fibers and the fact that they end almost exclusively in the inner aspect of the inner nuclear layer, we can reasonably expect the majority of the amacrines to be under direct centrifugal control if centrifugal fibers are the only input. The rest could be under indirect centrifugal control through the association amacrines which, according to Cajal, end on these fibers.

To the extent that the centrifugal fibers make different kinds of synapses on different kinds of amacrines, it can be expected that these should be affected in different ways by the centrifugal processes. Similarly, the different kinds of terminals can be expected to function differently.

The abundance of centrifugal fibers and their ordered and complex synapses suggest that their role in the visual processes is to exert localized influences in the retina, affecting in different ways the displaced ganglion cells and the various kinds of amacrines. They would thus constitute a system which would serve to not only enhance or diminish some of the different retinal functions (*7*), but also to do so locally, and perhaps in a different manner, in different retinal areas at the same time.

Although our observations are, in principle, relevant to the visual systems of all vertebrates, we are aware that the role of the centrifugal control of the retinal function may be different in different groups of animals. After all, sense organs are instruments of perception, and as such their functional organization can be expected to vary according to their purpose.

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References and Notes

1. S. R. Cajal, *La Cellule* **9**, 121 (1893).
2. A. S. Dogiel, *Arch. Mikrosk. Anat.* **44**, 622 (1895).
3. S. L. Polyak, *The Retina* (Univ. of Chicago Press, Chicago, 1941).
4. S. R. Cajal, *J. Anat. Physiol.* **32**, 481 (1896); H. R. Maturana, *J. Anat.* **92**, 21 (1958); W. M. Cowan and T. P. S. Powell, *Proc. Roy. Soc. London, Ser. B* **158**, 232 (1963).
5. R. Granit, *J. Neurophysiol.* **18**, 388 (1955); E. Dodt, *ibid.* **19**, 301 (1956).
6. J. I. McGill, *Nature* **204**, 395 (1964).
7. H. R. Maturana and S. Frenk, *Science* **142**, 977 (1963); H. R. Maturana, in *Information Processing in the Nervous System*, R. W. Gerard and J. W. Duytt, Eds. (Excerpta Medica Foundation, Amsterdam, 1964), p. 160.
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Macroglobulin-Producing Plasma-Cell Tumor in Mice: Identification of a New Light Chain

Abstract. *A transplantable plasma-cell tumor in mice produces both a γM-macroglobulin serum protein with a sedimentation coefficient of 17S and a kappa-type urinary protein. The reduced and alkylated macroglobulin, when examined by electrophoresis in acid-urea polyacrylamide gel, had a fast component which migrated in the same position as the urinary protein and also a slow component. These two components, as shown by exclusion chromatography, represent the light and heavy polypeptide chains of the γM-macroglobulin. The aforementioned macroglobulin was antigenically related to that in normal mouse serum.*

The high-molecular-weight γ*M* (*1*) antibodies (also called γ1M-, β2M-, 19*S*γ-, or γ-macroglobulin) have only recently been demonstrated in mice (*2*, *3*). Plasma-cell tumors in mice secrete proteins which are antigenically related to normal immunoglobulin counterparts (*4*), and because they each synthesize a single class of protein these tumors serve as convenient sources of material for study of the immunoglobulins. The mouse immunoglobulin classes thus far studied (γA, γG — Be1, γG — Be2, and γF) are composed of two types of polypeptide chain subunits, light and heavy (*5*, *6*); thus they have a similar pattern of chemical structure to that established for human immunoglobulins and immunoglobulins of other species (*7*). Clausen *et al.* described a transplantable plasma-cell leukemia in mice which was accompanied by high concentrations of macroglobulin in the serum (*8*). This macroglobulin migrates in two bands on acid-urea starch-gel electrophoresis after reduction and alkylation (*9*).

Investigation of the Bence Jones proteins produced in mice with transplantable plasma-cell tumors led to the identification (*10*) of three types based on antigenicity and structure: the light-chain type, the γA type, and the RPC-20 type. Proteins of the first type were related to the light chain most commonly found in γA-, γG-, and γF- myeloma proteins of the BALB/c mouse and are now designated as urinary λ (lambda)- globulins. Proteins of the second group represented combinations of one λ-chain and one γA- or heavy α-chain (*5*). The third (RPC-20) type was not immunologically or structurally related to the λ-chain or to any known heavy chain (*10*). The molecular weight of the RPC-20 urinary protein was 24,000 (*11*), which is in the size range of light-chain proteins. Three urinary proteins similar to the RPC-20 protein in antigenic and tryptic peptide structure have since been found, and this group is now designated κ (kappa). We now report a γM-macroglobulin, produced by a transplantable plasma-cell tumor in the BALB/c mouse, which has a light chain related to the κ-type of urinary globulins.

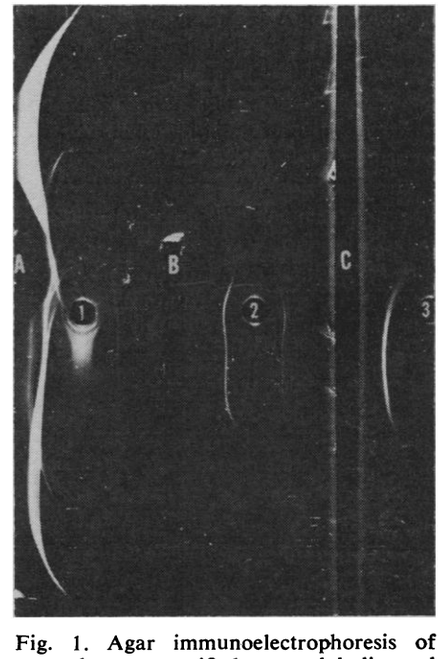

Fig. 1. Agar immunoelectrophoresis of normal serum, purified macroglobulin, and urinary κ-protein. Trench A contains rabbit antiserum against whole mouse serum, B contains rabbit antiserum against the MOPC-104 macroglobulin, and C contains rabbit antiserum against the κ-type protein (no cross reactivity with λ-type protein). Well 1 contains pooled serum from C57BL mice immunized with ovalbumin, well 2 contains purified MOPC-104 macroglobulin, and well 3 contains the urinary protein produced by MOPC-104.

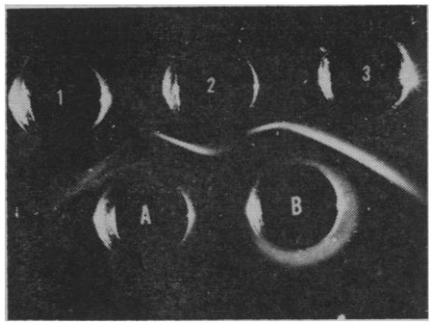

Fig. 2. Precipitin reactions (Ouchterlony technique) showing relation of the γM-macroglobulin associated with the MOPC-104 tumor to macroglobulin from a normal mouse and to the urinary κ-protein. Well A contains rabbit antiserum against the MOPC-104 macroglobulin and well B contains rabbit-antiserum against κ-protein. Well 1 contains serum of normal, immunized C57BL mice; well 2 contains purified MOPC-104 macroglobulin; and well 3 contains urinary κ-protein. There are reactions of identity between the γM-macroglobulin of normal mice and the purified macroglobulin and also between κ-protein and the tumor macroglobulin, indicating the presence of κ-polypeptide chains in the tumor macroglobulin.

One of the tumors (MOPC-104) producing a urinary κ-protein also produced an anomalous serum protein which migrated electrophoretically with γ-globulins. When examined by agar-gel immunoelectrophoresis the protein did not react with specific antiserums against mouse γF-, γG-, and γA-immunoglobulins, but it did react with antiserum specific for mouse γM-macroglobulin (*12*) and with antiserum specific for κ-protein (Fig. 1).

The tumor macroglobulin was purified from serum by a combination of zone electrophoresis with a supporting medium of Pevikon particles and gel filtration on Sephadex G-200 (*13*). The purified protein, at a concentration of 10 mg/ml, contained no contaminating γF-, γG-, or γA-globulins as measured by an immunological method capable of detecting 0.02 mg/ml (*14*). By means of the Ouchterlony technique, the tumor macroglobulin gave reactions of identity with urinary κ-protein and a serum protein, presumably γM-macroglobulin, of hyperimmune normal mice (Fig. 2). The purified protein (7.5 mg/ml), analyzed in a Spinco model E ultracentrifuge, was monodisperse, with a sedimentation coefficient ($s_{20,w}$) of 17*S* (Fig. 3).

The purified serum and urinary proteins from mice with tumor MOPC-104 and another urinary κ-protein from mice with RPC-20 were reduced with 0.1*M* dithioerythritol (*15*) in 7*M* guanidine at *p*H 8.2 and alkylated with 0.11*M* iodoacetamide (*16*). The reduced and alkylated γM-macroglobulin was then passed through a column of polyacrylamide (P-200) beads (*6*). A clear separation into two components differing in size was obtained. Electrophoresis on acid-urea polyacrylamide gel at *p*H 4.0 also dissociated the reduced and alkylated γM into two distinct components. The slower one had the same electrophoretic mobility as the larger (heavy chain) from the polyacrylamide column, and the faster component had the same mobility as the smaller component from the polyacrylamide column and the urinary proteins (Fig. 4).

To determine if the anomalous protein was synthesized by the tumor, incorporation of C^{14}-labeled amino acid into proteins was examined (*17*). Tumor and other tissues were minced and incubated in roller tubes for 24 hours in a medium containing C^{14}-lysine and C^{14}-isoleucine. Concentrated culture fluids were examined by radioimmunoelectrophoresis with both specific and polyvalent antiserums to mouse γ-globulin. Intensely labeled γM was found only in the tumor cultures. Other lymphoid tissues produced relatively little γM; γF, γG, and γA were not labeled in the tumor culture.

The MOPC-104 tumor yields, in quantity, a mouse γM-protein that is relatively pure as judged by acrylamide-gel electrophoresis, ultracentrifugation, and agar-gel immunoelectrophoresis.

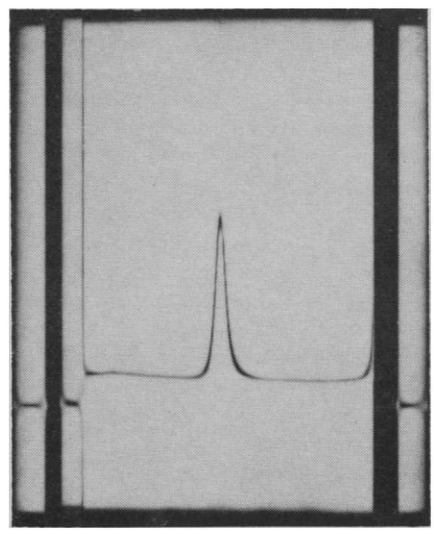

Fig. 3. Ultracentrifuge pattern of isolated MOPC-104 macroglobulin. The exposure was made 66 minutes after a speed of 37,020 rev/min was reached. The solvent was 0.125*M* ammonium bicarbonate, *p*H 8.2.

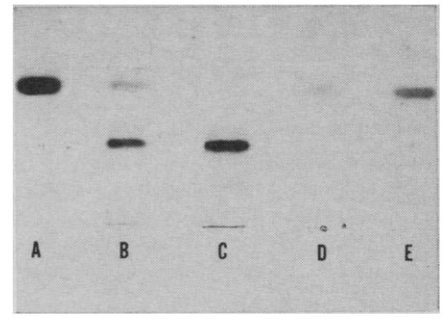

Fig. 4. Horizontal acrylamide-gel electrophoresis of (*A*) reduced and alkylated RPC-20 urinary κ-protein; (*B*) MOPC-104 γM-macroglobulin; (*C*) isolated heavy chain of γM; (*D*) isolated light chain of γM; and (*E*) MOPC-104 urinary κ-protein. The gel was equilibrated in 8*M urea*, 0.1*M* acetic acid, and 0.0014*M* β-mercaptoethanol.

The light chain of the γM is secreted by the tumor and excreted as a urinary κ-protein, a situation similar to that described for human macroglobulinemia (*18*).

The heavy chains of the mouse immunoglobulins have structural and antigenic differences among the different classes (*3, 6*), but heretofore only one type of light chain has been identified in the classes studied (*5*). The light chain (κ), which we have demonstrated only in γM-macroglobulin, represents a new class in the mouse.

There are two types of light chains in human immunoglobulins (*19*). These are controlled by two genetic loci both of which function in any individual so that the resulting immunoglobulin population (including the γM-macroglobulin) contains both types. However, only one type is identified in a single myeloma protein (*20*), indicating the selection of a genetic locus in the control of light-chain synthesis. That there are two different types in mice now makes possible an analogous selection of light-chain for combination with a selected heavy-chain type (*6*). The evidence thus far indicates that the κ-chain is selected infrequently in comparison to the λ-chain (*10*). The identification of a new type of light chain means that there are at least seven structural genes (those controlling the heavy chains of γA, γF, γG — Be1, γG — Be2, and γM and the light chains λ and κ) which participate in immunoglobulin synthesis in mice.

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References and Notes

1. The nomenclature used is that recommended for human immunoglobulins by C. R. Ceppelini *et al.*, *Bull. World Health Org.* **30**, 447 (1964), which has been applied with modification to mouse globulins by R. Lieberman, S. Dray, M. Potter, *Science* **148**, 640 (1965).
2. J. L. Fahey and J. H. Humphrey, *Immunology* **5**, 104 (1962).
3. J. L. Fahey, J. Wunderlich, R. Mishell, *J. Exp. Med.* **120**, 223 (1964).
4. M. Potter and E. L. Kuff, *J. Nat. Cancer Inst.* **26**, 1109 (1961); J. L. Fahey, *J. Exp. Med.* **114**, 385 (1961).
5. M. Potter and E. L. Kuff, *J. Mol. Biol.* **9**, 537 (1964).
6. M. Potter, E. Appella, S. Geiser, *ibid.* in press.
7. G. M. Edelman and M. D. Poulik, *J. Exp. Med.* **113**, 861 (1961); F. Franěk, *Biochem. Biophys. Res. Commun.* **4**, 28 (1961).
8. J. Clausen, R. Rask-Nielsen, H. E. Christensen, R. Lontie, J. Heremans, *Proc. Soc. Exp. Biol. Med.* **103**, 802 (1960).
9. J. P. Vaerman, R. Rask-Nielsen, J. F. Heremans, *Protides of the Biological Fluids* (Elsevier, Amsterdam, 1963), p. 99.
10. M. Potter, W. J. Dreyer, E. I. Kuff, K. R. McIntire, *J. Mol. Biol.* **8**, 814 (1964); K. R. McIntire and M. Potter, *J. Nat. Cancer Inst.* **33**, 631 (1964).
11. E. L. Kuff, M. Potter, K. R. McIntire, N. E. Roberts, *Biochemistry* **3**, 1707 (1964).
12. We thank J. L. Fahey for providing specific antiserum to γM-macroglobulin.
13. J. L. Fahey and C. McLaughlin, *J. Immunol.* **91**, 484 (1963).
14. J. L. Fahey and E. M. McKelvey, *ibid.* **94**, 84 (1965).
15. Available from Cyclo Chemical Corp., Los Angeles, Calif.
16. P. Small, in preparation.
17. G. M. Hochwald, G. J. Thorbecke, R. Asofsky, *J. Exp. Med.* **114**, 471 (1961).
18. D. Gross and W. V. Epstein, *J. Clin. Invest.* **43**, 83 (1964).
19. M. Mannik and H. G. Kunkel, *J. Exp. Med.* **116**, 859 (1962); J. L. Fahey, *J. Immunol.* **91**, 438 (1963).
20. J. L. Fahey, *J. Immunol.* **91**, 448 (1963).

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Lymphocytic-Choriomeningitis Virus in Hamster Tumor: Spread to Hamsters and Humans

Abstract. *A passage line of a spontaneous hamster fibrosarcoma is contaminated by the virus of lymphocytic choriomeningitis. Tumors from animals receiving implants when newborn contain high titers of infectious lymphocytic-choriomeningitis virus and complement-fixing antigen, and hamsters receiving implants when weanlings develop high titers of complement-fixing antibody against lymphocytic-choriomeningitis virus. In contrast with the specific reactions of tumorous hamsters to the initiating virus in virus-induced tumors, the development of complement-fixing antibody to lymphocytic-choriomeningitis virus does not depend on the development of tumors. Infant hamsters bearing the tumor have a generalized subclinical infection and seem able to spread virus to other hamsters and to humans.*

During studies to determine whether or not spontaneous hamster tumors can elicit complement-fixing antibody response to specific tumor antigens, comparable with the responses observed with virus-induced tumors (*1*), we found that hamsters bearing transplants of a spontaneous fibrosarcoma developed high-titer complement-fixing antibody to the homologous tumor. However, further study indicated that the reaction was due to lymphocytic-choriomeningitis (LCM) virus, which has been isolated from a number of primary and transplanted tumors of mice (*2*) and guinea pigs (*3*); its occurrence in hamsters, either normal or tumorous, has not been described.

Tissue fragments from the Fortner fibrosarcoma No. 2 (*4, 5*) were transplanted serially in newborn and weanling NIH Syrian hamsters that were free of LCM-virus infection. Serums were obtained from tumor-bearing hamsters that had been implanted as weanlings. Antigens were prepared from tumors that had been transplanted into newborn hamsters. Tumors were collected, and the antigens were prepared and tested in the complement-fixation test (*1*).

The LCM antigen was prepared by inoculation of LCM virus, strain CA 1371, passed through mouse brain (*6*), onto monolayers of kidney cultures from *Cercopithecus* monkeys. Cells and fluids were harvested on the 7th day. The cells were sedimented by centrifugation and resuspended in 5 percent of the original volume of supernatant. Control cells were prepared by a similar technique from the same lot of monkey-kidney cells (*7*). The resulting antigen was standardized in complement-fixation tests with specific guinea pig antiserum to LCM (*8*).

Serums from weanling hamsters bearing the Fortner fibrosarcoma in the first passage in our laboratory contained significant complement-fixing antibody to LCM virus. Sixteen individual serums collected 29 days after transplantation from animals having tumors ranging from 15 to 35 mm in diameter had titers of complement-fixing antibody from 1:20 to greater than 1:160 against both the LCM monkey-kidney antigen and Fortner-tumor antigen (20-percent extract of fibrosarcomas removed from hamsters that had received implants when newborn). Remarkably comparable titers were obtained with both antigens. These same serums gave no reactions when tested with the following groups of antigens: monkey-kidney control, extracts of hamster tumors induced with adenoviruses types 7, 12, and 18; Schmidt-Ruppin strain of Rous-sarcoma virus and simian virus 40; and potent viral antigens for mouse adenovirus, mouse hepatitis virus, Toolan H-1 agent, Kilham rat virus, K virus, polyoma, and simian virus 40.

The Fortner-fibrosarcoma No. 2 antigens from tumors transplanted to newborn hamsters had complement-fixing titers of 1:16 to 1:64 when tested against specific guinea pig antiserums to LCM. None of six serums from normal guinea pigs reacted with the Fortner-tumor antigens. No positive complement-fixing reactions were detected when antigens prepared from seven individual Fortner fibrosarcomas were tested with specific antiserums against the other viral and tumor antigens listed above. No positive reactions with the Fortner-tumor antigen have been detected in 300 serums obtained from normal adult hamsters in the NIH colony (*9*).

Intracerebral inoculation of NIH Swiss mice with extracts from three Fortner fibrosarcomas produced convulsive death, characteristic of LCM infection, in 5 to 10 days. The virus isolated from one of these tumors was designated the Fortner-fibrosarcoma No. 2 LCM virus and was established in serial passage in mouse brains. One tumor extract, from a hamster that had received an implant shortly after birth. had a titer of $10^{6.7}$ mouse median lethal doses per milliliter. Mice inoculated subcutaneously or intraperitoneally with Fortner-tumor antigens or with the Fortner-fibrosarcoma No. 2 LCM virus, passed through mouse brain, uniformly developed complement-fixing antibody titers from 1:40 to greater than 1:80 in 2 weeks; they were immune to intracerebral challenge with a dose of LCM virus, strain CA 1371, that killed all controls in 6 days. Conversely, mice immunized by subcutaneous injection of LCM virus, strain CA 1371, were immune to intracerebral challenge with the Fortner-fibrosarcoma No. 2 LCM virus.

Weanling hamsters inoculated intraperitoneally with cell-free filtrates of Fortner fibrosarcomas developed complement-fixing antibody to LCM mon-