The EMBO Journal vol.4 no.13B pp.3681-3688, 1985

The idiotypic network and the internal image: possible regulation of a germ-line network by paucigene encoded Ab2 (anti-idiotypic) antibodies in the GAT system

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Communicated by M.Fougereau

Heavy and light chain variable regions from eight monoclonal Ab2 (anti-idiotypic) antibodies of the GAT antigen, a $(Glu^{60}\ Ala^{30}\ Tyr^{10})_n$ co-polymer, have been analyzed by direct mRNA sequencing. Three mAb2s were directed against private idiotopes and used various V_H-D-J_H and V_k-J_k combinations. By contrast, the five 'anti-public' mAb2 antibodies used a very restricted repertoire, including all gene segments. Interestingly, within their D regions, Glu-Glu-Tyr or Tyr-Tyr-Glu sequences were reminiscent of the original (GAT) antigen and may act as possible internal images. A striking observation was that two mAb2 antibodies shared the same V-D-J sequence although derived from separate fusions. As this D sequence, 33 nucleotides long, has not been described so far, it is suggested that it may be encoded by a new germ-line D gene, acting as a crucial regulatory element in a GAT germ-line idiotypic network. An alternative model that may lead to the construction of this D segment by 'odd' rearrangements from pre-existing already reported sequences is also presented.

Key words: GAT antigen/germ-line network/anti-idiotypic antibodies

Introduction

The idiotypic network (Jerne, 1974) may be most simply written as the following cascade:

$$Ag \rightarrow Ab1 \rightarrow Ab2 \rightarrow Ab3 \rightarrow \ldots$$

in which Ag is the initial conventional antigen that elicits Ab1, functionally characterized by its antigen-specific combining sites or paratopes, and its idiotypic specificities (Oudin and Michel, 1963; Kunkel *et al.*, 1963), or idiotopes, which are specifically recognized by the Ab2 antibodies, induced by Ab1. Injection of Ab2 (anti-idiotypic) antibodies may in turn induce Ab3 (Cazenave, 1977; Urbain *et al.*, 1977), among which have been identified antibodies that resemble Ab1 in that they share similar idiotopes and, eventually, could directly recognize the original antigen (Ag). These antibodies were referred to as Ab1', and have been structurally characterized in a number of systems (Dildrop *et al.*, 1984; Legrain, 1985; Roth *et al.*, 1985a; Schiff *et al.*, in preparation). The possibility of inducing Ab3 antibodies of the Ab1' type is a consequence of the internal image concept, initially defined by Jerne (1974), and more recently refined (Jerne *et al.*, 1982), by considering that, within the Ab2 population of molecules, some could behave as true anti-idiotypic antibodies recognizing the Ab1 idiotopes (Ab2α), as opposed to another fraction, Ab2β, that would appear complementary to the Ab1 paratope, thus representing an internal image of the original antigen. Some of the latter kind may mimic physiological effects of the original antigen. This was first reported for insulin by Sege and Peterson (1978), and has been extended to a number of other systems (Leo *et al.*, 1981; Strosberg *et al.*, 1981). The structural bases for this functional mimicry evidently relies on similarity in three-dimensional structures (Erlanger, 1985). In most cases (haptens, non-proteic hormones, carbohydrates, etc.), a direct homology between the original antigen and a stretch of amino acid sequence within the Ab2 molecule simply cannot be found. Should the original antigen be a protein and the original epitope be at least partially sequential in nature, then it would be possible, in some cases, to detect short amino acid sequence homology between the antigen and the region bearing the internal image. To approach this problem, we have been working on the idiotypic cascade of the GAT system, in which the antigen GAT is a linear random terpolymer $(Glu^{60}\ Ala^{30}\ Tyr^{10})_n$, using hybridomas produced at all three levels, Ab1 (Leclercq *et al.*, 1982), Ab2 (Sommé *et al.*, 1983) and Ab3 (Roth *et al.*, 1985b), obtained in a syngeneic situation in BALB/c mice. Most anti-GAT antibodies (Ab1) express public idiotypic specificities, CGAT (Ju *et al.*, 1978) or pGAT (Thèse and Moreau, 1978), which strongly correlate with the preferential recognition of Glu,Tyr-containing epitopes (Thèze and Sommé, 1979). Ab2 hybridomas have been prepared either by immunization with GAT-specific ($pGAT^+$) mAb1s, or with polyclonal AB1s. Immunization with mAb1s led to the production of mAb2s that were strictly directed against private Ab1 idiotopes, whereas polyclonal Ab1s allowed production of Ab2s recognizing public idiotopes associated with the pGAT specificities, and falling into two discrete groups, termed 20 and 22, specific for distinct idiotopes (Sommé *et al.*, 1983). Several hybridomas of each category (20, 22 and 'private') have been selected. We report in this paper their H and L mRNA nucleotide sequences covering all or most of the V_H-D-J_H and V_k-J_k regions, allowing implications regarding the regulation of the germ-line idiotypic network, and suggesting a key role of the D region of Ab2 in acting as a potential internal image in this system.

Results and Discussion

It has been shown by Sommé *et al.* (1983) that the mAb2 hybridomas obtained from fusions using spleen cells from mice immunized with polyclonal Ab1s could be divided into two groups. Group 20 (HP 20, 21, 24 and 25) defined an idiotope common to GAT-specific Ab1s of all strains of mice tested. Group 22 (HP 22, 27 and 29) recognized an idiotope present on BALB/c Ab1s and only occasionally on Ab1s from other strains. Furthermore, these idiotopes were exclusively associated with those antibodies expressing the pGAT public idiotypic specificities. Ab2 antibodies of these two groups were thus considered as identifying public idiotopes. Immunization with monoclonal Ab1s only led to the synthesis of Ab2s specific of the immunizing Ab1, i.e., defining private idiotopes.

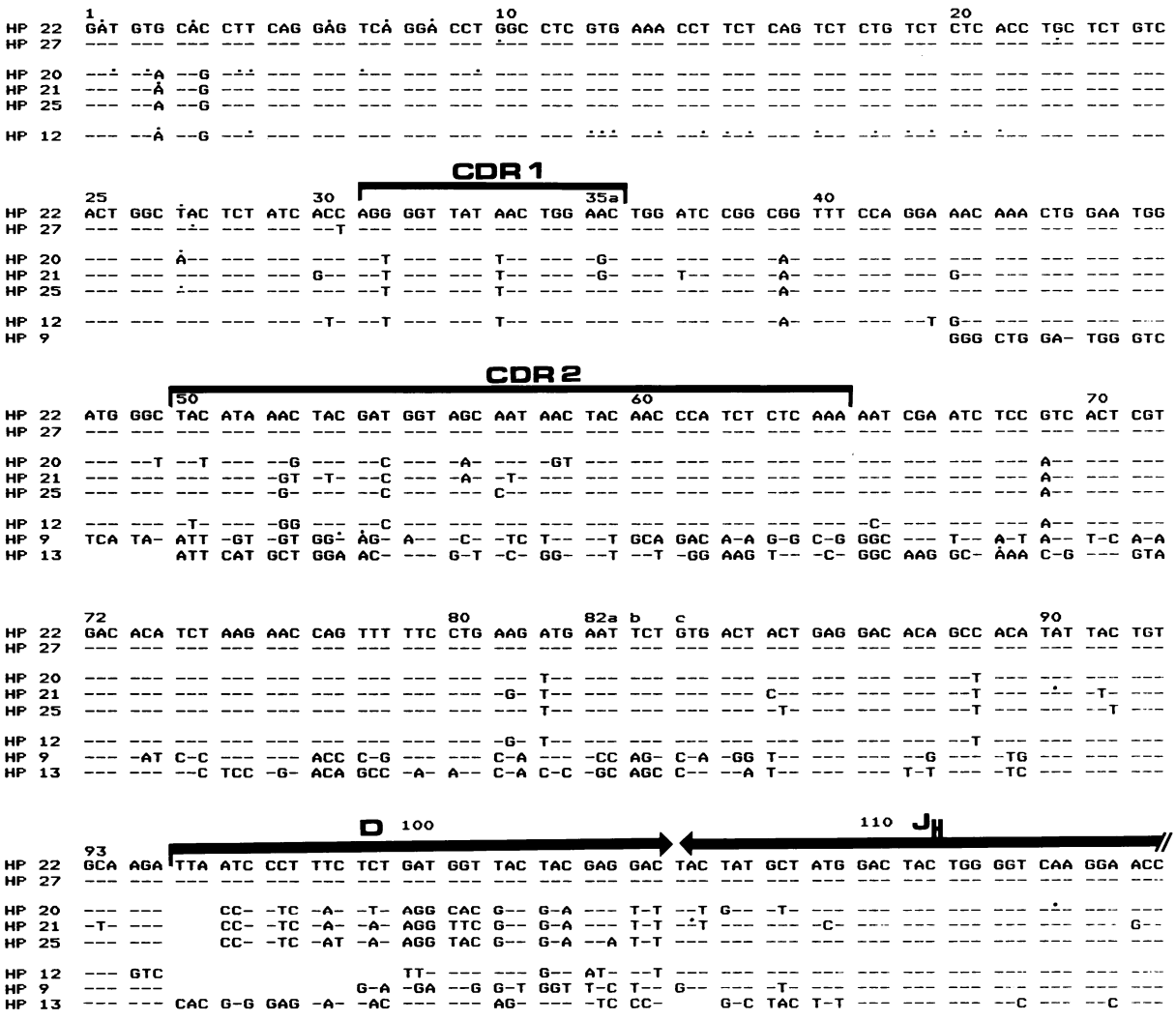

Fig. 1. Nucleotide sequences of mRNAs coding for the heavy chains of 'GAT' Ab2s recognizing the public specificities (groups 20 and 22) as compared with Ab2s directed against private idiotopes (HP9, 12, 13). Results are expressed by reference to the HP22 sequence. Dots indicate positions for which some ambiguity remained. Numbering is according to Kabat *et al.* (1983). All chains were $\gamma 1$.

The repertoire of mAb2s directed against public idiotopes of GAT-specific Ab1s is extremely restricted

Analysis of the heavy chains. In Figure 1 we compare the nucleotide sequences derived from the mRNA encoding five complete heavy chain variable regions pertaining to Ab2s of either group 20 or 22. The most striking result is that the two sequences of group 22 (HP22 and HP27) are completely identical all along the V_H, the D, and the J_H4 regions, with the exception of codon 30 which contains a silent C-T replacement. Since the hybridomas HP22 and HP27 were obtained from separate fusions, this observation strongly suggests that this V_H sequence is that of one germ-line gene, pertaining to the V_HI family (Kabat *et al.*, 1983). The complete homology of the D regions is even more puzzling and will be separately discussed below. The J_H4 germ-line gene is also used in totality and without mutations.

V_H sequences of group 20 heavy chains (HP20, 21, 25) are 94% homologous to that of group 22, and may be derived from the same germ-line gene. The presence in group 20 of conserved nucleotides that differ from the group 22 sequence (at codons 3, 31, 34, 39, 69) may, however, speak in favor of a second germ-line gene. As some germ-line genes may differ by as little as three nucleotides in their coding region (Schiff *et al.*, 1985), and some somatic mutants vary at >12 positions from the consensus sequence (Schiff *et al.*, in preparation), it cannot be decided which hypothesis is correct. The D regions differ from those of group 22, and are composed of 10 codons instead of 11. Here again, somatic mutations seem to have taken place rather extensively. The J_H4 gene is also used, although with several somatic mutations, which might be in favor of mutations occurring also on the V_H and the D genes (Crews *et al.*, 1982).

Light chain germ-line genes are group-specific. Partial nucleotide sequences of seven anti-public Ab2 light chains are represented in Figure 2, starting from codons 7 to 37, depending upon the antibody, and ending with the J_k region included. Sequences were clearly group-characteristic. Within group 22, light chains of HP27 and HP22 were identical, if one considers that the few undetermined positions are alike. All chains used the J_k2 gene. The situation is therefore identical to that reported above for the heavy chains, and supports the idea that HP22 and HP27 express the same germ-line gene sequence. HP29 may be derived from the same gene, differing from it at only 10 positions.

Group 20 light chains differed extensively from those of group 22, and were clearly encoded by a discrete germ-line gene. Differences within the group may be the result of somatic muta-

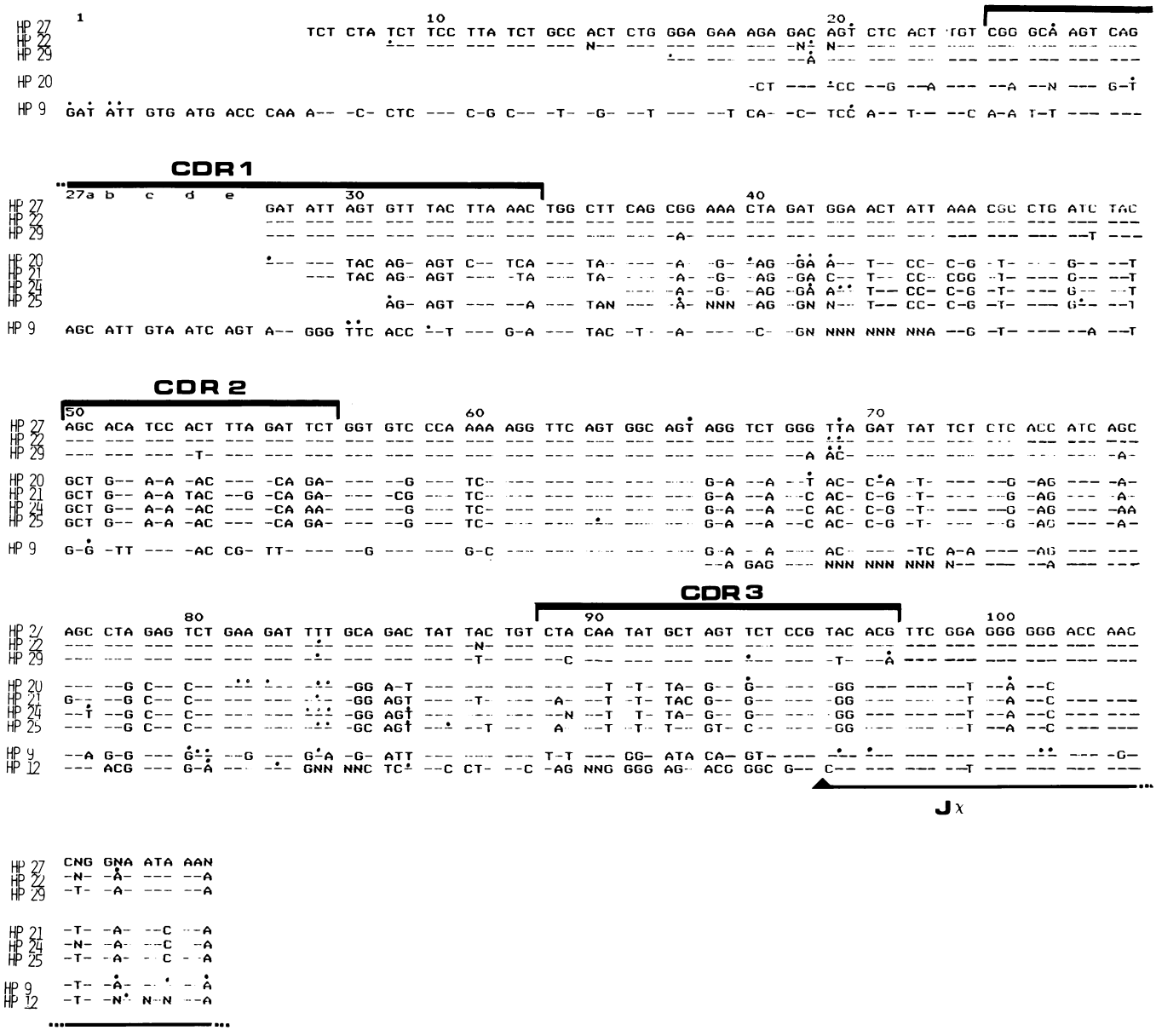

Fig. 2. Nucleotide sequences of mRNAs encoding the kappa chains of 'GAT' Ab2s recognizing the public idiotypic specificities (groups 20 and 22), as compared with Ab2s directed against private idiotopes (HP9, 12). Ambiguities are dotted. N: undetermined residue.

tions, although we cannot exclude, on the basis of the observed homology (~94%), that they alternatively represent expression of distinct but closely related germ-line genes. All group 20 light chains used the J_k1 gene.

Monoclonal Ab2s directed against private specificities are much more heterogeneous

Complete (HP12) or partial (HP9, HP13) sequences of mAb2 heavy chains are also reported in Figure 1, by comparison with the HP22 reference sequence. The three sequences differed extensively from each other, in several respects. (i) The V_H sequences showed a poor homology and belonged to different subgroups, V_HIII, V_HI and V_HII for HP9, 12 and 13, respectively (Kabat *et al.*, 1983). HP12 V_H was 94% homologous to the corresponding stretch of HP20 (group 20 'public'). (ii) The D regions had different lengths: 6, 7, and 11 for HP12, 9 and 13, respectively. Interestingly, however, the D sequence of HP13 had structural features in common with that of group 22, and this will be further discussed. (iii) The J_H region was either using the J_H4 (HP12 and 9) or J_H2 genes.

Sequencing of private light chains was completely determined for HP9 and partially for HP12 (Figure 2). These sequences differed profoundly from each other and from those of mAb2 antibodies associated with public idiotope recognition. The J_k2 gene was used with one mutation in HP9 and several in HP12.

Structural basis for the idiotypic network in the 'GAT' system

Structural correlates of idiotypes remain, so far, an unsettled problem (Capra and Fougereau, 1983; Rajewsky and Takemori, 1983; Rudikoff, 1983). In a limited number of cases, it has been possible to correlate idiotope expression with a precise position in the variable region of the corresponding idiotype (Johnson *et al.*, 1982; Lieberman *et al.*, 1977; Clevinger *et al.*, 1980; Rudikoff, 1983). Recently, a single amino acid substitution in the D region was shown to affect drastically idiotypic specificities of an anti-(4-hydroxy-3-nitrophenyl)acetyl group (Radbruch *et al.*, 1985).

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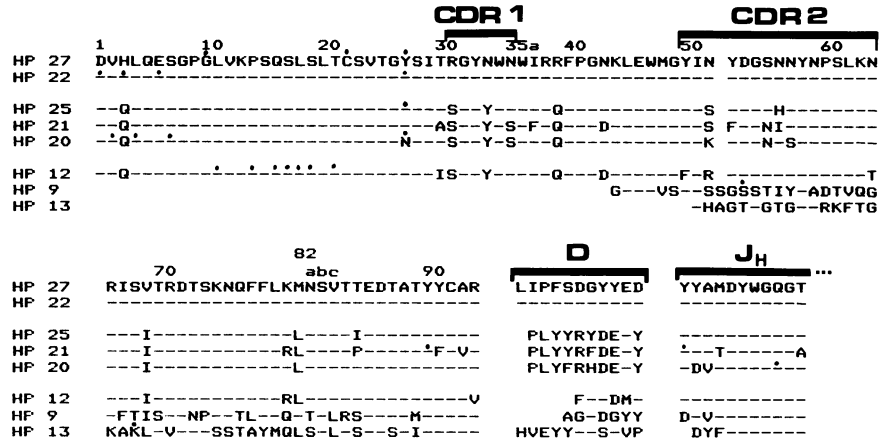

Fig. 3. Heavy chain variable region amino acid sequences derived from mRNA data. Possible internal image-like structures of the D regions are boxed. Amino acids are written in the one letter code according to IUPAC-IUB.

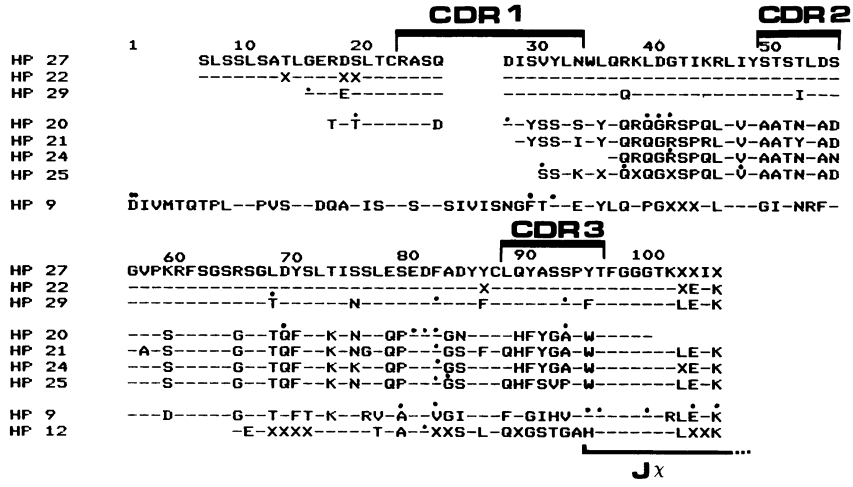

Fig. 4. Light chain variable region amino acid sequences derived from mRNA data.

Correlates should be best defined by analyzing the three-dimensional structure of idiotype-anti-idiotype complexes. All hypervariable regions are potential candidates (Poljak, 1984) including those residues of V_H and V_L that closely interact, and with a special reference to the D regions. Molecules at the three levels of the idiotypic network (Ab1, Ab2, Ab3) need to be characterized. This is being done in the GAT system. Already, mAb1s and mAb3s have been analyzed. It has been shown that heavy and light chains of Ab1 are under the control of a very small number of germ-line genes (Rocca-Serra *et al.*, 1983a; Tonnelle *et al.*, 1983; Schiff *et al.*, 1983a, 1983b, 1985 and in preparation). Most of the Ab3 molecules are of the Ab1′ type, i.e., they express the pGAT idiotopes and may be GAT-specific (Roth *et al.*, 1985a; Schiff *et al.*, 1985 and in preparation). The pGAT specificities are strictly dictated by V_H-V_L restricted associations (Sommé *et al.*, 1982). Apparently, the use of a specific D region is not correlated with the expression of the pGAT idiotype, that could rather appear as a combined V_H 'GAT'-V_k 'GAT' germ-line gene marker (Rocca-Serra *et al.*, 1983a; Tonnelle *et al.*, 1983; Roth *et al.*, 1985a). By contrast, the D seems very important for GAT recognition (Roth *et al.*, 1985b). As it was reported that the Ds of anti-GAT (Ab1) antibodies were often rich in basic and aromatic residues (Rocca-Serra *et al.*, 1983a), it was proposed that ionic interactions and stacking might be of importance in GAT binding. If the D regions were crucial in the part of the network responsible for antigen specificity, then it would be of interest to focus on their amino acid sequence in Ab2. On the other hand, if most of the idiotype-anti-idiotype interactions involved all or part of the complementarity determining regions (CDRs), it would also be of interest to look at possible obvious features of the primary structure of all CDRs, although extrapolation to three-dimensional structure cannot be simply obtained.

Amino acid sequence of Ab2 H and L variable regions, with special emphasis on a possible internal image-like structure within the D region

Amino acid sequences were derived from mRNA data both for the heavy and the light chains of the various Ab2 molecules. These data appear in Figures 3 and 4. Distinction between group 20 and 22 antibodies relies predominantly on pronounced differences in the light chain sequences, which are clearly group-specific, and also on the structure of the D regions which differ both in length (11 amino acids for group 22 and 10 for group 20) and in sequence. This would point to the crucial participation of the light chain (probably including the J_k-encoded section) and the D region of Ab2 for idiotope recognition on the Ab1 molecules. This is apparently in contrast to conclusions drawn for the Ab1/Ab3 levels, for which the D regions did not seem involved as part of the idiotypic specificities. The nucleotide

Table I. Hypervariable regions of the GAT system

			% of aromatic residues on the considered region	Net charge
Ab1/Ab1				
		31		
Heavy chains	CDR1	DTYMH	16	−1
		50		
	CDR2	RIDPANGNTKYDPKFQ	6	+1
	D	GWLRRD (Ab1)	16	+1
		24		
Light chains	CDR1	RSSQSIVHSNGNTYLY	12	+1
		50		
	CDR2	RVSNRFS	12	+2
		89		
	CDR3	FQGTHVPHT	11	0
		S		
Ab2				
		31		
Heavy chains	CDR1	RGYNWN (HP22)	40	+1
		SGYYWS (HP20)	50	0
		50		
	CDR2	YINYDGSNNYNPSLKN (HP22)	19	0
	D	LIPFSDGYYED (HP22)	27	−3
		PLYFRHDEEY (HP20)	30	−2
		Internal image - like sequences		
		24		
Light chains	CDR1	RASQDISVYLN (HP22)	9	0
		RASDDIYSSLS (HP20)	9	−1
		50		
	CDR2	STSTLDS (HP22)	0	−1
		AATNLAD (HP20)	0	−1
		89		
	CDR3	LQYASSPYT (HP22)	22	0
		LHFYGAPWT (HP20)	33	0

sequences of Ab2 which recognize private specificities were very different from those of groups 20 and 22, with the exception of the HP12 heavy chain, which contains a V_H amino acid sequence 94% homologous to that of HP20, but which possesses a completely different D region, both in size (six amino acids instead of 10), and in sequence, and a different V_k domain. This is another example where the same V_H may be used with different V_k and D genes (Rocca-Serra *et al.*, 1983b), leading to discrete antibody specificities, in this case within a given network.

An intriguing feature is the presence of the sequences Tyr-Tyr-Glu, and Glu-Glu-Tyr in the D regions of group 22 and 20, respectively (Figure 3). Since anti-GAT (Ab1), pGAT$^+$ antibodies recognize primarily Glu-Tyr determinants (Leclercq *et al.*, 1982), it is tempting to propose that these sequences may reflect the internal images of the original antigen. The possibility that group 20 or group 22 Ab2 may contain an internal image was implicit from the possibility of raising Ab3 of the Ab1′ type, possessing both the pGAT idiotopes and the GAT specificity (Roth *et al.*, 1985a). Obtaining anti-GAT antibodies upon immunization with synthetic peptides having one or the other of the D region sequences certainly strongly supports the hypothesis (Mazza *et al.*, in preparation). According to the nomenclature proposed by Jerne *et al.* (1982), the 'GAT' Ab2 would thus appear to be of the Ab2β type. Ab1-Ab2 or Ab2-Ab1′ interactions might thus involve, among several possible CDR-CDR interactions, D regions of the Ab2 antibodies. If one considers the possibility that the Ab1 D-region is implicated in the GAT binding, an idiotype-anti-idiotype recognition in the GAT system might involve a D-D interaction between Ab1 and Ab2, seen as a paratope-internal image recognition. In Table I all CDR sequences of H and L chains at all levels (Ab1, Ab2, Ab3/Ab1′) of the GAT idiotypic system are computed. It is remarkable that, taking into account the percentage of aromatic residues and the overall net charge of each CDR, some complementarities are clearly apparent. For instance, most CDRs of Ab2 are negatively charged, whereas most CDRs of the Ab1/Ab1′ set are positively charged. Taking into consideration the percentage of aromatic residues, it may be possible that some interactions are of crucial importance, such as Ab1 D-Ab2 D (already discussed), but also Ab1(L-CDR2)-Ab2(L-CDR2) or alternatively, the cross-combination. Elucidation of these interactions will obviously rely on three-dimensional analysis, but recent data obtained by Chen *et al.* (1985) and Goldfien *et al.* (1985), who succeeded in raising anti-idiotypic antibodies against rheumatoid factor by injecting synthetic CDRs, certainly is compatible with the importance of CDR-CDR interactions in the idiotypic network.

Finally, it may also be noted that HP13 contains a Glu-Tyr-Tyr tripeptide within the D region, although the V_H is very different from that of groups 20 and 22. It is thus possible that some private specificities are elicited by residues implicated in the Ab1-GAT interaction.

New genes or 'odd' rearrangements may account for 'GAT' Ab2 D regions

The central parts of group 20 Ab2 D regions probably result from

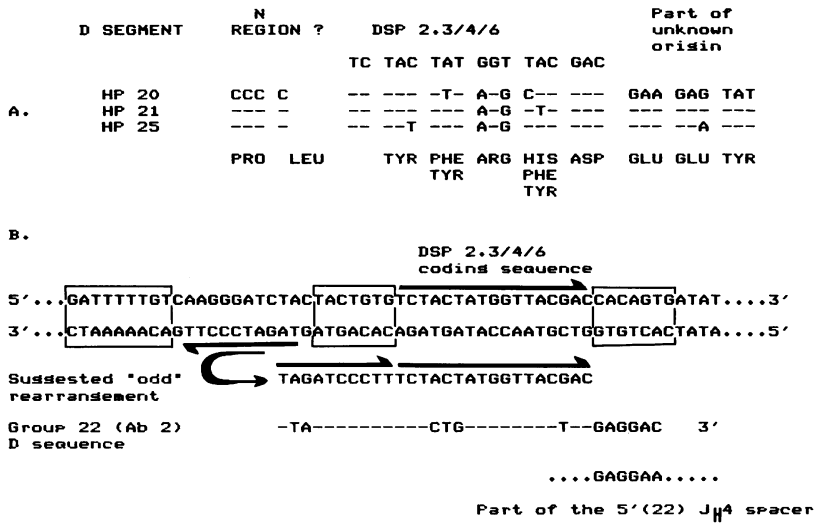

Fig. 5. **(A)** Homologies between the group 20 sequences and the DSP2.3/4 germ-line genes. **(B)** Homologies between the HP22/27 D region and coding and signalling sequences of DSP2.3/4 germ-line gene (sequence from Kurosawa and Tonegawa, 1982).

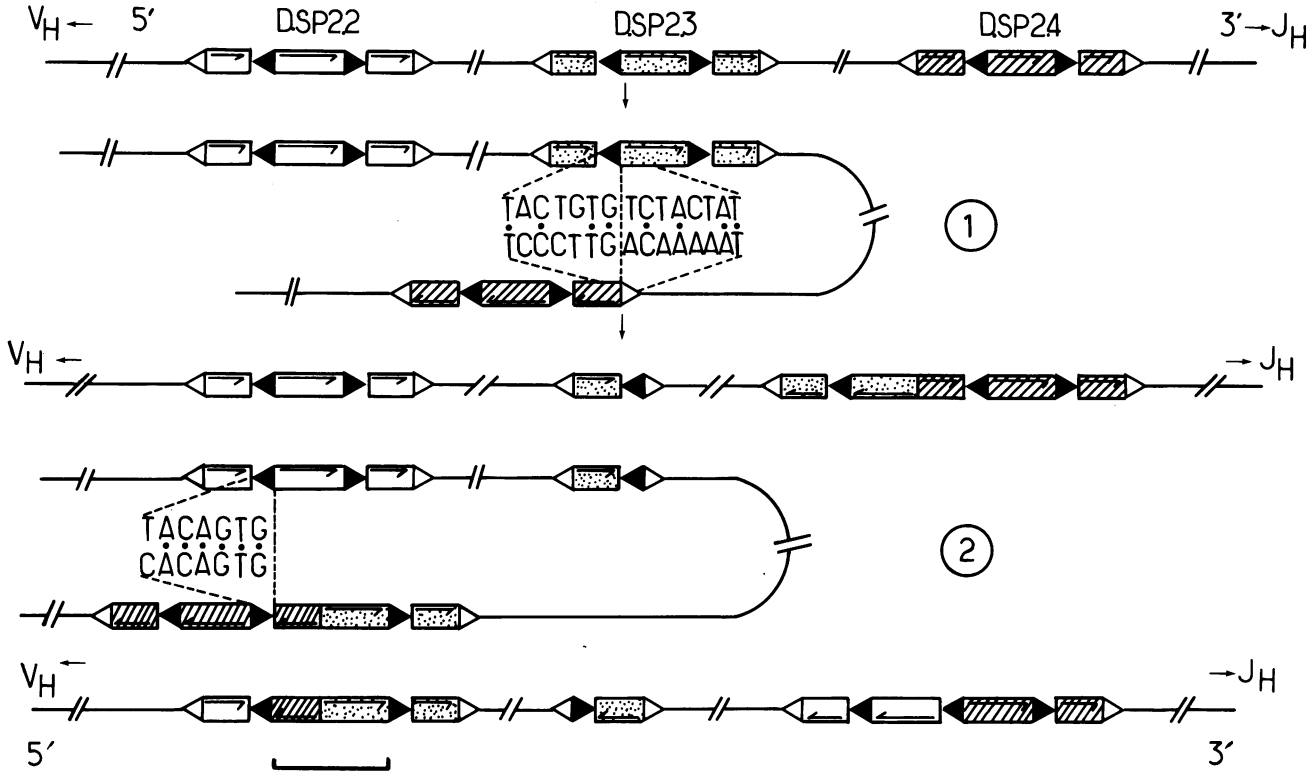

Fig. 6. A hypothetical mechanism that could account for the construction of the HP22/27 D gene (the choice of the three DSP2.2/3/4 adjacent genes is arbitrary). ▭ D coding region; □ spacers; △ nonamer and ▲ heptamer signals. The first inversion (1) would bring the inverted 5′ spacer of DSP2.4 adjacent to the coding region of DSP2.3. The second (2) leads to reinsertion of the 5′ 9-12-7 signal from DSP2.2 adjacent to the previously rearranged D genes.

the use of the DSP2.3/4 germ-line gene, with which they diverge by three nucleotides at different positions depending on the hybridoma (Figure 5a). The 5′ ends of these segments are exclusively composed of C, which suggests they arose through N diversity (Alt and Baltimore, 1982). Their nonanucleotidic 3′ ends are of interest since they can neither be accounted for by this latter mechanism, nor by the published sequences pertaining to the D-J_H locus; furthermore, they are homologous among all four heavy chains, except for a silent difference. This observation, coupled with the fact that the DSP2-related cores contain two conserved nucleotides among the group, corresponding to positions at which they differ from DSP2.3/4, suggests the existence of a germ-line gene including a DSP2-like 5′ end, and the consensus 3′ nonanucleotide, which would thus be germ-line encoded. The observation that the two conserved nucleotides (A and G, first and third positions of codon 100) were also identified in one anti-oxazolone antibody (Kaartinen *et al.*, 1983), in levan Ab1′ (Legrain *et al.*, 1985) and one anti-NP^a (17.2.25) antibody (Loh *et al.*, 1983), strengthens this hypothesis. It is worth noting that the 3′ nonanucleotide encodes a Glu-Glu-Tyr

amino acid sequence, corresponding to part of a synthetic peptide able to induce the Ab1′ polyclonal response in syngeneic immunization (Mazza *et al.*, in preparation), and thus considered as a possible internal image of the antigen.

The group 22 Ab2 D region is among the longest reported to date, with a length of 11 amino acids. This D region contains a stretch of 17 nucleotides, which is highly homologous to the coding section of the DSP 2.3/4 or of the DSP2.5 gene (Kurosawa and Tonegawa, 1982) from which it differs at only four positions in either case (Figure 5b). This heptadecanucleotide accounts for only half of the Ab2 D region. As two identical sequences have been identified for HP22 and HP27, although derived from separate fusions, this strongly suggests: (i) that this Ab2 D region is encoded by a so far unidentified D gene, (ii) that it has been constructed from other existing elements, or (iii) that the selective pressure within the network is such that this sequence was highly selected, whatever the origin of its diversity, including a number of somatic events. An intriguing feature emerges when the HP22/27 D segment is compared with the 5′-flanking signal region of the DSP2 family (Figure 5). Immediately 5′ upstream of the heptadecanucleotide there is a stretch of 10 nucleotides which are highly homologous to the inverted 3′-5′ strand of the 5′ spacer of the DSP2 germ-line genes. Only two substitutions were identified when these stretches were compared. In addition, a pentanucleotide, identical to part of the J_H4 spacer was present at the 3′ end of the HP22/27 D segment, and contributed to the Glu-Tyr sequence.

Rearrangements of Ig genes clearly do not operate only through excisions (Alt and Baltimore, 1982; Van Ness *et al.*, 1982; Höchtl and Zachau, 1983; Lewis *et al.*, 1985), and it seems difficult to account for the 5′ decanucleotide on the basis of the N diversity (Alt and Baltimore, 1982) since identical sequences occur in two separate fusions. We propose that this D segment results from an odd rearrangement including two successive inversions, as depicted in Figure 6, using for instance the adjacent DSP2.2/3/4 genes. The first one would bring the inverted 5′ spacer of DSP 2.4 adjacent to the coding region of DSP.3. The second would reinsert the 5′ 9-12-7 signal coming from DSP.2.2 to the previously rearranged and extended D gene. As junctions are known in the Ig system to be frequently imprecise (Alt and Baltimore, 1982), the two extreme nucleotides of the original inverted spacer would be easily lost. Adding the hexanucleotide (GAGGAC) at the 3′ end might be due to N diversity, although here again, identical sequences have been derived from two separate fusions. The presence of this hexanucleotide might, alternatively, implicate another odd rearrangement with a portion of the spacer of J_H4, possibly involving double strand cleavage in this region (Desiderio and Baltimore, 1984). Inversions have been described (Alt and Baltimore, 1982), as well as reciprocal recombination leading to a variety of rearranged segments on Ig genes (Van Ness *et al.*, 1982; Höchtl and Zachau, 1983; Lewis *et al.*, 1985). The degree of homology with which the first proposed inversion would operate may appear rather low, although the original proposals of hypothetical D-D rearrangements by Kurosawa and Tonegawa (1982) were given on the basis of less homology. Alternatively, the existence of a so far undescribed germ-line D gene should be looked for on the 5′ side of the previously reported D regions (Wood and Tonegawa, 1983). The existence of additional D genes have been invoked in other systems, such as oxazolone (Griffiths *et al.*, 1984) and lysozyme (Darsley and Rees, 1985) Ab1 antibodies. Should such a new D gene exist, with the described spacer homology, the proposed model might still be worth consideration, in a germ-line evolutionary context.

Materials and methods

Hybridomas

Preparation of the Ab2 hybridomas has been described in detail by Sommé *et al.* (1983). In brief, two types of fusions were prepared. One used spleen cells from BALB/c mice that had been immunized with monoclonal GAT-specific G5 Bb 2.2 Ab1, coupled to keyhole limpet haemocyanin (KLH). The fusion partner was the SP 2.0 Ag non-secreting cell-line (Shulman *et al.*, 1978). This type of fusion led to hybridomas 9, 12 and 13 which all produced Ab2 antibodies directed against private specificities of the immunizing Ab1 idiotype.

For the second type of fusions, lymphocytes originated from BALB/c mice immunized with polyclonal GAT-specific polyclonal Ab1 coupled to KLH, using the same fusion partner. All hybridomas were derived from the same fusion, with the exception of HP27. Hybridomas were serially transplanted subcutaneously as solid tumors, harvested and immediately frozen in liquid nitrogen.

mRNA sequencing

The poly(A)$^+$RNAs were extracted directly from the frozen hybridoma tumors by the LiCl-urea method (Auffray and Rougeon, 1980), purified on an oligo(dT)-cellulose column, and enriched for the H and the L coding fractions on a sucrose gradient. mRNA nucleotide sequencing was performed according to Hamlyn *et al.* (1978) as adapted from the orignal DNA dideoxy chain terminator procedure of Sanger *et al.* (1977). In most experiments, [^{35}S]nucleotide derivatives were used in place of [^{32}P]materials (Biggin *et al.*, 1983). Each sequence was determined at least three times by using radioactive αdATP, αdCTP and αdGTP, respectively.

For the sequencing of the light chains, a synthetic oligonucleotide, dTGGATGGTGGGAAGATG (prepared by A. Moulin and R. Sodoyer) complementary to the nucleotide sequence of the beginning of the C_k sequences was used as a primer for the AMV reverse transcriptase (Stehelin).

For the heavy chain sequencing, two synthetic primers, also prepared by A. Moulin and R. Sodoyer, were used. One was specific for the beginning of the C_γ sequence (dCAGGGGCCAGTGG), the other for a conserved region of the Ab2 V_H region corresponding to codons 59−63 (dGAGAGATGGGTTG).

Acknowledgements

We express our gratitude to R. Sodoyer and A. Moulin who prepared the oligonucleotide primers, and to C. Schiff for critical reading of the manuscript. This work was supported by CNRS (Centre National de la Recherche Scientifique) and ISERM (Institut National de la Santé et de la Recherche Médicale).

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Received on 2 October 1985