autochthonous tumors are few. The report by Thornes (*3*) of decreased motility and frequent cell death of V_2 carcinoma cells, observed in rabbits treated with dicumerol (*3*), may have relevance to the smaller primary tumors and decreased metastatic spread seen in our study.

JAMES J. RYAN
ALFRED S. KETCHAM
HILDA WEXLER

National Cancer Institute, Bethesda, Maryland 20014

References and Notes

1. R. O'Meara and R. Jackson, *Irish J. Med. Sci.* **391**, 327 (1958).
2. R. O'Meara, *ibid.* **394**, 474 (1958).
3. R. Thornes, in *Endogenous Factors Influencing Host-Tumor Balance*, R. Wissler, T. Dao, S. Wood, Ed. (Univ. of Chicago Press, Chicago, 1966), p. 255.
4. S. Wood, E. Holyoke, J. Yardley, *Proc. Can. Cancer Res. Conf.* **4**, 167 (1961).
5. E. Cliffton and D. Agostino, *Vasc. Dis.* **2**, 43 (1965).
6. D. Agostino, C. Cliffton, H. Cirolami, *Cancer* **19**, 284 (1966).
7. J. Ryan, A. Ketcham, H. Wexler, *Ann. Surg.* **168**, 163 (1968).
8. M. Michaels, *Lancet* **1964-II**.
9. R. O'Meara and M. O'Halloran, *ibid.* **1963-II**; L. Hughes, *ibid.* **1964-II**.
10. V. Laren, B. Mogensen, C. Amris, O. Storm, *Dan. Med. Bull.* **11**, 137 (1964).
11. E. O'Brien, R. Thomas, D. O'Brien, B. Hogan, *Lancet* **1968-I**.
12. G. Klein, *Cancer Res.* **19**, 343 (1959).
13. H. Wexler, *J. Nat. Cancer Inst.* **36**, 641 (1965).

12 August 1968; revised 12 November 1968

Immunologic Enhancement of Tumor Xenografts by Pepsin-Degraded Immunoglobulin

Abstract. *The serums from guinea pigs previously injected with mouse Ehrlich ascites tumor cells were fractionated to obtain γ_2-immunoglobulin. This immunoglobulin was degraded with pepsin to obtain an $F(ab')_2$ fragment. Fresh tumor cells were incubated with immunoglobulin or the fragment and injected into normal guinea pigs. The growth of these cells as tumor xenografts was inhibited by the γ_2-immunoglobulin and enhanced by the $F(ab')_2$ fragment. Similar incubation of tumor cells with normal guinea pig γ_2-globulin or its derived $F(ab')_2$ fragment did not alter subsequent tumor growth.*

Immunoglobulins are classically associated with protective host defense mechanisms. Rabbit immunoglobulin may be cytotoxic for tumor cells in the presence of complement in vitro, but the $F(ab')_2$ fragment prepared by pepsin degradation of the immunoglobulin is noncytotoxic (*1*). The guinea pig $F(ab')_2$ immunoglobulin fragment blocks the cytotoxic activity of intact immunoglobulin (*2*). Also, $F(ab')_2$ immunoglobulin fragments enhance the growth of mouse tumor isografts (*3*) and allografts (*4*). We present evidence that xenografts of mouse Ehrlich ascites tumor cells in guinea pigs are inhibited by γ_2-immunoglobulin, but are enhanced by the $F(ab')_2$ immunoglobulin fragment. We suggest that production of immunoglobulin fragments in vivo could similarly alter a variety of host immune responses.

γ_2-Immunoglobulin was obtained from the serums of guinea pigs previously injected with Ehrlich ascites tumor cells, and the $F(ab')_2$ immunoglobulin fragment was prepared by pepsin degradation (*2*). Normal γ_2-globulin from the serums of normal guinea pigs and its $F(ab')_2$ fragment were obtained in the same manner. The γ_2-immunoglobulin showed a single arc by immunoelectrophoresis when developed with rabbit antiserum to guinea pig serum. In concentrations of 0.06 mg/ml or more, the γ_2-immunoglobulin was cytotoxic for 3×10^6 Ehrlich ascites tumor cells in the presence of guinea pig complement, but was not cytotoxic with heat-inactivated complement. Normal γ_2-globulin and the $F(ab')_2$ fragments from immunoglobulin and normal globulin were not cytotoxic in the presence of complement (*2*).

Seven days after their intraperitoneal implantation into adult male ICR mice, Ehrlich ascites tumor cells were harvested and washed three times with 0.85 percent NaCl solution. Sixty million viable cells, as determined by Safranin O dye exclusion (*2*), were incubated with 1.5 mg of γ_2-globulin or $F(ab')_2$ fragments in the absence of complement in a total volume of 0.5 ml of 0.85 percent NaCl, or with 0.85 percent NaCl alone, for 30 minutes at 37°C. Then 0.4 ml of 0.85 percent NaCl was added, and 20×10^6 cells in 0.3 ml were injected intradermally into the shaved right lower lumbar region of three adult guinea pigs. Tumors were measured along two perpendicular axes, and the arithmetic mean was determined.

Tumors obtained from cells that had been incubated with $F(ab')_2$ immunoglobulin fragments were larger than controls (Fig. 1, A–C). Tumors produced by cells that had been incubated with γ_2-immunoglobulin were smaller than controls. Prior incubation with normal γ_2-globulin or its derived $F(ab')_2$ fragment produced essentially no change in tumor growth (Fig. 1D). Tumors in the $F(ab')_2$ immunoglobulin fragment groups were the last to be rejected (Fig. 1, A and B), although one exceptional tumor in the γ_2-immunoglobulin group showed initial inhibition followed by rapid growth and was not rejected until day 24 (Fig. 1C). In experiment D all tumors were rejected at the same time.

Two inferences can be made from these results. First, during incubation in vitro, γ_2-immunoglobulin combined with tumor cells. After inoculation of the cells, complement of the guinea pig host was fixed by the immuno-

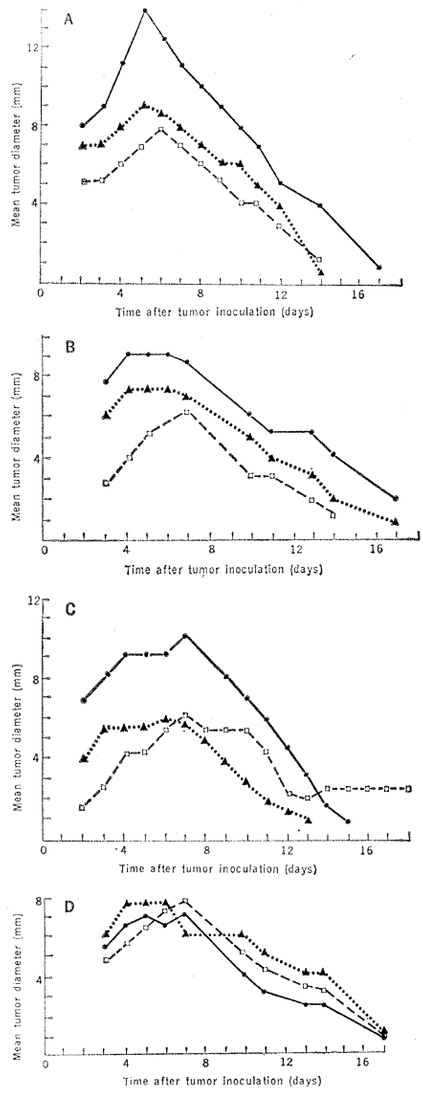

Fig. 1. Immunologic enhancement of mouse tumor xenografts in guinea pigs by pepsin-degraded immunoglobulin. Mouse Ehrlich ascites tumor cells (20×10^6) were incubated with guinea pig γ_2-globulin (□— —□), $F(ab')_2$ globulin fragments (●———●), or 0.85 percent NaCl (▲ · · · ▲) before intradermal inoculation into guinea pigs. Immunoglobulin and its fragment were used in experiments A to C; normal globulin and its fragment in experiment D.

globulin in vivo, with subsequent cytotoxicity and tumor inhibition. It is known that guinea pig γ_2-immunoglobulin binds homologous complement and destroys target cells in vitro (*5*). Antibody plus complement also appears to be important for rejection of renal xenografts in dogs (*6*) and rejection of ascites tumor allografts in mice (*7*). Second, incubation of tumor cells in vitro with $F(ab')_2$ immunoglobulin fragments enhanced subsequent tumor growth in vivo and delayed rejection by blocking antigenic sites. It is known that noncytotoxic $F(ab')_2$ immunoglobulin fragments combine with tumor cell antigens but do not bind complement (*1*); such fragments are divalent (*8*). Combination of tumor antigenic sites with whole immunoglobulin reduces the effectiveness of the host's immune response (*9*).

Regardless of the early effect of γ_2-globulins or $F(ab')_2$ fragments in vivo, tumor size in all groups was decreasing by day 8. Both the protective action of the $F(ab')_2$ immunoglobulin fragment and the inhibitory effect of γ_2-immunoglobulin would be expected to decrease as their concentration at cell surfaces was lowered by cell division.

As shown above, the divalent $F(ab')_2$ fragment from γ_2-immunoglobulin produces immunologic enhancement, probably by blocking antigenic sites. Univalent Fab fragments from mouse γ_2-immunoglobulin may act in a similar manner (*10*). Alternatively, the $F(ab')_2$ immunoglobulin fragment could possibly enhance tumors by stimulating cell growth or increasing resistance to the host, as previously suggested for some enhancing immunogobulins (*11*); $F(ab')_2$ immunoglobulin fragments stimulate incorporation of uridine in vitro by mouse thymus cells (*12*). Immunoglobulin and its $F(ab')_2$ fragment can also induce antigenic modulation of mouse leukemia cells (*13*).

Globulins resembling antibody fragments have been isolated from human serums (*14*), small lymphocytes (*15*), and canine renal allotransplants (*16*). In guinea pigs, $F(ab')_2$ fragments depress immunoglobulin formation (*17*). Theoretically such globulins produced in vivo by synthesis or degradation and possessing the properties of immunoglobulin fragments, could alter host immune responses to pathogens, transplants, and autochthonous neoplasm.

SAMUEL BRODER
FRANK WHITEHOUSE, JR.
Department of Microbiology, University of Michigan, Ann Arbor

References and Notes

1. F. Whitehouse, Jr., and J. Kilduff, *J. Immunol.* **97**, 386 (1966).
2. F. Whitehouse, Jr., and S. Broder, *Proc. Soc. Exp. Biol. Med.* **127**, 1064 (1968).
3. ———, S. Janners, *Bacteriol. Proc.* **1965**, 52 (1965).
4. T. Chard, *Immunology* **14**, 583 (1968).
5. K. Bloch, F. Kourilsky, Z. Ovary, B. Benacerraf, *J. Exp. Med.* **117**, 965 (1963); K. Bloch, *Fed. Proc.* **24**, 1030 (1965).
6. H. Gewurz, D. Clark, J. Finstad, W. Kelly, R. Varco, R. Good, A. Gabrielsen, *Ann. N.Y. Acad. Sci.* **129**, 673 (1966).
7. M. Tsoi and R. Weiser, *J. Nat. Cancer Inst.* **40**, 31 (1968); M. Phillips, U. Rother, K. Rother, *J. Immunol.* **100**, 493 (1968).
8. F. Whitehouse, Jr., and G. Ulrich, *Proc. Soc. Exp. Biol. Med.* **100**, 792 (1959); A. Nisonoff, F. C. Wissler, L. N. Lipman, D. L. Woernley, *Arch. Biochem. Biophys.* **89**, 230 (1960).
9. G. Moller, *J. Nat. Cancer Inst.* **30**, 1153, 1177, 1205 (1963).
10. T. Chard, M. French, J. Batchelor, *Transplantation* **5**, 1266 (1967).
11. M. Kaliss and B. Bryant, *J. Nat. Cancer Inst.* **20**, 691 (1958); M. Feldman and A. Globerson, *ibid.* **25**, 631 (1960).
12. G. Riethmuller, D. Riethmuller, H. Stein, P. Hausen, *J. Immunol.* **100**, 969 (1968).
13. M. Lamm *et al.*, *ibid.* **101**, 99 (1968).
14. T. Lawrence, Jr., and R. C. Williams, Jr., *J. Exp. Med.* **125**, 233 (1967).
15. E. Merler and C. Janeway, *Proc. Nat. Acad. Sci. U.S.* **59**, 393 (1968).
16. P. Kolker, C. L. Hampers, E. Hager, P. Lear, *Transplantation* **6**, 131 (1968).
17. T. Tao and J. W. Uhr, *Nature* **212**, 208 (1966).
18. Aided by Michigan Memorial Phoenix Project 180. S.B. is a fellow of the Life Insurance Medical Research Fund.

1 August 1968; revised 4 September 1968 ■

Sterol Precursors of Cholesterol in Adult Human Brain

Abstract. *Adult human brain contains cholestanol and two series of cholesterol precursors having 30, 29, 28, and 27 carbon atoms; one has an unsaturated steroid nucleus, and the other is unsaturated in both nucleus and side chain. The ability of preparations of brain to incorporate a specific precursor into cholesterol, as well as into these sterol metabolites in vitro, indicates that cholesterol synthesis continues long after brain maturation ceases.*

It is generally assumed that in adult nervous tissue cholesterol is the only sterol present and that its metabolism is negligible after brain maturation. Although the stability of cholesterol in brain can be accounted for by its structural role, some data show that there is some turnover of cholesterol in adult brain (*1*).

Simple radioactive precursors are incorporated into brain cholesterol of mature animals in vivo and in vitro (*2*). Early workers (*3*) detected saturated sterols (cholestanol) and sterols with conjugated double bonds (*4*). Fieser and co-workers detected "Δ^7-sterols," 7-ketocholesterol, and cholestane-3β,5α, 6β-triol in human brain samples fixed in formalin (*5*).

We have identified a number of sterols related to the synthesis of cholesterol in adult rat brain (*6*). The same techniques were used to analyze the sterols in samples of adult human brain. The isolation and identification of two homologous sterol series involved in the biosynthesis of cholesterol in other tissues (*7*) is reported. The intermediary role of these sterols is supported by the incorporation of a specific precursor 2-^{14}C-mevalonate in vitro.

A portion of the anterior region of the right temporal lobe of a 44-year-old female patient undergoing surgery for an aneurism of the posterior communicating artery was obtained at the Neurosurgical Clinic, University of Milan. The time between removal and incubation of the cleaned tissue was 20 minutes, during which the tissue was maintained in cold buffer solution. A 10-g sample of minced tissue was incubated with 20 μc of mevalonic acid-2-^{14}C (*8*) in 100 ml of Krebs-Ringer phosphate buffer, *p*H 7.4, devoid of calcium ions, and in an oxygen atmosphere for 2 hours at 37°C. The procedure used for the extraction of the unsaponifiable material, acetylation of the sterol mixture, and column chromatography on silver nitrate, kieselgel G, and Celite was carried out as described (*9*). The quantities of sterols were determined by gas-liquid chromatography (GLC) and identified by GLC-mass

Table 1. Sterols identified in adult human brain.

Sterol	Amount of total sterols (%)	Amount (μg/g tissue)
C_{30} Δ^8	0.003	0.596
C_{29} Δ^8	.030	5.642
C_{28} Δ^8	.020	3.857
C_{27} Δ^8	.082	15.480
C_{30} $\Delta^{8,24}$	.004	0.708
C_{29} $\Delta^{8,24}$	.019	3.588
C_{28} $\Delta^{8,24}$	.016	3.064
C_{27} $\Delta^{8,24}$	.011	2.157
C_{27} $\Delta^{5,24}$	.020	3.838
C_{27} Δ^5	99.090	18,660.000
C_{27} Δ^{14}	0.020	3.835
C_{27} Δ^5 (7 C=O)	.009	1.626
C_{27} Δ^0	.291	54.750
Unidentified and partially identified compounds	0.384	72.380