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Hybridoma Antibody-Producing Switch Variants: A Variant Lacking the CH1 Domain

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Immunoglobulin isotypes are biologically important because of the effector functions mediated by the different heavy chain constant regions. One of the primary roles of antibody molecules is to activate the complement cascade which initiates the inflammatory response and mediates cell lysis. In the mouse, IgM, IgG_3, IgG_{2b}, and IgG_{2a} immunoglobulin isotypes have been classified as complement-fixing antibodies. We are interested in determining whether antibody segmental flexibility, i.e., Fab movement relative to the Fc portion of the molecule, is correlated with the ability to fix complement.

Hybridoma antibodies were used in this study to avoid the inherent problems associated with heterogenous antibody populations. Anti-dansyl (DNS) hybridoma antibodies were selected because DNS bound in the antigen-combining sites provides fluorescence spectral and lifetime properties suited to fluorescence polarization experiments which measure molecular motions in the nanosecond time scale. Previous studies had shown that antibody segmental flexibility occurs in this time scale (5,7).

A comparison of different anti-DNS antibody isotypes would provide the most informative data to correlate segmental flexibility and complement-fixing ability. The problem of heterogeneity of the antigen-combining sites between different anti-DNS antibody isotypes was overcome by selecting rare variant cells from a monoclonally derived hybridoma cell line which have switched the heavy chain constant region of the antibody they produce but retained the same antigen combining site (i.e., V-region). This phenomenon has been described previously. Originally these cells were rescued by soft agar cloning techniques (1); more recently the fluorescence-activated cell sorter (FACS) has been used to select and clone these antibody "switch" variants (2).

OBSERVATIONS AND DISCUSSION

Antibody Switch Variants

Using the FACS, two families of hybridoma anti-DNS antibody-producing cell lines were obtained. The 27-44 family consists of IgG_1, IgG_{2b}, IgG_{2a}, IgE antibodies; the 44-45 family consists of IgG1 and IgG2b antibodies. The lineage of each family is shown in Fig. 1. These variant cells occur at a frequency of 1×10^{-6} to 1×10^{-5}, and were selected with directly fluoresceinated, affinity-purified rabbit and goat anti-mouse isotype antisera and directly fluoresceinated hybridoma anti-mouse allotype antibodies (3). Once selected and cloned, these variant cell lines stably retain their new phenotype. Each variant cell was selected from its immediate predecessor as shown in Fig. 1. The IgG_1 revertant cell line, designated 27-4F5, is shown to be different from the parental 27-44 cell line by DNA analyses of the immunoglobulin gene composition of each cell line using Southern blot techniques (data not shown).

The fluorescence emission spectra of DNS bound inside the combining sites of each switch variant antibody was used to characterize individual antigen-combining sites. DNS fluorescence is enhanced and shifted to shorter wavelengths when in a hydrophobic environment. Previously we had shown that independently derived anti-DNS hybridoma antibodies had unique DNS fluorescence spectra. Figure 2 shows the spectra obtained with the two parental IgG_1 anti-DNS antibodies. Each switch variant antibody had a fluorescence emission spectrum identical to the IgG_1 antibody produced by its parental cell line. This indicates that the environment of the antigen-combining site, e.g., the antigen-binding contact amino acid residues in the combining site, is identical in each antibody of the variant families. Moreover, two-dimensional gel electrophoresis patterns of the antibodies produced by each

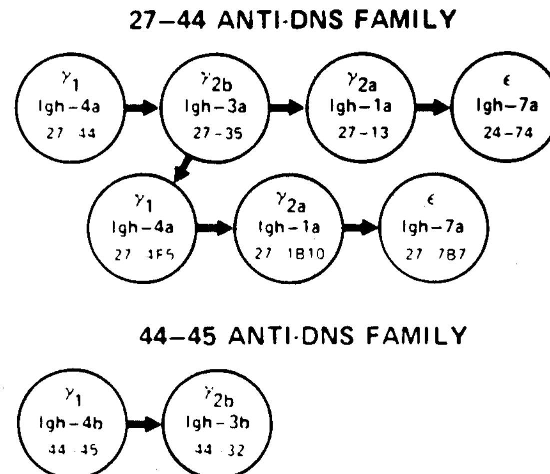

FIG. 1. Lineages of the 27-44 and 44-45 anti-DNS antibody-producing variant cell families.

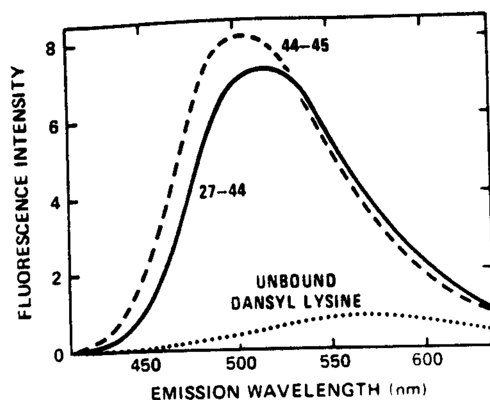

FIG. 2. Fluorescence emission spectra of unbound DNS and DNS bound by 27-44 and 44-45 anti-DNS antibodies.

switch variant of the 27-44 family have identical light chains (Fig. 3). The same is true of the 44-45 family (data not shown). Therefore, the antibodies produced by the family of switch variants shown in Fig. 1 represent different immunoglobulin isotypes sharing identical antigen-combining sites. Any differences in the segmental flexibility and complement-fixing ability between these antibodies should be due to the heavy chain isotype of the molecule.

The two-dimensional gel patterns of the heavy and light chains of the switch variant proteins appear normal (Fig. 3 and data not shown), except for 27-1B10 (see below). The glycosylation pattern of each heavy chain was characterized as normal by comparing the glycosylated immunoglobulin heavy chain with heavy chains that had been synthesized in the presence of tunicamycin, an inhibitor of asparagine-targeted glycosylation (6). The nonglycosylated heavy chains of each switch variant were of the expected apparent molecular weight (data not shown).

An unusual antibody switch variant is 27-1B10 (Figs. 1 and 3). The IgG_{2a} isotype of this antibody was determined by reactivity with a library of hybridoma anti-allotype antibodies (3); however, the apparent mass of the heavy chain of this antibody is 10,000 daltons smaller than that of a normal IgG_{2a} heavy chain (compare Panel A and F in Fig. 3). This short IgG_{2a} heavy chain is glycosylated normally and binds protein A from *Staphylococcus aureus*, indicating that the CH2 domain of the antibody is probably intact. The DNS emission spectra indicate that it has a combining site identical to the other antibodies of this variant family, indicating the V-region is intact. Figure 3 shows that this molecule has an intact light chain. However, there are free light chains and a heavy chain dimer present when this molecule is analyzed by nonreducing polyacrylamide gel electrophoresis. The presence of a heavy chain dimer indicates that the hinge region disulfide bridges are intact. Free light chains indicate that the interchain (H-L) disulfide bonds are absent. This suggests that certain cysteine residues in the CH1 domain of the heavy chain are absent. The size of the short heavy chain suggests that an entire domain is missing from this protein. Analysis of the mRNA produced by this variant cell line also indicates this possibility. We have determined, using domain-specific DNA

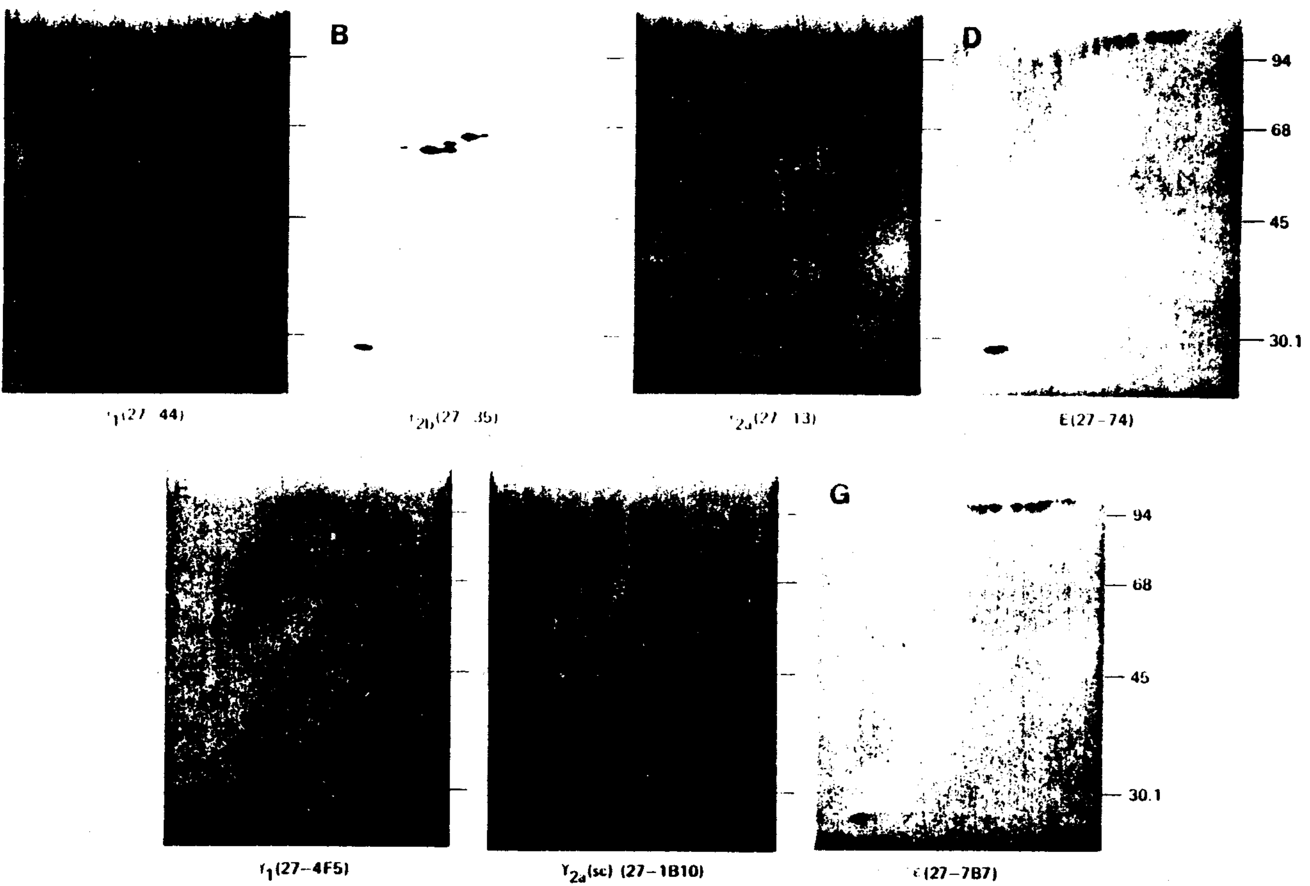

FIG. 3. Autoradiograms of two-dimensional gels of the 27-44 switch variant anti-DNS antibodies.

probes and Northern blot techniques, that most of, if not the entire, CH1 exon of mRNA from 27-1B10 has been deleted. Therefore, it would appear the heavy chain of this antibody molecule is missing the CH1 domain, but retains the hinge region of the molecule. This results in an unusual interaction between the shortened heavy chain and the normal light chain such as not to perturb the antigen-combining site.

Segmental Flexibility

Segmental flexibility in antibody molecules is the movement of the Fab arms relative to the Fc portion of the molecule. The dynamic aspect of antibody structure provides a means by which bivalent binding of both Fab arms is facilitated. The structural correlate of this action is thought to be the hinge region of the molecule. This part of the antibody is visualized as an extended structure (susceptible to proteolytic attack) separating the Fab and Fc portions of the antibody molecule. Although this particular dynamic picture of the antibody molecule has never been proven directly, internal molecular motion is generally accepted as a feature of antibody structure. Theoretical and technical limitations account for the lack of precision in existing models. With fluorescence polarization techniques, molecular motions can be measured. The type and extent of the motion is difficult to ascertain, and the structural correlate of the motion cannot be determined. However, comparison of the degree of motion between molecules can be measured. Since the antibody switch variant proteins are homologous structures sharing identical antigen-combining sites, a direct comparison of the molecular motions of these molecules is very informative.

IgG_1 and IgE molecules are rather rigid molecules, while IgG_{2a} and especially IgG_{2b} molecules are very flexible (4). The order of segmental flexibilities is $IgE < IgG_1 < IgG_{2a} < IgG_{2b}$ (Table 1). The fluorescence polarization measurements do not describe the mode of this motion; therefore, it may be that each antibody isotype has a different flexibility mode. This is highly unlikely, since the structural homology of the different isotypes is large. The dynamic differences

TABLE 1. *Segmental flexibility and complement fixation by the switch variant anti-DNS antibodies*

Cell line	Isotype	Segmental flexibility	Complement fixation
27-44	IgG_1	+ −	+ −
27-4F5	IgG_1	+/−	+ −
27-35	IgG_{2b}	+ + + +	+ + + +
27-13	IgG_{2a}	+ + +	+ + + +
27-1B10	IgG_{2a}[a]	−	+ + + +
27-74	IgE	—	—
27-7B7	IgE	—	—
44-45	IgG_1	+/−	+ −
44-32	IgG_{2b}	+ + + +	+ + + +

[a]The short chain IgG_{2a} switch variant antibody.

FIG. 3. Autoradiograms of two-dimensional gels of the 27-44 switch variant anti-DNS antibodies.

between isotypes, then, should be the degree of motion of the Fab arms relative to the different heavy chain isotypes.

The short chain IgG_{2a} switch variant protein has little, if any, segmental flexibility. This might imply that the hinge region is not the structural correlate of antibody motion, because the short chain protein does have a hinge. Since the short chain molecule is missing the CH1 domain, CH1 could be implicated as the structural correlate for motion. However, these correlations are not meaningful since this particular variant protein has an unusual three-dimensional structure.

Complement Fixation

Complement fixation by the antibody switch variants was measured with a microcomplement consumption assay. The ability to fix complement correlates with the degree of segmental flexibilities exhibited by the variant antibodies (4). The short chain IgG_{2a} molecule proves to be an exception (see below). The more flexible antibody isotypes are able to fix complement more efficiently (see Table 1). Since segmental flexibility of the Fab arms enhances bivalent antigen-binding, this kind of antigen binding could induce conformational changes (allostery) that affect complement binding sites in the Fc portion of the molecule. Another role for segmental flexibility may be that the mechanics of Fab movement undercover or reveal complement binding sites. Multivalent binding also could enhance antigen-antibody patching on target membranes, facilitating Fc-Fc interactions, which mediate complement activation. The present data cannot distinguish which of these molecular mechanisms is involved in activating the complement cascade.

The short chain IgG_{2a} molecule is an exception to the correlation between complement fixation and segmental flexibility. The CH1 deletion in this molecule has rendered it rather rigid, yet this molecule is able to fix complement as efficiently as intact IgG_{2a} molecules. However, the short chain molecule consistently has a low level of constitutive complement-activating activity in the absence of antigen. This suggests that this molecule has complement binding sites in a different conformation than the normal IgG_{2a} molecule. The absence of the CH1 domain may have sterically hindered segmental flexibility and locked the shortend Fab arms of this molecule in a partially active mode. Thus, the absence of segmental flexibility due to the absence of the CH1 domain may be correlated with a constitutive level of complement fixation by the protein in the absence of antigen.

SUMMARY

The comparison of individual anti-DNS antibody isotypes with identical antigen-combining sites has shown that each isotype has different segmental flexibilities. The exact nature of this molecular motion has yet to be determined. Segmental flexibility is correlated with the ability to fix complement. An unusual, short chain IgG_{2a} immunoglobulin variant, lacking the CH1 domain, has no segmental flexibility, but has a constitutive complement-activating activity. Dynamic aspects of antibody structure may play an important role in the biological effector functions

of antibody molecules. The next step in dissecting antibody function requires exploring techniques to construct novel immunoglobulin molecules with directed changes in protein structure.

REFERENCES

1. Coffino, P., and Scharff, M. D. (1971): Rate of somatic mutation in immunoglobulin production by mouse myeloma cells. *Proc. Natl. Acad. Sci. USA*, 68:219–223.
2. Dangl, J. L., Parks, D. R., Oi, V. T., and Herzenberg, L. A. (1982): Rapid isolation of clones isotype switch variants using fluorescence-activated cell screening. *Cytometry*, 2:395–401.
3. Oi, V. T., and Herzenberg, L. A. (1979): Localization of murine Ig-1b and Ig-1a (IgG_{2a}) allotypic determinants detected with monoclonal antibodies. *Mol. Immunol.*, 16:1005–1017.
4. Oi, V. T., Reidler, J., Dangl, J., Vuong, M., Herzenberg, L. A., and Stryer, L. (1980): Effector function and segmental flexibility of immunoglobulin isotypes containing identical anti-dansyl variable regions. *J. Cell Biol.*, 91:75 (abstr 4002).
5. Reidler, J., Oi, V. T., Carlsen, W., Mihn Vuong, T., Pecht, T., Herzenberg, L. A., and Stryer, L. (1982): Rotational dynamics of monoclonal antidansyl immunoglobulins. *J. Mol. Biol.*, 158:739–746.
6. Tkacz, J. S., and Lampen, L. O. (1975): Tunicamycin inhibition of polyisoprenyl *N*-acetylglucosamyl pyrophosphate formation in calf liver microsomes. *Biochem. Biophys. Res. Commun.*, 65:248–257.
7. Yguerabide, J., Epstein, E. F., and Stryer, L. (1970): Segmental flexibility in an antibody molecule. *J. Mol. Biol.*, 51:573–590.