Inv(1) Allotype: Effect of Immunoglobulin G Heavy Chain Subtype on Its Expression

Abstract. *More protein is required to detect the Inv(1) antigen carried in the light chain of immunoglobulin G molecules when the light chain is combined with a γ2 heavy chain than when it is combined with a γ1 or γ3 heavy chain. One of the four γ2 heavy chains used in the experiment, however, was as efficient as the γ1 and γ3 chains, indicating that there may be two subtypes of γ2. Inv(1) was more easily detected in one of the two light chains used in the experiment. This difference may be associated with the subtypes of the kappa chain derived from studies of the variable portion of the chain.*

Ropartz *et al.* (*1*) discovered the Inv(1) and Inv(2) antigens, and demonstrated that they are inherited independently of the Gm antigens. It was later shown that the Inv antigens are carried on the kappa subtype of the light chains of immunoglobulins (*2, 3*). Terry *et al.* (*3*) failed to detect Inv(1) or Inv(3) with certainty on myeloma proteins with heavy chains of the γ2 or γ4 subtypes, but they detected either Inv(1) or Inv(3) on most myeloma proteins of the γ1 or γ3 subtypes. Terry *et al.* therefore suggested that Inv antigenicity may not be as well expressed when the kappa light chains are combined with γ2 or γ4 heavy chains as they are when the kappa light chains are combined with γ1 or γ3 heavy chains. The suggestion seemed reasonable in the light of the demonstration that the expression of the Inv antigens is partially dependent upon the quaternary structure of the molecule (*4, 5*). We now report experiments designed to test the suggestion made by Terry *et al.*

The Inv(1) antigen was detected by a modification of the method reported earlier (*6*); the mixture of sensitized red blood cells, antibody to Inv, and protein to be tested were rotated at 60 rev/min during a 2-hour incubation period. All Inv tests were done with antiserum to Inv(1) Math diluted 1 : 4, and antiserum to D Roe diluted 9 : 10.

We obtained the following myeloma serum samples in sufficient quantity to permit their use in our experiments: four $(\gamma1)_2\ \kappa_2$; two $(\gamma2)_2\ \kappa_2$; two $(\gamma2)_2\ \lambda_2$; one $(\gamma3)_2\ \kappa_2$; and one $(\gamma3)_2\ \lambda_2$. The proteins were isolated by chromatography on diethylaminoethyl cellulose (DEAE) columns in 0.005*M* potassium phosphate buffer, *p*H 8.0; this isolation was followed in some instances by electrophoresis on a Pevikon block. The isolated proteins were tested for purity by immunoelectrophoresis against antiserum to whole normal human serum and by Gm (antigens carried on heavy chains) tests to detect contaminating normal IgG.

The polypeptide chains were reduced, alkylated, and separated as described (*7*). Purity of the isolated heavy chains was tested by double diffusion against antibody κ to λ, and to IgG antibodies. The heavy and light chains were recombined as described (*7*).

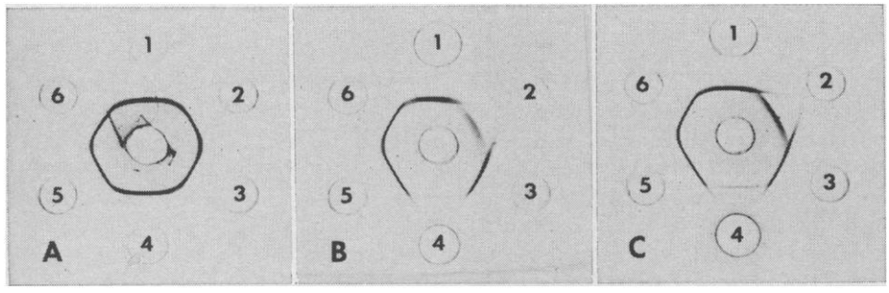

Fig. 1. Tests of reconstituted IgG molecules for completeness of recombination of the heavy and light chains. Horse antiserum to whole normal human serum is in the center well in each set of double diffusions. All proteins were used at 1 mg/ml. (A) Reconstituted IgG molecules are in wells 1 to 6. The light-chain donor is Per; the heavy-chain donors are as follows: 1, Hen (γ1); 2, Cin (γ2); 3, Pac (γ3); 4, Hug (γ1); 5, Nor (γ2); 6, Bri (γ3). The dark lines from the center well toward well 6, and at its edge toward well 3 are shadow artifacts due to chips that formed in the agar during washing, drying, and staining. (B) Reconstituted IgG molecules Cin, Hen, and Pac are in wells 1, 3, and 5, respectively. Isolated kappa light chain Per is in well 2, isolated γ1 chain Hug is in well 4, and normal IgG is in well 6. (C) Reconstituted IgG molecules Nor, Hug, and Bri are in wells 1, 3, and 5, respectively. Wells 2, 4, and 6 are as in (B).

Two (Per and Har) of the four $(\gamma1)_2\ \kappa_2$ proteins were Inv(1). Their light chains were combined with the heavy chains of each of the other proteins except that, because of limited materials, Per light chain was not combined with Har heavy chain and vice versa. The reconstituted molecules were tested at serial twofold dilutions to determine the lowest molar concentration at which Inv could be detected. The molecular weight for the IgG molecule was taken as 150,000 and that for the light chain as 20,000.

Considerably more protein is required to detect Inv(1) when the light chain is combined with a γ2 heavy chain than when it is combined with a γ1 or γ3 heavy chain (see the means, Table 1). This is in agreement with the hypothesis advanced above, namely, that the quaternary structure of IgG molecules with γ2 (and probably also with γ4) heavy chains is such as to suppress the expression of the Inv antigen, at least in part.

Inv(1) was detected in low molar concentrations of protein in all molecules in which the heavy chain was of the γ1 or γ3 subclass (Table 1). Such uniformity of response was not observed, however, when the heavy chain was of the γ2 subclass. Three of the γ2 molecules require high molar concentrations of protein (particularly with kappa chain Har), but the fourth (Nor) reveals Inv(1) activity as effectively as the γ1 and γ3 polypeptides.

Two possible explanations of this difference are that the Nor protein is not subtype γ2; and that, if it is, its H chain recombines with the light chain more efficiently than the H chains of the other γ2 proteins. The γ2 heavy chain subtype was determined by testing the whole molecule for Gm antigens 1, 3, 5, 6, 13, 14, and 21, none of which was present. This indicated that the heavy chain is of subclass γ2 or γ4 (*3*). Nor and the three other γ2 proteins are positive for Gm(8), which is present on γ2 molecules and on those γ1 molecules that are Gm(3), but not on other molecules (*8*). Finally, the proteins were found, by double diffusion, to be positive with an antiserum to both γ2 and γ3. This observation was confirmed for Nor protein by Terry.

The combination of the H chains with the light chains was checked by double diffusion with horse antiserum to whole normal human serum (*7, 11*). The results of simultaneous testing of two γ1, two γ2, and two γ3 reconsti-

Table 1. Minimum concentrations (micromoles) of reconstituted IgG at which Inv(1) was detected. For controls, the isolated light chains and the isolated untreated myeloma proteins were tested for Inv(1). The antigen was detected at 12.5 μmole/ml and 50 μmole/ml in Per and Har light chains, respectively, and at 0.2 and 0.4 μmole in Per and Har isolated myeloma proteins, respectively.

Name	Nature of the myeloma source of heavy chain		Source of kappa chain	
	γ Chain subclass	Light chain subclass	Per	Har
Hug	1	κ	0.2	0.4
Hen	1	κ	0.2	0.2
Per	1	κ	0.2	N.T.†
Har	1	κ	N.T.	0.4
Mean			0.20	0.33
Pac	3	κ	0.1	0.4
Bri	3	λ	0.2	0.2
Mean			0.15	0.30
Has	2	κ	1.7	6.7
Jon	2	κ	0.8	>6.7‡
Cin	2	λ	1.7	>6.7
Nor	2	λ	0.2	0.2
Mean			1.10	>5.08

* Initial concentration of all proteins: 1 mg/ml, that is, 6.7 μmole for IgG and 50 μmole for light chains. † N.T., not tested. ‡ Not detected at initial protein concentration of 6.7 μmole.

tuted proteins are shown in Fig. 1A. All the lines are equally intense and all meet as lines of identity. The proteins were also tested against horse antiserum to whole normal human serum with a kappa light chain, a γ1 heavy chain, and IgG from a normal donor as controls (Figs. 1, B and C). The reconstituted molecules with γ1 and γ2 heavy chains spur over the kappa chain, the reconstituted molecules with γ1 and γ3 heavy chains spur over the isolated γ1 chain and the reconstituted molecules with γ2 and γ3 chains show identity with the control IgG molecule. There is therefore no evidence to suggest that the Nor γ heavy chain combines more readily with the light chain than the Cin (γ2) heavy chain does.

The greater efficacy of the Nor γ2 chain in revealing the Inv(1) antigen on the light chain of the donor is probably not a function of the light chain with which the heavy chain was originally associated, because both Nor and Cin myeloma proteins have λ light chains, yet the latter required at least eight times the molar concentration of the former for the detection of Inv(1). It should be pointed out, however, that we do not know the Oz subtypes (*9*) of these light chains, hence it is possible that the observed difference is associated with the subtype of the light chain present in the molecule from which the heavy chain was obtained.

The data offer further confirmation that the Inv antigens are dependent upon the quaternary structure of the molecule (*4, 5*). It has also been demonstrated that the Gm antigens located on the Fd portion of the heavy chain [Gm(3) and Gm(17)] are dependent upon the quaternary structure of the molecule for their expression (*4, 5*). It seems likely, therefore, that all allotypic antigens in the Fab portion of the IgG molecule depend, at least in part, upon the quaternary structure of the molecule for their expression. The expression of the Gm antigens on the Fc portion of the heavy chain, on the other hand, seems to be independent of the quaternary structure of the molecule (*7*).

Four times as much protein was required to detect Inv(1) in the isolated light chain from myeloma protein Har as was required to detect it in the isolated light chain from myeloma protein Per (Table 1). In parallel with this observation, IgG molecules reconstituted with light chain Har tended to require more protein for the detection of Inv(1) than those IgG molecules formed with light chain Per. This was especially noticeable for the γ2 heavy chains. Nevertheless, γ2 heavy chain Nor was as effective with light chain Har as it was with light chain Per. We have no explanation for this observation, but the surprisingly great efficiency of γ2 heavy chain Nor suggests that the γ2 heavy chains may comprise at least two subtypes, one typified by heavy chain Nor, and a second type corresponding to the remaining three γ2 heavy chains shown in Table 1.

Possibly the difference between the reactions of light chains Per and Har is associated with the kappa chain subtypes based on differences in the variable portion of the molecule (*10*). Unfortunately, we do not have enough of either of these proteins to permit a test of this suggestion.

Our conclusion that γ2 heavy chains are less efficient than γ1 and γ3 heavy chains in enhancing Inv(1) activity seems to contradict that of Litwin and Kunkel (*5*). They concluded that, ". . . heavy chains of any γG type could restore the Inv(1)—Mo antigen. . . ." The contradiction may be more apparent than real, because as they showed, the antigen detected by Mo is markedly different from the antigen detected by Math (Math was called Matt by Litwin and Kunkel).

It would be of interest to test the same reconstituted molecules with antibody Math and Mo. Unfortunately, we do not have any antibody Mo at our disposal.

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12. We thank Drs. P. Bennett, J. T. Boyer, G. A. Spengler, L. G. Surhland, and W. D. Terry for supplying the serum samples from the myeloma patients; Dr. Terry for antiserums to determine the H chain subtypes and for confirming our typing of these chains, and Dr. C. Ropartz for reagents to determine GM(8). Our study was supported in part by NIH grant Gm 7412. I.R. is supported by funds from the Pan American Union and the Comision Nacional de Energia Nuclear, Mexico.

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Dihydroorotic Acid Dehydrogenase: Introduction into Erythrocyte by the Malaria Parasite

Abstract. *Mature, circulating mammalian erythrocytes lack dihydroorotic acid dehydrogenase activity. However, we have detected activity, which appears to reside in an enzyme, in malaria parasites purified from mammalian blood.*

Dihydroorotic acid dehydrogenase (DAD, E.C. 1.3.3.1) is one of the enzymes in the biosynthetic pathway leading to uridine-5′-monophosphate, and it catalyzes the reversible conversion of dihydroorotic acid to orotic acid. The