Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4788–4792, July 1985
Immunology

Gene repertoire of the anti-poly($Glu^{60}Ala^{30}Tyr^{10}$) (GAT) immune response: Comparison of V_H, V_κ, and D regions used by anti-GAT antibodies and monoclonal antibodies produced after anti-idiotypic immunization

(idiotope structure and expression/mRNA sequences)

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Communicated by Jacques Oudin, February 25, 1985

ABSTRACT **Eight monoclonal antibodies were selected from BALB/c mice immunized with two different monoclonal anti-idiotypic antibodies recognizing two discrete idiotopes characteristic of the anti-poly($Glu^{60}Ala^{30}Tyr^{10}$) (GAT) antibody response. These monoclonal antibodies were previously classified as Ab_1' (anti-GAT-like) and Ab_3 (anti-anti-idiotype) on the basis of expression of the public idiotypic specificity (*p.GAT*) studied with a xenogeneic serum, anti-GAT activity, and expression of various public idiotopes. All the heavy chain variable region (V_H) sequences from Ab_1' are nearly identical to the V_H sequences of Ab_1 anti-GAT monoclonal antibodies. The same type of results has been found with the Ab_1' κ light chain variable region (V_κ) sequences. Confirming our classification, Ab_3 V_H and V_κ sequences were found to be completely different from Ab_1 V_H and V_κ sequences. The Ab_1' diversity (D) regions are different from one another and different from the D regions found on monoclonal anti-GAT antibodies but function similarly. These D regions are not simply derived from already described D genes. Finally, our results suggest that in the anti-GAT response V_H and V_κ sequences are mainly responsible for idiotype expression.**

Antigen immunization leads to the production of specific antibodies (Ab_1), which can in turn be used as immunogens to produce anti-idiotypic reagents (1–3). Immunization with these reagents (Ab_2) can also elicit an immune response (4, 5). This response consists of immunoglobulins idiotypically related to Ab_1 with or without antibody activity (Ab_1') and of anti-anti-idiotypic antibodies (Ab_3). Structural analysis of the genes involved at the different steps of this "idiotypic cascade" is a major tool in elucidating the origin of the B-cell repertoire (6). More precisely, structural studies of antibodies obtained after anti-idiotypic (anti-Id) immunization should unequivocally establish the genetic origin of Ab_3 and Ab_1' and document the relationship between Ab_1' and Ab_1.

Various systems have been analyzed at the Ab_1 level, using either myelomas or hybridomas specific for well-defined antigens. The immune response against arsonate (Ars) (7), nitrophenyl acetyl (NP) (8), phosphocholine (PC) (9), oxazolone (ox) (10), dextran (dex) (11), and poly($Glu^{60}Ala^{30}$-Tyr^{10}) (GAT) (12) have been particularly studied. More recently several reports dealing with the study of Ab_1' and Ab_3 have been published (13, 14).

In the immune response against GAT different monoclonal anti-GAT antibodies (HP-GAT; HP, hybridoma product) have been characterized (15). Their partial amino acid sequences and complete nucleotide sequences have been reported. A limited number of sequences for the heavy chain variable region (V_H) (12, 16) and κ light chain variable region (V_κ) (17–19) are used. At the idiotypic level the anti-GAT response is characterized by the expression of the public idiotypic specificity *p.GAT* defined by a xenogeneic antiserum (20, 21) and expressed in all HP-GAT (15). More recently, two discrete idiotopes characteristic of the anti-GAT responses have been identified (22–25).

In this paper we report the nucleotide sequences of two sets of monoclonal antibodies obtained from BALB/c mice immunized with two different monoclonal anti-idiotypic antibodies (HP-Id) recognizing the two idiotopes previously defined (25). The V_H, V_κ, and diversity (D) sequences of these monoclonals are compared to the corresponding sequences previously reported for HP-GAT. The possible contribution of V_H, V_κ, and D segments to idiotope expression and antibody activity is discussed.

MATERIALS AND METHODS

Detection of Idiotypic Specificities and of Anti-GAT Activity. The detection of the *p.GAT* specificity and the percent maximal inhibition were calculated as described (20, 21). For the detection of the two idiotopes identified in the GAT response by $HP\text{-}Id_{20}$ and $HP\text{-}Id_{22}$ a solid-phase radioimmunoassay (RIA) was used (25). The idiotope binding inhibitory capacity (IBIC) of each Ab_1' was determined. IBIC was expressed as the percentage of the slope of the inhibition curves, taking the slope obtained with HP-GAT G5 as standard (IBIC = 100%).

The GAT-binding assay has been described (25). Results are expressed as the reciprocal of the dilution of supernatant giving 50% of maximal binding. Ig present in each fluid was measured and adjusted to 1 μg/ml before the assay. For the GAT-binding inhibition assay, radiolabeled $G5Bb_{2\text{-}2}$ (10 ng per well) was used to measure binding to GAT and various dilutions of ascitic fluids were tested for their inhibitory capacity. Results are expressed as percent of maximal inhibition obtained.

RNA Purification and Nucleotide Sequencing. The heavy- and light-chain-encoding fractions of poly(A)$^+$ RNAs were prepared as described (12, 26). Nucleotide sequences were

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Abbreviations: Ab_1, specific antibody; Ab_2, anti-idiotypic antibody; Ab_3, anti-anti-idiotypic antibody; Ab_1', antibody-like immunoglobulin generated after anti-idiotypic immunization; Id, idiotype; GAT, random terpolymer poly($Glu^{60}Ala^{30}Tyr^{10}$); V_H, variable region of immunoglobulin heavy chain; V_κ, variable region of immunoglobulin κ light chain; *D*, diversity region; *J*, joining region; HP, hybridoma product (monoclonal antibody); HP-Id, monoclonal anti-idiotypic antibody against public idiotopes; HP-GAT, monoclonal anti-GAT antibody; IBIC, idiotope binding inhibitory capacity; G5 or $G5Bb_{2\text{-}2}$, HP-GAT used as reference; *p.GAT*, public idiotypic specificity in the anti-GAT response; KLH, keyhole limpet hemocyanin.

determined according to the modification of the original dideoxy method using the mRNA as template (27). Synthetic oligonucleotides, d(CAGGGGCCAGTGG) specific for the constant region of the $\gamma1$ or $\gamma2a$ chain, d(GCTCTCGCAGGAGAC) specific for the constant region of the μ chain, or d(TGGATGGTGGGAAGATG) specific for the constant region of the κ light chain, were synthesized according to Gait and Sheppard (28) and used as primers. Each sequence was determined with 2 μg of enriched mRNA and 10 ng of oligonucleotide primer. The concentrations of nucleotides (unlabeled and labeled) and dideoxy analogs have already been reported (12). To minimize possible ambiguities in sequence determination on gels (27) labeling with each of the α-^{32}P-labeled deoxynucleotides (dCTP, dATP, and dGTP) was performed in separate experiments and each was repeated at least twice.

RESULTS

Characteristics of Monoclonal Antibodies Derived from HP-Id-Immunized BALB/c Mice. From BALB/c mice immunized with two different HP-Ids coupled to keyhole limpet hemocyanin (KLH), two fusion experiments were performed and eight hybridomas were studied. Three hybridomas were isolated from animals immunized with HP-Id$_{20}$-KLH and five hybridomas were from animals stimulated with HP-Id$_{22}$-KLH. The characteristics of the corresponding HP have been determined (25) and are summarized in Table 1.

The expression of the *p.GAT* idiotypic specificity characteristic of the anti-GAT antibody response was measured on these HPs by using the xenogeneic polyclonal antisera previously defined (20). Seven of these HPs were found to inhibit the binding of radiolabeled monoclonal anti-GAT antibody G5, which was taken as reference. One HP (22.134) was found not to inhibit in this assay. The anti-GAT activity of these reagents was tested with two different assays, and both assays gave the same results (25). Some HPs (20.8, 20.11, 20.33, and 22.186) did exhibit an anti-GAT activity comparable to G5, while others showed a low anti-GAT activity (22.162 and 22.8) or no activity (22.176, 22.134). We concluded that HP-22.134 is anti-anti-idiotypic (Ab$_3$), while the seven other HPs are Ab$'_1$.

In addition, we have tested the idiotope expression by these HPs, using two HP-Ids (HP-Id$_{20}$ and HP-Id$_{22}$) that recognize the two idiotopes characteristic of the anti-GAT response (Table 1). The seven Ab$'_1$s were able to inhibit the binding of G5 to these HP-Id. When compared to HP-GAT G5, the idiotopes expressed by these different Ab$'_1$s are not identical, since the inhibition curves were found to have different slopes. However, all the Ab$'_1$s isolated expressed the same characteristic idiotopes as all the BALB/c HP-GATs studied (22). As expected, Ab$_3$ inhibited only the binding of HP-Id$_{22}$ to G5 (25).

Primary Structure of V_H Regions of Ab$'_1$ and Ab$_3$ Monoclonal Antibody. The nucleotide sequence of seven heavy chain regions, encompassing most of the V_H, the entire *D*, and the beginning of the joining (J_H) segments was determined (Fig. 1). Within the limits of the analysis (four residues were undetermined), each of the five HPs has exactly the same V_H sequence as monoclonal anti-GAT G5. One (20.11) has only four substitutions, at position 56, 59, 67, and 68. These four changes do not lead to a net charge difference between the molecules (Gly → Val, Lys → Arg, Lys → Arg, and Ala → Gly). No silent substitution was identified.

While Ab$_1$ anti-GAT *D* regions usually code for five amino acids, the *D* regions of Ab$'_1$ are very heterogeneous. None of these *D* segments is identical to *D* segments of monoclonal anti-GAT antibodies, which were found to derive either from DSP-2 or from DFL-16 (12). The *D–J* border is very heterogeneous, and two Ab$'_1$ *D* segments are very short and encode only three amino acids. The seven J_H segments sequenced are $J_{H\text{-}4}$, which is also used by most monoclonal anti-GATs (G5, G8Ca$_{1\text{-}7}$, and G7Ab$_{2\text{-}9}$). At the *D*–V_H borders HP 20.33 has lost one triplet, while in all the other Ab$'_1$s there is a silent substitution.

HP 22.134 uses a completely different V_H chain; this is consistent with the fact that it has been identified as an Ab$_3$. However, it belongs to the same V_H subgroup as the Ab$'_1$ (29) and also uses $J_{H\text{-}4}$.

Primary Structure of Ab$'_1$ and Ab$_3$ V_κ Regions. Three types of sequences were already defined in the V_κ involved in the anti-GAT response. One was identified on HP-GAT G5 and on several other monoclonal anti-GAT antibodies derived from BALB/c mice. The other sequences were obtained from DBA/2 and (DBA/2 × BALB/c)F$_1$ hybridomas (H-56 and H-51 fusion experiments). V_κ chains from HPs H-51 and H-56 vary from the V_κ chain of G5 at seven positions (17).

The partial sequences of six V_κ chains from Ab$'_1$ are shown in Fig. 2. Strikingly, they are all identical and show the same substitutions as H-51 and H-56, when compared to the G5 sequence: at positions 50 (Arg → Lys), 83 (Met → Leu), 89 (Phe → Ser), and 91 (Gly → Ser). However, in HP 22.176 one substitution has been found at position 40 (Pro → Gln). The substitutions expressed only in H-51 and not in H-56 at positions 81, 87, 92, and 94 are not recovered on the Ab$'_1$ V_κ chains.

All the J_κ segment sequences are $J_{\kappa2}$, as in G5, H-51, and H-56. However, other J_κ segments are used in the anti-GAT response (17). Concerning the V_κ–J_κ junction, the same

Table 1. Characteristics of monoclonal antibodies derived from HP-Id-immunized BALB/c mice

Immunizing HP-Id	HP	*p.GAT* expression,* %	Type of HP	Idiotype expression,† %		Anti-GAT activity‡	Isotypes
				Group 20	Group 22		
HP-Id$_{20}$-KLH	20.8	75	Ab$'_1$	130	120	+	$\gamma1$ κ
	20.11	75	Ab$'_1$	100	100	+	$\gamma1$ κ
	20.33	80	Ab$'_1$	90	90	+	μ κ
HP-Id$_{22}$-KLH	22.186	80	Ab$'_1$	120	100	+	μ κ
	22.162	80	Ab$'_1$	40	40	+/−	$\gamma1$ κ
	22.8	90	Ab$'_1$	25	30	+/−	μ κ
	22.176	80	Ab$'_1$	50	40	−	μ κ
HP-Id$_{22}$-KLH	22.134	0	Ab$_3$	—	—	−	$\gamma2a$ κ

*Percent binding inhibition by hybridoma supernatants of rabbit anti-idiotypic antiserum to 5 ng of radiolabeled HP-GAT G5. G5 is taken as a probe of the public idiotypic specificity in the GAT response (*p.GAT*).

†IBIC determined by the inhibition of the binding of radiolabeled G5 to purified HP-Id$_{20}$- or HP-Id$_{22}$-coated plates.

‡Anti-GAT activity was determined by two independent assays. See Table 2.

```
                                            CDR 1
                                    30                                      40
        Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
G5Bb22  ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG
20.8
20.11                           --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -X-
20.33
22.186                                                                                  --- --- ---
22.8                            --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.176                          --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                Ser Pro Glu Lys Ser
22.134                                                                          TCT CCA GAA AAG AGT

                                                    CDR 2
                        50                                      60
        Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly Lys Ala
G5Bb22  GAG TGG ATT GGA AGG ATT GAT CCT GCG AAT GGT AAT ACT AAA TAT GAC CCG AAG TTC CAG GGC AAG GCC
20.8                                        --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                Val         Arg                             Arg Gly
20.11   --- --- --- --- --- --- --- --- --- --- -T- --- --- -G- --- --- --- --- --- --- --- -G- -GX
20.33               --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.186  --- --- --- --- --- --- --- --- -X- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.176  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
        Leu Glu Trp Val Ala Glu Ile Ser Ser Gly Gly Tyr Thr Tyr Tyr Leu Asp Thr Val Thr Gly Gln Phe
22.134  CTG GAG TGG GTT GCA GAA ATT AGT AGT GGT GGT TAC ACC TAC TAT CTA GAC ACT GTG ACG GGC CAA TTC

            70                                      80                                      90
        Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
G5Bb22  ACT ATA ACA GCA GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT
20.8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
20.11   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
20.33   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.186  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
22.176  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
        Thr Ile Ser Arg Asp Asn Ala Lys His Thr Leu Tyr Leu Glu Met Ser Ser Leu Arg Ser Glu Asp Thr
22.134  ACC ATC TCC AGA GAC AAT GCC AAG CAC ACC CTG TAC CTG GAA ATG AGC AGT CTG AGG TCT GAG GAC ACG

                    VH                      D                       JH
       ---------------------------><---------------------><----------------------------------
                                        100                                     110
        Ala Val Tyr Tyr Cys Ala Arg Gly Trp Leu Arg Arg Asp         Ala Met Asp Tyr Trp Gly Gln Gly
G5Bb22  GCC GTC TAT TAC TGT GCT AGG GGA TGG TTA CGA CGT G        AT GCT ATG GAC TAC TGG GGT CAA GGA
                                    Arg Leu Met Asp Tyr
20.8    --- --- --- --- --- --- --A AGG CTA ATG GAC TAC             --- --- --- --- --- --- --- ---
                                    Ser Leu Arg Val             Tyr
20.11   --- --- --- --- --- --- --A TCA CTA CGG GT            C T-- --- --- --- --- --- --- --- ---
                                    Ser Ser Thr Ser
20.33   --- --- --- --- --- ---     TCG TCT ACC TC            C T-- --- --- --- --- --- --- --- ---
                                    Gly Tyr Gly Leu Tyr     Tyr
22.186  --- --- --- --- --- --- --A GGG TAC GGC TTA TAT     TAC T-- --- --- --- --- --- --- --- ---
                                    Trp Glu Ser
22.8    -X- --- --- --- --- --- --A TGG GAA TC                              - --- --- --- --- --- ---
                                    Ser Asp Gly
22.176  --- --- --- --- --- --- --A TCG GAT GGT             TAC T-- --- --- --- --- --- --- --- ---
        Ala Met Tyr Tyr Cys Val Arg Gly Gly Tyr Gly Gly Val
22.134  GCT ATG TAT TAC TGT GTA AGG GGG GGA TAT GGG GGA GTA             --- --- --- --- --- --- ---
```

FIG. 1. Nucleotide and deduced amino acid sequences of seven V_H regions from BALB/c Ab'_1 and Ab_3 HPs. These sequences were determined from codon 115 to codon 29 to 56, according to a modification of the original dideoxy method (26). The nucleotide sequence of HP-GAT G5Bb$_{2-2}$ (12) is given as a reference. X, undetermined nucleotide. CDR, complementarity-determining region.

pattern of base substitution is found in all the Ab'_1s, and this pattern is similar to the patterns found in H-51 and H-56 HPs. A silent substitution from T to G is found at position 95 and a substitution from C to T is found at position 96.

Therefore, all the Ab'_1s have a V_κ sequence and a V_κ–J_κ junction very close to the H-51 and H-56 germ lines V_κ gene prototype. On the other hand, the V_κ sequence of Ab_3 22.134 does not show any similarities with H-51, H-56, or G5 V_κ chains.

DISCUSSION

Four main conclusions can be derived from the present work: (*i*) Idiotope-bearing molecules (Ab'_1) selected after immunization with monoclonal anti-idiotope reagents express V_H and V_κ sequences similar to those previously defined on some monoclonal anti-GAT molecules. (*ii*) These V_H and V_κ Ab'_1 sequences are more conserved than those found on anti-GAT monoclonal antibodies. (*iii*) The distinction between Ab'_1 and Ab_3 monoclonal antibodies previously made on the basis of idiotype expression and antibody activity has been confirmed at the sequence level. (*iv*) The *D* regions expressed by Ab'_1 were found to be heterogeneous in sequence and to differ from the *D* region expressed by anti-GAT reagents.

The V_H sequences determined for five Ab'_1s, including four μ chains and one $\gamma 1$ chain from two independent fusion experiments performed with spleen cells of animals immunized with two different HP-Id were found to be identical to the V_H sequences obtained from two Ab_1 anti-GAT HPs (G5Bb$_{2-2}$ and G8Ca$_{1-7}$), also obtained from two independent fusion experiments (12). This strongly suggests that these sequences represent direct expression of the V_H germ-line gene predominantly used in the anti-GAT (Ab_1) response. Five Ab'_1 V_κ sequences have also been determined. They are identical among themselves and very similar to the primary structure of two Ab_1 antibodies (H-51 and H-56). These sequences therefore presumably represent the expression of a V_κ germ-line gene that is common to BALB/c and DBA/2 mice. The conclusions concerning the homology of the different V_H and V_κ sequences were drawn from partial nucleotide sequences (down to position 30–35). Since the NH_2-terminal region is known to be particularly conserved, at least in the anti-GAT response (16), our conclusions can reasonably be extrapolated to the entire *V* sequence.

The comparison of the V_H and V_κ sequences from Ab'_1 and Ab_1 indicates that the expressed repertoire after anti-Id stimulation is much more homogeneous than after antigenic stimulation. At the V_H level, for instance, several amino acid substitutions are observed when different HP-GAT sequences are compared to the G5 basic and germ-line sequence (12). This suggests that optimal selection for idiotope

```
                                                                   CDR 2
                                                                   ____________________________
                            40                                      50
        Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly
G5Bb22  TGG TAC CTG CAG AAA CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AGG GTT TCC AAG CGA TTT TCT GGG

H51.5.2      _   _   _   _   _   _   _   _   _   _   _   _   _   _  Lys  _   _   _   _   _   _   _
                                                                    Lys
20.11               --- --- --- --- X-- --- --- --- --- --- --- --- -AA --- --- --- --- --- --- ---

22.186  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -AA --- --- --- --- --- --- ---

22.162  --- --- --- --- --X --- --- --- --- --- --- --- --- --- --- -AA --- --- --- --- --- --- ---

22.8    --- --- --- --- --- X-- --- --- --- --- --- --- --- --- --- -AA --- --- --- --- --- --- ---
                            Gln
22.176  --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- -AA --- --- --- --- --- --- ---
        Trp Tyr Gln Gln Lys Gln Gly     Thr Pro Glu Pro Leu Ile Tyr Ser Ala         Arg Tyr Ser Gly
22.134  TGG TAT CAA CAG AAA CAA GGG XAT ACT CCT GAA CCA CTG ATT TAC TCG GCA TXC TXC CGG TAC AGT GGG


                60                                      70                                      80
        Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala
G5Bb22  GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT

H51.5.2  _   _   _   _   _   _   _   _   _  --- --X --- --- --- --- --- --- --- --- --- --- --- --X

20.11   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

22.186  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --X --- ---

22.162  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --X --- ---

22.8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

22.176  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
        Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Thr Asn Val Gln Ser
22.134  GTC CCA GAT CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC TCT CTC ACC ATC ACC AAT GTG CAG TCT

                                         CDR 3
                Vκ                ________________________________                  Jκ

        ------------------------------------ 90 -------------------> <----------- 100 --------
        Glu Asp Met Gly Val Tyr Tyr Cys Phe Gln Gly Thr His Val Pro His Thr Phe Gly Gly Gly Thr Lys
G5Bb22  GAG GAT ATG GGA GTT TAT TAC TGC TTT CAA GGT ACA CAT GTT CCT CAC ACG TTC GGA GGG GGG ACC AAG
        Val     Leu             Phe     Ser     Ser Lys     Ile     Trp
H51.5.2 -T- --- C-- --X --- --- -T- --- -C- --- A-- -A- --- A-- --G TGG --- --- --T --A --C --- ---
                Leu                     Ser     Ser                 Tyr
20.11   --- --- C-- --- --- --- --- --- -C- --- A-- --- --- --- --G T-- --- --- --- --- --- --A ---

22.186  --- --- C-- --- --- --- --- --- -C- --- A-- --- --- --- --G T-- --- --- --- --- --- --A ---

22.162  --- --- C-- --- --- --- --- --- -C- --- A-- --- --- --- --G T-- --- ---
                                                                                Arg
22.8    --- --- C-- --- --- --- --- --- -C- --- A-- --- --- --- --G T-- --- --- --- A-- --A --- ---

22.176  --- --- C-- --- --- --- --- --- -C- --- A-- --- --- --- --G T-- --- --- --- --- --- --- ---
        Ala Val Gly Ala Asp Tyr Phe Cys Gln Leu Tyr Ile Arg
22.134  GCA GTC GGG GCC GAC TAC TTC TGT CAG CTA TAT ATT AGA --- --- --- --- --- --- --- --- --- ---
```

FIG. 2. Nucleotide and deduced amino acid sequences of six V_κ regions from BALB/c Ab_1' and Ab_3 HPs. The sequences of Ab_1 HP-GAT $G5Bb_{2\text{-}2}$ (BALB/c) (12) and Ab_1 HP-GAT H51.5.2 (BALB/c × DBA/2)F_1 are given in ref. 17. X, undetermined nucleotides. Horizontal arrows indicate sequence determined at the protein level.

expression, contrary to optimal selection for anti-GAT activity, does not require the selection of somatic variants. Therefore, the V_H and V_κ genes described are optimal for the expression of the two idiotopes previously defined (22), and these two structures appear to be strictly germ line encoded. Furthermore, since the *D* segments from the different Ab_1's are all different, one may conclude that *D* genetic segments are not involved in idiotype expression in this experimental model. However, optimal serological expression of these idiotopes may be influenced by *D* segments, since Ab_1's (22.161, 22.8, and 22.176) that have V_H and V_κ identical to all the other Ab_1' and to HP-GAT G5 and $G8Ca_{1\text{-}7}$ have a reduced IBIC (Table 1 and ref. 25).

Ab_1' antibodies have a large array of anti-GAT activity. Considering the identity of most of the V_H and V_κ regions involved in Ab_1', the role of the *D* region in anti-GAT activity needs to be addressed. Table 2 compares the anti-GAT activity and the amino acid sequences of three anti-GAT monoclonal antibodies to those of six Ab_1's. Some general features remain common between Ab_1 and Ab_1' *D* regions. They have similar length, at least for those antibodies recognizing GAT, and they contain some charged and aromatic amino acids. As already discussed (12), the *D* region of most anti-GAT monoclonal antibodies is rich in basic and aromatic amino acids. Ab_1's also have basic amino acids (arginine). The two Ab_1's with a low or no anti-GAT activity (22.8 and 22.176) have an acidic amino residue (glutamate or aspartate) not neutralized by a basic residue and have a very short sequence (three amino acids).

We did not find the Ab_1' *D* sequences comparable to the *D* regions of HP-GAT (12). By comparing the Ab_1' *D* segments with the three well-known germ-line *D* segments and their flanking sequences (30) some short homologous regions were identified (Fig. 3). Ab_1's 20.8, 20.33, and 22.176 as well as Ab_3 22.134 have sequences identical to short segments of the DSP.2 germ-line gene. Short sequences of DFL.16 and DQ.52 can also be found in Ab_1' 22.186, 20.11, and 22.8 *D* segments. In addition, all the Ab_1' and the Ab_3 *D* segments have short flanking sequences that are not found in the germ lines DSP.2, DFL.16, and DQ.52. Some of these additional sequences could represent *N* regions (31). The guanine content of *D* segments from 20.8, 22.134, and 22.186 seems to favor this hypothesis since guanine is thought to be the

Table 2. *D* region sequences and anti-GAT activity of Ab_1 and Ab_1' HPs

HP*	*D* region†	Anti-GAT activity‡	
		Binding	% maximal inhibition
$G5Bb_{2\text{-}2}$	$\text{Gly-}\underline{\text{Trp}}\text{-Leu-}\overset{+}{\text{Arg}}\text{-}\overset{+}{\text{Arg}}$	120	80
$G7Ab_{2\text{-}9}$	$\text{Gly-}\underline{\text{Trp}}\text{-}\underline{\text{Phe}}\text{-}\overset{+}{\text{Arg}}\text{-}\overset{+}{\text{Arg}}$	200	80
$G8Ca_{1\text{-}7}$	Gly-Thr-Thr-Val-Gly	200	80
20.8	$\overset{+}{\text{Arg}}\text{-Leu-Met-}\overset{-}{\text{Asp}}\text{-}\underline{\text{Tyr}}$	16	70
20.11	$\text{Ser-Leu-}\overset{+}{\text{Arg}}\text{-Val}$	10	65
20.33	Ser-Ser-Thr-Ser	64	80
22.186	$\text{Gly-}\underline{\text{Tyr}}\text{-Gly-Leu-}\underline{\text{Tyr}}$	120	80
22.8	$\underline{\text{Trp}}\text{-}\overset{-}{\text{Glu}}\text{-Ser}$	0	20
22.176	$\text{Ser-}\overset{-}{\text{Asp}}\text{-Gly}$	0	5
22.134	$\text{Gly-Gly-}\underline{\text{Tyr}}\text{-Gly-Gly-Val}$	0	0

*$G5Bb_{2\text{-}2}$, $G7Ab_{2\text{-}9}$, and $G8Ca_{1\text{-}7}$ are HP-GAT derived from BALB/c mice. $HP_{20.8}$ to $HP_{22.176}$ are Ab_1'; 22.134 is an Ab_3.

†Amino acid sequence derived from nucleotide sequence (Fig. 1). Aromatic amino acids are underlined and basic and acidic amino acids are indicated by $^+$ and $^-$, respectively.

‡Determined on hybridoma supernatant or ascitic fluid by two separate assays.

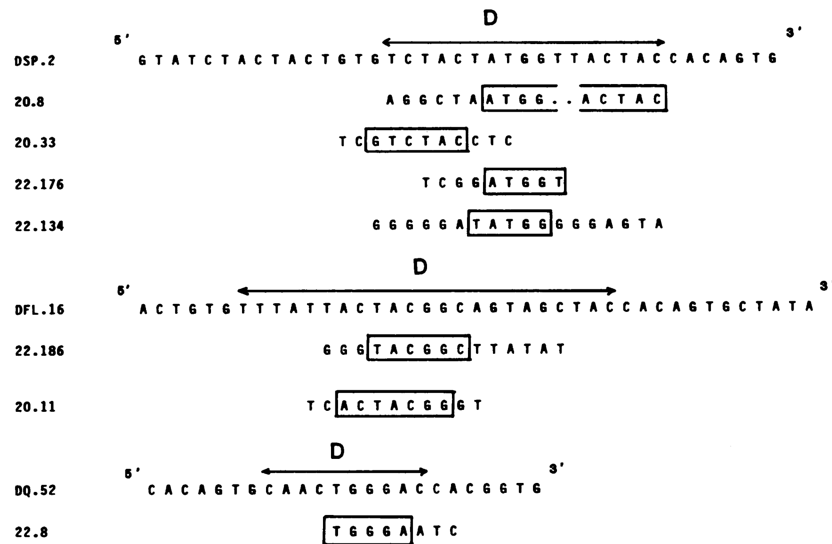

FIG. 3. Genetic origin of the Ab$_1'$ and Ab$_3$ *D* regions. Ab$_1'$ and Ab$_3$ *D* segments were compared with *D* germ-line sequences (30). Arrows indicate germ-line *D* coding sequences; boxes enclose nucleotides in common with germ-line sequences; and dots indicate hypothetical deleted nucleotides.

preferential substrate of terminal transferase (31). Alternatively, since the homologous regions between the Ab$_1'$ *D* segment and the germ-line *D* segments considered are very short, one may consider our *D* segments as the products of non-identified *D* genes.

Anti-idiotypic immunization has been used as a tool to derive monoclonal reagents after a selective pressure only on idiotype expression. These reagents have been characterized at the serological level (25) and their primary structures have been determined. These reagents have also permitted the further analysis of the relationship between V_H, V_κ, J_H, and *D* segments in the expression of idiotypic determinants and antibody activity. The germ-line V_H and V_κ are critical for determining conformations leading to idiotype expression, while in the context of these defined V_H and V_κ segments, *D* segments appear to be more involved in determining the antibody activity. In general terms the repertoire expressed after anti-Id immunization is comparable to the repertoire expressed after antigen stimulation. These results suggest that immunization with monoclonal anti-Id antibodies may be used for vaccinations in situations in which appropriate HP-Ids are available (32). HP-Ids recognizing idiotopes expressed by protective antibodies should be good candidates.

We thank Drs. C. Schiff, F. Rougeon, and Ph. Kourilsky for helpful discussion and Dr. P. Legrain for critical reading of this manuscript. Some of the tumors used in this work were prepared by M. Duphot. We gratefully acknowledge the secretarial assistance of M. Jabely. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (Program de Recherches Coordonnées 127021).

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