Relation between Certain Myeloma Proteins and Normal Gamma Globulin (18432)

H. G. KUNKEL, R. J. SLATER, AND R. A. GOOD.
From the Hospital of the Rockefeller Institute for Medical Research, New York City.

The recent purification of human gamma globulin by alcohol fractionation has led to studies of its immunological properties(1-3). The work has been hampered by the fact that the various gamma globulin preparations are not single proteins. Two materials, called gamma$_1$ globulin and gamma$_2$ globulin, have been separated that differ electrophoretically; however, each of these remains nonhomogeneous. Although each absorbs all of the antibody against the total normal gamma globulin, Kabat and Murray(2) have demonstrated immunological differences between these two preparations. The recent observations of Cohn, Deutsch and Wetter have shown clearly that the gamma globulin fractions contain more than one antigen. The present study represents an attempt to clarify the relationship between the fractions of gamma globulin through a study of the special readily purified proteins that exist in tremendous quantity in the serum of patients with multiple myeloma. Evidence was obtained for an immunological similarity between various myeloma proteins and a portion of normal human gamma globulin.

Two preparations of human gamma globulin (II-1,2 and II-3), obtained from the laboratory of Dr. E. J. Cohn, were injected into rabbits in the alum precipitated form according to the usual schedule(4). Good antisera were obtained in approximately 40% of the animals. This antiserum did not give a precipitin reaction with human albumin. An attempt was made to estimate the gamma globulin concentration in various sera by employing quantitative precipitin studies in the region of extreme antibody excess. A technic was used similar to that previously described for albumin(4). In confirmation of the work by Jager and coworkers(1), consistently higher values were obtained for gamma globulin by this procedure than by electrophoretic analysis. Values for normal serum ranged between 1.4 and 2.2 g/100 cc with several different antisera. The serum of patients with nephrosis, known to contain very small amounts of gamma globulin, gave very low values by the immunological method; the serum of patients with cirrhosis, containing large amounts of gamma globulin as determined electrophoretically, gave values higher than the normal range. There was a general correlation, although not a close one, between the immunological and the electrophoretic values for gamma globulin.

The sera of 5 different patients with multiple myeloma were also studied in a quantitative manner with the gamma globulin antisera. Each of these sera contained a very high total protein due to a single sharp electrophoretic peak which varied in mobility in the different sera (Table I). Fig. 1 shows the quantitative precipitin curves for 2 normal sera and myeloma serum D. It is apparent that the myeloma serum demonstrates an equivalence zone shifted to the

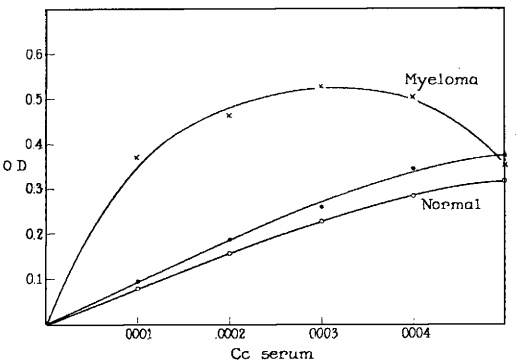

FIG. 1.

Quantitative precipitin curves showing the intense precipitin reaction given by myeloma serum D as compared to 2 normal sera. O.D. refers to ninhydrin color of the antigen-antibody precipitate.

1. Jager, B. V., Smith, E. L., Nickerson, M., and Brown, D. M., *J. Biol. Chem.*, 1948, v176, 1177.

2. Kabat, E. A., and Murray, J. P., *J. Biol. Chem.*, 1950, v182, 251.

3. Cohn, M., Deutsch, H. F., and Wetter, L. R., *J. Immunol.*, 1950, v64, 381.

4. Kunkel, H. G., and Ward, S. M., *J. Biol. Chem.*, 1950, v182, 597.

TABLE I. Electrophoretic Concentration and Mobility of the Myeloma Protein in the Serum of 5 Patients with Multiple Myeloma.*

	Total protein, g/100 cc	Myeloma protein, g/100 cc	Mobility U x 10^5
Myeloma D	13.5	10.20	2.15
" Y	17.2	13.70	1.20
" X	11.8	8.75	1.11
" S	11.0	6.13	2.15
" L	13.4	9.20	3.30

* Barbital buffer, pH 8.6, μ 0.1, descending boundaries.

left into the region of antibody excess relative to normal serum. This was also true of myeloma sera, Y, X, and S. However, serum S showed a somewhat different reaction. The quantitative curve showed a small but definite peak in the region of extreme antibody excess at dilutions where normal serum failed to give any reaction and where the other myeloma sera had given a more intense reaction. Similar results were obtained with 3 different antisera. It was possible to obtain a quantitative value for gamma globulin in each of these sera that approximated the high concentration of the abnormal myeloma component. Since each of these sera contained only a single electrophoretic component that was present in a concentration greater than normal, the only possible explanation for the immunological data was that the special myeloma protein reacted with the gamma globulin antisera. Myeloma serum L, despite a very high total protein, gave a curve resembling that of normal serum but with an even less intense precipitin reaction at low dilutions.

Quantitative precipitin studies of purified myeloma proteins and gamma globulin antiserum. The special myeloma proteins from each of the 5 sera were obtained in varying degrees of purity. Myeloma Y was obtained by ethanol fractionation in the cold; a procedure similar to that described by Deutsch and coworkers(5) was employed. The final preparation when tested electrophoretically showed a single sharp component at pH 8.6 that made up 96% of the total protein. It was not tested under other conditions or in the ultracentrifuge. The purified protein, myeloma X, was furnished by Dr. Mary L. Petermann and Dr. G. E. Cogin of the Sloan-Kettering Institute for Cancer Research. It was isolated from myeloma serum by ethanol and salt fractionation. Their data indicate that the purified protein was 98% one component electrophoretically and was homogeneous at ionic strength 0.1 between pH 4.0 and 10.0. Ultracentrifugal analysis showed 95% of S = 7 component and 5% of S = 10 component. The remaining myeloma proteins were purified by electrophoretic separation.

Fig. 2 illustrates the quantitative precipitin curves for myeloma Y and X as compared with II-3 gamma globulin when studied with II-3 antiserum. It is apparent that each of the myeloma proteins gives a definite precipitin reaction with the gamma globulin antiserum. However, the intensity of the reaction is not so great as for the normal gamma globulin. Myeloma Y gives a somewhat stronger reaction than myeloma X. Myeloma D, which is not illustrated in the chart, gave a curve almost identical with that for myeloma Y despite a distinct difference in electrophoretic mobility. It appeared as though the myeloma proteins reacted with only a portion of the total antibodies to normal gamma globulin. This was verified by absorption experiments. Antiserum against II-3 gamma globulin was carefully absorbed over a period of 6 days with progressively increasing amounts of myeloma Y until no further precipitate was obtained. This absorbed antiserum still gave a precipitin reaction with II-3 gamma globulin. The curves for II-3 gamma globulin with the whole antiserum and with that absorbed with myeloma Y are compared in Fig. 2. Myeloma X and D gave no precipitin reaction with the Y absorbed antiserum. The II-3 antiserum was also absorbed with myeloma X. This absorbed antiserum gave a strong reaction with II-3 gamma globulin and also with myeloma Y. It was apparent that, although myeloma Y removed all of the antibodies to myeloma X, the reverse was not true. This was to be expected from the slightly different quantitative precipitin curves. Myeloma D absorbed all of the antibody to myeloma Y and X. Similar results were obtained by absorption of II-1,2

5. Deutsch, H. F., Alberty, R. A., and Gosting, L. J., *J. Biol. Chem.*, 1946, v165, 21.

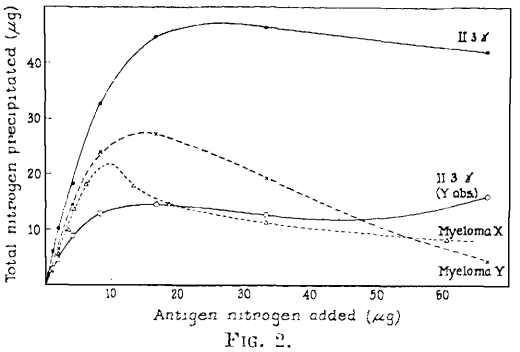

FIG. 2.

Quantitative precipitin curves of II-3 γ globulin, myeloma Y and myeloma X with antiserum to II-3 γ. The curve for II-3 γ with II-3 γ antiserum absorbed with myeloma Y is also illustrated.

antiserum. Purified myeloma protein L gave no reaction with gamma globulin antisera.

Reaction with antiserum to purified myeloma protein. Antiserum to myeloma X was obtained by employing the adjuvant technic of Freund. Previous attempts with alum precipitated myeloma proteins had failed to produce antibodies. A potent antiserum was obtained which, in addition to giving a precipitin reaction with myeloma X, also gave a strong reaction with II-1,2 gamma globulin as well as with normal serum. When quantitative precipitin curves were obtained, it was found that the II-1,2 gamma globulin gave a lower curve with this antiserum than did myeloma X protein when comparisons were made in terms of mg of nitrogen. Neither curve fell to the baseline in the region of antigen excess. It appeared that the II-1,2 gamma globulin contained protein which did not react with the myeloma antiserum. These observations correlated with those obtained with II-1,2 antiserum where both the quantitative precipitin curves and the absorption experiments indicated that the gamma globulin antiserum contained further antibodies in addition to those reacting with the myeloma protein. Absorption experiments with the myeloma antiserum indicated that the antibodies were entirely absorbed with the myeloma protein and no further precipitate was obtained with the II-1,2 gamma globulin. The latter also removed all of the antibodies to the myeloma protein. Despite the fact that some evidence was obtained indicating that the myeloma antiserum contained antibody to more than a single antigen, it definitely lacked certain of the antibody components found in the gamma globulin antiserum.

Discussion. The immunological data offer strong evidence for an antigenic relationship between the different myeloma proteins despite differences in electrophoretic mobility. Of particular interest is the fact that two of the myeloma proteins, D and Y, showed identical quantitative precipitin curves and each absorbed all of the antibody to the other, although one showed an electrophoretic mobility approximately twice that of the other. However, the myeloma protein showing the most rapid mobility of the 5 studied differed most markedly from the other proteins and gave no precipitin reaction with normal gamma globulin antiserum. Previous immunological studies with the proteins appearing in multiple myeloma have dealt chiefly with the Bence-Jones protein in the urine. This protein differs greatly in physical and chemical properties from normal serum proteins and from the myeloma proteins discussed in the present report and it has been found to produce a specific antiserum that does not react with normal proteins. Recently, Wuhrmann *et al.*(6) have reported that a myeloma protein showing a rapid electrophoretic mobility produced a specific antiserum that did not react with normal serum components. Myeloma L is possibly similar to that described by Wuhrmann as it failed to react with either normal gamma globulin antiserum or with myeloma X antiserum. Also, it showed a mobility similar to that of Wuhrmann's myeloma protein. It appears that the greater the mobility of the myeloma protein, the further removed antigenically it is from normal gamma globulin, as in myeloma L.

Both the precipitin curves and the absorption experiments indicate that the majority of myeloma proteins are immunologically related to a portion or normal gamma globulin. At least in the case of antisera to two different preparations of gamma globulin,

6. Wuhrmann, Von F., Wunderley, C., Hässig, A., and Hugentabler, F., *Helv. Med. Acta.*, 1949, v16, 279

similar results were obtained and the myeloma proteins absorbed a sizeable amount of the total antibody. This interpretation was confirmed by the reaction obtained between normal gamma globulin and myeloma X antiserum. Through the medium of absorption of gamma globulin antiserum with purified myeloma proteins, a method was obtained for dividing the normal gamma globulin antiserum into two portions, a myeloma related portion and a non-myeloma related portion. Studies are under way in which these two antisera are being used. Preliminary observations have indicated that the increased gamma globulin found in the serum of patients with liver disease is of the myeloma related type. Further subdivision of the gamma globulin antiserum was possible by absorption with different myeloma proteins.

Since the myeloma proteins are found in the serum in huge concentrations in a disease characterized by great proliferation of plasma cells, it would appear that these cells are involved in the production of these proteins. Therefore, the results obtained showing a relationship between myeloma proteins and a portion of normal gamma globulin adds further evidence for a role by plasma cells in the production of at least a component of normal gamma globulin.

Summary. 1. Antisera against two preparations of normal gamma globulin reacted quantitatively with the special myeloma protein found in the serum of 4 patients with multiple myeloma. 2. The myeloma protein from the serum of a fifth patient showing the most rapid electrophoretic mobility failed to react with gamma globulin antisera. 3. Absorption of gamma globulin antiserum with purified myeloma proteins removed only a portion of the total antiserum, thus dividing the antibody into fractions. 4. Antiserum prepared against a highly purified preparation of a gamma$_2$ myeloma protein gave a precipitin reaction with normal gamma globulin.

The authors are indebted to Dr. Mary L. Petermann for furnishing two of the myeloma sera in addition to a purified preparation of myeloma protein and to Dr. Merrill W. Chase for furnishing adjuvants for immunization.

Received December 1, 1950. P.S.E.B.M., 1951, v76.

Adsorption of Bovine Alkaline Phosphatase on Diatomaceous Silica. (18433)

CHARLES A. ZITTLE AND EDWARD S. DELLAMONICA.
(Introduced by G. W. Irving, Jr.)
From the Eastern Regional Research Laboratory, Philadelphia, Pa.*

Recent reports have described the adsorption of enzyme-carrying cell particles(1,2) and viruses(3,4) on various types of Celite (diatomaceous silica) from salt-containing solutions. Similarly, when salts are present, a partially purified alkaline phosphatase of cow's milk(5) is strongly adsorbed on the Celite Filter-Cel. The adsorption affords some increase in the purification of the enzyme, since the non-phosphatase protein is less strongly adsorbed. The results, however, are of more interest in explaining the adsorption of certain complex proteins on Celite. A comparison of adsorption on various Celites suggested that the presence of clay in its natural state was necessary for adsorption. The influence of various physical and chemical factors on the adsorption was also investigated.

Materials and methods. The preparation

* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

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