A NEW METHOD OF TYPING LIGHT CHAINS OF G-PARAPROTEINS

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A characteristic feature of the monoclonal immunoglobulinopathies is the presence of a homogeneous immunoglobulin (paraprotein) of a particular class (IgG, IgA, IgM, IgD, or IgE) and type (κ or λ) in the serum. A connection has been shown to existing [4, 6] between the class and type of paraprotein, on the one hand, and the clinical features of paraproteinemic reticulosis, on the other hand. The typing of the light chains of paraproteins is accordingly an essential element in the diagnosis of the paraproteinemias.

The usual methods of typing light chains of immunoglobulins are based on the use of monospecific antisera against light chains. Such methods include immunoelectrophoresis, gel diffusion, electroimmunodiffusion, and a combination of immunodiffusion with immunoelectrophoresis [8, 9, 12]. The recently suggested double-line method [4] requires special antisera containing antibodies against the γ-chain and the corresponding light chain (κ or λ) in the ratio of 1:2.

In this paper a new method of typing G-paraproteins, based on the use of unadsorbed antiserum against normal IgG is suggested.

In previous investigations [2] it was observed that some sera containing G-paraprotein form two precipitation rings around the well during radial immunodiffusion in gel with unadsorbed anti-IgG serum. The next stage in the work was to study the connection between the appearance of double rings and the type of the G-paraprotein in the serum.

EXPERIMENTAL METHOD

The sera from patients with G-myeloma and essential and reactive-G-monoclonal immunoglobulinopathies were studied.* The class and type of the paraprotein were determined by immunoelectrophoresis with monospecific antiserum against IgG and against κ and λ-chain. Of the 76 sera tested 48 (63.2%) contained G (κ)-paraprotein and 28 (38.8%) contained G(λ)-paraprotein. All the sera was studied by radial immunodiffusion in gel [1, 7, 10] with unadsorbed anti-IgG-serum obtained by immunizing rabbits with purified IgG; the latter was isolated from commercial γ-globulin on DEAE-cellulose with 0.005 M phosphate buffer, pH 8.0. On immunoelectrophoresis with normal human serum this antiserum gave one precipitation line corresponding in mobility to IgG.

EXPERIMENTAL RESULTS

All sera containing G(λ)-paraprotein gave the double ring phenomenon on radial immunodiffusion, whereas the sera with G(κ)-paraprotein gave one ring in more cases (Fig. 1).

To determine what antibodies contained in the unadsorbed anti-IgG-serum were responsible for the double-ring phenomenon, experiments were carried out to study absorption of antiserum by light chain of

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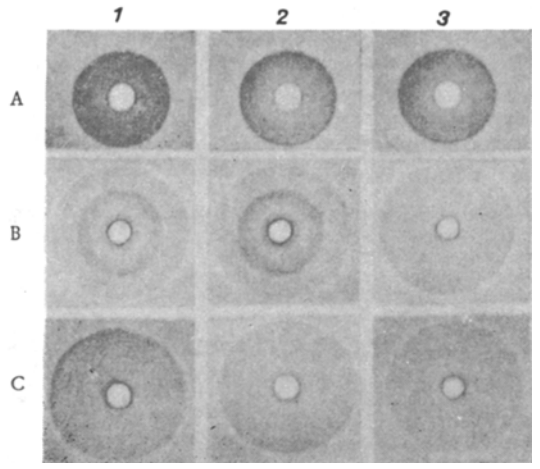

Fig. 1. Conditions of formation of double precipitation rings in radial immunodiffusion tests in gel. Wells contain normal serum (A) and sera with G(λ)-paraprotein (B) and G(ϰ)-paraprotein (C); agar contains unadsorbed antiserum against normal IgG (1), or the same after adsorption with Bence–Jones protein of lambda (2) and kappa (3) type.

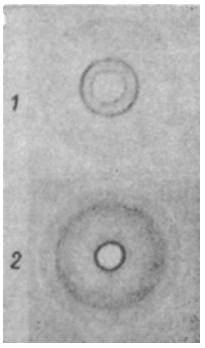

Fig. 2. Double precipitation rings with serum containing G(λ)-paraprotein before (1) and after (2) the addition of purified G(ϰ)-paraprotein to it. Agar contains unadsorbed antiserum against normal IgG.

the different types. Adsorption of antiserum by Bence–Jones protein of the lambda type did not change the ability of the antiserum to give double rings with serum containing G(λ)-paraprotein, whereas adsorption with Bence–Jones protein of kappa type abolished this ability (Fig. 1). The double ring phenomenon with serum containing G(λ)-paraprotein is thus due to the presence not only of antibodies against γ-chains in the unadsorbed antiserum against IgG, but also of antibodies against light chains of the kappa type. It must be emphasized that antibodies against kappa chains are always found in antiserum obtained by immunizing with normal IgG, for molecules of the kappa type constitute 60% of the total in normal IgG and kappa chains are more highly antigenic for rabbits than lambda chains [6, 11].

To determine the properties of antigens participating in the formation of precipitation rings, different quantities of purified G(ϰ)-paraprotein or of normal IgG were added to the serum containing G(λ)-paraprotein and giving the double ring phenomenon; an increase in the size of the inner ring was thereupon observed (Fig. 2). It thus followed that the inner ring in sera with G(λ)-paraprotein includes a protein immunologically identical with IgG(ϰ). This ring evidently arises on account of kappa-molecules of normal IgG, always contained in small quantities in the sera of patients with G-paraproteinemia.

Since antibodies of only one type participate in the formation of the outer ring the density of the resulting precipitate was relatively small and the denser inner ring, formed with the participation of two types of antibodies, could be clearly seen against its background. It is important to note that on the addition of an excess of purified G(ϰ)-paraprotein to serum containing G(λ)-paraprotein, when the diameter of the inner ring became larger than that of the outer ring, the latter appeared indistinguishable against the background of the dense precipitate formed by IgG(ϰ) and by antibodies against gamma and kappa chains. For the same reason, the double rings could not be seen in the sera of patients with G(λ)-paraprotein and in normal seria in which the number of IgG kappa molecules was significantly greater than the number of lambda molecules.

Final proof of the validity of the mechanism of double ring formation as described above was obtained with the aid of another immunologic system – antiserum against G(λ)-paraprotein containing antibodies against gamma and lambda chains [the antiserum was obtained by intensive immunization of a rabbit with G(λ)-paraprotein and, additionally with Bence–Jones protein of lambda type]. This antiserum revealed double rings with sera containing G(ϰ)-paraprotein and with normal sera, whereas sera containing G(λ)-paraprotein gave only one ring. It is evident that the outer ring in this immunologic system appeared on account of the reaction of G(ϰ)-paraprotein (in normal sera on account of kappa IgG molecules) with antibodies against gamma-chains, whereas the inner and denser ring appeared through the reaction of IgG lambda molecules with antibodies against gamma and lambda chains.

It can be concluded from these investigations that double ring formation in the radial immunodiffusion test in gel can serve for the reliable differentiation between sera containing G(λ)-paraprotein and

sera containing G(κ)-paraprotein. This method is simple, and it needs only unadsorbed antiserum against normal IgG, which is not difficult to obtain. The method enables G-paraprotein to be demonstrated and the type of its light chain to be determined simultaneously and quickly.

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