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Immunology

Antibody differentiation: Apparent sequence identity between variable regions shared by IgA and IgG immunoglobulins

(automatic sequenator/differentiation/variable-constant joining mechanisms/categories of biclonal immunoglobulins)

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ABSTRACT **We have analyzed a pair of human myeloma immunoglobulins (biclonal proteins) of the IgG and IgA classes from a single patient, GR. The light chains are identical in amino-acid sequence over 40 residues at their NH_2-terminus, whereas the heavy chains are identical throughout 45 residues of their NH_2-terminus. Additional chemical and serological studies suggest the light chains and variable regions of the heavy chain (V_H) are very similar, if not identical. The implications of these and of other published studies are discussed with regard to (*i*) the association of one V_H region with multiple constant regions of the heavy chain (C_H regions), (*ii*) two alternative types of V-C joining mechanisms, (*iii*) the differentiation of antibody-producing cells, and (*iv*) three categories of biclonal immunoglobulins.**

Structural and organizational features of antibody molecules and genes have provided fascinating insights into the molecular nature of differentiation as it must occur in antibody-producing cells (B-cells) (1, 2). The antibody molecule is comprised of two types of polypeptides, light (L) and heavy (H) chains. All antibody polypeptides can be divided into an NH_2-terminal variable (V) region and a COOH-terminal constant (C) region. The amino-acid sequence of the C region of the heavy chain (C_H region) determines the class of the immunoglobulin molecule (e.g., IgM, IgG, IgA, IgD, or IgE). Comparative analyses of the variable regions of light and heavy chains demonstrate three and four segments, respectively, of extreme amino-acid sequence variability, termed hypervariable regions. Five of these hypervariable regions fold to comprise the walls of the antigen-binding crevice. Three families of genes code for antibody molecules, two for light chains (λ and κ) and a third for heavy chains. The variable and constant regions in each family are coded by distinct genes. Thus, each immunoglobulin chain appears to be coded by two distinct genes (a variable and a constant gene) which are probably joined at the DNA level during the differentiation of each antibody-producing cell. Accordingly, four decisions must be made with respect to the antibody genes during differentiation; that is, individual V_L, V_H, C_L, and C_H genes must be expressed.

Antibody-producing cells, like other eukaryotic cells, have a discrete pathway of differentiation. In the mammalian fetus, stem cells for hematopoietic (blood) cells appear first in the yolk sac and then migrate to the liver and the bone marrow as some differentiate into lymphocytes (3, 4). As the animal matures, lymphocytes migrate to the peripheral lymphoid organs (spleen and lymph nodes) where they undergo their final differentiation steps upon contact with antigen. This process produces plasma cells which are specialized to produce thousands of antibody molecules per second and memory cells which are responsible for the enhanced secondary immune response.

In molecular terms three generalizations are commonly accepted about the differentiation of antibody-producing cells (see ref. 5). (*i*) All the antibody molecules on a particular lymphocyte have the same specificity (i.e., same V_L and V_H regions). (*ii*) Early in the differentiation cycle of lymphocytes the antibody receptor is an IgM molecule. Somewhat later IgM and IgD molecules, both potential receptors, may be expressed on the cell surface. (*iii*) Finally, individual lymphocytes may "switch" from the synthesis of IgM (and IgD) to IgG, IgA, or IgE.

Rare cancerous or neoplastic transformations can apparently occur at any stage in the differentiation of antibody-producing cells (2). Chronic lymphocytic leukemia and Burkitt's lymphoma involve B lymphocytes at an early stage of differentiation. The macroglobulinemia of Waldenström represents an accumulation of cells in transition from B lymphocytes to mature antibody-producing cells. Multiple myeloma results in the neoplastic proliferation of a single type of mature plasma cell and, indeed, is the source of homogeneous myeloma immunoglobulins, the structural analysis of which has led to many of the generalizations outlined above.

A special form of multiple myeloma is beginning to provide new insights into the differentiation process of B cells. Occasionally, individuals with multiple myeloma synthesize large quantities of two distinct myeloma immunoglobulins. Serological and chemical studies on the best-studied example of biclonal myeloma proteins from the human patient, Til, suggest that the light chains and V_H regions in the IgM and IgG myeloma proteins are very similar, if not identical (6–9). These molecular observations, together with those from a variety of cellular studies, suggest that a B lymphocyte initially has IgM receptors and that, in the course of differentiation, subclones arise which synthesize IgG molecules with precisely the same specificity. That is, the light chain expressed in this clone of antibody-producing cells remains the same throughout the differentiation process while the V_H gene is expressed first in association with a C_μ gene (IgM) and then in association with a C_γ gene (IgG). Thus, a fundamental aspect of differentiation in B cells is the association of one V_H gene with two or more C_H genes.

Preliminary reports have indicated that IgA and IgG immunoglobulins from patients with biclonal myeloma proteins also have similar light chains and V_H regions (10–12).

Abbreviations: L and H chains, light and heavy chains, respectively; V and C regions, variable and constant regions, respectively; B cell, antibody-producing cell.

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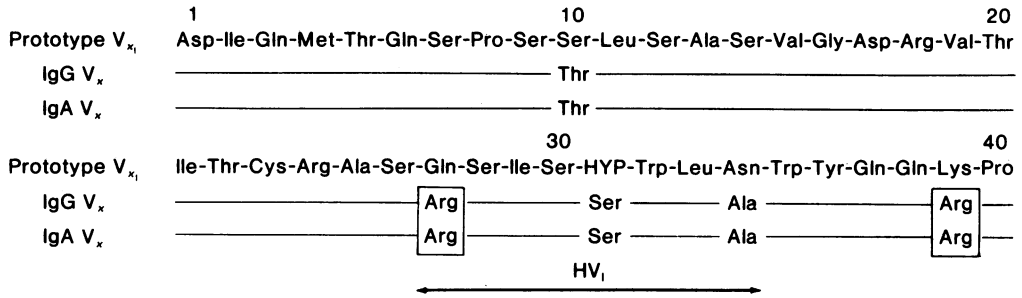

FIG. 1. The NH_2-terminal amino-acid sequences of V_κ regions from biclonal immunoglobulins of the human patient GR. These sequences are compared with a prototype sequence of the human V_κ subgroup I (17). Boxes indicate residues not previously seen in other human V_κ regions. HYP indicates a prototype position with many residue alternatives (*hyp*ervariable). HV_I indicates the extent of the first hypervariable region. The amide-acid distinctions have not been determined.

This study analyzes the amino-acid sequences of the light and heavy chains derived from IgG and IgA immunoglobulins from the biclonal patient, GR, through the first hypervariable regions.

MATERIALS AND METHODS

Techniques for the isolation and chain separation of biclonal immunoglobulins have been described (13). Each chain was subjected to 40–45 steps of automated Edman degradation on Beckman 890A protein sequenator (14). Yields of approximately 50% were noted at step 1, and the repetitive yields averaged 90% for all chains.

RESULTS

Variable Regions of GR Biclonal Immunoglobulins Appear to Be Very Similar and Possibly Identical to One Another. The V_κ regions from the IgA and IgG proteins are identical over their NH_2-terminal 40 residues (Fig. 1). The unique relationship of these light chains is emphasized by the fact they share two residues (i.e., positions 27 and 39) never before seen in more than 80 human V_κ sequences (15, 16) and have identical first hypervariable regions. Furthermore, amino-acid analyses, comparisons of chromatograms in three different systems, electrophoretic mobilities in five different systems, and isoelectric focusing are all consistent with the identity of these two light chains (13). Accordingly, this evidence strongly supports the identity of the light chains from the GR IgA and IgG immunoglobulins.

The V_H regions from the GR biclonal proteins are identical over their NH_2-terminal 45 residues (Fig. 2). The identity of these V_H regions throughout the extremely diverse first hypervariable region, as well as the observation that three residues are shared by these V_H regions that have not previously been seen in more than 25 human V_H regions (i.e., residues 33, 38, and 43), suggest that these V_H regions are uniquely related and very similar if not identical to one another (15, 17, 20). Supporting this supposition is the fact that both the IgA and IgG proteins from GR share their individual antigenic determinants (12).

Caution, however, must be used in attempts to extrapolate V region identity from partial amino-acid sequence data. Examples are known in which heavy chains identical through the first hypervariable region have residue differences at later positions in the variable region (see Fig. 7 in ref. 1). Indeed, the amino-acid sequence data from biclonal immunoglobulins accumulated to date on the presumptive identity of V_H regions are surprisingly meager (11, 18). Even with the immunoglobulins of the most thoroughly studied biclonal patient, Til, the sequences of the first and last hypervariable regions of the γ and μ heavy chains were not completely determined (compare the V_H sequences in refs. 7 and 17), and no sequence data are available for the second hypervariable region. Thus, apart from this study, the sequences of no active-site associated hypervariable regions have been completely determined in the V_H regions of a biclonal pair.

With the reservations above noted, we conclude that similar, if not identical, V_H regions can be associated with biclonal IgA and IgG immunoglobulins containing similar and probably identical light chains.

Our Study and Various Others Are Consistent with the Supposition That a Single V_H Gene Is Associated with Two or More C_H Genes During Differentiation of Antibody-Producing Cells. (*i*) The normal immune response undergoes a maturational process in which the predominant antibody type shifts from IgM to IgG ($C_\mu \rightarrow C_\gamma$) while retaining antigenic specificity. This implies that the same V regions are associated with different immunoglobulin classes. (*ii*) Antisera directed against the variable regions of specific antibodies (i.e., antiidiotype antisera) demonstrate

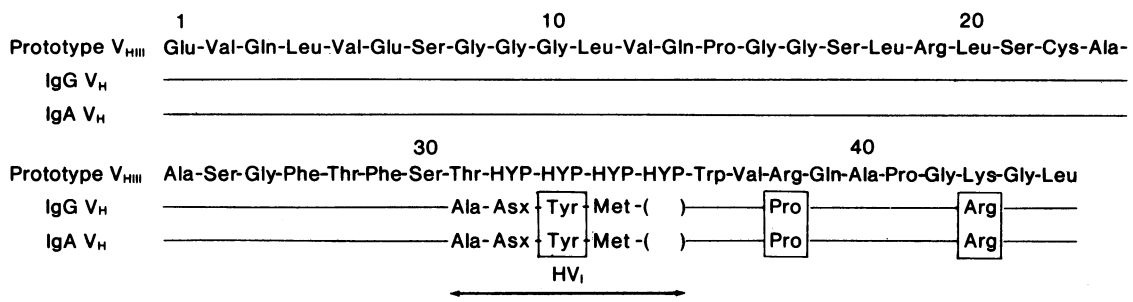

FIG. 2. The NH_2-terminal amino-acid sequences of V_H regions from biclonal immunoglobulins of the human patient GR. These sequences are compared with a prototype sequence of the human V_H subgroup III (20). The amide-acid distinctions have not been determined. Parentheses indicate an unidentified residue. See legend to Fig. 1.

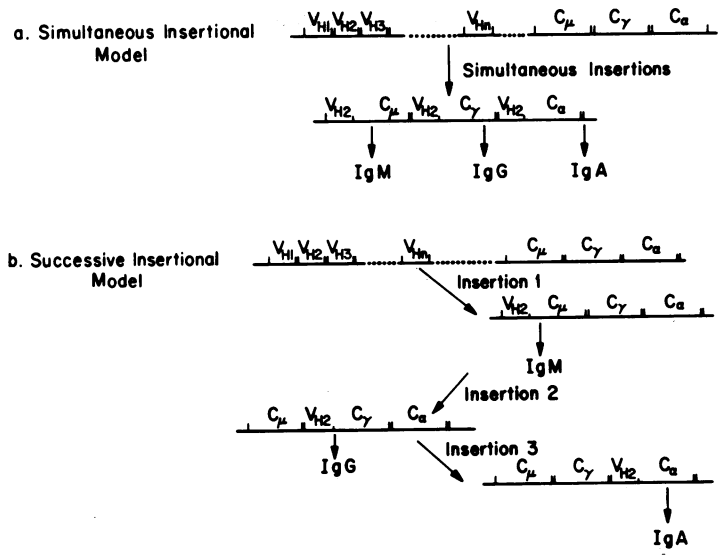

FIG. 3. Two models for the association of a V_H gene to multiple C_H genes. (a) The simultaneous insertional model. (b) The successive insertional model (see *text*).

that the IgM and IgG classes of antibodies in a given immune response may share V region idiotypes (i.e., V sequences) (19). (*iii*) Single lymphocytes may synthesize two or more classes of immunoglobulins. For example, adult and neonatal lymphocytes have both IgM and IgD on their cell surfaces (20–23). The identity of antigenic specificity (24, 25), allotype (26), or idiotype (27) in pairs of immunoglobulins produced by a single lymphocyte once again suggests the same V regions are associated with different C regions. (*iv*) A single lymphocyte may produce daughter cells that synthesize immunoglobulins of differing class while maintaining antigenic specificity and idiotype (28). (*v*) The treatment of lymphoid cells with anti-μ chain antisera early in development inhibits the production of IgG and IgA antibody-forming cells (29–31). This suggests that there is an orderly differentiation process and that IgG and IgA cells are derived from IgM precursors (32–34). (*vi*) Chemical and serological studies have suggested that identical V_H regions can be shared by two myeloma proteins derived from a patient with biclonal myeloma (ref. 7 and this study).

Two controversial questions arise from the association of one V_H region with multiple C_H regions. First, is the association of one V_H gene with multiple C_H genes a differentiation process that is antigen driven? Clearly the simultaneous expression of IgM and IgD on mouse lymphocytes is antigen independent (35). Other studies suggest the "switch" of certain immunoglobulin classes may be antigen driven (30, 33). Thus, certain "switches" are antigen independent whereas others may be antigen dependent. Second, is there a linear pathway of differentiation (e.g., IgM → IgD → IgG → IgA) or may IgM cells differentiate directly to produce any of the other immunoglobulin classes? The suppression of all immunoglobulin classes by the treatment of embryos with anti-μ antiserum suggests that IgM cells are the precursors to all other immunoglobulin-producing cells (29–31). Beyond this, very few conclusions can be drawn (see refs. 30, 32, and 33).

Two General Models May Account for Association of a Single V_H Gene with Two or More C_H Genes. First, the V_H gene may be copied many times over and separate copies be joined to each C_H gene in the heavy chain family (Fig. 3, model a). The differentiation process would then consist of the successive activation of complete V_H-C_H genes by conventional mechanisms of gene regulation (36). We have termed this the *simultaneous insertional model*. Second, a single V_H gene could, as others have postulated (9), "switch" from one C_H gene to a second (or even a third) during the differentiation process. Accordingly, each cell could only transcribe a single V_H-C_H gene at a particular point in time (Fig. 3, model b). We have termed this the *successive insertional model*. Although most immunologists have thought about the V-C joining process primarily in terms of the successive insertional model (9), some recent observations are consistent only with the simultaneous insertional model. The clear demonstration that a single antibody-producing cell can synthesize two or more classes of immunoglobulin with the same V regions over a long period of time (longer than the lifetime of H chain mRNA) would eliminate the successive insertional model because according to this model only a single V_H-C_H gene combination can be transcribed at any point in time. Three observations suggest a single antibody-producing cell can synthesize two immunoglobulin classes simultaneously. First, a patient with chronic lymphocytic leukemia has a monoclonal IgM that exhibits an anti-IgG activity (i.e., rheumatoid activity). This patient's leukemic lymphocytes exhibit IgD and IgM on their surface that can be cocapped with aggregated IgG (37). Accordingly, the specificity of the IgD and IgM molecules appears to be identical. Second, the idiotype of IgM and IgD molecules found on the majority of peripheral B lymphocytes in a patient with chronic lymphocytic leukemia and macroglobulinemia is the same (27). This suggests the variable regions of the two molecules are very similar, if not identical. Finally, a mouse myeloma tumor, TEPC 609, has been cloned in which single cells synthesize IgG2b and IgA molecules. These molecules have the same idiotype (H. C. Morse, III, personal communication). These studies demonstrate that a single cell can synthesize two classes of immunoglobulins over an extended period of time. Furthermore, the V regions produced by each cell are very similar, if not identical, by specificity or idiotype analysis. These data support the simultaneous insertional model. Two types of studies might unequivocally verify this model. First, the demonstration that a single cell can synthesize two or more classes of immunoglobulin with V regions of identical amino-acid sequence would be compelling evidence for the simultaneous insertional model. Second, perhaps nucleic acid hybridization or reassociation experi-

ments in the future may be able to differentiate between one or two V_H genes (successive insertional model) and ten or more V_H genes (simultaneous insertional model).

A variety of specific V-C joining mechanisms have been proposed (see ref. 38). The "copy-splice" model suggests that a given V gene may be copied and spliced onto one or more C genes (39). The "episomal" model suggests that a V gene may be converted into an episome and then reintegrated back into the genome adjacent to one or more C_H genes (40). The "looping out" model suggests that a V gene may be joined to a C gene by deleting as a circle all the intervening DNA. When this same V gene is joined to a new C gene, once again the intervening genetic material is lost as a circle (2, 41). Thus, a "looping out" process would successively join a V gene with different C genes. Finally, the "lateral gene duplication" model suggests a branching network of V and C genes which simultaneously places every V_H gene adjacent to every C_H gene (42). Control switches would then determine which V_H-C_H combination was transcribed. The first two mechanisms are compatible with either of the models described in the preceding paragraph, whereas the latter two are only possible with the successive insertional model. Because of the observations discussed in the preceding paragraph, one of the first two mechanisms appears more likely.

In the Biclonal Patient, GR, the Myeloma Process Probably Transformed a Precursor Lymphocyte That Synthesizes or Produces Daughter Cells Synthesizing Different Classes of Immunoglobulin. A number of observations suggest that the biclonal myeloma process generally transforms a single lymphocyte. In this regard the statistics of the myeloma process are interesting. Multiple myeloma occurs spontaneously in man with a frequency of approximately 1/20,000 individuals (43). If the biclonal myelomas were produced by two independent transformations, about 1/20,000 patients with multiple myeloma should exhibit two myeloma proteins. However, in extensive case study, about 1% of the individuals with multiple myeloma expressed two distinct immunoglobulins (44). The relative abundance of biclonal myeloma proteins is most easily explained by postulating that in about 1% of the myeloma cases, a precursor cell is transformed that expresses two immunoglobulins or is capable of differentiating into daughter clones that express different classes of immunoglobulins. This supposition is also consistent with the observation that the light chains and V_H regions in the few well-studied biclonal examples appear to be similar, if not identical (ref. 7 and this study).

At Least Three General Categories of Biclonal Proteins Exist, Each with Different Genetic and Developmental Implications. One category is the multiclonal IgM proteins produced by Waldenström's macroglobulinemia at a surprisingly high frequency (about 10%) (45, 46). The C regions of these immunoglobulins appear to be identical, whereas the V regions for one chain (light or heavy) are different while those for the second chain are similar. This category of biclonal proteins is consistent with the supposition that a single clone of cells selects a particular combination of V_H and V_L genes to be expressed as an IgM molecule. Subsequently, a subclone may be produced that expresses the same V_L (or V_H) region while shifting to the expression of a new V_H (or V_L) region. The new V region expressed might result from somatic mutation or the expression of new germ line genes. In the two best studied cases, the presence of multiple amino-acid differences renders somatic mutation less likely as an explanation (ref. 46; C. Sledge and L. Hood, unpublished data). Accordingly, early in the differentiation of antibody-producing cells, a diversity of antibody receptors (clones) may be generated by the combinatorial association of one V_L region with multiple V_H regions (or vice versa). Whether the readout of this information is programmed or random is an issue of enormous importance to developmental biology. Unfortunately, very little amino-acid sequence analysis has been carried out on proteins of this category (46).

In the second category, the light chains and V_H regions of both proteins appear identical, whereas the C_H regions are different. Biclonal proteins of this class, such as the GR immunoglobulins described in this paper, suggest that during the normal development of an antibody-producing cell the V_H gene is joined with many C_H genes which allows the corresponding cell or its clonal descendents to express two (or more) classes of immunoglobulins (refs. 9, 11, 22, and this study). Since it appears generally that two distinct clones produce these proteins (44), it is postulated that each clone represents a distinct stage of this differentiation process. Accordingly, these pairs of proteins may shed insight into the order and nature of the molecular switching events.

The third category of biclonal proteins comprises those which include pairs with apparently unrelated chains (e.g., λ and κ chains). This category may result from two independent neoplastic transformations. Little serological or sequence information is available on proteins of this category.

The different categories of biclonal proteins may also represent different stages in the development of antibody-producing cells. Perhaps the first category reflects the process of diversity generation in precursor IgM cells, whereas the second category reflects the subsequent differentiation of an IgM cell with a particular V_H-V_L combination into daughter cells capable of expressing the same active site (V_H-V_L) in association with other immunoglobulin classes. Too little information is available on proteins of the third category to speculate on their relationship, if any, to the differentiation process in antibody-producing cells.

As indicated above, the biclonal proteins may provide important insights into the process of antibody differentiation. However, before detailed conclusions can be reached, a large number of biclonal pairs must be studied with regard to three vital questions. (*i*) Into which of the three general categories do the biclonal proteins fall? Each category has very different developmental implications. (*ii*) Are the V regions in the appropriate chain (category 1) or chains (category 2) actually identical? If even a few substitutions are found, the significance of these proteins could change according to one's view of antibody diversity. (*iii*) Are the biclonal immunoglobulins produced by a single clone or by two clones of cells? This has, for example, important implications for the mechanism of V_H-C_H joining.

Biclonal immunoglobulin proteins have recently been detected among the myeloma tumor products of two inbred strains of mice, BALB/c and NZB (H. C. Morse, III, personal communication; M. Weigert, personal communication). These systems will offer many advantages over the human system for analysis of the developmental implications of these molecular curiosities.

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