The EMBO Journal Vol.2 No.6 pp.867–872, 1983

Analysis of antibody diversity: V-D-J mRNA nucleotide sequence of four anti-GAT monoclonal antibodies. A paucigene system using alternate D-J recombinations to generate functionally similar hypervariable regions

José Rocca-Serra*, Hans W.Matthes[1], Matti Kaartinen[2], César Milstein[1], Jacques Thèze[3] and Michel Fougereau

Centre d'Immunologie INSERM-CNRS de Marseille-Luminy Case 906, 13288 Marseille Cedex 9, France, [1]Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK, [2]Department of Bacteriology and Immunology, University of Helsinki, Finland, and [3]Institut Pasteur 25, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

Communicated by M.Fougereau
Received on 23 February 1983; revised on 2 April 1983

The nucleotide sequence of four anti-(Glu^{60}-Ala^{30}-Tyr^{10})$_n$ (GAT) monoclonal γ_1 heavy chain mRNAs was determined from codon 10 to 120. This sequence overlaps with the NH_2-terminal amino acid sequence, allowing elucidation of the complete protein sequence encompassing regions V_H, D and J_H. These sequences, which are highly conserved, indicate that anti-GAT antibodies expressing the same public idiotypic specificities represent a paucigene system, which uses at least two D-J combinations leading to functionally similar hypervariable regions involved in the recognition of the dominant Glu-Tyr determinant. D regions are encoded by D genes which are closely related either to the D-SP2 or the D.FL16 germ line gene cores.

Key words: antibody diversity/idiotypes/mRNA sequencing

Introduction

Immunoglobulin diversity has been extensively studied by amino acid sequence determination of heavy and light chains of myeloma proteins, and, more recently, by amino acid or nucleotide sequencing of hybridoma products (Bothwell *et al.*; Crews *et al.*, 1981; Estess *et al.*, 1980; Gearhart *et al.*, 1981; Kaartinen *et al.*, 1983; Kocher *et al.*, 1981; Rocca-Serra *et al.*, 1982; Schilling *et al.*, 1980; Tonnelle *et al.*, 1981) derived from hybridoma cells (Köhler and Milstein, 1975). The problem has also been approached through structural and genetic studies performed on idiotypes, regarded as V region markers (Cosenza and Köhler, 1972; Eichman, 1973; Ju *et al.*, 1979; Karjalainen and Mäkelä, 1978; Lieberman *et al.*, 1976; Pawlak *et al.*, 1973; Riblet *et al.*, 1975; Thèze and Sommé, 1979). Direct investigation at the gene level has provided insight on the origin of immunoglobulin diversity, which seems to result from three major events: (i) existence of a number of germ-line genes, which need to be rearranged before expression – V and J for the light chains (Hozumi and Tonegawa, 1976; Seidman *et al.*, 1978; Tonegawa *et al.*, 1978) and V, D and J for the heavy chains (Early *et al.*, 1980); (ii) combinatorial association of H and L chains (Edelman *et al.*, 1963; Metzger *et al.*, 1964; Amzel *et al.*, 1974; de Préval and Fougereau, 1976); and (iii) somatic events, including mutations, and affecting both chains (Sakano *et al.*, 1979, 1980; Max *et al.*, 1980). Among systems which are being actively studied, monoclonal antibodies and myeloma proteins directed against well-defined antigens such as dextran (Schilling *et al.*, 1980); phosphoryl choline (PC) (Gearhart *et al.*, 1981), inulin (Vrana *et al.*, 1978; Johnson *et al.*, 1982), arsonate (Estess *et al.*, 1980), nitrophenylacetyl (NP) (Bothwell *et al.*, 1981), or (Glu^{60} Ala^{30} Tyr^{10})$_n$ (GAT) (Rocca-Serra *et al.*, 1982; Tonnelle *et al.*, 1981), have provided ample information on the subject.

The synthetic random terpolymer (Glu^{60} Ala^{30} Tyr^{10})$_n$ known as 'GAT' elicits, in a number of inbred strains of mice, anti-GAT antibodies which express a variety of idiotypic specificities, among which public specificities, termed CGAT (Ju *et al.*, 1978), or pGAT (Thèze and Moreau, 1978), correlate with the recognition of Glu-Tyr containing antigenic determinants, but not with that of Glu-Ala containing epitopes (Thèze and Sommé, 1979). These idiotypic specificities require both the H and the L chains of the idiotype in order to be expressed (Sommé *et al.*, 1982). We recently reported (Rocca-Serra *et al.*, 1982; Tonnelle *et al.*, 1981) that these anti-GAT public idiotypic specificities may be considered a germ-line marker characterizing a paucigene system. In addition, private idiotypic specificities, defined on one given monoclonal antibody, and occasionally expressed on a very few others, have been reported (i_1 GAT), and were also shown to necessitate H-L interactions to be expressed on the idiotype (Sommé *et al.*, 1982).

We report here on the complete sequence of four BALB/c, $pGAT^+$, anti-GAT monoclonal heavy chain variable regions, encompassing the V, D and J segments. The approach used the combination of NH_2-terminal amino acid sequence determination already reported (Rocca-Serra *et al.*, 1982; Tonnelle *et al.*, 1981), with analysis of the nucleotide sequence, using mRNA as a template, a synthetic oligonucleotide as a primer and a modification (Hamlyn *et al.*, 1978) of the dideoxy method (Sanger *et al.*, 1977) of DNA sequencing.

Results

Primary structure of the entire variable region of four BALB/c monoclonal anti-GAT, $pGAT^+$ heavy chains

Four hybridomas, derived from BALB/c mice, immunized with the GAT random copolymer, were selected from three separate fusions, termed G5, G7 and G8 (Leclercq *et al.*, 1982). Antibodies, isolated on a GAT-Sepharose column, all expressed the pGAT public idiotypic specificities (Leclercq *et al.*, 1982) and had identical NH_2-terminal amino acid sequences (Rocca-Serra *et al.*, 1982). In addition, two monoclonal antibodies, G5 Bb 2.2 and G8 Ca 1.7, although derived from separate fusions, expressed identical private idiotypic specificities. Figure 1 presents the nucleotide sequences determined for the four heavy chains (all γ1), covering almost the complete variable regions (from codons 6–10), encompassing the D and the J_H segments. The overall number of ambiguous positions at the amino acid level, as determined from nucleotide sequence, was 0.5%. Partial sequence of the G8 Ca 1.7 cDNA allowed elimination of two ambiguities in the D region (C.Schiff *et al.*, in preparation). Extensive overlap with the previously determined NH_2-terminal amino acid sequence allowed derivation of the complete corresponding protein primary structure (Figure 1). The most striking feature that emerges from the data is the absolute identity of G5 Bb 2.2 and G8 Ca 1.7 heavy chain sequences, including

*To whom reprint requests should be sent.

© IRL Press Limited, Oxford, England.

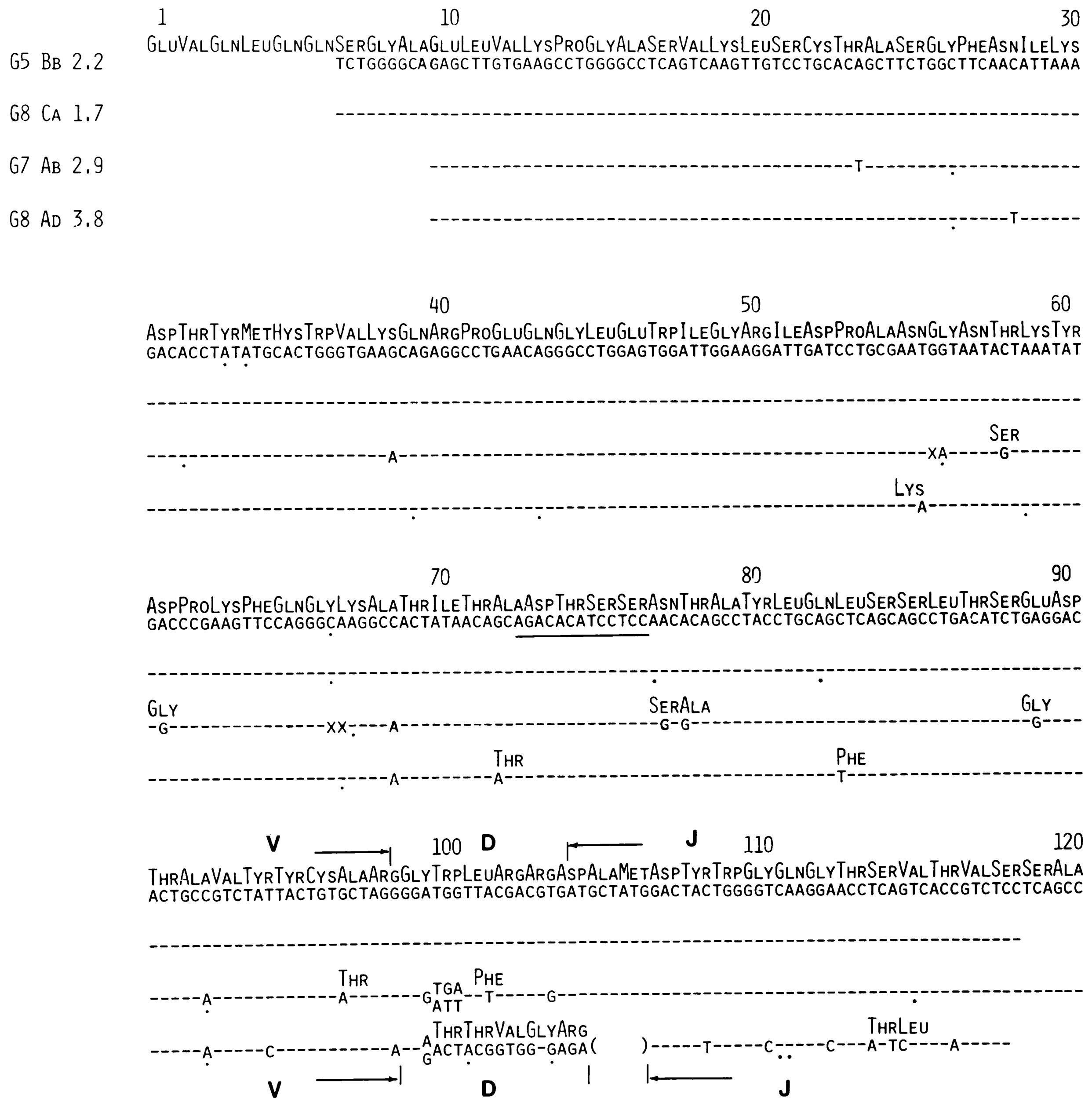

Fig. 1. Amino acid and nucleotide sequence of four V_H regions from four BALB/c anti-GAT, pGAT$^+$ monoclonal antibodies. Amino acid sequences were determined from the NH_2 terminus to residue 43 (Rocca-Serra *et al.*, 1982). Nucleotide sequences were determined from codon 120 to codon 6 – 10, according to a modification of the original dideoxy method (Sanger *et al.*, 1977) using the enriched H mRNA as a template (Hamlyn *et al.*, 1978), as described in Materials and methods. Parentheses at residues 105 – 106 of the G8 Ad 3.8 sequence represent a deletion introduced to ensure homology with the other chains. Dots indicate possible ambiguities. The location of the priming site of the V_H synthetic primer is underlined.

regions encoded by the V_H, the D and the J_H4 genes. These two antibodies express, in addition to the public pGAT specificities, identical private idiotopes. Since they were derived from separate fusions, it seems likely that these sequences represent the expression of identical germ-line genes. The two remaining heavy chains are identical to the G5 prototype up to residue 54, when the amino acid sequence is considered, but include three silent nucleotide differences at codons 23, 28 and 38. The possible occurrence of silent differences between codons 1 and 10 could not be substantiated because data in this section were only partial. Five silent differences were also observed between codons 55 and 98 of the V_H gene, grouped in four positions, at codons 66, 68, 92 and 94. Identical nucleotides were found for G7 Ab 2.9 and G8 Ad 3.8 at codons 68 and 92. Other nucleotide differences observed from position 55 led to amino acid substitutions, three in one case (G8 Ad 3.8), and eight in the other (G7 Ab 2.9). Substitutions were equally found in the second hypervariable region (four positions out of 15) and in the third framework region (seven positions out of 30). Twelve transitions were observed (four A→G, give G→A, two C→T and one T→C) *versus* seven transversions (one A→T, four C→A, one C→G and one T→A). Three D regions are very close, two being identical, and are joined to J_H4 segments. One heavy chain (G8 Ad 3.8) expressed a completely distinct D region, which was recombined to a J_H2 segment, the latter having one silent mutation as compared with the reported gene sequence (Sakano *et al.*, 1980).

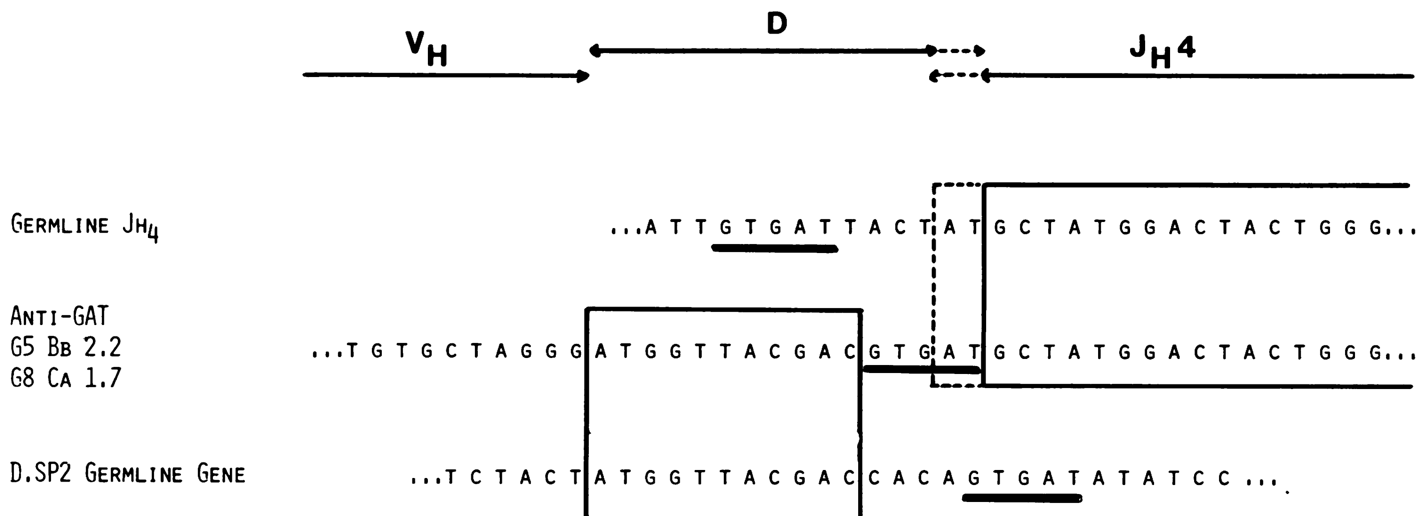

Fig. 2. Major nucleotide sequence of the anti-GAT heavy chains encompassing the third hypervariable region. Residues which are identical with the D.SP 2 (Kurosawa and Tonegawa, 1982) and with the J_H4 gene (Sakano *et al.*, 1980), are boxed. Uncertainty as to the D-J boundaries is boxed in dotted lines. The pentanucleotide sequence GTGAT is underlined to indicate possible derivation from either the D or the J 'untranslated' segments.

The D genes used in the analyzed anti-GAT antibodies are closely related either to the D.SP2 or to the D.FL 16 series

The existence of a 'Diversity' segment, encoded by a separate D gene, and located between the V_H and the J_H regions has been anticipated (Schilling *et al.*, 1980; Early *et al.*, 1980) and demonstrated (Sakano *et al.*, 1980; Kurosawa and Tonegawa, 1982). So far, three series of germ-line D genes have been described in the mouse, named D.Q 52, D.SP 2 and D.FL 16, respectively (Kurosawa and Tonegawa, 1982). The anti-GAT D regions are encoded by D genes that are clearly related to one or the other of the two latter. As shown in Figure 2, an undecamer of D.SP 2 is entirely contained in the 'D region prototype' found in G5 Bb 2.2 and G8 Ca 1.7 heavy chains. A third heavy chain, G7 Ab 2.9 contained a D region that was also close to D.SP 2, although at least three base substitutions occurred within the undecamer. All three D.SP 2 related D genes recombined with a J_H4 gene. In contrast, the fourth antibody, G8 Ad 3.8, used a D region which contained an octamer sequence (ACTACGGT) found in the D.FL 16 germ-line gene, and a J_H2 gene.

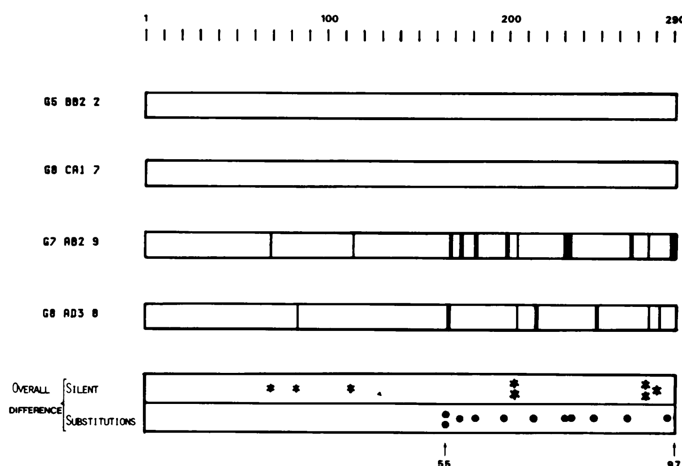

Fig. 3. Computation of silent (*) and substitution (.) differences on BALB/c V_H regions of anti-GAT, $pGAT^+$ antibodies. The four upper boxes represent V_H genes from bases 1 to 291 (amino acids 1–97). Thin bars indicate silent events, thick ones represent amino acid substitutions. The numbering of the upper scale refers to nucleotides, that of the lower boxes refers to amino acid positions.

Discussion

Origin of the diversity in the 'V_H GAT' system

Previous work (Tonnelle *et al.*, 1981; Rocca-Serra *et al.*, 1982; Ruf *et al.*, 1983) supported the idea that the public pGAT idiotypic specificities represented a germ-line marker, resulting from the expression of one V_H and two V_κ genes. Data presented here clearly confirm the overall conservation of the BALB/c V_H structures in the GAT system. The identification of two identical sequences for the V_H, D and J_H4 segments is a strong argument for the proposition that they represent the same germ-line gene. The two other amino acid sequences differed only from residue 55, in three and eight positions, for G8 Ad 3.8, and G7 Ab 2.9, respectively.

When the organization of fine diversity is looked at in various antibody – or myeloma – systems, several patterns may emerge: substitutions may be found: (i) in hypervariable regions (Bothwell *et al.*, 1981); (ii) framework regions (Johnson *et al.*, 1982); (iii) or both (Estess *et al.*, 1980; Gearhart *et al.*, 1981; Auffray *et al.*, 1981). In addition, they may be localized all along the chain (Estess *et al.*, 1980; Gearhart *et al.*, 1981) or accumulated in the second half of the V region (Schilling *et al.*, 1980; Bothwell *et al.*, 1981). It was also generally observed that silent and substitution differences follow the same pattern. In the GAT system, this is not the case (Figure 3). Silent differences are found within the first half (at least three), as well as in the second half (five, grouped in four positions), and are all present in framework codons. Amino acid substitutions are found exclusively from residue 55, and are present both in hypervariable (five positions) and framework (six positions) regions. This dissymmetry might be the result of a similar strong selective pressure, required to maintain a correct folding of the entire V_H domain, but which operates on a structure encoded by only one gene segment between residues 1 and 54, whereas three gene segments must recombine to account for the second half of the domain. Flexibility in V-D-J association may thus compensate some amino acid differences occurring in

the V_H framework which, otherwise, would be selected against. The lack of such a compensation within the first part of the V_H region may account for the absence of amino acid substitution before residue 55. In addition, selection of those V_H regions which contain CDR1 and CDR2 involved in the recognition of the GAT determinants may also be antigen driven.

As to the origin of the observed nucleotide differences several possibilities may exist, that have been proposed in other systems, such as different germ-line genes, recombinations between different genes, conversion or somatic mutations. The V_H GAT sequences are closely related to the V_HII subgroup family. Recently, several germ-line genes of this family have been characterized and sequenced (Estess *et al.*, 1980; Bothwell *et al.*, 1981; Givol *et al.*, 1981). No two genes were found identical, and the G5 prototype differs from all of them, although a clear homology was apparent with the NP^b genes and the dextran system (Bothwell *et al.*, 1981; Schilling *et al.*, 1980). The striking difference which emerges when looking at the pattern of substitution differences at the gene level *versus* the expression level, is that diversity is scattered all along the sequence whenever germ-line genes are compared. This observation would argue against the idea that the V_H GAT diversity is solely dictated by different germ-line genes. Analysis of the published sequences did not reveal structures that could be used either in recombination or conversion events. Therefore, we propose that the V_H GAT system is encoded mainly by one or a very few related germ-line genes.

Structural correlates for the expression of public (pGAT) and private (i_1 GAT) idiotypic specificities

Expression of the public (pGAT) as well as that of the private (i_1 GAT) idiotypic specificities require the presence of both the H and the L chains of the idiotype (Sommé *et al.*, 1982). Some correlation between amino acid sequence and expression of the various idiotypic specificities may thus be anticipated to be found on both chains, although this cannot provide in itself a very precise view of the actual three-dimensional organization of the idiotopes. It was therefore of special interest that the two antibodies that expressed the same private specificities, in addition to the public pGAT idiotopes, had completely identical V_H sequences, including the D and the J segments. It should be recalled that these two antibodies were derived from separate fusions, and preliminary data suggest that the light chains are also very close, differing by only two amino acid substitutions on 106 identified codons (J.Rocca-Serra and C.Tonnelle, unpublished data). The two other monoclonal antibodies, which did not share the i_1GAT private idiotopes, had an identical amino acid sequence only up to residue 54, whereas several substitutions were observed thereafter, affecting both the second hypervariable and the third framework regions (Figure 1). These observations would allow the suggestion that H structural correlates for the public specificities might lie within segment 1 – 54, whereas private specificities could depend upon amino acid residues located after position 55. This, of course, should be considered with caution, since conformational structures that most probably condition the expression of idiotopes cannot be simply deduced from amino acid sequence.

The third hypervariable region may be functionally conserved, as the result of two types of D-J associations

The third hypervariable region of the heavy chains is the most variable segment of immunoglobulins. Most of this region is contributed to by the D gene, but the last amino acid residues of the V_H and the first residues encoded by the J_H gene may also participate (Gough and Bernard, 1981; Sakano *et al.*, 1980). The D segment may vary extensively both in length and in sequence, even within a closely related set of antibodies which are supposed to recognize the same epitope. All four anti-GAT heavy chains analyzed here have D regions of similar length, corresponding to ~6 codons. The three D regions which are structurally related to D.SP 2 are all linked to J_H4, whereas the fourth region, closer to D.FL 16 is connected to J_H2. As often observed, the J_H germ-line genes are not used in full length, but the first two codons are deleted.

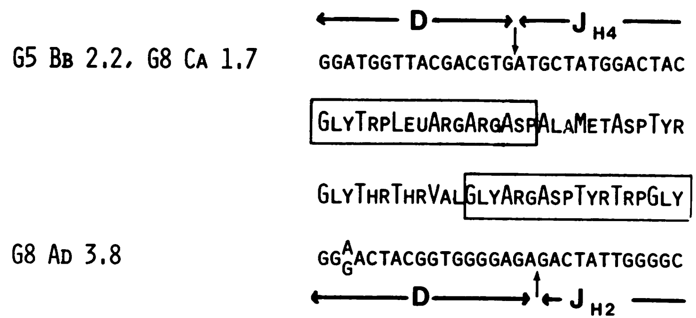

Fig. 4. Different combinations of D and J regions may lead to functionally similar HV3 involved in the recognition of the GT dominant antigenic determinant.

Kurosawa and Tonegawa (1982), considering that a D gene contained a heptamer (core sequence), flanked by one pentamer on the 5′ end (L or Left), and one pentamer on the 3′ end (R or Right), proposed that D gene diversity could be amplified by D-D recombinations, using nucleotide stretches that might yield new genes that would, for instance, contain two distinct 'R' segments attached together. The G5 prototype might be built on this model, with a deleted L stretch, a hexameric core (C) ATGGTT, a first R1 segment ACGAC, which is contained in the D.SP 2 germ-line structure, followed by a second R2 segment, which reads GTGAT. This pentamer was not identified on D gene coding structures published so far. It is worth noting, however, that this sequence can be found near the beginning of the 3′ non-coding stretch of the D gene and also on the non-coding sequence near the end of the 5′ side of the J_H4 gene (see Figure 2). An alternate possibility might be that the last two nucleotides of what we consider our D region pertain to the J_H4 sequence, which reads ...ATTACTATGCT. Depending upon which AT pair is used, a distinct deletion would be necessary. As underlined by Alt and Baltimore (1982), D-J boundaries in this recombinational process are not clearly marked. They suggested that an N segment, located between D and J might be inserted as the result of the addition of a few nucleotides, dictated by a terminal transferase. The presence of a G-rich sequence, possibly related to the intervention of terminal transferase, was not seen on the 3′ end of the D.SP 2 related D segments, but was seen on the 5′ side. A G-rich sequence was found on both the 5′ and the 3′ of the D.FL 16 related D region.

It has been established that anti-GAT, $pGAT^+$ antibodies recognized essentially Glu-Tyr determinants (Thèze and Sommé, 1979). The amino acid sequence contained in the D region of the G5 prototype reads Gly-Trp-Leu-Arg-Arg-Asp (Figure 4). In contrast, the D region of the fourth heavy chain, G8 Ad 3.8, contains only one Arg residue and no aromatic amino acid. In fact, it may be observed in Figure 4

that rearrangement which occurred between D and J_H2 allows definition of the sequence Gly-Arg-Asp-Tyr-Trp-Gly-, which may provide a third hypervariable region functionally similar to that previously described. This example would illustrate the enormous flexibility of the immune system to generate alternative similar recognition patterns. It is also worth noting that the sequence Lys-Tyr-Asp-Pro-Lys-Phe is found between residues 59 and 64, i.e., within the second hypervariable region of the four heavy chains, and HV_1 reads Phe-Asn-Ile-Lys-Asp-Thr-Tyr (positions 27 – 33) suggesting a similar complementarity for the antigen. It should be noted that these three hypervariable regions contain either one or two acidic amino acids the presence of which could be correlated with the need to stabilize the combining site structure in the absence of antigen by acid-base interaction. This internal interaction could be competed for in the presence of Glu-containing determinants, leading to fixation of the GAT antigen.

Materials and methods

Hybridomas and anti-GAT monoclonal antibodies

The four hybridomas were derived from three separate fusions, termed G5, G7 and G8, as described by Leclercq *et al.* (1982). The hybridoma cells were transplanted into BALB/c mice, and the resulting solid tumors were removed and stored at −70°C. The corresponding anti-GAT monoclonal antibodies were specifically isolated from ascitic fluids on a GAT-amino-hexyl-Sepharose column, as previously reported (Thèze *et al.*, 1977).

RNA purification and nucleotide sequencing

The poly(A)$^+$ RNAs were extracted directly from the frozen tumors by the LiCl-urea method (Auffray and Rougeon, 1980), purified on an oligo(dT)-cellulose column, and enriched for the H-coding fraction by ultracentrifugation on a sucrose gradient (5 – 20%), as identified upon analysis of fractions in a cell-free translation system. Heavy chain polypeptide represented 30 – 50% of the translation products in the pooled H mRNA fraction. A synthetic oligonucleotide, d(GGCCAGTGGATAGAC), complementary to a nucleotide sequence of the C_H1 domain close to the V-C joining, and chosen within a highly conserved stretch through the $\gamma 1$, $\gamma 2a$ and γb heavy chains (Kaartinen *et al.*, 1983) was synthesized according to Gait and Sheppard (1977), and was used as a primer for the reverse transcriptase (obtained from J.Beard). All chains were of the $\gamma 1$ isotype. Nucleotide sequences were determined towards the 5′ end of the mRNA, up to codon 60, on average, according to the modification of the original dideoxy method (Sanger *et al.*, 1977) using the mRNA as a template (Hamlyn *et al.*, 1978). Each sequence determination was carried out using 2 μg of enriched mRNA to which 100 ng of primer was added. Unlabeled and labeled deoxynucleotide concentrations were 60 μM and 4 μM, respectively. The concentration of dideoxy analogs of the deoxynucleotides were 10 μM and 0.2 μM for the analog of the labeling one. Incubations were performed for 15 min at 42°C in the presence of avian myeloblastosis virus reverse transcriptase (from J.Beard, Life Science Inc., St. Petersbourg, USA). A second oligonucleotide, d(GGAGGAGTGCT), complementary to codons 73 – 76, which sequence was found rather specific for anti-GAT heavy chain mRNA, was synthetized and used for a second sequencing run that ensured extensive overlap (up to codons 6 – 10) with the amino acid sequence previously determined from the NH_2 terminus (Rocca-Serra *et al.*, 1982). In order to minimize possible ambiguities in sequence determination (Hamlyn *et al.*, 1978) on electrophoresis gels, labelling with each of the three [α-^{32}P]deoxynucleotides (dCTP, dATP and dGTP) was performed in separate experiments each repeated at least in duplicate. Artefactual bands were found in different places with each label except for very few positions where some uncertainty remained (dotted on Figure 1).

Acknowledgements

We thank C.Schiff and M.Milili for making available to us unpublished results on cDNA sequencing. This work was supported by CNRS and INSERM and by an EMBO fellowship ASTF 3572 awarded to J.R-S.

References

Alt,F.W. and Baltimore,D. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4118-4122.

Amzel,L.M., Poljak,R., Saul,F., Varga,J. and Richards,F. (1974) *Proc. Natl. Acad. Sci. USA*, **71**, 1427-1431.

Auffray,C. and Rougeon,F. (1980) *Eur. J. Biochem.*, **170**, 303-314.

Auffray,C., Sikorav,J.-L., Ollo,R. and Rougeon,F. (1981) *Ann. Immunol. (Institut Pasteur)*, **132D**, 77-88.

Bothwell,A.L.M., Paskind,M., Reth,M., Imanishi-Kari,T., Rajewsky,K. and Baltimore,D. (1981) *Cell*, **24**, 625-637.

Cosenza,H. and Köhler,H. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 2701-2705.

Crews,S., Griffin,J., Huang,H., Calame,K. and Hood,L. (1981) *Cell*, **25**, 59-66.

de Préval,C. and Fougereau,M. (1976) *J. Mol. Biol.*, **102**, 657-678.

Early,P., Huang,H., Davis,M., Calame,K. and Hood,L. (1980) *Cell*, **19**, 981-992.

Edelman,G.M., Olins,D.E., Gally,J.A. and Zinder,N. (1963) *Proc. Natl. Acad. Sci. USA*, **50**, 753-761.

Eichman,K. (1973) *J. Exp. Med.*, **137**, 603-621.

Estess,P., McCumber,L., Siegelman,M., Waldschmidt,T., Pacifico,A. and Capra,D.J. (1980) in Fougereau,M. and Dausset,J. (eds.), *Progress in Immunology IV, Immunology 80*, Academic Press, NY, pp. 3-16.

Gait,M.J. and Sheppard,R.C. (1977) *Nucleic Acids Research*, **4**, 4391-4410.

Gearhart,P.J., Johnson,N.D., Douglas,R. and Hood,L. (1981) *Nature*, **291**, 29-34.

Givol,O., Zakut,R., Effron,K., Rechavi,G., Ram,D. and Cohen,J.B. (1981) *Nature*, **292**, 426-430.

Gough,N.M. and Bernard,O. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 509-513.

Hamlyn,P.H., Brownlee,G.G., Cheng,C.C., Gait,M.J. and Milstein,C. (1978) *Cell*, **15**, 1067-1075.

Hozumi,N. and Tonegawa,S. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 3628-3632.

Johnson,N., Slankard,J., Paul,L. and Hood,L. (1982) *J. Immunol.*, **128**, 302-307.

Ju,S.T., Kipps,T.J., Thèze,J., Benacerraf,B. and Dorf,M.E. (1978) *J. Immunol.*, **121**, 1034-1039.

Ju,S.T., Pierres,M., Germain,R.N., Benacerraf,B. and Dorf,M.E. (1979) *J. Immunol.*, **123**, 2505-2510.

Kaartinen,M., Griffith,G.M., Hamlyn,P.H., Markham,A.F., Karjalainen, K., Pelkonen,J.L.T., Mäkelä,O. and Milstein,C. (1983) *J. Immunol.*, in press.

Karjalainen,K. and Mäkelä,O. (1978) *Eur. J. Immunol.*, **8**, 105-112.

Kocher,H.P., Berek,C. and Jaton,J.C. (1981) *Mol. Immunol.*, **18**, 1027-1033.

Köhler,G. and Milstein,C. (1975) *Nature*, **256**, 495-497.

Kurosawa,Y. and Tonegawa,S. (1982) *J. Exp. Med.*, **155**, 201-218.

Leclercq,L., Mazie,J.C., Sommé,G. and Thèze,J. (1982) *Mol. Immunol.*, **19**, 1001-1009.

Lieberman,R., Potter,M., Humphrey,W.,Jr., Mushinski,E.B. and Vrana,M. (1976) *J. Immunol.*, **117**, 2105-2111.

Max,E.E., Seidman,J.G., Miller,H.I. and Leder,P. (1980) *Cell*, **21**, 793-799.

Metzger,H., Wofsy,L. and Singer,S.J. (1964) *Proc. Natl. Acad. Sci. USA*, **51**, 612-618.

Pawlak,L.L., Mushinski,E.B., Nisonoff,A. and Potter,M. (1973) *J. Exp. Med.*, **137**, 22-31.

Riblet,R., Blomberg,B., Weigert,M., Lieberman,R., Taylor,B.A. and Potter, M. (1975) *Eur. J. Immunol.*, **5**, 775-777.

Rocca-Serra,J., Mazie,J.C., Moinier,D., Leclercq,L., Sommé,G., Thèze,J. and Fougereau,M. (1982) *J. Immunol.*, **129**, 2554-2558.

Ruf,J., Tonnelle,C., Rocca-Serra,J., Moinier,D., Pierres,M., Ju,S.T., Dorf,M.E., Thèze,J. and Fougereau,M. (1983) *Proc. Natl. Acad. Sci. USA*, in press.

Sakano,H., Hüppi,K., Heinrich,G. and Tonegawa,S. (1979) *Nature*, **280**, 288-294.

Sakano,H., Maki,R., Kurosawa,Y., Roeder,W. and Tonegawa,S. (1980) *Nature*, **286**, 676-683.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.

Schilling,J., Clevinger,B., Davie,J.M. and Hood,L. (1980) *Nature*, **283**, 35-40.

Seidman,J.G., Leder,A., Nau,M., Norman,B. and Leder,P. (1978) *Science (Wash.)*, **202**, 11-17.

Sommé,G., Rocca-Serra,J., Leclercq,L., Moreau,J.L., Mazie,J.C., Moinier, D., Fougereau,M. and Thèze,J. (1982) *Mol. Immunol.*, **19**, 1011-1019.

Thèze,J., Kapp,J.A. and Benacerraf,B. (1977) *J. Exp. Med.*, **145**, 839-856.

Thèze,J. and Moreau,J.L. (1978) *Ann. Immunol. (Inst. Pasteur)*, **129**, 721-726.

Thèze,J. and Sommé,G. (1979) *Eur. J. Immunol.*, **9**, 294-301.

Tonegawa,S., Maxam,A.M., Tizard,R., Bernard,O. and Gilbert,W. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1485-1489.

Tonnelle,C., Pierres,M., Ju,S.T., Moinier,D. and Fougereau,M. (1981) *Mol. Immunol.,* **18**, 979-984.
Vrana,M., Rudikoff,S. and Potter,M. (1978) *Proc. Natl. Acad. Sci. USA,* **75**, 1957-1961.