MOLECULAR DISSECTION OF THE MURINE ANTIBODY RESPONSE TO STREPTOCOCCAL GROUP A CARBOHYDRATE

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Immunoglobulin genes are composed of several genetic elements (1, 2). In the mouse, a lymphocyte-specific rearrangement event juxtaposes one of a large number of V, gene segments (3) with one of 10–20 D gene segments (4) and one of four J, gene segments to encode the variable region of Ig heavy (H) genes. Similarly, one of a large number of V, gene segments (5) is combined with one of four functional J, gene segments to encode the variable region of λ light chains. Heavy and light chain gene products associate to form antibody molecules.

The variable regions of antibody molecules contain two functionally important structures, antigen binding sites and idiotypic determinants. Although antibody populations selected for individual specificities are structurally heterogeneous, it is not yet clear how much of V, D, and/or J gene segment diversity may be associated with antibodies of a single antigen-binding specificity or bearing a single idiotypic determinant.

Antibodies to streptococcal group A carbohydrate (GAC) provide a particularly interesting model to study the relationship between gene segment diversity and variable region structures. Although essentially all anti-GAC antibodies are specific for the hapten sugar, N-acetyl glucosamine (6), these antibodies are strikingly more diverse than antibodies to other polysaccharide antigens, such as C-polysaccharide (7, 8) and α(1 → 3) dextran (9). Furthermore, anti-GAC antibodies express a multiplicity of idiotypes, allowing them to be divided into four groups: (a) IdX+, Idl-1+, Ids-; (b) IdX+, Idl-1-, Ids-; (c) IdX-, Idl-1+, Ids+; (d) IdX-, Idl-1-, Ids-. The IdX, Idl-1, and Ids determinants are all expressed by free κ chains (10, 11, and our unpublished data). In contrast to IdX and Idl-1, which are expressed in an all-or-none fashion by anti-GAC mAb, other idiotypic determinants (e.g., Idl-3a) are expressed to a varying degree by...
most anti-GAC mAb (12). Unlike IdX and IdI-1, IdI-3a is intimately associated with the antigen-binding site, in that binding of anti-IdI-3a to anti-GAC mAb is fully inhibitable by N-acetyl glucosamine (10).

In this paper we show that at least four Vᵦ, one Vₜ, two Vₜᵦ, three Jₜ, one Jₜᵦ, and three Jₜₜ gene segments encode anti-GAC antibodies. Each pattern of IdX, IdI-1, and Id5 public idiotypes identifies antibodies using a single Vᵦ gene segment and does not correlate with expression of particular Vₜ gene segments. Thus, in the murine antibody response to GAC, a multiplicity of V and J gene segments is compatible with anti-GAC binding structures and with the IdI-3a idiotope, but a single Vᵦ gene segment appears to encode each set of public idiotypic determinants.

Materials and Methods

Anti-GAC and idiotype-specific hybridomas were produced as described (12, 13). Isotype and GAC binding were determined by radioimmunoassay as described elsewhere (13).

Genomic DNA was isolated from liver or hybridoma cells, by standard techniques (14). 10 µg of liver DNA or 20 µg of hybridoma DNA was digested for 1.5-3 h with 2-3 U of restriction endonuclease per microgram of DNA, followed by a second digestion with an equal amount of enzyme (New England Biolabs, Beverly, MA; Bethesda Research Laboratories, Gaithersburg, MD; Boehringer Mannheim, Indianapolis, IN; International Biotechnologies, New Haven, CT; or Pharmacia Fine Chemicals, Piscataway, NJ). Digested DNA was subjected to Southern analysis using the procedure described by Maniatis et al. (14).

The 5'Vₛ-25-39 probe is a 536 bp Eco RI-Nsi I fragment or a 420 bp Dra I-Nsi I fragment, both consisting of leader and noncoding sequence from the HGAC 39 rearranged k gene (15 and Fig. 1). The J₄ probe is a Hae III-Sau 3AI fragment (positions 1,995-2,328, 16 and Fig. 1). The J₅ₕ probe is a Hind III-Eco RI fragment, or subfragments, Hind III-Xba I or Xba I–Eco RI (positions 727-2,316, 727-1,639, and 1,639-2,316, respectively; 17 and Fig. 1). J₄ₕ and J₅ₜ probes were subcloned from J₉ₕ and J₉ₜ BALB/c DNA in pBR322; the 5'Vₛ-9 probe, which is identical to the 5' HGAC 9 probe (18 and Fig. 1) was excised from M13mp8. Probes were labeled by nick translation (14) or by random hexamer primer extension (19).

After hybridization, nitrocellulose filters were washed three times in 0.4 X SSC (14) at 68°C and exposed to Kodak XAR-5 film with or without intensifying screens. Nitrocellulose filters were reused after washing at room temperature, twice in 0.25 M NaOH for 15 min, once in 0.1 M Tris-HCl (pH 7.5) for 10 min, and once in 0.01 M Tris-HCl for 10 min. Filters were then soaked in 5X SSC, prehybridized, and hybridized. In all cases, radioactive probe from the previous hybridization was completely removed by this washing protocol.

Results

At Least Four Vₛ and One Vₜ Gene Segments Encode Anti-GAC Antibodies. Preliminary experiments suggested that the GAC-specific hybridomas HGAC 39 and HGAC 47 rearranged distinct Vₛ gene segments. The rearranged Vₛ gene segments from these two cell lines and their germline counterparts were cloned and sequenced (15), confirming that HGAC 39 and HGAC 47 rearranged distinct, albeit related, Vₛ gene segments. These Vₛ gene segments are closely related to the Vₛ24B gene segment of Joho et al. (20) and to the Vₛ25 proteins

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reported by Herbst et al. (20a). Because all these sequences belong to the \textit{V}_25 group of Potter et al. (20b and L. A. D’Hoostelaere, personal communication), we name them \textit{V}_9-25-39 and \textit{V}_9-25-47. We examined other anti-GAC hybridoma cell lines using a \textit{J}_5 probe, which hybridizes to the germline \textit{J}_5 region and to all rearranged \textit{V}_\textit{J} \textit{J}_5 gene segments. These results suggested that HGAC 8, 39, 47, and 91 have each rearranged distinct \textit{V}_\textit{J} gene segments. For example, Bgl II digestion produced three \textit{J}_5-hybridizing bands for each hybridoma: two are shared by the SP2/0 fusion partner and one is a rearranged band specific to each hybridoma (Fig. 2). HGAC 8, 39, 47, 91, showed specific rearranged bands of 14, 5.0, 2.9, and 3.9 kb, respectively (Fig. 2, Table I). Each of the four hybridomas differed from the others following at least two restriction endonuclease digestions (Table I), consistent with use of four distinct \textit{V}_\textit{J} gene segments. Furthermore, the 5’\textit{V}_9-25-39 probe hybridized to the rearranged band in both HGAC 39 and HGAC 47 (which are \texttext ~92\% homologous in this probe region\texttext), but not to a rearranged band in HGAC 8 or HGAC 91 (Fig. 2). In addition, some anti-GAC mAb contain \text\lambda light chains (13). Thus, at least five distinct light chain \textit{V} gene segments appear to encode anti-GAC antibodies.

We analyzed \textit{V} \textit{J} gene segments used by 25 additional anti-GAC hybridomas. In this analysis we have adopted the following assumptions: \textit{(a)} When a hybridoma displayed two non-SP2/0 rearranged bands, the rearranged band similar to those of other anti-GAC hybridomas was expressed and the other dissimilar band was nonproductively rearranged. \textit{V} and \textit{J} gene segments often rearrange nonproductively in B cells (21, 22). \textit{(b)} When rearranged bands from two
hybridomas differed by a small consistent amount with several different restriction endonucleases, the same V gene segment was paired with different J gene segments. Use of different J gene segments by the same V can result in discrete size shifts of up to 1.3 kb. (c) When rearranged bands from two hybridomas migrate similarly with all restriction endonucleases except one, the change is due to somatic mutation. Somatic mutation occurs frequently in the vicinity of rearranged V gene segments (23).

The results of the Southern analysis of V\(_k\) gene segments permitted the
## Table I

**Southern Blot Analysis of Specific J,5-hybridizing Bands**

| Hybridomas (by V, group) | Relative mobility of bands after digestion of DNA with: |  |
|--------------------------|-------------------------------------------------------|---|
|                           | BglII | Hind III | Hind III and EcoRI | SstI | XbaI |
|                           | kb    |          |                    |      |      |
| **V,25-39**              |       |          |                    |      |      |
| HGAC 39                   | 5.0   | 3.5      | 3.4                | 2.6  | 6.4  |
| HGAC 34                   | 5.0   | 3.5      | 3.4                | 2.6  | ND   |
| HGAC 54                   | 5.0/1.3 | 3.5/17   | 3.4/8.0            | 2.6/2.3 | 6.4/9.5 |
| HGAC 58                   | 5.0   | 3.5      | 3.4                | 2.6  | 6.4  |
| HGAC 89                   | 4.4/5.0 | 3.0/3.5  | 2.6/3.2            | 2.0/1.1 | 5.6/4.9/3.9 |
| **V,25-47**              |       |          |                    |      |      |
| HGAC 47                   | 2.9   | ~11      | 2.9                | 2.1  | 0.6  |
| HGAC 44                   | 2.9   | ~11      | 2.9                | 2.1  | ND   |
| HGAC 50                   | 2.9   | ~11      | 2.9                | 2.1  | ND   |
| HGAC 92                   | 4.0   | ~11      | 4.0                | 3.2  | 1.5  |
| HGAC 103                  | 4.0   | ~11      | 4.0                | 3.2  | ND   |
| HGAC 125                  | 3.6   | ~11      | 3.6                | 2.7  | 1.5  |
| HGAC 128                  | 3.6   | ~11      | 3.6                | 2.7  | ND   |
| HGAC 129                  | ND    | ~11      | 3.6                | 2.7  | ND   |
| IdXP-1                    | 3.9/1.5 | ~11/7.4  | 3.9/7.9            | 3.1/3.8 | ND   |
| IdXP-2                    | 3.5   | ~11      | 3.6                | 2.8  | ND   |
| **V,24A-8**              |       |          |                    |      |      |
| HGAC 8                    | ~14   | ~11      | 8.2                | 1.7  |      |
| HGAC 9                    | ~14   | ~11      | 8.2                | 1.7  | ND   |
| HGAC 11                   | ~14/2.9 | ~11/5.8  | 8.2                | 1.7/2.6 | ND   |
| HGAC 35                   | ~14/2.2 | ~11/3.9  | 8.2/3.5            | 1.7/3.3 | ND   |
| HGAC 55                   | ~14/3.2 | ~11/3.0  | 8.2                | 1.7/3.0 | ND   |
| HGAC 57                   | ~17/3.1 | ~11/5.8  | 8.2                | 1.7/2.7 | ND   |
| HGAC 62                   | ~14   | ~11      | 8.9                | 2.4  | 3.9  |
| HGAC 85                   | ~14   | ~11      | 8.2                | 1.7  | ND   |
| HGAC 86                   | ~14   | ~11      | 8.2                | 1.7  | ND   |
| HGAC 96                   | ~14/~11 | ~11  | 8.2/2.5           | 1.7/3.2 | ND   |
| HGAC 99                   | ~14/3.3 | ~11/5.0  | 8.2/4.8            | 1.7/6.9 | 3.2  |
| Id5P-8                    | ~14   | ~11      | 9.2                | 2.7  | 1.25 |
| Id5P-11                   | ~14   | ~11      | 9.2                | 1.7  |      |
| Id5P-13                   | ~14   | ~11      | 9.2                | 2.7  | 1.25 |
| Id5P-24                   | ~14/2.9 | ~11/3.5  | 9.2/3.6            | 2.7/4.2 | 1.25/2.9 |
| Id5P-40                   | ~14/1.6 | ~11  | 9.2                | 2.7/6.2 | 1.25/1.5 |
| **V,2-91**               |       |          |                    |      |      |
| HGAC 91                   | 3.9   | 5.9      | 3.9                | 7.7  | 8.1  |
| HGAC 93                   | 3.9   | 5.9      | 3.9/2.0            | 7.7  | 8.1  |
| HGAC 98                   | 3.9   | 5.9      | 3.9                | 7.7  | ND   |
| HGAC 101                  | 3.9   | 5.9      | 3.9                | 7.7  | ND   |
| HGAC 102                  | 3.9   | 5.9      | 3.9                | 7.7  | ND   |

When more than one J,5-hybridizing band appears in addition to germline- and SP2/0-derived bands, both are listed. When one of these bands migrates similarly to bands in other hybridomas, it is listed first. Low relative mobilities can be estimated only approximately, denoted by tildes.

* Denotes a rearranged band that hybridized to the 5'V,-25-39 probe for each member of the group; in all cases it matched the specific J,5-hybridizing band from that hybridoma.
clustering of the anti-GAC panel into four groups (Table I). One group of five hybridomas showed rearranged J5-hybridizing bands similar to HGAC 39 that also hybridized to the 5' V5-25-39 probe, consistent with use of V5-25-39. A second group of eight hybridomas gave hybridization patterns similar to that of HGAC 47 with both the J5 and the 5' V5-25-39 probes and likely use V5-25-47. 11 hybridomas displayed rearranged J5-hybridizing bands similar to HGAC 8, which failed to hybridize to the 5' V5-25-39 probe. Because one member of this group, HGAC 9, shows NH3-terminal amino acid homology to the deduced amino acid sequence of V24A (18, 20, and data not shown), we label the gene segment rearranged by this group of hybridomas V5-24A-8. These sequences are also homologous to the 7S34.1 protein designated V27 by Chang et al. (23a). Five hybridomas displayed rearranged J5-hybridizing bands, which comigrated with the specific band of HGAC 91 and which did not hybridize to the 5' V5-25-39 probe, consistent with the use of the same V5 gene segment. Because these sequences are homologous to the V52 group (20b and N. J. Phillips, personal communication), we name them V5-2-91.

The simplest interpretation of the data presented thus far is that four V5 gene segments encode anti-GAC antibodies in these 29 hybridomas. Other interpretations of the data would increase the number of V5 gene segments that are used in the anti-GAC response. In addition, small differences in size of the rearranged bands within the groups suggest that at least three J5 segments are used by these antibodies.

At Least Two V5 Gene Segments Encode Anti-GAC Antibodies. Perlmutter et al. (18) cloned and sequenced the rearranged VH gene segment from HGAC 9 and demonstrated that at least two V5 gene segments were used by nine anti-GAC hybridomas. To extend this observation we examined 28 anti-GAC hybridomas with Jn4 and 5' V5p-9 probes. The Jn4 probe detects the germline Jn region and all productively rearranged Jn regions. The anti-GAC hybridomas fell into two groups, the HGAC 9 and HGAC 39 groups (Fig. 3 and Table II). Eight hybridomas displayed hybridization patterns like HGAC 9, and likely have rearranged V5-9. Five additional hybridomas displayed hybridization patterns similar to HGAC 9 after all digestions except Eco RI and Eco RI plus Bgl I. These reflect allelic differences surrounding the V5p-9 locus, because each subgroup is derived from strains that differ at the Igh-1 locus (24). Therefore 13 hybridomas appear to have rearranged V5p-9. Because HGAC 9 has rearranged V5p-9 to Jn2, we can deduce from the Mr of rearranged bands that other hybridomas have paired V5p-9 with Jn1, Jn2, Jn3, and Jn4 (Table II).

15 anti-GAC hybridomas had Jn4 and 5' V5p-9 hybridization patterns similar to HGAC 39, but different from HGAC 9 (Fig. 3 and Table II); these hybridomas thus appear to have rearranged the same V5n, which we label V5n-39. Small, consistent differences in the size of restriction endonuclease fragments suggest that this group of hybridomas has paired V5n-39 with three different Jn gene segments.

V5 Gene Segment Correlates with k Chain-associated Public Idiotype. Id5, IdX, and Idl-1 public idiotypic determinants are expressed by free k chains (10, 11, and data not shown). Table III shows that all IdX+, Idl-1+, anti-GAC mAb are encoded by V5-25-39; all IdX+, Idl-1-, anti-GAC mAb are encoded by V5-25-47;
Anti-GAC antibodies are encoded by two germline $V_h$ gene segments. Shown are Southern blots using Pvu II, with the $J_h$ (Hind III-Xba I) probe. Arrowheads denote the location of germline or SP2/0 hybridizing bands. Brackets indicate the location of rearranged $V_h$ gene segments. Molecular mass standard is $^{32}$P-labeled Hind III fragments of lambda DNA. HGAC 8, 9, 34, 35, 53, 57, 58, 62, 96, 125, 128, and 129 have specific bands that migrate similarly to the germline $J_h$ band (4.9 kb). However, because all of these hybridomas have lost their A/J, C57BL/6, or C.B20 germline $J_h$ regions, as seen on all other digests, the 4.9 kb $J_h$-hybridizing bands seen here cannot be ascribed to germline $J_h$ DNA. Unmarked lanes represent irrelevant hybridoma DNA.

and all Id5$^+$ anti-GAC mAb are encoded by $V_{\nu}$-24A-8. The perfect correlation between idiotypic pattern and $V_{\nu}$ gene segment is broken only by HGAC 85, which has rearranged $V_{\nu}$-24A-8, but which secretes an Id5$^-$ anti-GAC antibody. We suspect that somatic mutation destroyed the Id5 idiotypic determinant in HGAC 85; single amino acid substitutions can destroy idiotypes without changing hapten-binding affinity (25). The IdX$^-$, IdI-1$, Id5$^-$ idiotypes are associated with secretion of a $\lambda$ light chain (HGAC 43), and with the $V_{\nu}$-2-91 gene segment. Finally, there is no correlation of any of these determinants with $V_{\nu}$ gene segment. For example, the $V_{\nu}$-39 gene segment encodes anti-GAC antibody with each of the four idiotypic patterns.

The correlation between idiotype and $V_{\nu}$ gene segments extends to immunoglobulins that do not bind GAC. We injected A/J mice with pokeweed mitogen, fused their spleen cells with SP2/0, and screened the resulting hybridomas for idiotype-positive Ig. Two independent hybridomas, IdXP-1 and IdXP-2, secrete IdX$^+$, Id1-1$, Id5$^-$ products that do not bind GAC; IdXP-1 secretes a complete
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### Table II

**Southern Blot Analysis of Specific JH4-hybridizing Bands**

| Hybridomas (by Vh group) | Relative mobility of bands after digestion of DNA with: |  |
|-------------------------|--------------------------------------------------------|---|
|                         | Pvu II | Sst I | Xba I | Eco RI | Eco RI | Bgl I | Dra I |
| **Vh-39**               |        |      |      |        |        |        |      |
| HGAC 39                 | 7.2    | 5.1  | 2.9  | 7.1    | 7.1    | 2.2    |      |
| HGAC 43                 | 7.2    | 5.1  | 2.9  | 7.1    | 7.1    | 2.2    |      |
| HGAC 44                 | 6.6/9.2| 4.5  | 2.3/6.1| 6.6/4.0| 6.6/4.0| 1.5/1.7|      |
| HGAC 47                 | 6.6    | 4.5  | 2.3  | 6.6    | 6.6    | 1.5    |      |
| HGAC 50                 | 6.6/9.6| 4.5/3.5| 2.3/6.2| 6.6/7.0| 6.6    | 1.8/2.4|      |
| HGAC 54                 | 7.6    | 5.4  | 5.4  | 7.5    | 7.5    | 2.7    |      |
| HGAC 85                 | 7.2    | 5.1  | 2.9  | 7.1    | 7.1    | 2.2    |      |
| HGAC 86                 | 7.2    | 5.1  | 2.9  | 7.1    | 7.1    | 1.8    |      |
| HGAC 91                 | 6.6    | 4.5  | 2.3  | 6.6    | 6.6    | 1.8    |      |
| HGAC 92                 | 6.6    | 4.5  | 2.3  | 6.6    | 6.6    | 1.5    |      |
| HGAC 93                 | 6.6/8.5| 4.5/3.2| 2.3/3.6| 6.6/4.7| 6.6/4.7| 1.5/1.7|      |
| HGAC 98                 | 6.6    | 4.5  | 2.3  | 6.6    | 6.6    | 1.5    |      |
| HGAC 101                | 6.6    | 4.5  | 2.3  | 6.6    | 6.6    | 1.5    |      |
| HGAC 102                | 6.6/8.4| 4.5/3.2| 2.3/3.6| 6.6/4.7| 6.6/4.7| 1.5/1.7|      |
| HGAC 103                | 6.6/8.4| 4.5/3.2| 2.3/3.6| 6.6/4.7| 6.6/4.7| 1.5/1.7|      |
| **Vh-9**                |        |      |      |        |        |        |      |
| HGAC 8                  | 5.2/2.1| 3.8/6.1| 4.0/2.7| 5.6/3.9| 5.6/3.9| 1.7/3.3|      |
| HGAC 9                  | 5.2/8.7| 3.8/4.4| 4.0/6.0| 5.6/3.6| 5.6/3.6| 1.7/1.8|      |
| HGAC 11                 | 5.2    | 3.8  | 4.0  | 5.6    | 5.6    | 1.7    |      |
| HGAC 35                 | 4.8/8.8| 3.4/4.4| 3.7/1.6| 5.2/4.4| 5.2/4.4| 1.3/1.5|      |
| HGAC 53                 | 5.6    | 4.3  | 4.3  | 5.9    | 5.9    | 2.0    |      |
| HGAC 57                 | 5.2/4.2| 6.1/2.4| 4.0/4.5| 5.6/4.5| 5.6/1.8| 1.8/1.9|      |
| HGAC 58                 | 4.8    | 3.4  | 3.7  | 5.2    | 5.2    | 1.3    |      |
| HGAC 96                 | 4.8    | 3.4  | 3.7  | 5.2    | 5.2    | 1.3    |      |
| HGAC 34†                | 5.2/5.5| 3.8  | 4.0/5.6| 8.6/5.3| 8.6/2.8| 2.0/3.0|      |
| HGAC 62†                | 4.2    | 2.8/4.7| 3.2/2.6| 8.0/5.7| 8.0/2.6| 0.8/1.7|      |
| HGAC 125†               | 4.6    | 3.4  | 3.8  | 8.3    | 8.3    | 1.3    |      |
| HGAC 128†               | 4.6/2.3| 3.4/3.6| 3.8/6.0| 8.3/4.1| 8.3/4.1| 1.3/3.2|      |
| HGAC 129†               | 4.6/25 | 3.4/5.0| 3.8/6.0| 8.3~16 | 8.3/5.3| 1.3/−11|      |
| Id5P-11                 | 4.3    | 3.0  | 3.3  | 4.8    | 4.8    | 0.8    |      |
| Id5P-1.1                | 4.8/1.8| 3.4/3.0| 3.7/5.1| 5.2/5.8| 5.2/5.3| 1.3    |      |
| **Unrelated**           |        |      |      |        |        |        |      |
| IdXP-1                  | 2.2/2.0| 9.5/2.6| 4.2/2.2| 4.8/3.3| 4.8/3.3| 1.5    |      |
| IdXP-2                  | 2.4    | 4.1  | 4.0  | 5.4    | 5.4    | 2.1    |      |
| Id5P-8                  | 1.8/4.4| 9.0/8.7| 1.8/4.4| 5.5/5.7| 5.5/5.7| 1.2/2.6|      |
| Id5P-13                 | 1.8    | 9.0  | 1.8  | 5.5    | 5.5    | 1.2    |      |
| Id5P-24                 | 3.6/1.7| 5.9/4.1| 22/1.9 | 3.1/2.4| 3.1/2.4| 4.2/3.5|      |
| Id5P-40                 | ~14/1.2| 3.9/3.4| 4.5/2.5| 9.2/5.5| 9.2/5.5| 4.0/1.6|      |

When more than one JH4-hybridizing band appeared in addition to germline- and SP2/0-derived bands, both are listed as in Table I. Tildes denote approximate (low) mobilities.

* Denotes a rearranged band that hybridized to the 5' Vh-9 probe for each member of the Vh-9 group; in all cases it matched the specific JH4-hybridizing band from that hybridoma.

† Denotes C.B20 or C57BL/6 origin; all other hybridomas were derived from A/J.
| Hybridoma group | Idl-3a idiotpe expression* | V<sub>s</sub> | V<sub>H</sub> |
|-----------------|---------------------------|-------------|-------------|
| HGAC 39 (IdX<sup>+</sup>, Idl-1*)<sup>2</sup> | +++ | V<sub>s</sub>, 25-39 | 39 |
| HGAC 39 | +++ | V<sub>s</sub>, 25-39 | 39 |
| HGAC 34 | - | 25-39 | 9 |
| HGAC 54 | ++ | 25-39 | 9 |
| HGAC 58 | +++ | 25-39 | 9 |
| HGAC 89 | +++ | 25-39 | ND |
| HGAC 47 (IdX<sup>+</sup>, Idl-1*)<sup>3</sup> | - | V<sub>s</sub>, 25-47 | 39 |
| HGAC 47 | - | V<sub>s</sub>, 25-47 | 39 |
| HGAC 50 | - | 25-47 | 39 |
| HGAC 92 | - | 25-47 | 39 |
| HGAC 103 | - | 25-47 | 39 |
| HGAC 125 | ++ | 25-47 | 9 |
| HGAC 128 | - | 25-47 | 9 |
| HGAC 129 | +++ | 25-47 | 9 |
| IdXP-1 | - | 25-47 | Unrelated |
| IdXP-2 | - | 25-47 | Unrelated |
| HGAC 8 (Id5<sup>+</sup>)<sup>1</sup> | +/- | V<sub>s</sub>, 24A-8 | 9 |
| HGAC 9 | + | 24A-8 | 9 |
| HGAC 11 | ++ | 24A-8 | 9 |
| HGAC 35 | ++ | 24A-8 | 9 |
| HGAC 53 | ++ | 24A-8 | 9 |
| HGAC 57 | - | 24A-8 | 9 |
| HGAC 62 | +++ | 24A-8 | 9 |
| HGAC 86 | ND | 24A-8 | 39 |
| HGAC 96 | +/- | 24A-8 | 9 |
| HGAC 99 | - | 24A-8 | ND |
| Id5P-8 | ND | 24A-8 | Unrelated |
| Id5P-11 | - | 24A-8 | 9 |
| Id5P-13 | - | 24A-8 | Unrelated |
| Id5P-24 | - | 24A-8 | Unrelated |
| Id5P-40 | - | 24A-8 | Unrelated |
| Others | ++ | None-λ | 39 |
| HGAC 43 | ++ | None-λ | 39 |
| HGAC 85 | - | V<sub>s</sub>, 24A-8 | 39 |
| HGAC 91 | - | V<sub>s</sub>, 2-91 | 39 |
| HGAC 93 | ++ | 2-91 | 39 |
| HGAC 98 | ++ | 2-91 | 39 |
| HGAC 101 | +++ | 2-91 | 39 |
| HGAC 102 | ++ | 2-91 | 39 |
| Idl-3aP-1.1 | ++ | Unrelated | 9 |

All HGAC mAb bind GAC antigen; none of Id mAb bind GAC except Id5P-11.

* The level of expression of the Idl-3a idiotpe, as measured in competition radioimmunoassay with HGAC 54 antibody as the labeled proband (12).

† From four independent fusion experiments.

‡ From five independent fusion experiments.

§ From nine independent fusion experiments.
**Germline Diversity, Antigen Binding, and Idiotype**

Ig molecule, whereas IdXP-2 secretes k light chain in the absence of detectable Ig heavy chain (data not shown). IdXP-1 and IdXP-2 had Jk.5 and 5'V,-25-39 hybridization patterns similar to HGAC 47, consistent with rearrangement of V,-25-47 to Jk.1 and Jk.2, respectively (Table I). The 5'V,-9- and Jk.4-hybridizing bands of IdXP-1 and IdXP-2 did not resemble one another or the V,-39 or V,-9 gene segments (Table II). This observation further strengthens the association of the IdX+, IdI-1 - idiotype with the V,-25-47 gene segment, but not with any V,- gene segment.

We also isolated several hybridomas that secrete Id5+ Ig. These hybridomas secrete IgM, IgG2a, IgG2b, and IgG3, in contrast to members of our anti-GAC panel, which only secrete IgM or IgG3. We tested five hybridomas derived from three separate animals that showed distinct Ig IEF patterns (data not shown); three secrete IgG2a (Id5P-8, -13, -24) and two secrete IgG3 (Id5P-11 and -40). Table I shows that rearranged, Jk.5-hybridizing bands of all five resembled V,-24A-8. Only one hybridoma, Id5P-11, secretes a GAC-binding antibody; it displayed 5'V,-9- and Jk.4-hybridizing bands similar to HGAC 9, consistent with V,-9 rearranged to Jk.4. The other four Id5+ hybridomas do not bind GAC and their hybridization patterns did not resemble the V,-39 or V,-9 rearranged gene segments (Table II). This observation strengthens the correlation between the Id5 idiotype and the V,-24A-8 gene segment.

*The IdI-3a Idiotope Is Associated with Several V Gene Segments.* The IdI-3a idiotope is expressed to a varying degree by most anti-GAC mAb (12) and is intimately associated with the GAC binding site (10). Not surprisingly, we found no correlation between IdI-3a and either V,- or V, (Table III). IdI-3a was associated with all possible V gene segments, including V,. HGAC 128 and 129 rearranged the same V,-, Jk,-, V,-, and Jk,- gene segments, yet expressed totally different levels of IdI-3a (Table III). Further, IdI-3aP-1.1, an IdI-3a+, non-GAC-binding hybridoma, had a light chain hybridization pattern unlike any of the anti-GAC hybridomas (Table I). In contrast, IdI-3aP-1.1 had a V,-9- and Jk.4-hybridizing pattern similar to HGAC 9 (Table III). Thus, expression of IdI-3a is compatible with extensive diversity of V and J gene segments.

**Discussion**

*Multiple V, and V, Gene Segments Encode Murine Anti-GAC Antibodies.* A hallmark of anti-GAC antibodies is extensive structural diversity. Comparison of the IEF profiles of 17 IgG3a mAb (11 of which are included in the present study) showed no duplicate spectrotypes (13). Indeed, based on IEF patterns, Briles and Carroll (26) estimated that the A/J mouse strain can produce ~200 distinct clonotypes in response to GAC. Our results provide at least a partial explanation for this extensive diversity. 30 GAC-specific hybridoma cell lines rearranged four V,-, one V,,-, two V,-, three Jk,-, one Jk,-, and four Jk,- gene segments. This is a minimal estimate because highly homologous gene segments may share the same restriction endonuclease patterns (27). Also, we have interpreted isolated differences with single restriction endonucleases as being due to somatic mutation, and assumed that the rearranged band that was shared by several other hybridomas was always the expressed gene segment. Interestingly, there appears to be no restriction in the pairing of V,- and V,- gene segments (Fig. 4 and Table
FIGURE 4. The pairing of \( V_\mu \) and \( V_\lambda \) gene segments observed in anti-GAC hybridomas and their relationship to IdX, IdI-1, and Id5 public idiotypic determinants.

III). Thus, all five \( V_\lambda \) gene segments are paired with \( V_\mu-39 \), and three of the five are paired with \( V_\mu-9 \). Assuming that combinatorial joining of GAC-specific antibody \( V \) and \( J \) gene segments occurs randomly, and that combinatorial association of GAC-specific heavy and light chains is similarly promiscuous, at least 104 distinct germline antibody species contribute to the murine GAC-specific repertoire. Thus, much of the molecular heterogeneity in this antibody response as defined by Briles and Carroll can be explained without recourse to somatic hypermutation or D gene segments.

Although the \( V \) region gene segments of anti-GAC antibodies are distinct, some are clearly related. Rearranged \( V_\mu-39 \) (HGAC 39) and \( V_\mu-9 \) (HGAC 9) gene segments share 57 of 59 NH\(_2\)-terminal amino acids (18). The germline \( V_\nu-25-39 \) and \( V_\nu-25-47 \) nucleotide sequences are 95% homologous.\(^2\) The NH\(_2\)-terminal 74 amino acids of \( V_\nu-24A-8 \) rearranged by HGAC 9 are 86% homologous to the \( V_\nu-25-39 \) of HGAC 39 (18). In contrast, \( V_\nu-2-91 \) rearranged by HGAC 91 and HGAC 101 is only 75% homologous to \( V_\nu-25-39 \) in nucleotide sequence encoding mature protein (N. J. Phillips, personal communication).\(^2\) Moreover, both germline \( V_\lambda \) gene segments are <50% homologous to \( V_\nu-25-39 \) (data not shown). Thus, the two \( V_\nu \) gene segments are very homologous, and three out of five of the \( V_\lambda \) gene segments are homologous. Antibodies to other naturally occurring carbohydrate haptens such as \( C \) polysaccharide (28), \( \alpha(1 \rightarrow 3) \) dextran (29), and \( \beta(1 \rightarrow 6) \) galactan (30–32) are encoded by few, relatively homologous \( V \) gene segments. In contrast, antibodies to synthetic haptens such as 2-phenyl-5-oxazolone (33–35) and azophenylarsonate (36–38) are encoded by a relatively large number of nonhomologous \( V \) gene segments. The dichotomy between naturally occurring and synthetic haptens is consistent with the hypothesis that bacterial pathogens provide selective pressure to maintain germline \( V \) gene segments that encode antibody specific for naturally occurring haptens (28, 39). The availability of a germline-encoded close fit may allow one or a few germline \( V \) gene segments to dominate the immune response to naturally occurring haptens. An alternative hypothesis is that there are a limited number of antibody sequences that can effectively compete with water to bind hydrophilic, naturally occurring carbohydrate haptens, whereas a large number of antibody sequences may effectively bind hydrophobic synthetic haptens.

**Molecular Basis of Idiotype in Anti-GAC Antibodies.** It has been proposed that public idiotypic determinants may be directly encoded by germline elements (8, 40). Our data on IdX, IdI-1, and Id5, which are expressed by free \( \kappa \) chains, are clearly consistent with this hypothesis. \( V_\nu-25-39 \) encodes IdX\(^*\), IdI-1\(^*\) Ig, \( V_\nu-25-\)
47 encodes IdX+, IdI-1 Ig, and V,-24A-8 encodes Id5+ Ig, apparently without the requirement for particular J μ, V μ, or J n gene segments (Tables I-III and Fig. 4), or for somatic mutation. The IdX and IdI-1 idiotypic determinants are encoded by rearranged V, gene segments identical in sequence to the germline V,,-25-47 and V,,-25-39 (N. J. Phillips, unpublished data).

In contrast to IdX and IdI-1, the IdI-3a idiotypic is intimately associated with the N-acetyl glucosamine-binding site (10). In addition, anti-GAC mAb express IdX or IdI-1 in an all-or-nothing fashion but express IdI-3a to varying degrees (12). As might be predicted, IdI-3a expression did not correlate with any V or J gene segment (Table III). However, because all IdI-3a+ Igs are encoded by the closely related V,,-39 and V,,-9 gene segments, IdI-3a expression may require these gene segments with additional contributions provided by D regions, junctional sequences, or somatic hypermutation. More non-GAC-binding, IdI-3a+ hybridomas will have to be analyzed to answer this question.

Selective Expression of Individual GAC-specific Antibody Clones. Finally, we note that diversity of GAC-specific antibody gene segments makes the phenomenon of clonal dominance more complex (41). Although the murine anti-GAC repertoire includes hundreds of chemically distinct species with >100 potentially accountable by germline V and J gene segments, individual mice typically express one, or at most a few antibody species distinguishable by IEF analysis. With a germline-encoded repertoire of perhaps 10^8 distinct antibodies, any individual clone is likely represented only once in the preimmune murine B cell population.

GAC-specific antibodies, by virtue of their germline diversity, may well be represented in 100 or more preimmune B cells. Perhaps somatic hypermutation is responsible for the dominance of unique B cell clones that produce antibodies with high affinity for GAC. However, the continued maintenance of clonal dominance in animals exposed to GAC may require more than simple competition for antigen and might additionally reflect biases in rearrangement frequencies (42) or regulatory effects of an underlying immune network (43). The availability of V, and V, probes for anti-GAC antibodies will permit the analysis of the physiology of clonal dominance in molecular detail.

Summary

Monoclonal antibodies (mAb) to streptococcal group A carbohydrate (GAC) are encoded by a minimum of two V μ, four J μ, four V α, three J α, one V λ, and one J λ gene segments. The IdX, IdI-1, and Id5 idiotypic determinants are expressed by anti-GAC mAb and are found on free κ chains. Each pattern of these determinants is encoded by a distinct V, gene segment, apparently without the requirement for a particular J μ, V μ, or J n gene segment, or somatic mutation. In contrast, the binding site-associated idiotypic determinant IdI-3a does not correlate with any single V or J gene segment.

We thank Dr. Nancy Jean Phillips and Dr. L. A. D’Hoostelaere for sharing unpublished data. We acknowledge the expert technical assistance of Mr. Alexander Shaffer and Mrs. Kris Sterbenz and the cheerful secretarial services of Mrs. Janice Cole. Finally, we thank Drs. Weissman, Gershenfeld, and Joho for their help in generating probes.

Received for publication 8 September 1986.
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