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Immunology

Demonstration of an idiotypic antigen on a monoclonal cold agglutinin and on its isolated heavy and light chains

(hypervariable regions/gene insertion theory/affinity chromatography)

LESTER KOBZIK, MICHAEL C. BROWN, AND AMIEL G. COOPER

Department of Pathology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111

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ABSTRACT **A potent anti-idiotype serum produced in a rabbit immunized with the isolated heavy chains of an IgM cold agglutinin "Col" was rendered specific by solid-state adsorptions. The anti-Col idiotype was shown to bind specifically to both isolated Col heavy (mu) and light (kappa) chains as well as to intact Col IgM by three methods: (*i*) reversal of anti-idiotype inhibition of Col cold agglutinin in an automated hemagglutination-inhibition assay system; (*ii*) adsorption of the anti-idiotype by affinity gels consisting of Col IgM, mu, or kappa chains covalently coupled to Sepharose 2B; (*iii*) binding of Col IgM and its isolated chains by an anti-idiotype affinity gel. Fragments of Col light chain lacking constant region determinants but still capable of inhibiting anti-idiotype were produced by limited pepsin digestion of the light chains. The finding of shared idiotypic determinants on isolated heavy and light chains of a monoclonal antibody suggests that these chains share a common sequence in a hypervariable region. As an extension of the gene insertion theory of Wu and Kabat, we postulate that genes coding for hypervariable regions may be available for insertion into the DNA for both heavy and light chains.**

Interest in idiotypic determinants as markers of the variable region of immunoglobulins and as probes of the antigen-binding site has been generated by recent studies demonstrating: (*i*) localization of an idiotypic determinant to the variable region (1), (*ii*) hapten inhibition of anti-idiotype reactions (2–4), and (*iii*) a correlation of shared or cross-idiotypy with specificity (5, 6). Most anti-idiotypic sera used in such studies have been produced against the intact antibody. We report here the characterization of an anti-idiotype serum produced by immunization with the heavy chain isolated from a monoclonal IgM kappa cold agglutinin. This anti-idiotype is shown to bind specifically to the intact cold agglutinin and to its separated mu chains and kappa chains. Furthermore, the feasibility of isolating small fragments of the variable region bearing the idiotypic determinant is explored by: (*i*) showing retention of the idiotypic determinant in the variable-region-enriched fraction of a pepsin digest of light chain, and (*ii*) preparation of an anti-idiotype affinity gel that specifically binds the idiotypic cold agglutinin and its separated chains.

MATERIALS AND METHODS

Immunoglobulin Preparations. The principal cold agglutinin used in this study was purified from the serum of a patient "Col" with long-standing chronic cold hemagglutinin disease (7). Purification involved 4° adsorption and 37° elution from red cell stroma followed by Sephadex G-200 filtration (8). The purified Col cold agglutinin is a monoclonal 19S IgM-κ immunoglobulin of a relative anti-i specificity [reacts better with cord than with adult red blood cells (8–10)].

Abbreviations: Col and similar three-letter symbols refer to cold agglutinins from patients.

Four Waldenström IgM proteins (three IgM-κ, one IgM-λ) were purified as described (11). The normal IgG was prepared from a pool of the sera from 50 normal donors by ammonium sulfate precipitation and DEAE-cellulose chromatography (12). IgG from patient Col was similarly purified. The Col IgG showed no hemagglutinating activity and was free of IgM (<0.1%) as judged by an automated assay for mu chains (13).

Isolation of Heavy and Light Chains. Immunoglobulins were reduced by the method of Miller and Metzger (14); chains were separated by gel chromatography on Sephadex G-100 in 1 M acetic acid (15). To radiolabel the heavy and light chains of Col cold agglutinin, iodo[^{14}C]acetamide was used for alkylation. Protein, measured by A_{280} absorbance or by a modified automated Lowry method (16), and radioactivity were monitored throughout.

Antisera. Two rabbits were hyperimmunized with isolated mu heavy chains from purified Col cold agglutinin, twice in complete Freund's adjuvant (footpads), and twice with alum-precipitated heavy chains (subcutaneous). Anti-kappa and anti-mu were prepared as described elsewhere (13).

Preparation of Affinity Gels. Affinity gels were prepared by coupling proteins to Sepharose 2B activated with CNBr by the method of Porath *et al.* (17). Approximately 90% of each respective protein was bound, resulting in approximately 1 mg of coupled protein per ml of packed gel.

Automated Hemagglutination-Inhibition Assay. Anti-idiotypic activity or inhibition of this activity was quantitated by a continuous-flow automated hemagglutination system employing the Technicon AutoAnalyzer (13). This method is based on the ability of small amounts of the anti-idiotype to inhibit specifically and quantitatively hemagglutination of human erythrocytes by Col cold agglutinin at 4°. The percent inhibition of a standard amount (100 ng) of purified Col cold agglutinin by serial dilutions of anti-idiotype was quantitated by comparing the residual hemagglutination with that given by serial dilutions of the Col standard alone.

That dilution of rabbit anti-idiotype serum that gave approximately 80% inhibition of the 100 ng of Col cold agglutinin was used in the reversal of inhibition assay to measure the idiotypic antigen on soluble or affinity-gel-bound proteins. This was measured by pre-incubating the diluted anti-idiotype antibody with the test and control soluble or gel-bound proteins. An aliquot of each solution was then tested for the remaining anti-idiotype activity, using the hemagglutination-inhibition assay. Reduction of inhibition by proteins bearing the idiotypic antigen could be measured accurately and was proportional to the amount of these proteins present in the pre-incubation stage of the assay. Mu and kappa chains were quantitated by analogous hemagglutination-inhibition methods previously described (13).

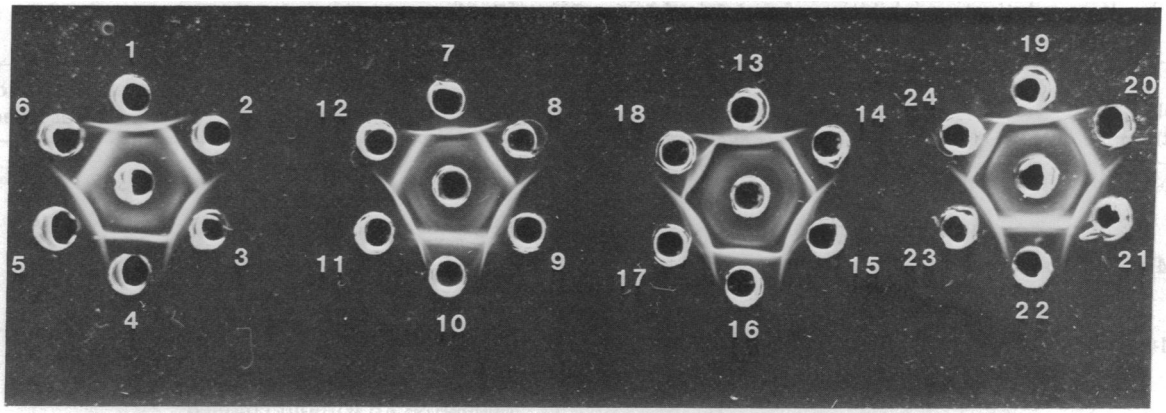

FIG. 1. Demonstration of the anti-idiotypic antibodies in unabsorbed serum of rabbit immunized with the mu chains from purified cold agglutinin Col. Each central well contains the rabbit antiserum. Odd-number peripheral wells (1, 3, 5 . . . 23) contain serum from patient Col; wells 6, 8, 10, 20, and 22 contain Waldenström sera (Ell, Clu, Kin, Oco, Bro); wells 2, 4, 12, 14, 16, and 24 contain other cold agglutinin sera (Dew, She, Ada, Rei, Wor, Sil); well 18 contains normal serum. Not shown is the testing of approximately 20 other cold agglutinin and Waldenström proteins that gave similar results.

Pepsin Digestion. Col light chains at 0.8 mg/ml in 0.025 M sodium acetate buffer (pH 4.5) were digested with pepsin at a substrate-to-enzyme ratio of 175:1 (wt/wt) at 37° (18). Aliquots of the digest were taken at intervals and the digestion was stopped by adding a small amount of saturated Na_3PO_4 solution to raise the pH to 8.2 and by cooling the solutions to 4°.

RESULTS

Preparation of Anti-Idiotype. The presence of anti-idiotypic antibodies in the serum of one of two rabbits immunized with heavy chains from the IgM cold agglutinin of patient Col was first indicated by the marked spurring obtained when this unabsorbed serum was tested by the Ouchterlony immunodiffusion assay against Col serum or purified cold agglutinin and a battery of approximately 30 other cold agglutinins and Waldenström IgM proteins, some of which are shown in Fig. 1. Specific anti-idiotypic serum was produced by successive adsorption of the rabbit serum with affinity gels to which the following proteins were covalently bound: (*a*) pooled purified Waldenström IgM proteins, (*b*) pooled Waldenström isolated mu chains, (*c*) cold-agglutinin Ada (of anti-I specificity, reacts better with adult than cord red blood cells), and (*d*) cold agglutinin Mac (an anti-i). The gel purification of anti-idiotype was monitored at each step by the Ouchterlony immunodiffusion test. The final adsorbed anti-idiotypic serum gave precipitation with only Col cold agglutinin.

Hemagglutination-Inhibition by Anti-Idiotype. The adsorbed anti-idiotype was tested for binding to Col and other cold agglutinins (Table 1), as measured by the effect of the anti-idiotype on their ability to agglutinate red cells in the automated quantitative hemagglutination-inhibition assay (13). Each cold agglutininin was tested at a final dilution giving hemagglutination comparable to that given by 100 ng of Col IgM (9). The anti-idiotype was found to be a potent inhibitor of Col cold agglutinin, 0.1 ml of a 1/1600 dilution of the anti-idiotype causing 50% inhibition of the purified Col (Table 1). Of the other cold agglutinins tested only two others, Mac and Tea, both IgM anti-i antibodies, were partially inhibited by the anti-idiotype, but equivalent inhibition required 50 to 100 times more of the anti-idiotype for these cold agglutinins as compared with Col. Among the other cold agglutinins not appreciably inhibited was Rob, an IgA cold agglutinin which, like Col, has kappa IV light chains (19).

Purification of Heavy and Light Chains. The reduction and alkylation of Col cold agglutinin consistently gave a clean separation of heavy and light chains (Fig. 2). In an alkylation performed with iodo[^{14}C]acetamide, the plot of radioactivity matches closely the protein distribution, giving a ratio of radioactivity in the heavy chain peak to that in the light chain peak of 3.3:1. This ratio is close to the 4:1 expected from selective cleavage of interchain disulfide bonds; moreover, any incomplete separation of heavy and light chains or inclusion of light chains in the mu pool would raise the anticipated ratio rather than lower it. Pools from each peak were analyzed for purity by sodium dodecyl sulfate polyacrylamide electrophoresis (20). A single sharp band for protein and radioactivity was observed for the respective pools in the anticipated positions. Furthermore, using an automated anti-kappa assay, we found only 0.25% contamination of the heavy chain pool by kappa chains. Isolated Col kappa chains were used as standards and gave results comparable to other kappa chains. This assay was capable of accurately measuring nanogram amounts of Col kappa chains in artificial mixtures with 3 μg of Col heavy chains, in addition to being able to detect kappa chains as part of intact IgM.

Binding of Anti-Idiotype to Soluble Immunoglobulins. To attempt to detect and measure the Col idiotypic antigen on various immunoglobulins, cold agglutinins, and isolated heavy and light chains, we used an assay described above based on reversal of the anti-idiotype inhibition of Col IgM cold agglutinin.

The results summarized in Fig. 3 show potent reversal of the hemagglutination-inhibition by the heat-inactivated homologous Col IgM and by the isolated Col mu and kappa chains. In contrast, much larger amounts of other heat-inactivated cold agglutinins and their isolated mu and kappa chains essentially lacked the idiotypic antigen, as did the pool of four Waldenström proteins and their isolated heavy and light chains (Fig. 3). One hundred micrograms of the kappa IV Bence-Jones protein Len (21), provided by Drs. Donald Capra and Alan Solomon, also failed to inhibit the anti-idiotype (data not shown in Fig. 3), whereas this protein gave the expected inhibition of the anti-kappa in our automated anti-kappa assay (13). The idiotypic antigen was absent from the IgG purified from a pool of 50 serum donors

Table 1. Hemagglutination-inhibition of various cold agglutinins by anti-Col-idiotype

Cold agglutinin*	Relative amount anti-idiotype serum†	% Inhibition of cold agglutination
Col (IgM-κ; i > I)	1	50
	2	80
	3.3	100
Mac (IgM-κ; i)	14	0
	26	20
	140	72
Tea (IgM-κ; i)	14	20
	26	37
	146	70
Den (IgM-λ; i)	14	0
Dud (IgM-κ; i)	14	0
Maw (IgM-κ; i > I)	14	0
	26	5
	140	15
Dew (IgM-κ; i, I)	15	0
Ada (IgM-κ; I)	14	0
	26	9
	140	16
Rei (IgM-κ; I)	140	0
Kah (IgM-κ; I)	15	0
Dun (IgM-κ; I)	15	0
Coo (IgM-? κ + λ; I)	140	0
Wor (IgM-? κ + λ; I)	140	0
She (IgM-λ; PR)	140	0
Rob (IgA-κ; PR)	14	0
	140	15

* All of the cold agglutinins were from patients with high titer cold hemagglutinin disease except for Coo and Wor, which were normal low titer cold agglutinins. Class and antigenic specificity for each cold agglutinin are given in parentheses. The specificity of each cold agglutinin was determined by the relative binding to cord and adult red cells and by the inhibition of hemagglutination by a purified i antigen glycoprotein (9). Cold agglutinins reacting with the PR red cell antigen system are destroyed by neuraminidase treatment.

† The amount of anti-idiotype added to each cold agglutinin relative to that amount (0.1 ml of a 1/1600 dilution) of the anti-idiotype that gave 50% inhibition of the homologous Col cold agglutinin.

and was also lacking in an aliquot (14,000 μg of protein) of pooled normal serum (not shown in Fig. 3). In contrast, some idiotypic antigens were present in the IgG purified from Col serum. Separation of this IgG into gamma and light chains showed a small amount of idiotypic antigen on the light chains but none on the gamma chains (Fig. 3).

Binding of Anti-Idiotype to Affinity Gels. To confirm the presence of the idiotypic antigen on the isolated mu and kappa chains of the purified Col cold agglutinin and the absence of the antigen on other purified cold agglutinin and Waldenström IgM proteins, affinity gels were made by coupling each of the proteins to Sepharose 2B. These gels were then used along with control uncoupled gel to attempt to bind the specific anti-idiotype. The results (Table 2) again showed the idiotypic antigen on purified Col cold agglutinin IgM and on its isolated mu and kappa chains and its absence on purified Mac, Rei, and Ada cold agglutinins and pooled Waldenström Igm, Mu, and light chains.

Adsorptions Using Anti-Idiotype Affinity Gel. An affinity gel was prepared by coupling the globulin fraction (ammonium sulfate precipitate) of the specific adsorbed anti-

Table 2. Binding of anti-idiotype to Col IgM, mu, and kappa affinity gels

Affinity gel*	% Anti-idiotype adsorbed†
Col Igm	98
Col mu chains	98
Col kappa chains	87
Mac IgM	<3
Ada IgM	<3
Rei IgM	<3
Waldenström pool IgM	<3
Waldenström mu chains	<3
Waldenström light chains	<3
Sepharose 2B without coupled protein	<3

* Each affinity gel contained approximately 1 mg of coupled protein per ml of gel. The coupled proteins were: Col purified IgM cold agglutinin and its isolated mu and kappa chains; purified cold agglutinins Mac, Ada, and Rei; a pool of four purified Waldenström IgM proteins; and the mu and light chains from the Waldenström proteins.

† Experiments were performed by mixing 250 μl of a 1/100 dilution of anti-idiotype with 25 μl of the respective gels overnight at 4°. Supernatants were tested for residual anti-idiotype activity by the automated hemagglutination-inhibition of intact Col cold agglutinin.

idiotype serum to Sepharose 2B. The ability of this gel to bind Col and other Cold agglutinins was tested as shown in Table 3. The anti-idiotype gel bound 67% of the added (500 μg) of Col, 10% of the Mac, and insignificant amounts of the Ada or Rob cold agglutinins. Direct binding of the Col mu and kappa chains to the anti-idiotype gel using the ^{14}C-radiolabeled chains (Fig. 2) was not possible because the specific activity of these chains was too low. We therefore used

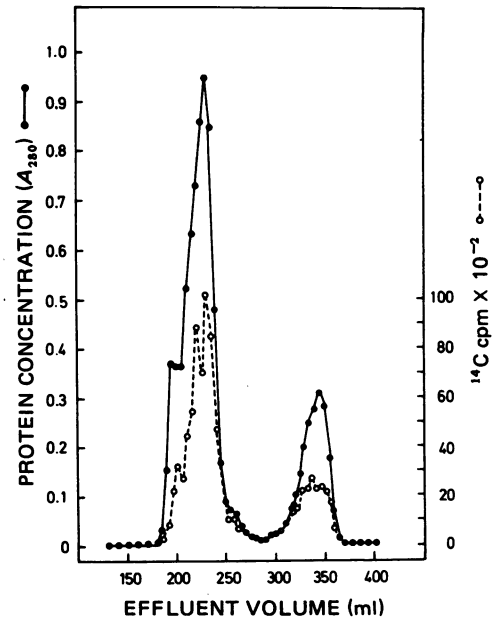

FIG. 2. Separation of mu and kappa chains of reduced and radioalkylated Col cold agglutinin. The purified IgM Col was reduced with dithiothreitol and alkylated with iodo[^{14}C]acetamide. After dialysis, the chains were separated on a 110 × 2.5 cm column of Sephadex G-100 equilibrated with 1 M acetic acid. The sharp separation of the mu and the kappa chains is demonstrated by both the A_{280} readings and the radioactivity counts.

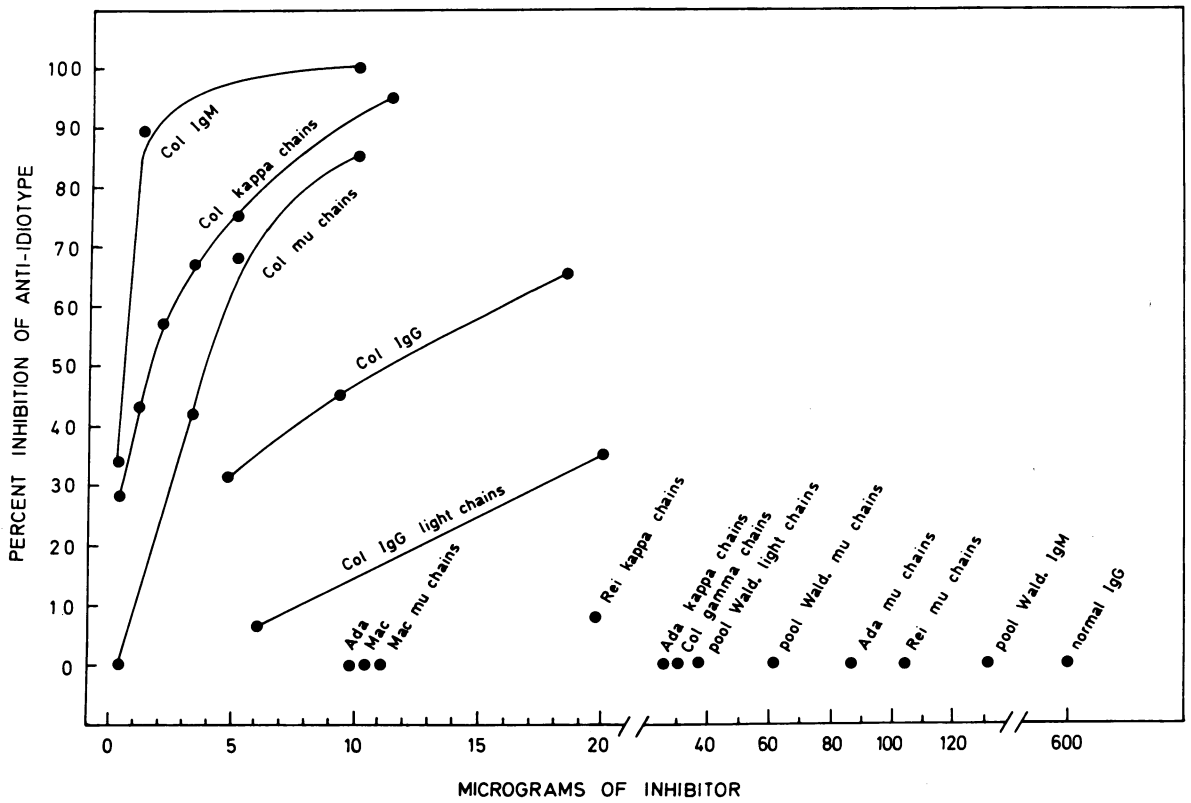

FIG. 3. Idiotypic antigen on soluble immunoglobulins was measured by the inhibition of the anti-idiotype potency in the subsequent hemagglutination-inhibition assay. The idiotypic antigen was present on purified Col IgM and on each of its chains, mu and kappa, but was absent from other cold agglutinin (Mac, Ada, Rei) IgM, mu, and kappa preparations; from pooled Waldenström (Wald.) IgM, mu, and light chains; and from normal human purified IgM. Some idiotypic antigen was present on IgG purified from Col serum and on the light chains, but not the gamma chains, obtained from this IgG. Gentle heating (65°, 5 min) was used to activate the cold agglutinins used.

an indirect approach by pre-loading aliquots of the anti-idiotype gel with Col cold agglutinin mu or kappa chains and with Waldenström mu and light chains. After pre-loading, Col cold agglutinin was added, the samples were incubated, and an aliquot of each supernatant was then used to measure the amount of unbound Col cold agglutinin, using the AutoAnalyzer hemagglutination assay. Use of 50 μg of the Col mu chains resulted in 85% inhibition of binding, whereas 9 μg of Col kappa chains completely blocked the Col binding. In contrast, there was no blocking of Col binding when 60 μg of Waldenström mu chains or 38 μg of Waldenström light chains were used.

Pepsin Digestion of Col Light Chains. In an attempt to produce variable-region fragments bearing the idiotypic antigen, we used a limited pepsin digestion of the light chains (18). Fractionation of a 30 min digest on Sephadex G-50 in phosphate-buffered saline (pH 7.4) gave three peaks based on Lowry analysis. The first peak corresponded to the known elution position of intact kappa chains and contained both idiotypic and kappa constant region antigens, as monitored by their automated hemagglutination-inhibition assays. The third peak possessed neither antigen. The second peak contained idiotypic antigen but not the kappa antigen. These preliminary data suggest that the second peak fraction is enriched in the variable region and that localization of the idiotypic antigen to variable region fragments may be possible.

Table 3. Binding of cold agglutinins by anti-idiotype affinity gel

Cold agglutinin	Specificity	Nanograms of adsorbed cold agglutinin*
Col	i > I	335
Mac	i	50
Ada	I	10
Rob	Pr	0

* Experiments were performed by mixing 500 ng of each cold agglutinin (200 μl) with 10 μl of anti-idiotype gel overnight at 4°. Supernatants were tested for remaining cold agglutinin by quantitative hemagglutination using the AutoAnalyzer. Control noncoupled Sepharose 2B gel bound no cold agglutinin.

DISCUSSION

Studies of idiotypic determinants on human monoclonal cold agglutinins using anti-idiotype serum generated against whole immunoglobulin led to the demonstration of cross-reactive or shared idiotypic determinants (5). Subsequent investigations have shown that there are different groups of crossreactivity related to the iI versus PR specificity systems (22). The thrust of these studies has been to demonstrate sharing of idiotypic antigens in relation to structural similarities in the antigen combining sites. In our work, we have used a different approach. By immunizing a rabbit with the isolated heavy chain of anti-i cold agglutinin Col, rather than the intact antibody, and by rendering the anti-idiotype as specific as possible by adsorbing not only with Waldenström IgM and Waldenström mu affinity gels but also with affinity gels containing an anti-I and an anti-i cold agglutin-

in, we have produced a potent anti-idiotype antiserum highly specific for Col cold agglutinin.

Our conclusive finding that this anti-idiotype reacts strongly with both the isolated mu and kappa chains of the purified antibody is a new observation. This was demonstrated by showing: (*i*) the Col heavy and the light chain preparations each completely reversed the inhibition by the anti-idiotype of IgM Col cold hemagglutination. Control heavy and light chains showed no inhibition. (*ii*) Affinity gels containing Col mu chains or Col kappa chains covalently bound to Sepharose 2B were capable of adsorbing all anti-idiotype activity against the parent Col cold agglutinin. Control affinity gels removed none of the activity. (*iii*) Soluble Col mu or kappa chains could prevent the binding of the IgM Col cold agglutinin to an anti-idiotype affinity gel. Control chains failed to block the binding. The fact that isolated Col mu and kappa chains, or affinity gels made from each chain type, can completely bind the anti-idiotype excludes the possibility that our antiserum consists of a mixture of anti-idiotypes specific for the Col mu and kappa chains, respectively. Also numerous structural studies (8, 10, 23, 24, and unpublished studies) all discount any bizarre chain structure for the Col cold agglutinin.

We did find some idiotypic antigen on purified Col IgG light chains. It is possible that excess cold agglutinin kappa chains have combined with some gamma chains or that a clone of cells has arisen which is producing the homologous kappa chains along with gamma chains lacking the idiotype. It is of interest that Cazenave *et al.* report a similar finding of idiotypic antigen on immunoglobulin without detectable antibody function (25).

Our interpretation of these unique findings is as follows: The Col cold agglutinin mu and kappa chains have a very similar or identical segment of amino acids, absent in the majority of all other immunoglobulin chains, which determines the shared idiotypic antigen. We favor the attractive hypothesis that this shared variable region sequence is in one of the hypervariable regions of both the mu and the kappa chains. There has been increasing support (26–35) recently for the gene insertion hypothesis of Wu and Kabat (36). This hypothesis postulates that the hypervariable regions of immunoglobulins are encoded by distinct genes that are inserted into the DNA coding for the relatively invariant "framework" portions of the variable region. Since the constant region is encoded by another group of genes, this hypothesis envisions at least three groups of genes for each chain. By extending this hypothesis to allow access of both heavy and light chain variable regions to a common dictionary of hypervariable genes, we would explain our findings and reduce the necessary number of entries in the total hypervariable gene dictionary.

Our hypothesis is susceptible to direct analysis. We have large amounts of this purified cold agglutinin, sufficient for sequencing the entire variable region of each chain. In addition, it may be possible to isolate subfragments of each chain bearing the idiotypic antigen, using our anti-idiotype affinity gel. Through the use of these approaches it should be possible to characterize the Col idiotypic antigen chemically and to define its position in both the heavy and light chains.

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1. Wells, J. V., Fudenberg, H. H. & Givol, D. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1585–1587.
2. Brient, B. W. & Nisonoff, A. (1970) *J. Exp. Med.* **132,** 951–962.
3. Sirisinha, S. & Eisen, H. N. (1971) *Proc. Natl. Acad. Sci USA* **68,** 3130–3135.
4. Sher, A. & Cohn, M. (1972) *Eur. J. Immunol.* **2,** 319–326.
5. Williams, R. C., Jr., Kunkel, H. G. & Capra, J. D. (1968) *Science* **161,** 379–381.
6. Kunkel, H. G., Agnello, V., Joslin, F. G., Winchester, R. J. & Capra, J. D. (1973) *J. Exp. Med.* **137,** 331–342.
7. Cooper, A. G. & Hobbs, J. R. (1970) *Br. J. Haematol.* **19,** 383–396.
8. Cooper, A. G. (1968) *Clin. Exp. Immunol.* **3,** 691–702.
9. Cooper, A. G. & Brown, M. C. (1973) *Biochem. Biophys. Res. Commun.* **55,** 297–304.
10. Cooper, A. G., Chavin, S. I. & Franklin, E. C. (1970) *Immunochemistry* **7,** 479–482.
11. Franklin, E. C. & Frangione, B. (1967) *J. Immunol.* **99,** 810–814.
12. Fahey, J. L. & Horbett, A. P. (1959) *J. Biol. Chem.* **234,** 2645–2651.
13. Cooper, A. G., Brown, M. C., Derby, H. A. & Wortis, H. H. (1973) *Clin. Exp. Immunol.* **13,** 487–496.
14. Miller, F. & Metzger, H. (1965) *J. Biol. Chem.* **240,** 4740–4745.
15. Fleischman, J. G., Porter, R. R. & Press, E. M. (1963) *Biochem. J.* **88,** 220–228.
16. Wilson, L. A. & Amos, D. B. (1972) *Tissue Antigens* **2,** 105–111.
17. Porath, J., Axen, R. & Ernback, S. (1967) *Nature* **215,** 1491–1492.
18. Seon, B. K., Roholt, O. A. & Pressman, D. (1972) *J. Immunol.* **109,** 1201–1209.
19. Wang, A. C., Fudenberg, H. H., Wells, J. V. & Roelcke, D. (1973) *Nature* **243,** 126–128.
20. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10,** 2606–2616.
21. Schneider, M. & Hilschmann, N. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* **355,** 1164–1168.
22. Feizi, T., Kunkel, H. G. & Roelcke, D. (1974) *Clin. Exp. Immunol.* **18,** 283–293.
23. Cohen, S. & Cooper, A. G. (1968) *Immunology* **15,** 93–100.
24. Cooper, A. G. (1967) *Science* **157,** 933–985.
25. Cazenave, P.-A., Ternynck, T. & Avrameas, S. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4500–4502.
26. Capra, J. D. & Kehoe, J. M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4032–4036.
27. Kindt, T. J., Klapper, D. G. & Waterfield, M. D. (1973) *J. Exp. Med.* **137,** 663–648.
28. Thunberg, A. L. & Kindt, T. J. (1974) *Eur. J. Immunol.* **4,** 478–483.
29. Huser, H., Haimovich, J. & Jaton, J. C. (1975) *Eur. J. Immunol.* **5,** 206–210.
30. Claflin, J. L. & Davie, J. M. (1975) *J. Exp. Med.* **141,** 1073–1083.
31. Hopper, J. E. (1975) *J. Immunol.* **115,** 1101–1107.
32. Kluskens, L., Lee, W. & Kohler, H. (1975) *Eur. J. Immunol.* **5,** 489–496.
33. Urbain, J., Tasiaaux, N., Leuwenkroon, R., Van Acker, A. & Mariame, B. (1975) *Eur. J. Immunol.* **5,** 570–575.
34. Capra, J. D. & Kindt, T. J. (1975) *Immunogenetics* **1,** 417–427.
35. Capra, J. D. & Kehoe, J. M. (1975) *Adv. Immunol.* **20,** 1–40.
36. Wu, T. T. & Kabat, E. A. (1970) *J. Exp. Med.* **132,** 211–250.