice], IgE\textsubscript{m}) was prepared using spleen cells from an A/J mouse that had been irradiated (600 rad) and had received 5 x 10\textsuperscript{7} splenic leukocytes, pooled from two donor A/J mice. The donors had been immunized three times at 2-wk intervals with 5 μg KLH-Ars in alum and were sacrificed 4 wk after the last inoculation. Recipients were immunized i.p. immediately after the adoptive transfer with 5 μg KLH-Ars in alum, and fusions were carried out 7 d later. The hybridomas secreting mAb SE20.2 or SE1.3 were cloned by limiting dilution. Hybridomas were grown i.p. in CAF\textsubscript{1} mice. Pristane (0.5 ml) was given 1–3 wk before injecting tumor cells. For mRNA sequencing, the tumor cells were grown in culture medium (DMEM containing 10% FCS).

Assay for Total or IgE Anti-Ars. The total concentration of anti-Ars antibodies was determined by using a polyvinylchloride microtiter plate, the wells of which were coated with BSA-Ars (26). Final development was carried out with \textsuperscript{125}I-labeled, affinity-purified rabbit anti–mouse Fab. For IgE anti-Ars, a similar assay was used, but \textsuperscript{125}I-labeled, affinity-purified rabbit anti–mouse IgE was used as the developing reagent (27). Since mAb or hybridoma culture supernatants were assayed, competition by large amounts of non-IgE anti-Ars (28) was not a significant factor in the assays.

Assays for CRI\textsubscript{a} and CRI\textsubscript{m}. The RIA for CRI\textsubscript{a} (12) made use of 10 ng of \textsuperscript{125}I-labeled mAb R16.7 (CRI\textsubscript{a}), and enough rabbit anti-Id to bind 50–60% of the labeled ligand. Complexes were precipitated with excess goat anti–rabbit IgG that had previously been adsorbed with mouse IgG. The concentration of CRI\textsubscript{a} in unknown, unlabeled samples was quantified through their capacity to inhibit binding of the labeled ligand. Unlabeled mAb R16.7 was used as the standard; 7–12 ng was required for 50% inhibition.

Antibodies expressing CRI\textsubscript{m} are defined as those that share some but not all idiotopes with antibodies expressing CRI\textsubscript{a}. Since some idiotopes are shared, CRI\textsubscript{m} antibodies are bound by anti-CRI\textsubscript{a}, but CRI\textsubscript{m}\textsuperscript{+} antibodies cannot displace CRI\textsubscript{a}\textsuperscript{+} antibodies completely from anti-CRI\textsubscript{a}. The IgE mAb expressing CRI\textsubscript{m} (SE1.3) was identified by its binding to anti-Id (R16.7), and by the failure of a large amount to cause 50% inhibition in the assay for CRI\textsubscript{a} (see Results). R16.7 is a prototype of the CRI\textsubscript{a} family.

Affinity Purification of Antibodies. Anti-Ars antibodies were purified by passage over a column of bovine gamma globulin (BGG)-Ars conjugated to Sepharose 4 B, followed by elution with 0.5 M sodium p-arsanilate, in Tris HCl buffer, pH 8.0 (23).

Recombination of H and L Chains. The method of Bridges and Little (29) was used with some modification. Antibodies were reduced with 0.01 M DTT (Calbiochem-Behring, San Diego, CA) for 2 h at room temperature, then alkylated, in an ice bath, for 15 min with 0.06 M iodoacetamide, (Sigma Chemical Co., St. Louis, MO) that had been further recrystallized from distilled water. This was followed by dialysis against neutral buffer. To separate H and L chains, the protein was dialyzed against 1 M propionic acid and 4.5 M urea, then 20–30 mg was applied to a column of Sephadex G-100, equilibrated with the same solution. The solvent used for elution was 1 M propionic acid (without urea). Good separation of H and L chains was achieved; the separation was monitored by electrophoresis on a 10% polyacrylamide gel in the presence of SDS under nonreducing conditions. To recombine chains, an optical absorbance ratio (280 nm) of 1.8:1 (H/L chain) was used. The proteins were mixed while in 1 M propionic acid, dialyzed twice against distilled water, then against neutral Tris HCl buffer.

Sequencing of V Regions of mRNA for H and L Chains. mRNA was isolated from hybridomas essentially as described by Palmiter (30), who used other tissues. Briefly, 1–3 x 10\textsuperscript{6} cells were suspended in neutral Tris buffer containing 2% Triton X-100, and lysed in a Dounce homogenizer. Cell debris was removed by centrifugation. Polysomes were precipitated by adjusting the MgCl\textsubscript{2} concentration to 100 mM, and were isolated by
Oligonucleotide Primers Used for mRNA Sequencing

| Amino acid position of first 5' nucleotide in oligomer | Sequence of primer | Source |
|------------------------------------------------------|------------------|--------|
| 131 in C, | 5'-d(AGTGCCTTTACAGGGCT) | OCS Laboratories, Denton, TX (32) |
| 99 in V, | 5'-d(GGATCTTGCACAGAAATA) | Prepared by K. Meek* |
| 89 in V, | 5'-d(CTCAGATGTCAGGCT) | C. Milstein and C. Berek |
| 122 in C, | 5'-d(TGGATGGTGGGAAGATG) | OCS Laboratories (33) |
| 86 in V, | 5'-d(ACCCTGTTGGCAAAAGTA) | Prepared by K. Meek* |
| 66 in V, | 5'-d(CCCACTGCCACTGTT) | C. Milstein and C. Berek |
| 45 in V, | 5'-d(TTTAACAAGTTCCATCTGG) | Prepared by K. Meek* |

* Prepared using an Biosearch, Inc. (San Rafael, CA) oligonucleotide synthesizer.

Results

Fig. 1 shows nucleotide and amino acid sequences of the V, regions of IgE anti-Ars mAb SE20.2 and SE1.3. For SE1.3, only nucleotide sequencing was carried out. For SE20.2, the complete nucleotide sequences were obtained for V,JH and V,JL. In addition, the N-terminal sequences of both chains of SE20.2 were obtained by protein sequencing (positions 1–25 of both V, and V,). There was complete agreement between the peptide and nucleic acid sequences. For SE20.2 (but not SE1.3), the mRNA sequence for large segments of both chains was determined independently in two laboratories. This resulted in the elimination of a few uncertainties that would otherwise have been present. In addition, there was disagreement with respect to one nucleotide, corresponding to amino acid 96 of the light chain.

We will discuss the V, segments (positions 1–98) first. They are compared with the corresponding sequence of mAb 36–65, which has been shown (6) to have a V, segment encoded by an unmutated germline gene. This gene (generally in a mutated form) is believed to encode the V, segment of all CR1A+ anti-Ars mAb studied so far. When the nucleotide sequence of SE20.2 is compared to the germline sequence, there are only three nucleotide substitutions in the V, segment (positions 1–98), two of which occur in complementarity-determining
FIGURE 1. Nucleotide and deduced amino acid sequences of the V\textsubscript{H} regions of two IgE anti-Ars mAbs, SE20.2 and SE1.3. They are compared with the corresponding sequences of mAb 36–65, whose V\textsubscript{H} segment (1–98) is virtually identical in nucleotide sequence to that of a putative germline gene (6). A solid line indicates identity with the 36–65 nucleotide sequence. An uncertainty in the nucleotide or amino acid sequence is indicated by an X or by a question mark, respectively. Amino acid differences are boxed.

region 2 (CDR2) and cause amino acid substitutions. The third is a silent substitution at amino acid position 72.

There are more substitutions in the V\textsubscript{H} segment (positions 1–98) of mAb SE1.3 (CRI\textsubscript{m}+), which differs from the germline-encoded amino acid sequence at five positions, three of which are in CDR2. There is uncertainty for one amino acid at position 43. Again, all differences from the germline-encoded amino acid sequence involve a single nucleotide substitution. The first 17 amino acids of the V\textsubscript{H} segment of SE1.3 were not determined.

As indicated in Fig. 1, the D\textsubscript{H} segments of 36–65 and SE20.2 have the same length (eight amino acids), which is characteristic of the CRI\textsubscript{A}+ mAb sequenced so far, with very few exceptions (35 and E. Rosen and P. Robbins, unpublished results). There is one amino acid substitution (position 100) in SE20.2, as compared to 36–65; this involves all three nucleotides of the codon. The D\textsubscript{H} segment of SE1.3 is one amino acid longer than that of 36–65 or SE20.2 (shown as an insertion of phenylalanine between positions 105 and 106). In addition,
there is a substitution (His for Val) at position 100, again involving all three nucleotides.

The partial sequences obtained for the JH regions of SE20.2 and SE1.3 indicate that they are JH1 and JH2, respectively. With rare exceptions (36), CRI\(_A^+\) mAb use JH2. Thus, SE20.2, which is CRI\(_A^+\), constitutes another exception to this general rule. The partial JH sequence of SE20.2 agrees with the germline BALB/c JH1 sequence (37 and data not shown), except for two nucleotide substitutions, one of which is at the D-J junction.

Fig. 2 shows nucleotide sequences and corresponding amino acid sequences of the entire V\(_L\) region of the IgE anti-Ars mAb, SE20.2 (CRI\(_A^+\)) and all but the first eight amino acids of SE1.3 (CRI\(_M^+\)). The deduced amino acid sequences are compared with that of the V\(_L\) region of mAb R16.7 (38), a prototypical CRI\(_A^+\) anti-Ars mAb. In addition, nucleotide sequences of SE1.3 and SE20.2 are compared with one another. Nucleotide sequences are not available for the V\(_L\) of R16.7. For SE20.2 there are three differences as compared with the V\(_L\) segment of R16.7 (positions 1–95), two of which are in CDRs. In addition, there is one uncertainty in the nucleotide sequence of SE20.2.

A comparison of the amino acid sequence of the V\(_L\) segment of SE1.3 (positions 1–95) with that of R16.7 shows only one definite amino acid substitution,
### Table II

**In Vitro Recombination of H and L Chains from Anti-Ars mAb**

| Exp. | Protein | Apparent concentration (μg/ml) | From OD280 | From anti-Ars titer* | From anti-Ars titer‡ | CRIα assay§ |
|------|---------|--------------------------------|------------|----------------------|----------------------|-------------|
| Intact |         |                                |            |                      |                      |             |
| 1    | R16.7   | 300                            | 302        | 0                    | 8                    |             |
| 2    | SE1.3   | 300                            | 230        | 301                  | >3,000 (9)           |             |
| 3    | SE20.2  | 300                            | 285        | 300                  | 10                   |             |
| 4    | Nonspecific IgG | 300 | 0 | 0 | >3,000 (13) | | |
| Reduced, alkylated | | | | | | |
| 5    | R16.7   | 300                            | 278        | 0                    | 6                    |             |
| 6    | SE1.3   | 300                            | 66         | 92                   | >3,000 (26)          |             |
| Recombinant | | | | | | |
| 7    | H16.7L16.7 | 300 | 283 | 0 | 7 | |
| 8    | H16.7L1.3 | 300 | 271 | 1 | 8 | |
| 9    | H16.7LN  | 300 | 20 | 0 | 340 | |
| 10   | H15L16.7 | 300 | 35 | 55 | 250 | |
| 11   | H15L1.3  | 300 | 28 | 52 | >3,100 (3) | |
| 12   | H15LN    | 300 | 2 | 5 | >3,300 (4) | |
| 13   | H15L16.7 | 300 | 2 | 0 | 385 | |
| 14   | H15L1.3  | 300 | 3 | 1 | >3,000 (8) | |
| 15   | H15LN    | 300 | 0 | 0 | >3,100 (17) | |

* Using 125I-labeled, affinity-purified rabbit anti–mouse Fab as the developing reagent. R16.7 was used as the standard.

‡ Using 125I-labeled, affinity-purified rabbit anti–mouse IgE as the developing reagent. SE20.2 was used as the standard.

§ Amount (ng) required for 50% inhibition in the RIA for CRIα. Numbers in parentheses refer to percent inhibition by weight (in ng) specified.

1 Note that these are the standards used.

(position 93 in CDR3), and two uncertainties. 8 of the 95 positions in the Vα segment of SE1.3 were not sequenced.

The Vα nucleotide sequences of SE20.2 and SE1.3 are strikingly similar; there are only three differences (and four uncertainties), strongly supporting their derivation from the same Vα germline gene.

The Jκ regions of SE1.3 and SE20.2 are both Jκ1. In comparison with the Jκ1 amino acid sequence of R16.7, there are no substitutions (but three uncertainties) in SE1.3 and one substitution, as well as one uncertainty, in SE20.2.

**Idiotypic Properties of mAb SE20.2 and SE1.3.** Table II shows data on inhibition by SE20.2 and SE1.3 in the RIA for CRIα. SE20.2 expresses CRIα; its inhibitory capacity is comparable to that of R16.7, a prototype of CRIα+ mAb. In contrast, SE1.3 is a very poor inhibitor, causing only 9% inhibition when 3,000 ng was tested. SE1.3 is, however, bound by anti-CRIα. It was completely removed upon passage through a column of Sepharose 4 B, to which an IgG fraction of anti-Id, specific for mAb R16.7, was conjugated. This experiment was done with a culture supernatant of the SE1.3 hybridoma; all anti-Ars activity was bound by the column. Another experiment made use of 125I-labeled, affinity-purified SE1.3. 10 ng was added to varying dilutions of rabbit anti-Id (R16.7) antiserum, and complexes were precipitated with goat anti–rabbit IgG; rabbit antiovalbumin
antiserum was present as carrier. 92% of the radioactivity was bound by a 1:80 dilution of the anti-Id antiserum.

The fact that SE1.3 is bound by anti-CRI\textsubscript{A} but fails to inhibit strongly in the RIA for CRI\textsubscript{A} indicates that it possesses one or more, but not all idiotopes that constitute the CRI\textsubscript{A} idiotype. We have referred to such molecules as expressing a minor idiotype, or CRI\textsubscript{m} (39).²

Recombination of H and L Chains. The amino acid sequence data indicate that mAb SE20.2 and SE1.3 both have V\textsubscript{H} (1-98) and V\textsubscript{L} (1-95) regions that are very similar to those associated with CRI\textsubscript{A}. As indicated above, SE1.3 is a very poor inhibitor in the RIA for CRI\textsubscript{A}. In an effort to ascertain whether this is attributable to its H chain, L chain, or both, we carried out chain recombination experiments. The results are shown in Table II. The following points emerge from the data.

The most significant conclusion is that the L chain of SE1.3 (CRI\textsubscript{m}+\textsuperscript{7}) is equivalent to that of R16.7 (CRI\textsubscript{A}+\textsuperscript{7}) with respect to mediating expression of CRI\textsubscript{A}. The recombinant molecule, H\textsubscript{16.7}L\textsubscript{SE1.3} (the prefixes R and SE are omitted), is nearly equivalent to R16.7, or to the recombinant, H\textsubscript{16.7}L\textsubscript{16.7} in inhibitory capacity in the assay for CRI\textsubscript{A} (Exps. 1, 7, and 8). Conversely, the recombinant of the L chain of R16.7 with the H chain of SE1.3 expresses CRI\textsubscript{A} very poorly (250 ng required for 50% inhibition as compared to 7 ng for the autologous recombinant, H\textsubscript{16.7}L\textsubscript{16.7}; Exps. 7 and 10). These results indicate that the failure of SE1.3 to express CRI\textsubscript{A} is attributable to its H chain rather than to its L chain. The weak inhibition by H\textsubscript{1.3}L\textsubscript{16.7} in the assay for CRI\textsubscript{A} (Exp. 10) may be due to slight residual contamination of L\textsubscript{16.7} by H\textsubscript{16.7}. This is supported by the fact that the recombinant H\textsubscript{N}L\textsubscript{16.7} (where N denotes nonspecific IgG) has a similar, very weak inhibitory capacity (385 ng required for 50% inhibition; Exp. 13).

Thus, the data strongly suggest that the L chain of SE1.3 is idiotypically equivalent to that of R16.7. The conclusion that H\textsubscript{1.3} is not idiotypically equivalent to H\textsubscript{16.7} is weakened by evidence that the procedures used modified the properties of the L chain of SE1.3. First, ~70% of the Ars-binding activity of SE1.3 was lost simply as a consequence of reduction and alkylation (Exps. 2 and 6). In addition, the autologous recombinant H\textsubscript{1.3}L\textsubscript{1.3} expressed only ~12% of the Ars-binding activity of the intact molecule (Exps. 2 and 11). It thus appears that L chains may be more easily denatured than H chains under the conditions used. Nevertheless, the virtual identity of L\textsubscript{1.3} and L\textsubscript{16.7} in their serological properties and amino acid sequences, and the fact that SE1.3 is idiotypically deficient with respect to CRI\textsubscript{A}, indicate that the defect resides in the V\textsubscript{H} and/or D\textsubscript{H} segments.

Discussion

The results presented here provide the first data on the primary structure of V regions of IgE antibodies that express a major idiotype (CRI\textsubscript{A}). This permits a comparison with the corresponding germline-encoded sequence of the CRI\textsubscript{A}-associated V\textsubscript{H} segment. The corresponding germline V\textsubscript{H} sequence is not known, but comparisons among known amino acid sequences are informative (see below). The principal question we wanted to address was the extent of somatic mutation that would be found in V regions of IgE antibodies. This, in turn, might reflect the stage of development of the B cell when the switch to IgE synthesis occurred.

²The primary structure of SE1.3, described in this paper, indicates that it may be a member of the 91A3 V\textsubscript{H} family described by Milner and Capra. (40).
There is now strong evidence (2, 3, 9, 18) that antibodies produced very early in a primary response exhibit very few or no mutations, and that mutations tend to accumulate with time after immunization. Although mutations were found to be more prevalent in IgG than in IgM antibodies expressing the T15 idiotype, a direct association of mutations with a class switch is unproven. For example, very early antiphenyloxazolone antibodies of the IgG class exhibit very few mutations (18).

Our results indicate that an IgE CR1A+ antibody may express a very limited number of somatic mutations. The V\mu sequence of the CR1A+ IgE antibody, SE20.2, is particularly informative. In the V\mu segment (positions 1–98) there are only three nucleotide differences (and one uncertainty) as compared to the germline nucleotide sequence. This results in two amino acid substitutions, both in CDR2. It is remarkable that mAb 93G7 (CR1A+) has precisely the same two nucleotide substitutions in CDR2 (41). Since SE20.2 is a relatively late mAb, prepared from a spleen taken after four inoculations of antigen over an 8-wk period, it is evident that the switch to IgE biosynthesis can be accomplished with very few associated somatic mutations. This is supported by the amino acid sequence of the V\kappa segment (1–95), which shows only three definite substitutions (at positions 30, 76, and 93) with respect to R16.7, a prototype of the CR1A family. It should be noted, however, that the V\kappa amino acid sequences of a number of CR1A+ mAb show very few differences (38, 42).

For SE1.3, which also has a V\mu sequence very similar to that encoded by the putative germline gene, there are five nucleotide substitutions among 243 nucleotides for which a comparison with the germline sequence of the V\mu segment is possible. In the V\kappa region (1–95), there is one amino acid difference from R16.7, at position 93 (and two uncertainties) among the 87 positions for which a comparison can be made. D and J sequences are discussed below. It is evident, then, that our IgE antibodies exhibit somatic mutations, but that they are no more frequent, particularly for SE20.2, than is typically seen in CR1A+ IgG anti-Ars mAb prepared after repeated inoculation of antigen (34, 38, 43). These results are of interest with respect to mechanisms of immunoglobulin class switching. Mongini et al. (44) have presented evidence suggesting that the IgM to IgE switch may proceed by a direct pathway, without an intermediate switch to an IgG subclass. This is consistent with the small number of somatic mutations that we observed in IgE, either on the basis that somatic mutation is associated with a class switch (2), or that mutations occur simply as a function of time or number of cell divisions (9).

Some other features of the amino acid sequences are of interest. One is the presence of a J\kappa1 sequence in SE20.2, a strongly CR1A+ anti-Ars antibody. Nearly all other anti-Ars CR1A+ antibodies sequenced have proven to be J\kappa2, although at least one exception (with J\kappa4) has been reported previously (36).

Another question of interest is the basis for the deficiency of CR1A-associated idiotopes in SE1.3. An obvious possibility is the presence of an extra amino acid in the D region of SE1.3 (Fig. 1). The presence of eight amino acids in the D region is an almost constant feature of CR1A+ anti-Ars antibodies, although a weakly CR1A+ mAb (123E6) with a nine–amino acid D region has been described previously (45). It is also possible that amino acid substitutions in the V\mu segment are in part responsible; there are five known substitutions in SE1.3 with respect
to the germline-encoded sequence, of which three are in CDR2. The presence of arginine at position 54 has not previously been observed. The deficiency of idiotopes in SE1.3 is evidently not attributable to V\textsubscript{i} or J\textsubscript{i}, since the recombinant molecule H\textsubscript{16.7} L\textsubscript{1.3} fully expressed CRI\textsubscript{A} and anti-Ars activity.

On this basis, one would predict that the H chains of SE1.3 would fail to reconstitute CRI\textsubscript{A} when combined with L\textsubscript{16.7}. This was in fact observed, but the results are not germane because of evidence for partial denaturation of \(\epsilon\) chains upon reduction and alkylation (see Results).

A novel observation is the presence of a cysteine (or half-cystine) residue in the J\textsubscript{n}1 region (of SE20.2). It will be of interest to ascertain whether this amino acid is present in a disulfide-bonded form.

**Summary**

We have obtained amino acid sequences (by mRNA and amino acid sequencing) for two IgE mAb that have specificity for the Ars hapten group and are related to the major idiotypic family, CRI\textsubscript{A} (crossreactive idiotype A), in the A strain of mouse. One mAb, SE20.2, fully expresses CRI\textsubscript{A}; the other, SE1.3, possesses some but not all of the characteristic idiotopes. Both IgE proteins contain V\textsubscript{H} and V\textsubscript{L} segments that are closely related to those associated with CRI\textsubscript{A}. The D segment of SE20.2 is also typical of CRI\textsubscript{A}\textsuperscript{*} mAb, but that of SE1.3 is one amino acid residue longer. Chain recombination experiments indicated that the L chain of SE1.3 is fully capable of supporting CRI\textsubscript{A} expression. Its deficiency with respect to idiotopes of CRI\textsubscript{A} was attributed to the extra amino acid in the D region and/or substitutions in the V\textsubscript{H} segment.

A major objective was to ascertain the frequency of somatic mutations in IgE. For the V\textsubscript{H} segment (amino acids 1–98) of SE20.2, there are only three nucleotide differences and one uncertainty with respect to the nucleotide sequence of the germline gene associated with CRI\textsubscript{A}. A somewhat higher frequency of substitutions is present in the V\textsubscript{H} segment of SE1.3. The V\textsubscript{H} amino acid sequences of the IgE proteins are nearly identical to those of a prototype of the CRI\textsubscript{A} family, mAb R16.7. The results are discussed with reference to the mechanism of the IgM to IgE switch.

We thank Dr. J. D. Capra for helpful discussions.

*Received for publication 17 March 1986.*

**References**

1. Schilling, J., B. Clevinger, J. M. Davie, and L. Hood. 1980. Amino acid sequence of homogeneous antibodies to dextran and DNA rearrangements in heavy chain V-region gene segments. *Nature (Lond.)* 283:35.

2. Gearhart, P. J., N. D. Johnson, R. Douglas, and L. Hood. 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. *Nature (Lond.)* 291:29.

3. Crewes, S., J. Griffin, H. Huang, K. Calame, and L. Hood. 1981. A single V\textsubscript{H} gene segment encodes the immune response to phosphorylcholine: somatic mutation is correlated with the class of the antibody. *Cell.* 25:59.

4. Bothwell, A. L. M., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D.
Baltimore. 1981. Heavy chain variable region contribution to the NPb family of antibodies: somatic mutation evident in a γ2a variable region. *Cell.* 24:625.

5. Estess, P. E., Lamoyi, A. Nisonoff, and J. D. Capra. 1980. Structural studies on induced antibodies with defined idiotypic specificities. IX. Framework differences in heavy and light chain variable regions of monoclonal anti-p-azophenylarsonate antibodies from A/J mice differing with respect to a cross-reactive idiotype. *J. Exp. Med.* 154:863.

6. Siekevitz, M., S. Y. Huang, and M. L. Gefter. 1983. Genetic basis of antibody production: a single heavy chain variable region gene encodes all molecules bearing the dominant anti-arsenate idiotype in the strain A mouse. *Eur. J. Immunol.* 13:123.

7. Schiff, C., M. Mili, and M. Fougereau. 1983. Immunoglobulin diversity: analysis of the germ line V, gene repertoire of the murine anti-GAT response. *Nucleic Acids Res.* 11:4007.

8. Claflin, J. L., J. Wolfe, A. Maddalena, and S. Hudak. 1984. The murine antibody response to phosphocholine. Idiotypes, structures, and binding sites. In *The Biology of Idiotypes.* M. I. Greene and A. Nisonoff, editors. Plenum Press, New York. 171.

9. Berek, C., G. M. Griffiths, and C. Milstein. 1985. Molecular events during maturation of the immune response to oxazolone. *Nature.* 316:412.

10. Rudikoff, S., M. Pavlita, J. Pumpheyre, E. Mushinski, and M. Potter. 1983. Galactan-binding antibodies; diversity and structure of idiotypes. *J. Exp. Med.* 158:1385.

11. Rajewsky, K., and T. Takemori. 1983. Genetics, expression and function of idiotypes. *Ann. Rev. Immunol.* 1:569.

12. Kuettner, M. G., A. Wang, and A. Nisonoff. 1972. Quantitative investigations of idiotypic antibodies. IV. Idiotype specificity as a potential genetic marker for the variable regions of mouse immunoglobulin peptide chains. *J. Exp. Med.* 135:579.

13. Ju, S-T., A. Gray, and A. Nisonoff. 1977. Frequency of occurrence of idiotypes associated with anti-p-azophenylarsonate antibodies arising in mice immunologically suppressed with respect to a cross-reactive idiotype. *J. Exp. Med.* 145:540.

14. Dessein, A., S-T. Ju, M. E. Dorf, B. Benacerraf, and R. N. Germain. 1980. IgE response to synthetic polypeptide antigens. II. Idiotype analysis of the IgE response to t-glutamic acidser-t-alanine30-L-tyrosine10 (GAT). *J. Immunol.* 124:71.

15. Lowy, I. A., A. Prouvost-Danon, A. Ahadie, and J. Theze. 1980. Fine specificity and idiotype analysis of the IgE response to the synthetic terpolymer t-glutamic acidser-t-alanine30-L-tyrosine10 (GAT) and its dinitrophenyl conjugate (DNP-GAT). *Mol. Immunol.* 17:1033.

16. Hirano, T., S. Kojima, N. Hirayama, M. J. Nelles, T. Inada, A. Nisonoff, and Z. Ovary. Presence of an intrastrain cross-reactive idiotype on A/J antibodies of the IgE class specific for the p-azophenylarsonate group. *J. Immunol.* 130:1300.

17. Shigemoto, S., T. Kishimoto, and Y. Yamanura. 1981. Characterization of phosphorylcholine (PC)-specific IgE B cells in CBA/N or (CBA/N X BALB/c)F1 male mice. *J. Immunol.* 127:1070.

18. Kaartinen, M., G. Griffiths, A. F. Markham, and C. Milstein. 1985. mRNA sequences define an unusually restricted IgG response to 2-phenyloxazolone and its early diversification. *Nature.* 304:320.

19. Griffiths, G. M., C. Berek, M. Kaartinen, and C. Milstein. 1984. Somatic mutation and the maturation of immune response to 2-phenyloxazolone. *Nature.* 312:271.

20. Rocca-Sera, J., J.-C. Mazie, D. Moinier, L. LeClercq, G. Somme, J. Theze, and M. Fougereau. 1982. Limited diversity of mouse gamma chain anti-GAT repertoire does not seem to be noticeably amplified upon class switch. *J. Immunol.* 129:2554.

21. Köhler, G., and C. Milstein. 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511.

22. Gefter, M. L., D. Margolies, and M. D. Scharff. 1977. A simple method for polyeth-
ylene glycol-promoted hybridization of mouse myeloma cells. *Somatic Cell Genet.* 3:231.

23. Lamoyi, E., P. Estess, J. D. Capra, and A. Nisonoff. 1980. Heterogeneity of an intrastrain cross-reactive idiotype associated with anti-p-azophenylarsonate antibodies of A/J mice. *J. Immunol.* 124:2834.

24. Haba, S., Z. Ovary, and A. Nisonoff. 1985. Clearance of IgE from serum of normal and hybridoma-bearing mice. *J. Immunol.* 134:3291.

25. Shulman, M., D. C. Wilde, and G. Köhler. 1978. A better cell line for making hybridomas secreting specific antibodies. *Nature.* 276:269.

26. Klinman, N. R., A. R. Pickard, N. H. Sigal, P. J. Gearhart, E. S. Metcalf, and S. K. Pierce. 1976. Assessing B cell diversification by antigen receptor and precursor cell analysis. *Ann. Immunol. (Paris).* 127:489.

27. Haba, S., T. Inada, and A. Nisonoff. 1984. Quantitative measurements of an intrastrain cross-reactive idiotype in IgE antibodies. *J. Immunol. Methods.* 73:97.

28. Haba, S., and A. Nisonoff. 1985. Quantitation of IgE antibodies by radioimmunoassay in the presence of high concentrations of non-IgE antibodies of the same specificity. *J. Immunol. Methods.* 85:39.

29. Bridges, S. H., and J. R. Little. 1971. Recovery of binding activity in reconstituted mouse myeloma proteins. *Biochemistry.* 10:2525.

30. Palmiter, R. D. 1974. Magnesium precipitation of ribonucleoprotein complexes. Expedient techniques for the isolation of undegraded polysomes and messenger ribonucleic acid. *Biochemistry.* 13:3605.

31. Hamlyn, P. H., G. G. Brownlee, C.-C. Cheng, M. J. Gait, and C. Milstein. 1978. Complete sequence of constant and 3' noncoding regions of an immunoglobulin mRNA using the diideoxynucleotide method of RNA sequencing. *Cell.* 15:1067.

32. Ishida, N., S. Ueda, H. Hayashida, T. Miyata, and T. Honjo. 1980. The nucleotide sequence of mouse immunoglobulin epsilon gene: comparison with the human epsilon gene sequence. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1117.

33. Kaartinen, M., G. M. Griffiths, P. H. Hamlyn, A. F. Markham, K. Karjalainen, J. L. T. Pelkonen, O. Mäkelä, and C. Milstein. 1983. Anti-oxazolone hybridomas and the structure of the oxazolone idiotype. *J. Immunol.* 130:997.

34. Slaughter, C. A., and J. D. Capra. 1983. Amino acid sequence diversity within the family of antibodies bearing the major anti-arsonate cross-reactive idiotype of the A strain mouse. *J. Exp. Med.* 158:1650.

35. Gridley, T., M. W. Margolies, and M. L. Gefter. 1985. The association of various D elements with a single-immunoglobulin V, gene segment: Influence on the expression of a major cross-reactive idiotype. *J. Immunol.* 134:1236.

36. Slaughter, C. A., D. J. Jeske, W. A. Kuziel, E. C. B. Milner, and J. D. Capra. 1984. Use of J,4 joining segment gene by an anti-arsonate antibody that bears the major A-strain cross-reactive idiotype but displays diminished antigen binding. *J. Immunol.* 132:5164.

37. Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa. 1980. Two types of somatic recombinations are necessary for the generation of complete immunoglobulin heavy-chain genes. *Nature.* 286:676.

38. Seligman, M., and J. D. Capra. 1981. Complete amino acid sequence of light chain variable regions derived from 5 monoclonal p-azophenylarsonate antibodies differing with respect to cross-reactive idiotype. *Proc. Natl. Acad. Sci. USA.* 78:7679.

39. Gill-Pazaris, L. A., E. Lamoyi, A. R. Brown, and A. Nisonoff. 1981. Properties of a minor cross-reactive idiotype associated with anti-p-azophenylarsonate antibodies of A/J mice. *J. Immunol.* 126:75.

40. Milner, E. C. B., and J. D. Capra. 1982. V,11 families in the antibody response to p-
azophenylarsonate: Correlation between serology and amino acid sequence. *J. Immunol.* 129:193.

41. Capra, J. D., C. A. Slaughter, E. C. B. Milner, P. Estess, and P. W. Tucker. 1982. The cross-reactive idiotype of A strain mice. Serological and structural analysis. *Immunol. Today.* 3:332.

42. Ball, R. K., J. Y. Chang, S. S. Alkan, and D. G. Braun. 1983. The complete amino acid sequence of the light chain variable region of two monoclonal anti-β-azophenylarsonate antibodies bearing the cross-reactive idiotype. *Mol. Immunol.* 20:197.

43. Margolies, M. N., A. Marshak-Rothstein, and M. C. Gefter. 1981. Structural diversity among anti-β-arsenate monoclonal antibodies from A/J mice: Comparison of Id⁺ and Id⁻ sequences. *Mol. Immunol.* 18:1065.

44. Mongini, P. K. A., W. E. Paul, and E. S. Metcalf. 1983. IgG subclass, IgE and IgA anti-trinitrophenyl antibody production within the trinitrophenyllicoll-responsive B cell clones. Evidence in support of three distinct switching pathways. *J. Exp. Med.* 157:69.

45. Slaughter, C. A., and J. D. Capra. 1984. Structural and genetic basis of the major cross-reactive idiotype of the A-strain mouse. In *The Biology of Idiotypes.* M. I. Greene and A. Nisonoff, editors. Plenum Press, New York. 35–58.