IMMUNE AND NATURAL ANTIBODIES TO SYNGENEIC MURINE PLASMA CELL TUMORS

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Plasma cell tumors have been induced with high frequency in BALB/c (BALB) mice by the introduction of plastic chambers (1) or mineral oil (2) into the peritoneal cavity. The etiology of these tumors remains unclear. Intracisternal type-A viral particles have been found by electron microscopy in several of the plasma cell tumors (3–5), and one group has recently found C-type viral particles in some of their lines (6). It has not been possible to prove a viral etiology for these tumors, however, since all attempts at induction of the tumors with cell-free extracts have been unsuccessful (4, 5, 7–9). Others have suggested that neoplasia of plasma cells is due to chronic antigenic stimulation (10) or to the immunosuppressive effects of mineral oil (11).

In contrast to the extensive studies on the induction of plasma cell tumors (1–11) and on the proteins produced by these tumors (12), the search for tumor-associated antigens on the plasma cell tumors has received little attention. Some recent studies have indicated that mice can be immunized against subsequent challenge of syngeneic plasma cell tumors (11, 13, 14). The finding of transplantation antigens common to several plasma cell tumors (13) is consistent with a viral etiology for these tumors.

In addition to the in vivo studies, there have been recent reports of antibodies produced in BALB mice by immunization against plasma cell tumors (14, 15). However, the specificity of these reactions was not well defined.

Cytotoxic antibodies have also been produced against BALB plasma cell tumors by immunization of DBA/2 (DBA) mice (16). These antibodies appeared to demonstrate an alloantigen, PC.1, which was present on normal plasma cells and on cells of the liver, kidney, brain, and lymph nodes, as well as on plasma cell tumors.

In the present study, cytotoxic antibodies were produced by immunization of BALB mice with syngeneic plasma cell tumors. An unexpected observation was that the sera of normal BALB mice also contained antibodies reactive with plasma cell tumors. The specificity of the natural and immune syngeneic antibodies was compared with the reported specificity of the allogeneic anti-PC.1

1 Abbreviations used in this paper: B6, C3HBL/6; BALB, BALB/c; BSS, Hanks' balanced salt solution; VEA, viral envelope antigen on MOPC-70A and MPC-113 cells; DBA, DBA/2; FBS, fetal bovine serum; GCSA, Gross cell surface antigen; GMuLV, Gross murine leukemia virus; IEM, immunoelectron microscopy; MEM, Eagle's minimal essential medium; RI, recombinant inbred strain; SBMV, southern bean mosaic virus.
antibody. The presence of naturally occurring antibody reacting with an antigen common to all plasma cell tumors suggested a possible relationship to a virus in these cells. Some evidence which supports this hypothesis is presented here.

**Materials and Methods**

**Mice.**—Male inbred mice were obtained from the Animal Production Branch, Division of Research Services, National Institutes of Health. The recombinant inbred (RI) strains, C × BD, C × BE, C × BG, C × BJ, C × BK, and C × B, derived from the cross of BALB and C57BL/6 (B6) mice by Dr. D. W. Bailey (17) were supplied by Dr. Michael Potter, National Cancer Institute.

**Tumors.**—The transplantable BALB (18) and C3H/He (19) plasma cell tumors were supplied by Doctors Michael Potter, K. Robert McIntryre, and Ruth Merwin, National Cancer Institute. All were maintained in ascites form. Dr. Potter also supplied two oil-induced primary plasma cell tumors of C × BD and C × BJ. The plasma cell tissue culture lines, derived from C3H tumor X5563 (6), were obtained from Dr. Robert Hyman, Salk Institute, La Jolla, Calif. The virus-induced leukemias, RBL-3, RBL-5, MCDV-12, and LSTRA, were supplied by the late Dr. John Glynn and by Dr. Michael Chirigos, NCI. The EoG2 lymphoma in B6, induced by passage A Gross virus, and the transplantable AKR spontaneous leukemia, K36, were obtained from Dr. E. A. Boyse and Miss Gayla Geering, Sloan-Kettering Institute for Cancer Research, New York. The BALB reticulum cell sarcomas, RCS-A, MC-1934, and MC-2103, were produced by Dr. Richard Asofsky, National Institute of Allergy and Infectious Diseases and by Dr. K. Robert McIntire, NCI. The NZB tumors were obtained from Dr. Robert Mellors, Hospital for Special Surgery, New York.

**Antisera.**—Groups of 20–30 mice were immunized against plasma cell tumors, according to the following schedules: (a) eight weekly subcutaneous inoculations of viable MPC-110 or MPC-113 cells, at doses producing tumors in 20% or less of the recipients; (b) four weekly subcutaneous doses of MPC-110 or MPC-113 cells, treated with mitomycin C. 1 × 10⁷ cells were incubated for 3 hr at 37°C with 100 μg of mitomycin C and were then washed twice. This procedure was previously found to prevent tumor growth of as many as 1 × 10⁵ MPC-110 or MPC-113 cells. (c) Tumorigenic doses of plasma cell tumors were inoculated subcutaneously. At various times after immunization, the recipients were bled from the retroorbital sinus under ether anesthesia. The serum of each group was pooled and stored at −70°C until tested.

**Normal Sera.**—Normal BALB and other mice of various ages were bled. The sera from most of these mice were stored as individual specimens rather than as pools.

**Cell Suspensions.**—(a) Spleens, lymph nodes, and thymuses were finely minced with scalpels in a few milliliters of Hank's balanced salt solution ([BSS], Media Production Unit, NIH) supplemented with 20% heat-inactivated (at 56°C for 30 min) fetal bovine serum ([FBS] Grand Island Biological Co., Grand Island, N. Y.). (b) Bone marrow was obtained from the femurs by rinsing of the marrow cavities with approximately 2 ml of BSS-FBS. (c) Tumor cells were obtained by harvesting ascites fluid or by mincing the solid tumor or spleen. The tissue culture cells were harvested by incubation with 0.1% crystalline trypsin for 5 min at 37°C.

Each of the cell suspensions were washed twice in 25 ml of BSS-FBS and then adjusted to the final desired viable concentration in tissue culture medium, Eagle's minimal essential medium (MEM) with 20% FBS. Viability of cell suspensions was determined by exclusion of trypan blue dye.

**Tissue Homogenates.**—Tissues from exsanguinated mice were minced and 25% (w/v) homogenates were prepared with a Teflon tissue homogenizer in MEM-FBS. The protein concentration of the homogenates was determined by the Lowry method (20).

**Assay for Cytotoxic Antibody.**—The method used was described in reference 21. 0.1 ml of ⁵¹Cr-labeled target cells was added to 0.1 ml of serial twofold dilutions of antiserum. After incubation at 37°C for 30 min, 0.1 ml of undiluted rabbit serum was added as the source of
complement and the incubation continued for 30 min. The supernatant from each tube was then counted for released radioactivity. The titer of serum was defined as the reciprocal of the serum dilution producing cytotoxicity twice that of the background controls. The control tubes contained cells alone or cells plus complement and were 5-15% of the maximum amount of $^{51}$Cr released by 3X freezing and thawing of the cells.

Quantitative Determination of Antigen by Absorption of Cytotoxic Antibody.—Absorption studies were performed with the final dilution of serum producing 50-75% lysis. Serial twofold dilutions of 0.1 ml of the cells or tissue homogenates to be tested for antigenicity were incubated with 0.2 ml of serum for 30 min at 37°C. Labeled target cells were then added and the mixtures tested for residual cytotoxic activity. An antigen unit was defined as the number of

| Immunizing cell | No. and condition of cells inoculated | Time of bleeding | Cytotoxic titer against |
|-----------------|-------------------------------------|-----------------|------------------------|
|                 |                                     |                 | MPC-110 | MPC-113 |
| MPC-110         | $1 \times 10^6$, viable*             | 1 wk after 8 weekly doses | 32      | 64      |
| MPC-113         | $1 \times 10^5$, "                | "              | 96      | 128     |
| MPC-110         | $1 \times 10^5$, treated with mitomycin C | 1 wk after 4 weekly doses | 32      | 48      |
| MPC-113         | $5 \times 10^4$, "                | "              | 32      | 64      |
| MPC-110         | $1 \times 10^4$, viable†            | After 4 wks     | 16      | 24      |
| MPC-113         | $1 \times 10^4$, "                | "              | 16      | 24      |
| MPC-86          | $1 \times 10^5$, "                | "              | 8       | 12      |
| RPC-20          | $1 \times 10^5$, "                | "              | 64      | 64      |
| MOPC-28A        | $1 \times 10^5$, "                | "              | 16      | 16      |
| RCS-A§          | $1 \times 10^6$, "                | "              | 64      | 64      |

* Subthreshold dose of cells.
† Tumorigenic dose of cells.
§ Immunization with this reticulum cell sarcoma was performed when it was found that it contained the PC antigen.

Immuneelectron Microscopy (IEM) (22).—Briefly, 5 X 10$^6$ washed test cells were mixed with BALB serum. After washing, the cells were incubated with anti-mouse IgG/anti-southern bean mosaic virus (SBMV) F(ab')$_2$ hybrid antibody, and then with SBMV. The final pellets of viable cells were fixed with glutaraldehyde and osmium tetroxide. After incubation overnight in cold 0.5% uranyl acetate, they were dehydrated and embedded in Epon, as usual. Thin sections were stained with uranyl acetate and lead citrate.

To examine the ability of various tumors to absorb out reactivity by IEM, the undiluted test serum was mixed with an equal volume of packed washed cells, incubated for 30 min on ice, and recovered by centrifugation in the cold. The same absorption procedure was repeated with fresh cells. The absorbed sera were then tested for reactivity in the usual manner.

To control for binding of SBMV due to production of immunoglobulins on the surface of the myeloma cells, the reagent alone was reacted with the cells. To control for reactivity of the mouse sera with other antigens, the GCSA$^{+}$ PC$^{+}$ cells, EL-4, were studied.

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**TABLE I**

Cytotoxic Activity of Antisera Prepared by Immunization of BALB Mice with Plasma Cell Tumors
RESULTS

Cytotoxicity of BALB Immune Sera against Plasma Cell Tumors.—Antisera produced by immunization with syngeneic plasma cell tumors or reticulum cell sarcoma were tested for cytotoxic reactivity against MPC-110 and MPC-113 cells. The results obtained with some of these sera are shown in Table I. Titers as high as 128 were obtained by multiple inoculations of tumor cells. High-titered sera were also obtained from mice with progressively growing tumors.

TABLE II

| Description of serum donors | Type          | Age | No. of mice tested | % of mice positive* | Mean titer† |
|-----------------------------|---------------|-----|--------------------|---------------------|-------------|
|                             | Conventional  | months |                     |                     |             |
|                             | 1             | 20   | 70                 | 3.6                 |             |
|                             | 2             | 40   | 85                 | 5.7                 |             |
|                             | 3             | 40   | 93                 | 6.8                 |             |
|                             | 4             | 30   | 90                 | 7.4                 |             |
|                             | 5             | 10   | 90                 | 6.4                 |             |
|                             | 6             | 20   | 80                 | 5.8                 |             |
|                             | 7-8           | 10   | 80                 | 5.2                 |             |
|                             | 9-12          | 20   | 70                 | 4.6                 |             |
|                             | Germfree      | 1    | 10                 | 20                  | 2.0         |
|                             | 2             | 10   | 50                 | 3.3                 |             |
|                             | 3-4           | 15   | 60                 | 4.1                 |             |
|                             | Germfree, transferred to conventional conditions at 1 month of age | 3 | 10 | 90 | 6.9 |

* Serial twofold dilutions of sera, beginning at 1:2, were tested. Sera were considered negative if the 1:2 dilution did not show reactivity.

† Geometric mean of the titers of the positive sera.

Similar titers were seen against both of the target cells, with the tendency for the results with MPC-113 to be somewhat higher. MPC-113 was therefore used as the target cell for most of the subsequent studies. None of the sera had detectable cytotoxic activity against normal BALB spleen cells.

Cytotoxicity of Normal BALB Sera against Plasma Cell Tumors.—During the studies with the immune BALB sera, it was noted that sera from control mice, which had not been inoculated with plasma cell tumors, had low levels of cytotoxic reactivity. This unexpected and interesting finding was investigated further. Sera from individual normal BALB mice of various ages were tested for cytotoxic reactivity against MPC-113 cells (Table II). Most of the 190 normal BALB mice had positive reactivity, with titers ranging from 2–32. Peak reactivi-
ity was seen in sera of 3–4-month old mice, with frequency of positive reactions and mean titers significantly higher ($P < 0.05$, Student’s $t$ test) than those of the 1-month old and 9–12-month old mice. Some of the older mice were retired exbreeders, but this background did not appear to influence reactivity.

To determine the serial changes in cytotoxic reactivity, a group of 10 1-month old mice were bled every 4 wk for 6 months. Four of these mice had no detectable cytotoxic reactivity when they were 1 month old; all developed reactivity by 4 months of age. Three of the mice, which were previously reactive, lost detectable serum cytotoxicity by the last bleeding.

The finding of cytotoxic reactivity in the sera of most normal BALB, which tended to increase with age, indicated that the reactivity may have been related to sensitization by environmental agents. Therefore, similar studies were performed with germfree mice (Table II). The frequency and amount of reactivity at all ages tested was significantly lower than those of animals raised under conventional conditions. Germfree mice which were transferred to conventional conditions, at 1 month of age, developed reactivity which was indistinguishable from conventional mice.

**Characteristics of the Cytotoxic Reactivity in Normal Sera.**—The cytotoxic reactivity of the normal BALB sera was presumed to be due to natural antibodies. It was important, however, to obtain supportive evidence for this. A pool of normal BALB serum, from 3–4-month old mice with a titer of eight, was used in these studies. The cytotoxic reactions were shown to be dependent on complement. Incubation of the sera with target cells, without the addition of rabbit complement, resulted in no lysis. A variety of procedures known to inactivate or inhibit C' activity was performed with normal rabbit serum before, or during, the incubation with target cells (23). In each case, inhibition of complement activity was associated with loss of cytotoxic reactivity.

To determine whether the material responsible for the cytotoxic activity behaved like an immunoglobulin, assays were performed with serum fractions. The cytotoxic activity was precipitable by 33% saturated ammonium sulfate. Gel filtration of immune BALB serum on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) yielded activity mainly in the 7S region. In the normal BALB serum, activity was also found in the void volume, in those fractions containing IgM. The antibody nature of the cytotoxic activity was further confirmed by its specificity, as indicated by the absorption experiments and by the reaction with the anti-mouse IgG reagent in the IEM studies.

**Cytotoxicity of Sera of Mice from Other Strains.**—The occurrence of natural antibodies reactive with the plasma cell tumors was reminiscent of the natural antibodies to the Gross leukemia antigen (24). With the Gross system, antibodies were only found in some strains of mice. It was of interest to determine whether the antibody reactive with plasma cell tumors also had a characteristic strain distribution. Sera of normal mice from seven different inbred mouse strains were tested for cytotoxic activity against MPC-113 cells (Table III).
All strains were found to be reactive. Initially, sera from young A/He mice were tested and found to be negative. However, sera from older mice gave positive results. With the small number of sera tested from each strain, it is difficult to state whether there are characteristic differences in reactivity among the strains.

**Specificity of Antibodies Reactive with Plasma Cell Tumors.**—A pool of normal BALB serum, which was reactive with MPC-113, was tested against a series of plasma cell tumors, and against other tumor cells and normal BALB cells (Table IV). The serum was cytotoxic against all of the BALB plasma cell tumors, including TEPC-18 and BFPC-3511 which were solid tumors in the first transplant generation. There was no relationship between reactivity and the type of immunoglobulin produced by the tumor cells. The serum also reacted with three reticulum cell sarcomas which did not produce paraproteins. Positive results were also obtained with the C3H plasma cell tumor, X5563, and with the cell line, C1, derived from it. It was of interest, that the cell line, XC1, which did not produce immunoglobulin and lacked detectable virus particles (6), was negative. The serum also did not react against leukemias induced by Moloney or Rauscher virus or against normal BALB cells.

Absorption tests were performed to further demonstrate the specificity of the cytotoxic reaction, and to determine whether an antigen common to all plasma cell tumors was responsible for the observed reactions. All of the plasma cell tumors, except XC1, were able to remove activity (Table V), although there were varying amounts of antigen on the different cells. The BALB reticulum cell sarcomas were positive, but SJL/J and NZB reticulum cell sarcomas had no detectable antigen. The AKR leukemia, K36, had a small amount of detect-
able antigen. Negative results were also obtained with other leukemias, induced by viruses or by carcinogens. The PC.1 antigen has been found on all plasma cell tumors tested and was not detected on other BALB leukemias (16). It was therefore of interest to compare the specificity of the normal BALB serum antibody with the reported specificity of the anti-PC.1 antiserum. The tissue and strain distribution of anti-

| Target cell                        | Cytotoxic titer |
|------------------------------------|-----------------|
| BALB plasma cell tumors             |                 |
| MPC-110                            | 6               |
| MPC-113                            | 8               |
| MPC-80                             | 4               |
| MPC-86                             | 3               |
| MPC-105                            | 2               |
| RPC-20                             | 8               |
| MOPC-28A                           | 8               |
| MOPC-70A                           | 6               |
| MOPC-104E                          | 6               |
| TEPIC-18                           | 2               |
| BFPC-3511                          | 4               |
| BALB reticulum cell sarcomas       |                 |
| RCS-A                              | 8               |
| MC-1934                            | 4               |
| MC-2103                            | 2               |
| BALB leukemias, induced by leukemia virus |           |
| LSTRA                              | 0               |
| MCDV-12                            | 0               |
| BALB normal cells                  |                 |
| Spleen cells                       | 0               |
| Lymph node cells                   | 0               |
| C3H plasma cell tumors             |                 |
| X563                               | 4               |
| Cl                                 | 4               |
| XC1                                | 0               |

* Pool of serum from 20 BALB mice, 3 months of age.

gen reactive with the normal BALB serum was found to be identical with that of PC.1 (Table VI). In BALB mice the antigen was detected on spleen cells and lymph node cells and in homogenates of liver, kidney, and brain. The other positive strains were C3H/He, A/He, NZB, and SJL/J. No antigen was found in spleen cells from DBA, B6, or 129 mice.

Most of these absorptions were performed with tissues from 2-month old mice. A possible explanation for the decreased serum reactivity in older mice was that the antigen content of the tissues increased with age and removed
### TABLE V

Antigenic Activity of Plasma Cell Tumors and of Other Tumor Cells

| Cells used for absorption | Origin of cells                          | Antigenic activity* |
|---------------------------|------------------------------------------|---------------------|
|                           |                                          | Normal BALB/c serum | Immune BALB/c serum |
|                           |                                          | cells/antigen unit  | cells/antigen unit  |
| BALB plasma cell tumors   | Chamber induced                         | 5 x 10⁶             | 1 x 10⁸             |
| MPC-110                   |                                         |                     |                     |
| MPC-113                   |                                         | 1 x 10⁶             | 2 x 10⁶             |
| MPC-80                    |                                         | 1 x 10⁷             | 5 x 10⁶             |
| MPC-105                   |                                         |                     |                     |
| RPC-20                    | Oil induced                              | 1 x 10⁷             |                     |
| MOPC-28A                  |                                         | 5 x 10⁵             | 5 x 10⁵             |
| MOPC-70A                  |                                         | 2 x 10⁶             | 1 x 10⁶             |
| C3H plasma cell tumors    | Spontaneous                              | 2 x 10⁶             | 1 x 10⁸             |
| X5563                     | Tissue culture line derived from X5563   | 1 x 10⁶             |                     |
| C1                        |                                         |                     | >1 x 10⁸            |
| XC1                       |                                         |                     |                     |
| Plasma cell tumors, primary, from RI strains | Oil induced                | 1 x 10⁶             |                     |
| C X BD                    |                                         |                     |                     |
| C X BJ                    |                                         |                     |                     |
| BALB reticulum cell sarcomas | Spontaneous in germfree mice             | 1 x 10⁶             | 1 x 10⁶             |
| RCS-A                     |                                         |                     |                     |
| MC-1934                   | Oil induced                              | 1 x 10⁷             |                     |
| MC-2103                   |                                         |                     |                     |
| S.J.L./J reticulum cell sarcomas (6 primary tumors tested) | Spontaneous               | >1 x 10⁸            |                     |
| NZB reticulum cell sarcomas (2 transplanted tumors tested) | Spontaneous (Gross virus positive)     |                     |                     |
| Mouse leukemias           | Moloney virus induced                    | >1 x 10⁸            | >1 x 10⁸            |
| BALB LSTRA                | Rauscher virus induced                   | >1 x 10⁸            | >1 x 10⁸            |
| BALB/MCDV-12              |                                         | >1 x 10⁸            | >1 x 10⁸            |
| B6 KBL-5                  | Gross virus induced                      | >1 x 10⁸            | >1 x 10⁸            |
| B6 E.G2                   | Benzo(a)pyrene induced                   | >1 x 10⁸            | >1 x 10⁸            |
| B6 EL-4                   | 3-Methylcholanthrene induced             | >1 x 10⁸            |                     |
| DBA L210                  | Spontaneous (Gross virus positive)       | 1 x 10⁸             | 1 x 10³             |
| AKR K36                   |                                         |                     |                     |

* Method: 0.2 ml of serum incubated with 0.1 ml of serial twofold dilutions of cells for 30 min at 37°C. Serum tested for residual cytotoxicity against MPC-113 cells. One unit of antigen = number of cells needed to reduce cytotoxic activity by 50%.

† Normal BALB serum pool, from 3-month old mice, tested at 1:6 dilution.

§ Serum produced by eight weekly injections of 1 x 10⁶ viable MPC-113 cells, tested at 1:80 dilution.
some of the antibody. Absorption was therefore performed with spleen cells from 10-month old BALB mice. No increase in antigen was found; in fact, a decreased quantity was present (Table VI). Spleen cells from 10-month B6 mice had no detectable activity. Therefore it seemed unlikely that absorp-

TABLE VI

| Cells used for absorption | Normal BALB serum | Immune BALB serum | Presence of PC.1 antigen, from (16) |
|--------------------------|------------------|------------------|----------------------------------|
| BALB normal tissues      |                  |                  |                                  |
| Spleen cells             | $1 \times 10^7$ cells | $2 \times 10^7$ cells | +                               |
| Liver homogenate         | 1 mg             | +                |                                  |
| Kidney homogenate        | 0.5 mg           | +                |                                  |
| Brain homogenate         | 0.25 mg          | +                |                                  |
| Lymph node cells         | $1 \times 10^5$ cells | $>1 \times 10^6$ cells | -                               |
| Cells from thymus or bone marrow; erythrocytes | $>5 \times 10^7$ cells | $2 \times 10^7$ cells | +                               |
| Spleen cells from 1-month old germfree mice | $1 \times 10^6$ cells | NT§              |                                  |
| Spleen cells from 10-month old mice | $5 \times 10^7$ cells | NT              |                                  |
| Liver homogenate from 14-day fetuses | 0.5 mg          | NT              |                                  |
| Kidney homogenate from 14-day fetuses | 0.25 mg          | NT              |                                  |
| DBA normal tissues       |                  |                  |                                  |
| Spleen and lymph node cells | $>1 \times 10^5$ cells | $1 \times 10^6$ cells | -                               |
| Homogenates of liver, kidney, and brain | $>5$ mg          | NT §             | -                                |
| C3H/He spleen cells      | $4 \times 10^7$ cells | $2 \times 10^7$ cells | +                               |
| A/He                     | $2 \times 10^7$ cells | $2 \times 10^7$ cells | +                               |
| NZB                      | $1 \times 10^7$ cells | $4 \times 10^7$ cells | +                               |
| SJL/J                    | $2 \times 10^7$ cells | $2 \times 10^7$ cells | +                               |
| B6                       | $>1 \times 10^6$ cells | $>1 \times 10^6$ cells | -                               |
| 129                      | $>1 \times 10^6$ cells | $>1 \times 10^6$ cells | -                               |

* Absorption with 0.1 ml of serial twofold dilutions of cells or tissue homogenate. One unit of antigen = number of cells or amount of protein (mg) in homogenate needed to reduce cytotoxic activity by 50%.

† Cells from 2-month old and 10-month old mice were tested.

§ NT, not tested.

tion of antibody by tissue antigens could account for the decreased serum activity.

Since the sera of germfree BALB mice were less reactive against the plasma cell tumors, it was possible that the tissues lacked the antigen. However, antigen was detected in the spleen cells of 1-month old germfree BALB mice, although
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in lower quantity compared with conventional 1-month old mice. Tissues from 14-day BALB fetuses were also antigen positive.

As a further test of whether the natural BALB antibody and the anti-PC.1 antiserum both reacted with PC.1 antigen, absorption studies were done with spleen cells from RI strains (17). These strains were derived from a cross between BALB (PC.1+) and B6 (PC.1-) strains and had been previously typed for distribution of PC.1 antigen (Itakura, unpublished observations). The absorption studies gave the same pattern as that obtained for PC.1 (Table VII), Dr. Michael Potter has been able to induce plasma cell tumors in C × BD, C × BG, and C × BJ mice (unpublished observations). Two primary tumors were tested for absorption with the normal BALB serum (Table V); both had activity.

When absorption studies were performed with one of the BALB antisera

| Strain  | H-2 type (If) | Antigenic activity | Presence of PC.1 antigen* |
|---------|---------------|--------------------|---------------------------|
| C × BD  | d             | 2.5 × 10⁷           | +                         |
| C × BK  | b             | 5.0 × 10⁷           | +                         |
| C × BE  | b             | >1 × 10⁸            | -                         |
| C × BG  | b             | >1 × 10⁸            | -                         |
| C × BH  | d             | >1 × 10⁸            | -                         |
| C × BI  | b             | >1 × 10⁸            | -                         |
| C × BJ  | b             | >1 × 10⁸            | -                         |

* Data of Dr. Itakura (unpublished).

produced by immunization with plasma cell tumors (anti-MPC-113 viable cells), a similar pattern of results was obtained (Table V). Activity against MPC-113 cells was removed by all of the plasma cell tumors tested and by normal BALB and A/He spleen cells. The AKR leukemia, K36, had some antigenic activity. Other tumor cells and DBA and B6 spleen cells gave negative results.

Absorption studies were also performed with pooled serum from 3-month old B6 mice to determine whether it had the same specificity as the antibody in the BALB sera. The same pattern of absorption was found. Activity against MPC-113 cells was removed by MPC-113 and MOPC-70A and by normal spleen cells. The B6 leukemia Eo G2 and B6 spleen cells had no detectable activity.

Intracisternal A particles have been observed by electron microscopy in plasma cell tumors (3–5) and in thoracic duct lymphocytes (25) of BALB mice. It was of interest to determine whether there was a direct relationship between...
these particles and the antigen under study. 1 mg of isolated A particles (9) was obtained from Dr. E. Kuff, National Cancer Institute. This preparation was unable to remove cytotoxic activity from the normal BALB serum pool.

**Immunoelectron Microscope Studies.**—In previous studies by IEM the typing serum for PC.1 was shown to contain antibodies to virus-related antigens in addition to antibody to PC.1 (22). The antiserum reacted with GCSA and PC.1, and with a new viral envelope antigen, xVEA, on the virus in MOPC-70A cells. Since the antiserum was produced by immunization with MOPC-70A, it was possible that the detected viral envelope antigen was particularly associated with that tumor cell rather than widely distributed on other plasma cell tumors or in the environment. The pooled serum of untreated BALB mice, which had been shown to have cytotoxic antibody to PC antigen, was examined by TEM and compared with the standard PC antiserum (B6 × DBA)F1 anti-MOPC-70A. A summary of the results are given in Fig. 1. The BALB serum reacted with both the viral envelope and the cell surface of MOPC-70A (GCSA+ PC+) (Fig. 2) as well as MPC-113 (GCSA-PC+) (Fig. 3) but not with those of E3G2 leukemia (GCSA+PC-) which was induced by passage A Gross leukemia virus (GMuLV). In addition, cells of the transplanted AKR spontaneous leukemia K36 (GCSA+PC+) were labeled with the BALB serum on very small restricted areas of the cell surface, but not on the envelope of wild-type GMuLV. Absorp-
tion of the BALB serum with K36 cells removed reactivity with the MOPC-70A cell surface, but not with the viral envelope. The main difference of these results from those previously obtained with the standard PC.1 antiserum is that the BALB serum did not contain antibody to GCSA. The absence of antibody to GMuLV-related antigens in the BALB serum was confirmed by absorption tests with E\textsuperscript{o}\textsuperscript{G2} cells. No removal of activity with the surface of MOPC-70A virus and cells was seen.

**DISCUSSION**

The present study confirmed the previous observation that murine plasma cell tumors contain common cell surface antigens which can be detected by antibodies (15, 16). The finding of common antigens on tumor cells, rather than individually specific antigens, could be due to association with a virus or to the presence of tissue-specific antigens. The present data indicate that the antigen is not really tumor specific, since it was found in some normal tissues as well as plasma tumor cells. This does not, however, rule out an association of the antigen with a virus, since leukemia virus-related antigens have been found in normal tissues (26, 27).

The distribution of the antigen reactive with the immune and natural antibodies was identical with that reported for the PC.1 antigen (16) and presumably the two antigens are the same. The antisera to PC.1 were produced by immunization of an antigen negative strain, DBA with a BALB plasma cell tumor. No tests with normal DBA or BALB sera were reported (16). Yamada et al. (14) obtained negative results with normal BALB sera. Our finding of cytotoxic reactivity of normal BALB and other sera was unexpected. It is likely that positive results were obtained because of the use of undiluted rabbit serum as the source of complement, which has been shown to be more effective than guinea pig serum for lysis of murine nucleated cells (28, 29). The strict dependence on complement, the fractionation of the cytotoxic factor with immunoglobulins, the reactivity with anti-mouse IgG reagent in IEM, and the specificity of the reactions strongly indicate that natural antibodies were responsible for the activity.

The finding of widespread natural antibodies to PC.1 needs to be accounted for. To our knowledge, this has not been observed with the major histocompatibility antigens or other alloantigens. The presence of antibody in the sera of both PC.1\textsuperscript{+} and PC.1\textsuperscript{-} strains was particularly surprising. The absorption studies indicated that the antibodies in normal BALB and B6 serum had the same specificity. One possible explanation is that the antibodies were evoked by an environmental agent or by an occult antigen in the PC.1 negative strains. Sensitization by exposure to an environmental antigen, even in the PC.1\textsuperscript{+} mice, could explain most of the findings. Germfree BALB mice had lower reactivity than conventional mice. This reactivity rapidly increased after the transfer of germfree mice to conventional conditions. The presence or absence of antigen
Fig. 2. BALB myeloma MOPC-70A cells were reacted with the serum from untreated BALB mice and labeled with SBMV. (a) The entire surface of extracellular C-type viruses was labeled with SBMV, × 100,000. (b) Very small restricted areas of the cell surface were labeled with SBMV, × 80,000.
Fig. 3. BALB myeloma MPC-113 cells were reacted with the pooled serum of untreated BALB mice and labeled with SBMV. $\times$ 70,000. Amount of surface antigen is larger than that on MOPC-70A cells (cf. Fig. 2 b).
in tissues of the animals did not appear to have an important role in the time of appearance or the titer of antibody. It is possible that the exogenous route or the amount of exposure may be important factors in the development of antibodies against the PC.1 antigen. Inoculation of BALB mice with plasma cell tumors resulted in higher titers than those found in any normal sera. The decline in natural antibody titers with age could not be accounted for by absorption by an increased level of tissue antigen. The spleens of 10-month old BALB mice did not contain as much antigen as those of younger mice, and antigen did not appear in the spleens of 10-month old B6 mice.

It seems likely that there are one or more viruses closely associated with plasma cell tumors. Several electron microscope studies have shown virus particles in plasma cell tumors (3-6). Gross leukemia virus-related antigens have been found in some plasma cell tumors (22, 30, 31). However, other plasma cell tumors lack the Gross specific cell surface antigen but have the GCSA(h) antigen, which appears to be common to tumors infected by all murine leukemia viruses (31). The MOPC-70A tumor contains GCSA and reacts with sera from aged NZB mice, indicating the probable presence of the Gross viral envelope antigen (22). The IEM studies indicate that the virus in this tumor also contains a viral envelope antigen, VEKA, distinct from that of Gross leukemia virus (22). Several possible explanations could be offered for these findings. Plasma cell tumors may contain different variants of Gross leukemia virus, with expression of some or all of its associated antigens. An alternative explanation is that the presence of Gross virus-related antigens in some plasma cell tumors is due to chance contamination by the virus, which appears to occur with other types of tumors (26). A third, but somewhat unlikely, possibility is that the virus of MOPC-70A is a hybrid between GMuLV and the virus associated with VEKA. A reasonable hypothesis to account for the natural antibodies to PC.1 and the virologic data is that there is a virus, distinct from the previously described leukemia viruses, which induces the formation of PC.1 antigen. The finding of PC.1 antigen in some normal tissues could be due to the presence of the latent virus. Aaronson et al. (32) have recently provided evidence to support the hypothesis of latent virus in some normal BALB cells.

If PC.1 antigen is indeed induced by a virus, the characteristic strain distribution of the antigen and the results of the immunogenetic studies (16) must be explained. As Boyse and Old (33) have discussed in regard to TL antigen, and as predicted by the Huebner and Todaro oncogene theory (34), integration of viral genetic information into the cell genome of some strains is a possibility. Alternatively, host genetic factors could determine the stable persistence of virus or expression of latent virus in normal cells. It is possible that this virus is intimately associated with the induction of plasma cell tumors. Oils and plastic chambers might activate latent virus. The RI strains are of interest in this regard. Potter has successfully induced plasma cell tumors in the PC.1- strains, C × BG, and C × BJ. However, a plasma cell tumor arising
in C × BJ was found to contain the PC.1 antigen. This indicates that even PC.1⁻ strains have the information needed to code for antigen expression. This observation is similar to the appearance of TL antigen in some leukemias of TL⁻ strains (33).

The relationship of the PC.1 antigen to the intracisternal A particles remains unclear. Antigen was not found on isolated A particles, but this was not surprising since PC.1 is a cell surface antigen rather than a viral envelope antigen. It will be of interest to determine whether isolated A particles can induce PC.1 antigen in negative strains.

There is precedence for the occurrence of natural antibodies to murine tumor viruses. Aoki et al. (24) have found antibodies to Gross leukemia virus–induced cell surface antigen in the sera of many normal mice. There are some major differences, however, between the Gross system and the plasma cell system. Antibody to Gross antigen was only found in strains with a low incidence of spontaneous leukemia, and the antibody was found mainly in old mice. IEM showed reactivity with Gross antigen in sectors on the cell surface but not with virus particles (35). In contrast, antibody to PC.1 antigen was present in the sera of all strains examined, including those with a high incidence of inducible plasma cell tumors. Peak antibody activity was found at 3–4 months of age and then declined in later life. IEM study of normal BALB serum showed reactivity with viral envelope as well as cell surface antigen. The biological role of the natural antibody to PC.1 and the reasons for its differences from Gross antibody remain to be determined.

SUMMARY

Cytotoxic antibody to a plasma cell tumor antigen was produced in syngeneic BALB mice by immunization with viable or inactivated plasma cell tumors. Antibody with the same specificity was found in the sera of normal BALB and other strains of mice. This natural antibody reacted with an antigen with characteristics indistinguishable from the previously described alloantigen, PC.1, and with viral envelope antigen, xVEA. The incidence of cytotoxic reactivity and the antibody titers reached a peak in normal BALB mice at 3–4 months of age, and were lower in 9–12-month old mice. The sera of germfree mice had lower reactivity; but when the mice were transferred to conventional conditions, their sera soon became as active as those of conventional mice.

A virus common to all plasma cell tumors, which is present in latent form in some normal tissues of BALB and other PC.1 positive strains, is suggested as the cause for the PC.1 antigen and for the appearance of natural antibody to it. The considerable evidence for the close association of a virus with plasma cell tumors is presented.

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