Evidence for Control of Synthesis of the Variable Regions of the Heavy Chains of Immunoglobulins G and M by the Same Gene*

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Abstract. Previous work indicated that the light chains of a monotypic immunoglobulins G2-K and M-K from a single patient (Tii) are identical. Our present data show that the monotypic immunoglobulins G and M share idiotypic determinants not present in their isolated light chains or in any of a large number of other immunoglobulins tested, and that amino acid sequences of the first 27 residues from the NH₂-terminal end of the γ- and μ-chains are identical. These results support the hypothesis that at least two genes control the synthesis of each heavy and light chain and suggest that the monotypic immunoglobulin G and monotypic immunoglobulin M of this patient share three of the four genes involved. It is proposed that, during normal immunoglobulin synthesis, different cells of a single clone synthesize immunoglobulins M and G, and that the light chains and the variable segments of the heavy chains of the proteins of the two classes are identical within the clone. A genetic switching mechanism is suggested.

A patient (Tii) with multiple myeloma, having two serum paraproteins (IgG2-K and IgM-K), was recently investigated by Wang et al. The light chains of the two monotypic proteins appeared identical by the following criteria: amino acid composition, peptide maps, electrophoretic mobility in starch gel containing urea at pH 3 and pH 8, optical rotary dispersion, and circular dichroism measurements. We have now compared these two monotypic proteins in terms of "idiotypic"² antigenic determinants (i.e., determinants specific for a given myeloma protein³) and amino-terminal amino acid sequences.

Materials and Methods. The monotypic IgG and IgM used for sequence analysis were isolated as previously described. For use in immunization the IgG was isolated by precipitation with sodium sulfate, passage through DEAE-cellulose in 0.04 M phosphate, pH 6.9, and further fractionation in an NaCl concentration gradient on carboxymethyl cellulose, pH 6.7. IgM was eluted from the DEAE-cellulose with 0.04 M phosphate containing 0.5 M NaCl and further purified by gel filtration on Sephadex G-200. The proteins were characterized by immunoelectrophoresis and by their capacity to react specifically in agar gel with monospecific antisera to pooled IgG or IgM. The sedimentation coefficients (S₂₀,w) of the isolated IgG and IgM were 6.6 and 15.1, respectively, at a concentration of approximately 7 mg/ml.
Antisera against T11 IgG and T11 IgM were prepared in rabbits by inoculations of 3–5 mg of either protein in complete Freund’s adjuvant, followed by periodic intravenous inoculations. Antisera of high titer from individual rabbits were pooled separately and absorbed sequentially with nonspecific human IgG and IgM by precipitation at equivalence; excess antigen was then added to the supernatant. The absorbed antisera will be designated anti-G( abs) and anti-M( abs).

F'(ab')2 fragments were prepared from monotypic T11 IgG by treatment with 2% by weight of pepsin at pH 4.3,4 followed by gel filtration. Fab fragments were prepared from T11 IgM by the method of Goodman and Inman.5 Fab fragments of T11 IgG were made by digestion with papain for 4 hr at pH 7, then were purified by passage through DEAE-cellulose in 0.01 M phosphate, pH 8.0, and gel filtration. Purity of the F'(ab')2 and Fab was shown by immunoelectrophoresis. Proteins were labeled with 125I (less than 1 atom per molecule) by the method of McFarlane,7 utilizing ICl.

Quantitation of proteins reactive with antiidiotypic antisera: Percentages of T11 proteins reactive with absorbed antisera were determined by indirect precipitation. 125I-labeled F'(ab')2 fragments from T11 IgG or 125I-Fab fragments from T11 IgM were mixed with a 20-fold excess of nonspecific IgG or a 40-fold excess of nonspecific IgM, respectively, and allowed to react at 37°C with excess anti-G( abs) or anti-M( abs). After 1 hr, excess goat antiserum to rabbit fragment Fc was added. The percentage of labeled fragments precipitated was determined by measurement of the radioactivities of each redissolved, washed precipitate, and of the combined supernatant and washes. A typical test contained 0.5 μg 125I-F'(ab')2, 50 μg bovine serum albumin, 6 μl anti-G( abs) or anti-M( abs), and 0.3–0.4 ml goat anti-rabbit Fc. Various unlabeled substances (see Table 1), to be tested as inhibitors, were mixed with the labeled fragments prior to addition of anti-G( abs) or anti-M( abs). Controls were run with rabbit antiovalbumin serum replacing the anti-G or anti-M; less than 1.5% of the 125I was precipitated in each case. Heavy and light chains were isolated from IgG or IgM by the method of Fleischman et al.8 Recombination of heavy and light chains was performed as described elsewhere,9 utilizing approximately a 1.5 to 1 molar ratio of light and heavy chains.

NH2-terminal residues of heavy chains were determined by the cyanate procedure of Stark.10,11 Amino acid sequences were determined by the automatic Edman degradation procedure,12 using a Beckman peptide sequencer. After each cycle of degradation, the cleaved residue was analyzed by gas chromatography,13 amino acid analysis, thin-layer chromatography,14 or a combination of two methods.

Results. Analyses of specificity were first performed by double diffusion in agar gel (Ouchterlony method). The anti-G( abs) gave a single line against T11 serum which showed identity with both T11 IgG and T11 IgM. The anti-M( abs) gave two lines with T11 serum; both antigenic components were present in the IgM but only one in the IgG. The anti-G( abs) failed to form any lines with a large variety of other sera tested (listed in Table 1). In several instances faint lines were obtained with the anti-M( abs) reacting with sera having elevated IgM concentrations. These must represent weak cross-reactions or unrelated reactions, since these sera failed to react with anti-G( abs) or anti-M( abs) in the quantitative tests described below.

By the indirect precipitation method an excess (5 μl) of the anti-G( abs) or anti-M( abs) reacted with 91–93% of the 125I-(Fab')2 fragments (0.5 μg) of the T11 IgG, and with 80 and 85%, respectively, of the 125I-Fab fragments of T11 IgM.

Table 1 shows the effects of various unlabeled competitors on the binding of 0.5 μg of 125I-F'(ab')2 fragments of IgG by anti-G( abs) or anti-M( abs). In each system, 100 μg of T11 IgG displaced essentially all of the labeled reference fragments; the same weight of T11 IgM displaced 79% from anti-G( abs) and 90%
from anti-M(abs). In contrast, 150 μg of unlabeled nonspecific human IgG or IgM or 1200 μg of the IgG had no significant effect. Two other purified IgG2 immunoglobulins, belonging to the same subclass as Ti1 IgG (Table 1), also produced no inhibition.

Whole Ti1 serum was very effective as a competitor. Ten μl gave complete inhibition and 0.2 μl partial inhibition in both systems. Ten μl of a large variety of other sera from patients with elevated serum levels of monotypic IgG, IgM, or IgA were ineffective (Table 1).

Similar results were obtained with the anti-M(abs) or anti-G(abs) reacting with 

<table>
<thead>
<tr>
<th>Patient</th>
<th>Competitors</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti1</td>
<td>IgG2-K</td>
<td>20 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgG2-K</td>
<td>100 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgM-K</td>
<td>20 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgM-K</td>
<td>100 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>Light chains</td>
<td>100 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>Aut.-recomb.</td>
<td>50 μg</td>
</tr>
<tr>
<td>C1a/Ti1</td>
<td>Het.-recomb.</td>
<td>50 μg</td>
</tr>
<tr>
<td>Wat</td>
<td>IgG2-K</td>
<td>250 μg</td>
</tr>
<tr>
<td>Win</td>
<td>IgG2-K</td>
<td>250 μg</td>
</tr>
<tr>
<td>Pooled human</td>
<td>IgG</td>
<td>150 μg</td>
</tr>
<tr>
<td>Pooled human</td>
<td>IgG</td>
<td>1200 μg</td>
</tr>
<tr>
<td>Pooled human</td>
<td>IgM</td>
<td>150 μg</td>
</tr>
<tr>
<td>Ti1</td>
<td>Whole serum</td>
<td>10 μl</td>
</tr>
<tr>
<td>Ti1</td>
<td>Whole serum</td>
<td>0.2 μl</td>
</tr>
<tr>
<td>Pooled human</td>
<td>Whole serum</td>
<td>10 μl</td>
</tr>
<tr>
<td>Thirty-two patients</td>
<td>sera§</td>
<td>10 μl each**</td>
</tr>
</tbody>
</table>

Test mixtures contained 0.5 μg 125I-F(ab')2 fragments and 5 μl of anti-G(abs) or anti-M(abs). Experiments were obtained by the method of indirect precipitation.

** Containing a minimum of 300 μg of immunoglobulin.

## Table 1. Inhibition of the reaction of 125I-F(ab')2 fragments (0.5 μg) of Ti1 IgG with excess anti-G(abs)* or anti-M(abs)*.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Competitors</th>
<th>Quantity</th>
<th>125I-F(ab')2 Bound (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti1</td>
<td>IgG2-K</td>
<td>20 μg</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgG2-K</td>
<td>100 μg</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgM-K</td>
<td>20 μg</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
<td>IgM-K</td>
<td>100 μg</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
<td>Light chains</td>
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<td>By anti-G(abs)</td>
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</tr>
<tr>
<td>Pooled human</td>
<td>IgM</td>
<td>150 μg</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
<td>Whole serum</td>
<td>10 μl</td>
<td>By anti-G(abs)</td>
</tr>
<tr>
<td>Ti1</td>
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<td>0.2 μl</td>
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<td>Thirty-two patients</td>
<td>sera§</td>
<td>10 μl each**</td>
<td>By anti-G(abs)</td>
</tr>
</tbody>
</table>

Additional remarks:

- Excess light chains were added to each serum and used as a negative control.
- The number and class of the sera were: 12 IgG, 6 IgA, and 14 IgM. Values given represent the range of results for the entire panel.
- The panel of sera tested contained monotypic proteins of both light chain types.
- The method of indirect precipitation was used to determine the inhibition.

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** Notes:

* Antisera prepared against Ti1 IgG or Ti1 IgM and absorbed with excess nonspecific IgG and IgM. Experiments were obtained by the method of indirect precipitation.

† Recombinant of light and heavy chains of Ti1 IgG (see text).

‡ Recombinant of Ti1 light chains with heavy chains of monotypic IgM of patient C1a.

§ The panel of sera tested contained monotypic proteins of both light chain types.

** Containing a minimum of 300 μg of immunoglobulin.
Data on sequence analysis: By the cyanate procedure, 84 nmoles of glutamic acid were obtained from 100 nmoles of Ti1 IgM \(\mu\) chain, and 89 nmoles of glutamic acid were obtained from 100 nmoles of Ti1 IgG \(\gamma\)-chain. Thus, both heavy chains have an unblocked glutamic acid as the NH\(_2\)-terminal residue.

The NH\(_2\)-terminal sequences of Ti1 IgM and Ti1 IgG heavy chains, as determined with a Beckman peptide sequencer, are shown in Figure 1. The first 27 amino acid residues are identical in the two proteins. (More extensive structural studies will be performed in collaboration with Dr. William Dreyer.) Preliminary sequence data indicate that the Ti1 IgG light chain and Ti1 IgM light chain are identical up to the first 20 residues from their NH\(_2\)-terminal ends.\(^{16}\)

Discussion. The results on shared idiotypic determinants and identity of amino acid sequence of the first 27 residues at the NH\(_2\)-terminus of the Ti1 \(\gamma\)- and \(\mu\)-chains strongly suggest that most or all of the variable regions of the two chains are identical. (This region comprises approximately 118 residues.\(^{16}\)) Previous studies had already indicated identity of the light chains of monotypic Ti1 IgG and IgM.\(^{1}\)

Ti1 IgG and IgM share idiotypic determinants not detectable by quantitative tests in any of a large number of heterologous proteins and sera tested, although a few weak cross reactions were observed by the Ouchterlonly test with anti-M(abs). The IgM possessed nearly all idiotypic determinants of the IgG, recognized by anti-G(abs), but the converse was not true; Ti1 IgG displaced a maximum of 45\% of the Fab fragments of Ti1 IgM from anti-M(abs). This does not prove nonidentity of the variable regions of the heavy chains (V\(H\)), however, since factors such as steric hindrance, or a shared contribution of parts of the constant and variable regions to certain idiotypic determinants, could account for the observed idiotypic differences between Ti1 IgG and IgM.

Despite the identity of light chains in Ti1 IgG and IgM, isolated light chains failed to react with anti-idiotypic antiserum by Ouchterlonly or competitive binding tests. This is in contrast to the reaction of isolated light chains with antisera to whole immunoglobulins\(^{17, 18}\) and suggests that the heavy chain may contribute to idiotypic determinants. Since it is highly probable that \(V\) regions largely control idiotypic determinants, this argues for a common structure in the \(V_H\) regions of Ti1 \(\gamma\)- and \(\mu\)-chains. In support of this, a recombinant of Ti1 light chains with \(\mu\)-chains of a heterologous macroglobulin failed to react with anti-idiotypic antibody, whereas activity was largely restored when the light chains were recombined with \(\gamma\)-chains of Ti1 IgG (Table 1). Experiments with other heterologous recombinants will provide a further test of this hypothesis.

Strong additional evidence that the same \(V_H\) gene contributes to Ti1 \(\gamma\)- and \(\mu\)-chains is provided by the NH\(_2\)-terminal sequences of the two proteins, which are identical up to residue 27 (Fig. 1). Another important resemblance is the presence of an unblocked glutamic acid residue (rather than pyrrolidone carboxylic acid) at the NH\(_2\)-terminus of each protein. It seems highly unlikely that this identity of sequences is coincidental since the \(\gamma\)- and \(\mu\)-chains of other monotypic proteins have shown multiple differences among the first 20 residues.\(^{16, 19–21}\)

Thus, the combined evidence on shared idiotypic determinants and sequences
strongly suggests that the same $V_H$ gene contributes to the IgG and IgM. That the light chains of Ti1 IgG and IgM are identical has already been indicated by several criteria.\textsuperscript{1, 15}

\begin{align*}
1 & \quad 5 & \quad 10 & \quad 15 & \quad * \\
\text{Glu-Val-Gln-Leu-Leu-Glu-Ser-Gly-Gly-Leu-Val-Gln-Pro-Gly-Gly-Ser-Leu-Arg-Leu-Ser-} \\
\text{Cys-Ala-Ala-Ser-Gly-Phe-}
\end{align*}

Fig. 1.—Amino-terminal amino acid sequence of Ti1 IgG and Ti1 IgM heavy chains. Number 1 (Glu) is the NH$_2$-terminal residue.

* There is a possibility that residue 17 is threonine rather than serine in both Ti1 IgG and IgM.

On the basis of these results, and those of other investigators described below, we propose that the observations made with the serum of patient Ti1 reflect the events occurring during normal immunoglobulin synthesis; i.e., that different cells of a single normal clone synthesize IgG and IgM and that the light chains and the variable segments of the heavy chains of the two classes are identical within the clone. A corollary is that the active combining sites of the IgG and IgM produced within a clone may be identical. Evidence supporting this hypothesis may be summarized as follows.

That two genes may contribute to the synthesis of a single polypeptide chain in a normal immunoglobulin was proposed by Dreyer and Bennett\textsuperscript{22} to account for the variable and invariant regions of the light chain.\textsuperscript{23} They suggested the presence of multiple germ-line genes controlling the $V_L$ regions, a single gene for the $C_L$ (constant or invariant) region of a light chain, and the fusion of the $C_L$ gene with one of the $V_L$ genes at the DNA level. The concept that two genes control a single polypeptide chain was supported by Milstein,\textsuperscript{24} Dreyer et al.,\textsuperscript{25} and Hood and Ein,\textsuperscript{26} who demonstrated that different subtypes of the $V$ region of light chains share the same $C$ regions.

Todd,\textsuperscript{27} and Todd and Inman\textsuperscript{28} suggested that the same $V_H$ gene may contribute to the synthesis of heavy chains of IgG, IgM, and IgA on the basis of the presence of similar allotypic determinants on the heavy chains of each class of immunoglobulins. They questioned why divergence of allotypes through mutation had not occurred during evolution after the divergence into classes and suggested, as a solution to this dilemma, that the same $V_H$ genes contribute to each class. These and other arguments supporting this concept were reviewed and extended by Lennox and Cohn.\textsuperscript{29} The similarity in sequences in the $V_H$ regions of a macroglobulin and certain IgG1 myeloma proteins\textsuperscript{30} is also consistent with this hypothesis. The finding by Koshland\textsuperscript{31} and Wilkinson\textsuperscript{32} that amino acid differences characterizing heavy chain allotypes in the rabbit are largely localized to the NH$_2$-terminal 34 residues (i.e., the $V_H$ region) provided strong experimental support for these arguments. More recently, Koshland et al.\textsuperscript{33} have shown that the same differences in average amino acid composition characterize allotypes a1 and a3 in both rabbit IgG and IgM, thus providing additional evidence that the same $V_H$ genes contribute to both classes.

It seems highly probable, from the apparent identity of their light chains and $V_H$ regions, that Ti1 IgG and IgM are products of cells belonging to the same
clone, i.e., derived from the same precursor cell. That IgG and IgM are synthesized by cells of a single normal clone was indicated first by the data of Nossal et al., who observed synthesis of both classes by a single isolated cell 4 days after immunization. Sterzl also has presented data suggesting a genetic shift from IgM to IgG production during the early phase of the immune response. Perhaps the most convincing evidence that cells of the same clone synthesize IgG and IgM is that of Oudin and Michel, who showed that anti-Salmonella antibodies of the IgG and IgM classes from an individual rabbit share idiotypic determinants. These data support the concept that the chemical relationship between Til IgG and IgM also exists in normal immunoglobulins.

There is less direct evidence for our hypothesis that the identity of light chains of Til IgG and IgM has its normal counterpart. The argument based on allotype is not applicable, since all classes share the same types of light chains. Some support comes from the experiments of Roholt et al., who found that the light chains of an antibody of the IgG class recombine with μ-chains of antibody of the same specificity, and from the same rabbit, to give a product with a somewhat higher degree of specific activity than a recombinant of μ-chains with non-specific light chains.

Til IgG and IgM have been shown by immunofluorescence, using antisera rendered specific for Fc determinants, to be synthesized in different plasma cells. It seems possible that a clone of cells originally synthesized only Til IgM and that a switching mechanism took place, in one or more of these cells, which suppressed the CH, gene and simultaneously derepressed the CH, gene. Such an event may well occur in normal immunoglobulin synthesis. Our data suggest that this switching mechanism does not affect the expression of the VH gene.

Thus, IgG and IgM appear to be under the control of at least four genes: VL, CL, VH, and CH. Our data indicate that the same VL, CL, and VH genes, but different CH genes, control the synthesis of Til IgG and IgM, and suggest that the same genetic mechanisms may apply in normal immunoglobulin synthesis. This does not preclude the possible participation of more than two genes in the control of a single heavy chain. It also seems possible that additional classes of immunoglobulin (e.g., IgA) may be produced by cells of a single clone and that analogous principles may apply in their biosynthesis.

It is of additional interest that the two Til proteins define a third class of variable region genes in immunoglobulin heavy chains. (This and other variable region gene products can combine with constant regions of either IgM, IgA, or of the various subclasses of IgG. Thus the genetic mechanism regulating synthesis of the heavy chains of immunoglobulins differ from those regulating immunoglobulin light chains.) A feature unique to heavy chain variable regions of the Til class is an unblocked amino terminal as first shown for some IgA proteins by Wang, Goodman, and Fudenberg.

Abbreviations: The nomenclatures for immunoglobins, their fragments, and chains are standard abbreviations recommended by the World Health Organization; Bull. World Health Organ., 30, 447 (1964); and Bull. World Health Organ., 33, 721 (1965).
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§ Recipient of a postdoctoral fellowship of the National Institutes of Health.


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