# The Correlation between Plasma Cell Tumor Development and Antibody Response in Inbred Strains of Mice* (34016)

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Potter and his associates (1, 2) demonstrated that intraperitoneal administration of mineral oil, or mineral oil plus other antigens, led to the development of plasma cell tumors when BALB/c mice were used. The tumor could be induced by Millipore membranes or plastics in the peritoneal cavity of the same strain of mice (3). Similar treatment of other strains failed to develop such tumors (3–5). It is possible that plasma cell tumors arise in BALB/c mice because this strain of mouse responds to an antigenic stimulus with strong and uncontrolled antibody formation with accompanying proliferation of plasma cells. Support for this concept was obtained from two lines of evidence. BALB/c and A mice developed antibody toward bovine serum albumin more rapidly and to a greater degree than mice which never develop plasmacytomas. The early rise in serum globulin which followed intraperitoneal administration of mineral oil also separated these strains.

The BALB/c mice were obtained from Microbiological Associates, Bethesda, Md. The DBA/2J, C3H/HeJ, C57BL/6J, and A/J mice were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Antibody production was measured after intraperitoneal injections of 0.2 and 0.4 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) in 0.1 ml of saline emulsified with 0.1 ml of Freund's complete adjuvant at 2-week intervals. Blood was collected from the tail at weekly intervals. The antibody titers were assessed by the double diffusion method of Ouchterlong (6). The titer was expressed as the antigen dilution at which equivalence was reached with the test serum.

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The response to mineral oil (Prime Oil 355, white, heavy, USP, Humble & Refining Co., New York, N. Y.) was established by interperitoneal injection of 0.5 ml at 2, 4, and 6 months of age. Blood was collected from the tail after 2 weeks and at monthly intervals thereafter. Serum protein was determined by a refractometric method (National Instrument Co., Inc., Baltimore, Md.). Serum electrophoresis was carried out on cellulose acetate strips (Sepraphore III, Gelman Instrument Co., Ann Arbor, Mich.) with 0.05 *M* barbital buffer, pH 8.6, for 60 min at 175 V. The strips were stained with Ponceau 2R and scanned (Chromoscan J288, Joyce Loebl and Co., Ltd., Gateshead on Tyne, England).

Plasma cell tumors were diagnosed by finding characteristic neoplastic plasma cells in the peritoneal fluid (7) and by the electrophoretic pattern of serum demonstrating a myeloma peak (2).

Purified mineral oil was used in some experiments. When the mineral oil (USP) was shaken with concentrated sulfuric acid, a considerable darkening of the acid took place. On subsequent shaking with acid no further color was extracted. Such oil was washed free of sulfuric acid with water before it was injected into mice.

Figure 1 shows the results of immunization with bovine serum albumin. The BALB/c mice showed an earlier and greater response than C3H, DBA, or C57BL mice. Merwin and Redman (3) reported that C57BL, DBA, and C3H mice failed to develop plasma cell tumors following introduction of diffusion chambers into the peritoneal cavity. However, the A strain of mice showed a response equal to that of BALB/c. This result led us to test the idea that A mice like BALB/c mice would develop plasma cell tu-

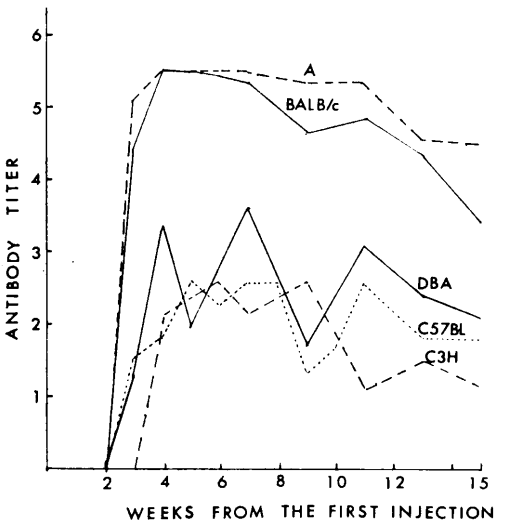


FIG. 1. Antibody production to bovine serum albumin in strains of mice: bovine serum albumin was injected intraperitoneally with Freund's complete adjuvant at 0 and 2 weeks (0.4 and 0.2 mg, respectively). The antibody titer refers to the antigen dilution at which equivalence was reached with the test serum by double diffusion in agar-gel: +6 = 5 mg of bovine serum albumin/ml, undiluted; +5 = 1:10 dilution; +4 = 1:100 dilution, etc. Each point represents the average of 5 female animals.

mors. Accordingly, the five strains of mice were treated with intraperitoneal mineral oil as described above. Total globulin and the electrophoretic pattern in the development of plasma cell tumors were followed.

Figure 2 shows that some of the mice tested developed hyperglobulinemia within 2 weeks of the administration of the first dose of intraperitoneal mineral oil. However, in 2 months a distinct difference on the part of BALB/c and A mice was apparent. When the serum globulin of other strains of mice had returned to normal, the BALB/c and A-mice continued to show a slow but steady rise of serum globulins. During this period careful examination of the peritoneal fluid failed to disclose tumor cells. We have never seen a plasma cell tumor develop within 4 months of the first administration of oil.

Figure 3 illustrates the electrophoretic pat-

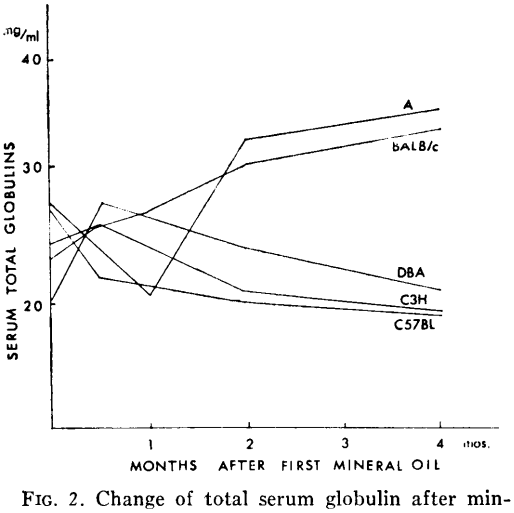


FIG. 2. Change of total serum globulin after mineral oil treatment (USP mineral oil): average of 6–12 mice.

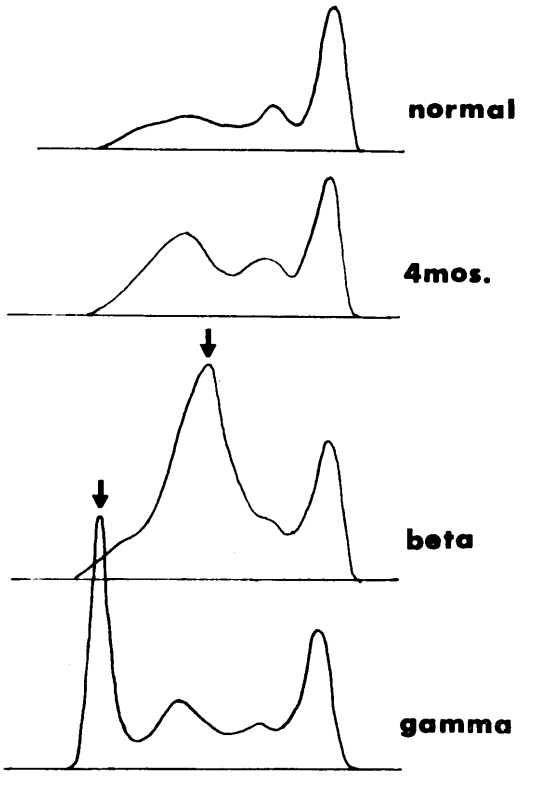


FIG. 3. Electrophoretic patterns of sera from USP mineral oil-injected BALB/c mice: (normal), normal BALB/c serum; (4 mos), 4 months after the first mineral oil injection. Shows increase in all of the globulins. A-mice showed similar pattern of serum protein; (beta), serum from a mouse bearing primary plasma cell tumor (beta$_{2A}$ type); (gamma) same (gamma type). The arrows indicate myeloma protein-peaks.

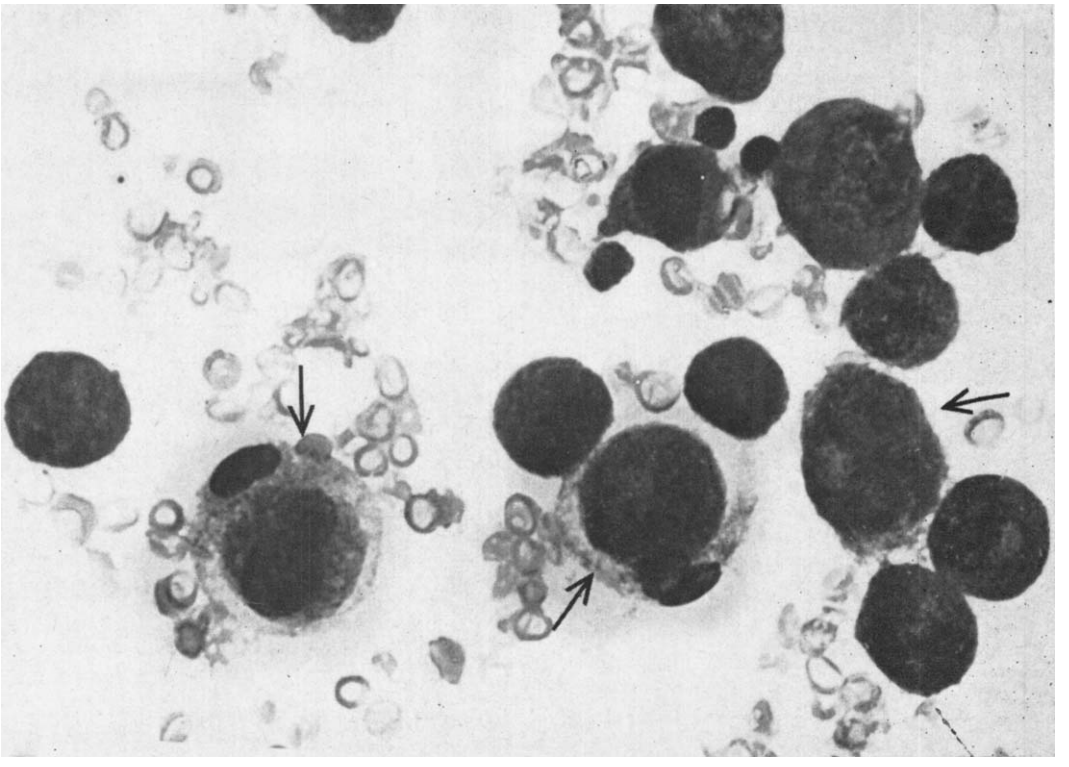

FIG. 4. Ascites smear of A-mouse bearing plasma cell tumor: the arrow indicates phagocytosed tumor cells; Wright–Giemsa staining; 1200×.

tern of serum, 4 months after the first administration of mineral oil in BALB/c mice. The pattern of serum protein at this time shows only a heterogenous increase in all of the globulins. The A-mice showed also similar patterns of serum protein. These mice which eventually develop tumor developed homogeneous globulin peaks at later times as shown in Fig. 3.

TABLE I. Incidence of Plasma Cell Tumors (PCT) in Strains of Mice after Intraperitoneal Injection of USP Mineral Oil.[a]

| Group | Strain | Sex | No. of mice | Incidence of PCT |
|---|---|---|---|---|
| 1 | BALB/c | M | 30 | 28 |
| 2 | BALB/c | F | 30 | 21 |
| 3 | DBA/2J | F | 12 | 0 |
| 4 | C57Bl/6J | F | 8 | 0 |
| 5 | C3H/J | F | 10 | 0 |
| 6 | A/J | M | 10 | 1 |

[a] USP mineral oil (0.5 ml) was injected intraperitoneally at 2, 4, and 6 months of age.

Table I shows the incidence of plasma cell tumors in these animals. The BALB/c mice developed a high incidence of plasma cell tumors. The DBA, C57Bl, and C3H mice failed to develop a single plasma cell tumor. A plasma cell tumor did develop in an A-strain mouse. This low incidence of plasma cell tumor development in A-mice, despite equally good response to antibody formation compared to BALB/c mice, was perplexing. Study of the ascitic fluid of the single A mouse to develop tumor partially explained the discrepancy. Figure 4 shows the ascites smear from the A-mouse which developed plasma cell tumor. Typical neoplastic plasma cells are seen abundantly. However, Fig. 4 also shows fairly frequent phagocytosis of tumor cells by macrophages. The picture is very similar to our finding in BALB/c mice where development of plasma cell tumors was

suppressed by administration of glycoprotein pituitary hormones (8). This frequent phagocytosis of tumor cells is never seen in BALB/c mice unless they receive hormone treatment. In these A-mice, it seems that plasma cell tumors do develop but are subsequently suppressed by the hosts.

In the development of plasma cell tumors, the development of nascent tumor cells on the basis of uncontrolled antibody formation is opposed by a "seek and destroy" phagocytic protective mechanism. The BALB/c mice not only lack antibody control but in the absence of treatment by glycoprotein hormones they also lack this protective mechanism.

*Summary.* The antibody response to bovine serum albumin and the development of hyperglobulinemia following administration of intraperitoneal mineral oil separated BALB/c and A-mice from DBA, C57B1, and C3H mice. BALB/c mice developed many plasma cell tumors following intraperitoneal mineral oil injections. The A-mice developed 1/10 tumors but the peritoneal contents showed extensive phagocytosis of tumor cells. This phenomenon is observed in BALB/c mice only when tumor development is inhibited by administration of glycoprotein pituitary hormones. A hypothesis is developed to explain these observations.

1. Potter, M. and Boyce, R. C., Nature **193**, 1086 (1962).

2. Potter, M. and Robertson, C. L., J. Natl. Cancer Inst. **25**, 847 (1960).

3. Merwin, R. M. and Redmon, L. W., J. Natl. Cancer Inst. **31**, 997 (1963).

4. Potter, M., *in* "Methods in Cancer Research" (H. Basch, ed.), Vol. 2, p. 105. Academic Press, New York (1967).

5. Cohn, M., Cold Spring Harbor Symp. Quant. Biol. **32**, 211 (1967).

6. Ouchterlony, O., Progr. Allergy **6**, 30 (1962).

7. Potter, M. and MacCardle, R. C., J. Natl. Cancer Inst. **33**, 497 (1964).

8. Takakura, K., Yamada, H., and Hollander, V. P., Cancer Res. **26**, 2464 (1966).

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