PRIMARY STRUCTURE OF IgE MONOCLONAL ANTIBODIES EXPRESSING AN INTRASTRAIN CROSSREACTIVE IDIOTYPE

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It is now evident that most if not all major intrastrain crossreactive idiotypes (CRI)[1] 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)F_1 (CAF_1) 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,

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[1] *Abbreviations used in this paper:* CDR, complementarity-determining region; CRI, crossreactive idiotype; CRI_A, major intrastrain CRI of anti-Ars in A/J mice; CRI_m, minor intrastrain CRI of anti-Ars in A/J mice.

CRI_A^+ [major intrastrain CRI of anti-Ars in A/J mice], $IgG1_\lambda$) was described previously (23). A hybridoma producing mAb SE20.2 (anti-Ars, CRI_A^+, IgE_λ) (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_A by the RIA described below. A hybridoma secreting mAb SE1.3 (anti-Ars, CRI_m^+ [minor intrastrain CRI of anti-Ars in A/J mice], IgE_λ) was prepared using spleen cells from an A/J mouse that had been irradiated (600 rad) and had received 5×10^7 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_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 ^{125}I-labeled, affinity-purified rabbit anti–mouse Fab. For IgE anti-Ars, a similar assay was used, but ^{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_A and CRI_m. The RIA for CRI_A (12) made use of 10 ng of ^{125}I-labeled mAb R16.7 (CRI_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_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_m are defined as those that share some but not all idiotopes with antibodies expressing CRI_A. Since some idiotopes are shared, CRI_m^+ antibodies are bound by anti-CRI_A, but CRI_m^+ antibodies cannot displace CRI_A^+ antibodies completely from anti-CRI_A. The IgE mAb expressing CRI_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_A (see Results). R16.7 is a prototype of the CRI_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 obtained; 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, $1–3 \times 10^8$ 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 $MgCl_2$ concentration to 100 mM, and were isolated by

TABLE I
Oligonucleotide Primers Used for mRNA Sequencing

Amino acid position of first 5′ nucleotide in oligomer	Sequence of primer	Source
131 in C_H1	5′-d(AGTGCCTTTACAGGGCT)	OCS Laboratories, Denton, TX (32)
99 in V_H	5′-d(GGATCTTGCACAGAAATA)	Prepared by K. Meek*
89 in V_H	5′-d(CTCAGATGTCAGGCT)	C. Milstein and C. Berek
122 in C_κ	5′-d(TGGATGGTGGGAAGATG)	OCS Laboratories (33)
86 in V_κ	5′-d(ACCCTGTTGGCAAAAGTA)	Prepared by K. Meek*
66 in V_κ	5′-d(CCCACTGCCACTGTT)	C. Milstein and C. Berek
45 in V_κ	5′-d(TTTAACAGTTCCATCTGG)	Prepared by K. Meek*

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

centrifugation through 100 mM sucrose at 12,000 *g*. Polysomes were resuspended in 20 mM Hepes buffer, multiply extracted with a phenol/chloroform mixture, and finally extracted with chloroform. Nucleic acids were precipitated from the aqueous phase with 2.5 volumes of ethanol. Poly-A^+ mRNA was then isolated on oligo-dT-cellulose (New England BioLabs, Beverly, MA).

mRNA for V_L or V_H regions was sequenced by the chain-termination method, as described by Hamlyn et al. (31). In brief, a synthetic oligodeoxynucleotide primer is used, which hybridizes to an mRNA sequence a short distance beyond the 3′ end of the V region. One or more primers hybridizing within the V region are needed to extend the sequence further toward the *N*-terminus. Primer extension with reverse transcriptase is carried out in the presence of the four unlabeled dideoxynucleotides, which act as chain terminators. ^{32}P-labeled ATP or GTP is also present in the mixture. After a 30-min chase with an excess of the four deoxynucleotides, the mRNA is hydrolyzed with NaOH, diluted 1:1 with formamide, and subjected to electrophoresis on an 8% polyacrylamide gel, followed by autoradiography. Information on the primers used is given in Table I.

Protein Sequencing of SE20.2 H and L Chains. Protein sequencing was carried out for the *N*-terminal regions of the H and L chains (positions 1–25) as described (34).

Results

Fig. 1 shows nucleotide and amino acid sequences of the V_H 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 $V_HD_HJ_H$ and V_LJ_L. In addition, the *N*-terminal sequences of both chains of SE20.2 were obtained by protein sequencing (positions 1–25 of both V_H and V_L). 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_H 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_H segment encoded by an unmutated germline gene. This gene (generally in a mutated form) is believed to encode the V_H segment of all CRI_A^+ 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_H segment (positions 1–98), two of which occur in complementarity-determining

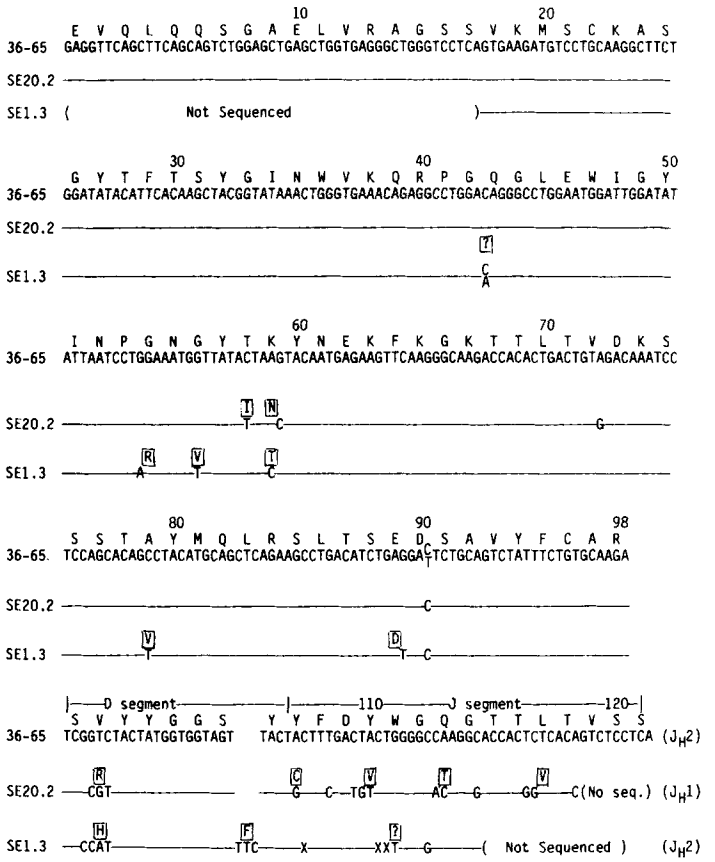

FIGURE 1. Nucleotide and deduced amino acid sequences of the V_H regions of two IgE anti-Ars mAb, SE20.2 and SE1.3. They are compared with the corresponding sequences of mAb 36–65, whose V_H segment (1–98) is virtually identical in nucleotide sequence to that of a putative germline gene (6). A solid 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_H segment (positions 1–98) of mAb SE1.3 (CRI_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_H segment of SE1.3 were not determined.

As indicated in Fig. 1, the D_H segments of 36–65 and SE20.2 have the same length (eight amino acids), which is characteristic of the CRI_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_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,

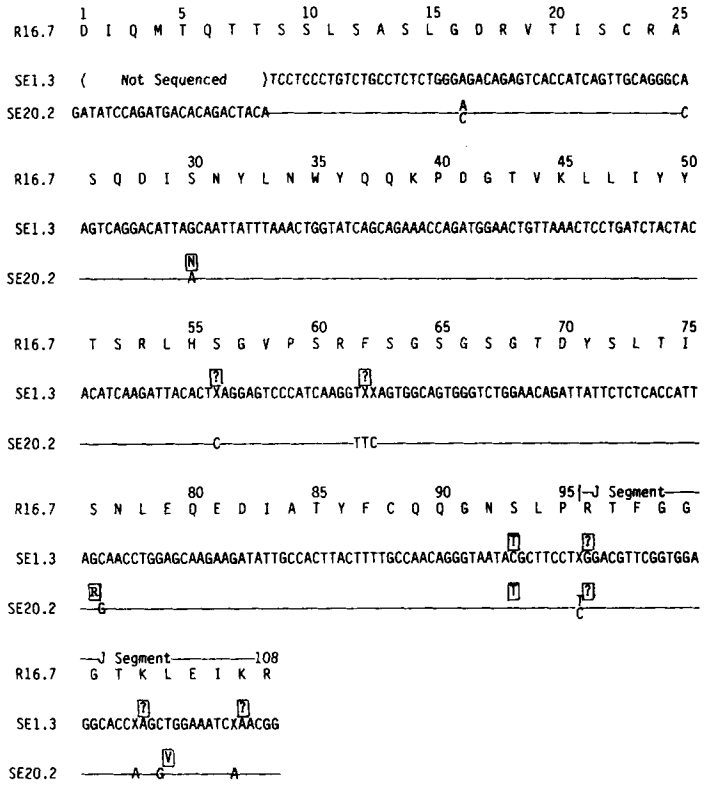

FIGURE 2. Comparison of the V_L amino acid sequences of mAb SE1.3 and SE20.2 (both IgE_κ anti-Ars), with that of R16.7 ($IgG1_\kappa$), a strongly CRI_A^+ anti-Ars mAb (23). An uncertainty in the nucleotide or amino acid sequence is indicated by an X or by a question mark, respectively. Amino acid differences are boxed.

there is a substitution (His for Val) at position 100, again involving all three nucleotides.

The partial sequences obtained for the J_H regions of SE20.2 and SE1.3 indicate that they are J_H1 and J_H2, respectively. With rare exceptions (36), CRI_A^+ mAb use J_H2. Thus, SE20.2, which is CRI_A^+, constitutes another exception to this general rule. The partial J_H sequence of SE20.2 agrees with the germline BALB/c J_H1 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_κ 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_κ 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_κ of R16.7. For SE20.2 there are three differences as compared with the V_κ 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_κ 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)			CRI_A assay[§]
		From OD_{280}	From anti-Ars titer*	From anti-Ars titer‡	
	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	$H_{16.7}L_{16.7}$	300	283	0	7
8	$H_{16.7}L_{1.3}$	300	271	1	8
9	$H_{16.7}L_N$	300	2	0	340
10	$H_{1.3}L_{16.7}$	300	35	53	250
11	$H_{1.3}L_{1.3}$	300	28	52	>3,100 (3)
12	$H_{1.3}L_N$	300	2	5	>3,300 (4)
13	$H_NL_{16.7}$	300	2	0	385
14	$H_NL_{1.3}$	300	3	1	>3,000 (8)
15	H_NL_N	300	0	0	>3,100 (17)

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

‡ Using ^{125}I-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 CRI_A. Numbers in parentheses refer to percent inhibition by weight (in ng) specified.

‖ Note that these are the standards used.

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

The V_κ 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 V_κ germline gene.

The J_κ regions of SE1.3 and SE20.2 are both $J_\kappa 1$. In comparison with the $J_\kappa 1$ 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 CRI_A. SE20.2 expresses CRI_A; its inhibitory capacity is comparable to that of R16.7, a prototype of CRI_A^+ 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-CRI_A. 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 ^{125}I-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_A but fails to inhibit strongly in the RIA for CRI_A indicates that it possesses one or more, but not all idiotopes that constitute the CRI_A idiotype. We have referred to such molecules as expressing a minor idiotype, or CRI_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_H (1–98) and V_L (1–95) regions that are very similar to those associated with CRI_A. As indicated above, SE1.3 is a very poor inhibitor in the RIA for CRI_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_m^+) is equivalent to that of R16.7 (CRI_A^+) with respect to mediating expression of CRI_A. The recombinant molecule, $H_{16.7}L_{1.3}$ (the prefixes R and SE are omitted), is nearly equivalent to R16.7, or to the recombinant, $H_{16.7}L_{16.7}$ in inhibitory capacity in the assay for CRI_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_A very poorly (250 ng required for 50% inhibition as compared to 7 ng for the autologous recombinant, $H_{16.7}L_{16.7}$; Exps. 7 and 10). These results indicate that the failure of SE1.3 to express CRI_A is attributable to its H chain rather than to its L chain. The weak inhibition by $H_{1.3}L_{16.7}$ in the assay for CRI_A (Exp. 10) may be due to slight residual contamination of $L_{16.7}$ by $H_{16.7}$. This is supported by the fact that the recombinant $H_NL_{16.7}$ (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 $H_{1.3}$ is not idiotypicaly equivalent to $H_{16.7}$ is weakened by evidence that the procedures used modified the properties of the ϵ 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 $H_{1.3}L_{1.3}$ expressed only ~12% of the Ars-binding activity of the intact molecule (Exps. 2 and 11). It thus appears that ϵ chains may be more easily denatured than γ chains under the conditions used. Nevertheless, the virtual identity of $L_{1.3}$ and $L_{16.7}$ in their serological properties and amino acid sequences, and the fact that SE1.3 is idiotypically deficient with respect to CRI_A, indicate that the defect resides in the V_H and/or D_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_A). This permits a comparison with the corresponding germline-encoded sequence of the CRI_A-associated V_H segment. The corresponding germline V_κ 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.

[2] The primary structure of SE1.3, described in this paper, indicates that it may be a member of the 91A3 V_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 CRI_A^+ antibody may express a very limited number of somatic mutations. The V_H sequence of the CRI_A^+ IgE antibody, SE20.2, is particularly informative. In the V_H 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 (CRI_A^+) 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_κ segment (1–95), which shows only three definite substitutions (at positions 30, 76, and 93) with respect to R16.7, a prototype of the CRI_A family. It should be noted, however, that the V_κ amino acid sequences of a number of CRI_A^+ mAb show very few differences (38, 42).

For SE1.3, which also has a V_H 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_H segment is possible. In the V_κ 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 CRI_A^+ 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_H1 sequence in SE20.2, a strongly CRI_A^+ anti-Ars antibody. Nearly all other anti-Ars CRI_A^+ antibodies sequenced have proven to be J_H2, although at least one exception (with J_H4) has been reported previously (36).

Another question of interest is the basis for the deficiency of CRI_A-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 CRI_A^+ anti-Ars antibodies, although a weakly CRI_A^+ 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_H 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_L or J_L, since the recombinant molecule $H_{16.7}$ $L_{1.3}$ fully expressed CRI_A and anti-Ars activity.

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

A novel observation is the presence of a cysteine (or half-cystine) residue in the J_H1 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_κ mAb that have specificity for the Ars hapten group and are related to the major idiotypic family, CRI_A (crossreactive idiotype A), in the A strain of mouse. One mAb, SE20.2, fully expresses CRI_A; the other, SE1.3, possesses some but not all of the characteristic idiotopes. Both IgE proteins contain V_H and V_κ segments that are closely related to those associated with CRI_A. The D segment of SE20.2 is also typical of CRI_A^+ 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_A expression. Its deficiency with respect to idiotopes of CRI_A was attributed to the extra amino acid in the D region and/or substitutions in the V_H segment.

A major objective was to ascertain the frequency of somatic mutations in IgE. For the V_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_A. A somewhat higher frequency of substitutions is present in the V_H segment of SE1.3. The V_κ amino acid sequences of the IgE proteins are nearly identical to those of a prototype of the CRI_A family, mAb R16.7. The results are discussed with reference to the mechanism of the IgM to IgE switch.

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