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Localization of Idiotypic Antigenic Determinants in the Fv Region of Murine Myeloma Protein MOPC-315

(heavy and light chains/variable and constant regions)

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ABSTRACT Rabbit antisera were prepared against the idiotypic determinants of the mouse IgA myeloma protein-315, its purified heavy and light chains, and the Fv fragment comprising the variable region of both heavy and light chains. Agar diffusion demonstrated a line of identity between protein-315 and its Fv fragment against either homologous antiserum. Protein-315 and Fv fragment were labeled with ^{125}I and reacted with their anti-idiotypic antisera. Inhibition studies confirmed that the Fv fragment contained all the idiotypic specificities present in the intact protein. Fv was as effective as protein-315 on a weight basis in inhibiting the reaction between anti-idiotypic antiserum and protein-315. Protein-315 has high affinity for the 2,4-dinitrophenyl group, and such ligands can inhibit the reaction between protein-315 and its anti-idiotypic antibodies. Hapten inhibition was also obtained with the Fv fragment and its anti-idiotypic antiserum, implying that Fv contains an intact combining site. In this system with protein-315 the antigenic determinants expressed as idiotypic specificities are entirely within the variable region and are not influenced in their expression by the constant region. We therefore suggest that, in general, idiotypic determinants are antigenic determinants of the Fv portions of immunoglobulins.

Idiotypic determinants are individually specific antigenic determinants on monoclonal immunoglobulins (1) or populations of specific antibody (2). These determinants are located in the Fab fragment (3–5), which is composed of the N-terminal half of the heavy chain together with the light chain. Studies with rabbit antibenzoate antibodies first clearly established the active antigen-binding site as an important idiotypic determinant (6). This finding was then confirmed with murine IgA myeloma proteins that bind Dnp (7, 8) or phosphoryl choline (9).

The idiotypic determinants of the ligand-binding sites are presumably located within the variable (V)-region, but published studies have all been performed with proteins containing 1, 2, or all 3 constant (C)-region domains (6–8, 10). The anti-idiotypic antibodies for the 2,4-dinitrophenyl (Dnp)-binding IgA myeloma protein MOPC-315 from BALB/c mice produced in rabbits (7) or in other BALB/c mice (8, 11) were all studied with the 7S protein or the Fab′ fragment, both of which contain part of the C-region. A method was recently reported for the preparation of a fragment from MOPC-315 Fab′ by pepsin digestion and affinity chromatography on Dnp-lysine–Sepharose (12, 13).

The fragment with univalent anti-Dnp activity had a molecular weight of 25,000 and sedimentation coefficient of 2.6 S. Amino-acid sequence studies confirmed that the fragment, designated Fv or the variable fragment, was composed of the N-terminal variable portions of both heavy (H) and light (L) chains (V_H and V_L).

The present studies were therefore performed to determine whether the idiotypic determinants are located exclusively in the Fv fragment.

MATERIALS AND METHODS

Protein-315 from BALB/c mice bearing the tumor MOPC-315 was studied in the mildly reduced and carboxamidomethylated 7S form (13). H and L chains were obtained by reduction with 0.2 M 2-mercaptoethanol, alkylation with iodoacetamide, and gel filtration over Sephadex G-100 in 1 M acetic acid (14). Methods for the preparation of Fab′ and Fv fragments from protein 315-7S have been described in detail (12, 13). In summary, the Fv is prepared by digestion of Fab′ with pepsin at 37° for 4 hr at pH 3.7 followed by affinity chromatography on Dnp-lysine–Sepharose and elution with 0.05 M Dnp-glycine. The proteins were labeled with ^{125}I by the iodine monochloride method (15) to yield a product with less than one atom of iodine per protein molecule and with more than 98% of the protein-bound radioactivity precipitable by 5% trichloroacetic acid. In the radio-inhibition precipitin tests (see below), an average of 4000 net cpm was used per supernatant–precipitate pair.

Antisera were prepared in California white female rabbits against 315-7S, 315-H chains, 315-L chains, and Fv fragment. The immunization regime consisted of 200 μg of protein in 1 ml of 0.15 M NaCl emulsified in 1 ml of Freund's complete adjuvant and injected in multiple subcutaneous sites. 7–10 Days later 100 μg of protein in Freund's incomplete adjuvant was injected; subsequent booster injections each 2 weeks were given intravenously (100 μg). A total of about 1 mg of protein was injected into each rabbit. Sera from different bleedings were tested for precipitating antibody by Ouchterlony immunodiffusion and pooled. The antiserum for each protein was absorbed with normal BALB/c serum.

Interactions of ^{125}I-labeled 315 proteins and the rabbit antisera were studied by inhibition of indirect precipitation (7, 8, 11). To each tube (6 × 50 mm) was added (in order) 10 μl of ovalbumin (50 μg) to reduce nonspecific adsorption of

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

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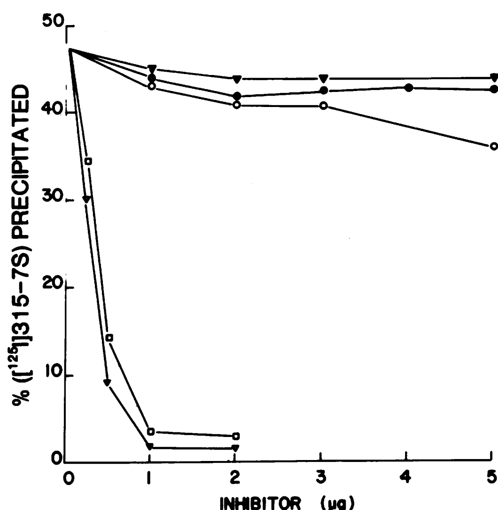

FIG. 1. Patterns of inhibition of binding of ^{125}I-labeled protein 315-7S to rabbit anti-idiotypic antiserum to 315-7S. In each case, the unlabeled inhibitor was added before the anti-idiotypic antiserum. The final reaction volume was 250 μl, and each experiment was performed in duplicate. Inhibitors: □——□, protein 315-7S; ▽——▽, Fv; ○——○, 315-H chain; ●——●, 315-L chain; ▼——▼, protein 460.

radioactivity to the glass tube, 5 μl of normal rabbit serum, and 5 μl of ^{125}I-labeled protein (0.1 μg). If inhibitors were being tested in this particular reaction, they were added at this stage and incubated for 1 hr at 37°. After 5-20 μl of diluted rabbit anti-idiotypic antiserum were added, the reaction mixtures were incubated for 2 hr at 37°. Then 150-300 μl of goat antibody to rabbit Fc fragment were added. After 16 hr at 4° the precipitates were harvested and washed three times with 0.15 M NaCl, and the radioactivities of both supernatant and precipitate were measured in a scintillation counter to determine the percentage of ^{125}I-labeled protein in the precipitate (10). The goat antiserum, generously provided by Dr. A. Nisonoff, Chicago, was absorbed with normal rabbit F(ab')$_2$, mouse IgG, and normal BALB/c serum.

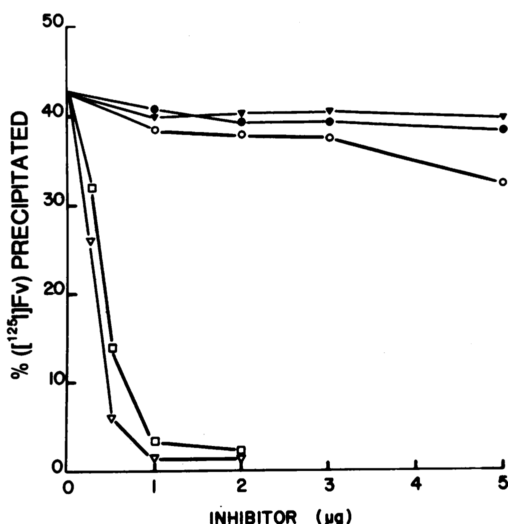

FIG. 2. Patterns of inhibition of binding of ^{125}I-labeled protein 315 Fv fragment to rabbit anti-idiotypic antiserum to Fv. See legend to Fig. 1.

TABLE 1. *The effects of haptens on the reactions between ^{125}I-labeled 315-7S and Fv fragment and their rabbit anti-idiotypic antisera*

^{125}I-labeled antigen (0.1 μg)	Anti-idiotypic antiserum	Hapten (final concentration 3 mM)	^{125}I-labeled antigen in precipitate (% of control)
315-7S	Ra 315-7S	Dnp-L-lysine	24
315-7S	Ra 315-7S	p-aminobenzoate	103
315-7S	Ra Fv	Dnp-L-lysine	30
315-7S	Ra Fv	p-aminobenzoate	108
Fv	Ra 315-7S	Dnp-L-lysine	33
Fv	Ra 315-7S	p-aminobenzoate	98
Fv	Ra Fv	Dnp-L-lysine	26
Fv	Ra Fv	p-aminobenzoate	102

Inhibition with haptens was tested by addition of Dnp-L-lysine or p-aminobenzoate before the anti-idiotypic antiserum. The hapten molar concentration was calculated on the final reaction volume. Control experiments included replacement of rabbit antisera to 315 proteins with rabbit antiserum to bovine-serum albumin to measure nonspecific precipitation of radioactivity. In all quoted results, this figure was less than 6% (average 3%). All experiments were performed in duplicate.

RESULTS

Before absorption of the rabbit antisera (Ra315-7S, RaFv, Ra315-H, and Ra315-L), Ra315-7S formed a spur over RaFv against 315-7S in agar diffusion. After absorption, the antisera formed lines of identity with both 315-7S and Fv fragment. No precipitin lines were formed against normal BALB/c serum or MOPC proteins 460 and 511. The agar precipitin reactions with Ra315-H and Ra315-L and their homologous antigens were weak, especially those with the light chain. No precipitin lines were observed between Ra315-L and the proteins 315-7S, 315-H, and Fv fragment.

The studies with labeled proteins were performed in greatest detail with [^{125}I]315-7S and [^{125}I]Fv. Initial experiments determined the amounts, dilutions, and conditions of antisera to achieve maximal binding of the antigen. Figs. 1 and 2 present the data on the inhibition of these reactions by the proteins. In each case, both 315-7S and the Fv fragment caused almost complete inhibition in higher concentration. Neither protein-460 nor isolated L chains (16) from 315-7S had any significant inhibitory activity at 50-fold weight excess over labeled antigen, while isolated H chains at this concentration caused about 25% inhibition. These experiments were performed with each labeled antigen and its homologous anti-idiotypic antiserum ([^{125}I]315-7S and Ra315-7S). However, a generally similar pattern was obtained with [^{125}I]315-7S and RaFv and with [^{125}I]Fv and Ra315-7S.

The hapten Dnp-L-lysine inhibited reactions between the labeled antigens and the anti-idiotypic antisera over a range of hapten concentrations, whereas p-aminobenzoate did not inhibit the reactions. Table 1 summarizes the data on experiments at 3 mM final hapten concentration, where Dnp-L-lysine produced inhibition. There were no significant differences in hapten inhibition of the reaction between either the Fv fragment or the intact protein-315 and the anti-idiotypic antisera.

DISCUSSION

The present experiments on inhibition of anti-idiotypic antibodies to protein-315 provide direct evidence that the idiotypic determinants of the whole protein are located entirely in the V-region, represented by the Fv fragment. Moreover, the similar inhibition efficiency of Fv and the intact protein indicates that the C-region of protein-315 has no significant effect on the expression of these V-region determinants. Neither the isolated H nor L chains of protein-315 contained the full set of idiotypic determinants, although about 25% inhibition was obtained with H chains at 50-fold excess. In a reported study with protein-315 and an anti-idiotypic antiserum prepared in BALB/c mice, the isolated H and L chains were about 100- and 400-fold less effective in inhibition studies than the intact protein-315 (8). The Fab fragment was only slightly less effective than the whole protein (8). Clearly, the V-regions of both H and L chains are required in association for the full expression of the idiotypic determinants of protein-315. This is a corollary to the fact that the formation of the antibody-combining site also requires the association of the V-regions of both peptide chains. Indeed, the data from the experiments with hapten inhibition of protein-315 and the Fv fragment support conclusions that the ligand-binding site is a major idiotypic determinant (6–9). They also demonstrate that Fv has an intact binding site similar to that of the 7S protein. The use of a limiting antibody concentration is crucial in such experiments since hapten inhibition may not be obtained if the studies are performed in antibody excess (17). Maximum inhibition was in the range of 70%, comparable to results reported from two studies with antisera prepared in rabbits (7) and BALB/c mice (8). The specificity of the hapten inhibition was confirmed by the inability of haptens not bound by protein-315 to inhibit the idiotypic reaction, e.g., *p*-aminobenzoate.

This confirmation of the detection of the idiotypic determinants of the whole protein molcule within the Fv fragment is an important adjunct to studies of the nature of the surface immunoglobulin receptors on lymphocytes in tumor-bearing animals. The surface immunoglobulin may be oriented so that only the Fv region is accessible and capable of combining with anti-idiotypic antibodies of the appropriate specificity. The immunization of normal BALB/c mice with protein-315 is capable of suppressing the development of the tumor MOPC-315 in these animals (18). The sequence is thought to be the elicitation of anti-idiotypic antibodies to protein-315, their combination with the specific tumor immunoglobulin on the lymphocyte membrane (19), and suppression of the tumor cells by an unexplained mechanism. A tumor variant may be produced in those cells that do not bind the protein-315 (20). We suggest that immunization with Fv fragment alone would protect specifically against subsequent inoculation of MOPC-315 tumor cells, and antibodies to allotypes and other antigenic determinants in the C-region would not be formed. The absence of the latter antibodies would be an important asset in studies of idiotype suppression in human malignancies.

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