Concanavalin A Reaction with Human Normal Immunoglobulin G and Myeloma Immunoglobulin G

Abstract. *Concanavalin A precipitated less than 5 percent of immunoglobulin G from human serum. It reacted with all of 42 myeloma serums of the immunoglobulin G type tested, but no more than approximately 50 percent of the total myeloma protein was ever precipitated. The fact that not all of the protein was precipitated and that the amounts precipitated varied from serum to serum may be interpreted as demonstrating heterogeneity of the carbohydrate in these myeloma proteins. Other glycoproteins precipitated by concanavalin A were identified, and subsequently separated from concanavalin A by chromatography.*

Concanavalin A, the globulin from jack-bean meal, reacts with serum glycoproteins (*1*, *2*), presumably because of its specificity for their nonreducing mannosyl- and *N*-acetylglucosaminyl end groups (*3*). Harris and Robson demonstrated that crude jack-bean meal extracts precipitated α_2-macroglobulin, γ_1-macroglobulin (IgM), β-lipoprotein, and ceruloplasmin; as judged by electrophoresis haptoglobins were also precipitated (*2*). Since no reaction with 7*S* γ-globulin (IgG) was noted, I attempted to use concanavalin A to separate IgM from IgG. I now present data demonstrating that, depending on the serum, variable amounts of IgG from normal and myeloma serums precipitated with concanavalin A.

Concanavalin A was prepared by a modification of Agrawal and Goldstein's procedure (*4*). The crude saline extract of commercial, defatted jack-bean meal was clarified by centrifugation at 25,000*g* (0°C) for 1 hour. After removal of the lipid layer, the supernatant was applied directly to a Sephadex G-75 column. Subsequent procedures were as described (*4*). The purified product was dialyzed against 0.1*M* phosphate buffer, *p*H 6.2, and kept in small vials at −70°C to prevent the precipitation noted on storage at 4°C.

To precipitate serum glycoproteins, 7 to 8 mg of concanavalin A were added to 1 ml of serum, and the mixture was incubated at 37°C for 1 hour. The flocculent precipitate was centrifuged at 4°C and then washed three times with cold saline. Overnight incubation at 4°C prior to centrifugation gave only slightly increased yields of glycoproteins. The washed concanavalin-glycoprotein complexes were dissociated at 37°C with 1 ml of saline containing 0.5*M* methyl α-D-glucoside.

Immunoelectrophoretic and immunodiffusion tests on the dissociated material were performed in agarose gels which contained 0.25*M* methyl α-D-glucoside to prevent recombination of concanavalin A with glycoproteins. Components in the dissociated precipitate, confirmed by immunodiffusion tests with specific antiserums or by specific biological activities, were IgM, IgA, α_1-antitrypsin, α_2-macroglobulin, transferrin, β_2-lipoprotein, β_2-glycoprotein, haptoglobin, ceruloplasmin, group-specific components, the serum inhibitor of C'1 esterase (*5*) and all the components of complement.

Although, by immunoelectrophoresis, no IgG was observed in the dissociated precipitate, both immunodiffusion and Gm typing (*6*) showed that a small amount (<5 percent) of IgG had precipitated with the concanavalin A. These data may be attributed to (i) the known heterogeneity of immunoglobulin carbohydrate (*7*) or (ii) differences in availability of IgG carbohydrate for reaction with concanavalian A as a result of conformational differences in IgG molecules with different amino acid sequences. Since homogeneous IgG of both reactive and nonreactive types would facilitate testing of these hypotheses, 42 IgG myeloma serums were examined for reactivity with concanavalin A (*8*). Precipitation, washing, and dissociation were performed as already described, except that 10, 30, or 60 mg of concanavalin A were added per milliliter of each serum.

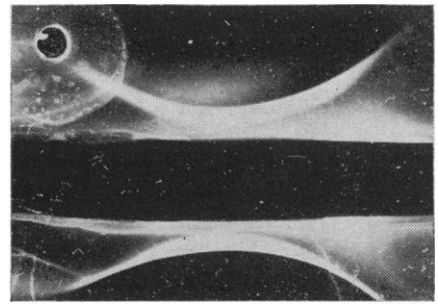

Fig. 1. Immunoelectrophoresis of precipitable (top) and nonprecipitable (bottom) fractions obtained from reaction of concanavalin A with serum of a patient with IgG myeloma of heavy-chain subtype γ_3. Both fractions are diluted 1 to 3 with respect to original serum. The antiserum is horse antiserum to human IgG.

Precipitable and nonprecipitable fractions were analyzed electrophoretically. Depending on the IgG myeloma serum, the quantity of IgG precipitated by concanavalin A varied from approximately 50 percent to mere traces of the total present. Immunoelectrophoretic patterns of the most reactive, an IgG myeloma serum of heavy-chain subtype γ_3, are shown in Fig. 1. Our limited data do not yet permit correlation of heavy-chain subtype with reactivity towards concanavalin A. In all reactive serums, the dissociated myeloma protein showed the same electrophoretic mobility as the myeloma protein of the original serum. Increasing the ratio of concanavalin to serum above 30 mg/ml did not significantly increase the amount of myeloma protein precipitated. If one accepts the theory that monoclonal myeloma protein in an individual serum is homogeneous with respect to amino acid sequence, then all molecules of myeloma protein have the same range of conformational possibilities in equilibrium. Carbohydrate chains attached to these molecules should show similar reactivity with concanavalin A provided that the carbohydrate chains are identical and are attached at the same position in the peptide chain. The partial precipitation of IgG myeloma proteins by concanavalin A, demonstrates existence of some type of heterogeneity of each IgG myeloma carbohydrate. The data further suggest that a given sequence of amino acids either does not uniquely determine the structure, or does not uniquely determine the location, of the carbohydrate attached to the peptide chain. These findings extend, by entirely different techniques, those reported of carbohydrate heterogeneity in an IgA myeloma globulin (*7*).

Despite the observation that small amounts of IgG are precipitated by concanavalin A, the preparation of IgM is greatly facilitated by a preliminary precipitation of the serum with concanavalin A. The chromatographic behavior of concanavalin A makes it readily separable from IgM and from most other serum glycoproteins. When the dissociated mixture of concanavalian A and glycoproteins is applied to diethylaminoethyl Sephadex at ionic strength above 0.02, in tris buffer at *p*H 8.0, the concanavalian A is not bound. A com-

petitive inhibitor of concanavalin A such as 0.2*M* methyl α-D-glucoside must be added to block recombination of concanavalin A with serum glycoproteins in the column.

MYRON A. LEON
Pathology Research Department,
St. Luke's Hospital,
Cleveland, Ohio 44104

References and Notes

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5. The serum inhibitor of C'1 (first component of complement) esterase was measured by Dr. J. Pensky.
6. I thank Miss Janet Wilson, in Dr. A. Steinberg's laboratory, for performing the Gm titrations.
7. J. R. Clamp, G. Dawson, L. Hough, *Biochem. J.* **100**, 35C (1966). References to earlier papers on IgG carbohydrate are given here.
8. Dr. G. Bernier, Dr. W. D. Terry, and Dr. G. A. Spengler provided the typed myeloma serums. Supported by NIH grant 5-R01-A103104-07.

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Hormone-Dependent Differentiation of Immature Mouse Mammary Gland in vitro

Abstract. *Explants from the mammary glands of 3-week-old mice can be induced to synthesize casein in vitro in the absence of lobuloalveolar development. Maximum biochemical differentiation requires the presence of insulin, hydrocortisone, and prolactin in the culture medium. In contrast to explants from adult mice, the mammary epithelium of immature animals undergoes DNA synthesis and mitosis in vitro in the absence of exogenous insulin; however, such proliferation does not lead to the formation of differentiated daughter cells. Insulin acts in at least two ways during the proliferative phases of the cell cycle of differentiating mammary epithelial cells.*

Mammary gland epithelial cells in explants derived from pregnant or nonpregnant mature mice proliferate, assume a secretory appearance, and begin to synthesize certain milk proteins when cultured in the presence of insulin, hydrocortisone, and prolactin (*1–4*). This functional differentiation of the epithelial cells has been found to be necessarily coupled to their proliferation (*5–7*). Synthesis of DNA and subsequent cell division require only insulin; however, proliferation does not lead to differentiation unless hydrocortisone and prolactin are present in the culture medium. The sequence of action of the hormones in relation to the cell cycle in explants of mammary gland from pregnant mice has been described (*8*).

Although the relationships between hormone-dependent proliferation and differentiation have been characterized in the mature gland, the biochemical behavior of the immature gland in vitro has been less clear. We examined the capacity of the immature tissue to differentiate in vitro and compared the requirements for and responses to the hormones with those of the mature tissue.

Although the onset of "maturity" is variable, 21-day-old female weanling mice of the C3H/HeN strain are termed immature in our report. The glands of the 21-day-old mouse consist principally of branching ducts several cell layers thick with terminal buds. No alveoli are seen in serial sections, nor have any been seen in whole mounts in other laboratories (*9*).

Mammary epithelial cells in immature animals proliferate into the mammary fat; yet, as the animal matures, proliferation virtually ceases and does not resume until pregnancy. We confirmed this by injecting thymidine-H^3 (1 μc per gram of body weight) intraperitoneally and preparing autoradiographs of the mammary gland 24 hours later. Only about 1.5 percent of the epithelial cells from 3-month-old mice were labeled, whereas 18 percent of the epithelial cells from 3-week-old animals were labeled. It is unlikely that the results reflect a difference in pool size, because almost all the labeled nuclei had approximately 35 to 40 grains.

Synthesis of DNA in vitro by mammary epithelial cells from mature mice is completely dependent on the presence of insulin in the culture medium (*5–7*). In contrast, our studies indicate that DNA synthesis in vitro by mammary gland explants derived from immature mice [as reflected by the amount of thymidine-H^3 incorporated during 4-hour periods as previously described (*5*)] is quantitatively independent of the addition of insulin. Since these explants contain large numbers of fibroblasts whose DNA synthesis is insulin-independent, data on total incorporation of thymidine-H^3 must be interpreted with caution. To determine whether such incorporation of thymidine-H^3 into DNA reflected epithelial cell activity, we made autoradiographs of explants exposed to thymidine-H^3 (1 μc/ml) for 72 hours. Similar explants were cultured in the presence of colchicine (0.05 μg/ml) for 72 hours and were examined for mitotic activity.

The duct epithelium, especially the epithelium of smaller terminal branches, was heavily labeled, and mitotic figures were numerous in either the presence or absence of insulin in the cultures. In explants cultured without the addition of hormones, 12 percent of the cells were in metaphase (5000 epithelial cells counted); in those cultured in the presence of insulin, 9 percent were in metaphase (5000 epithelial cells counted); and in those cultured in the presence of hydrocortisone and prolactin, 11.5 percent were in metaphase (1000 epithelial cells counted). In contrast to tissue from mature mice (*5–7*) then, proliferation of mammary epithelial cells from immature animals occurs in vitro in the absence of exogenous insulin. However, proliferation in the absence of exogenous insulin does not lead to functional differentiation.

Figure 1 shows that explants of mammary glands of immature mice can be induced to synthesize the major casein components when cultured in the presence of insulin, hydrocortisone, and prolactin. During the first 24 hours, the synthesis of casein bands 2, 3, and 4 is virtually undetectable, but by the 4th or 5th day these proteins are synthesized at greatly accelerated rates in ratios similar to those observed in tissue from mature animals (*10*). Casein band 1, a more heavily phosphorylated component (*11*), is present from the start and is least affected by culture. This component persists longest in mature tissue cultured in incomplete hormone systems (*10*).

Figure 2 shows patterns of casein production during the 5th day of culture in several media. The full differentiative response is elicited only when the three hormones are present. Of the incomplete systems, only those containing insulin are even partially effective, and insulin alone is as effective as it is in combination with hydrocortisone or prolactin. In the absence of insulin, not even band 1 is sythesized.

The hydrocortisone-prolactin system (Fig. 2), in which proliferation occurs