Humoral Immunosuppressive Substance in Mice Bearing Plasmacytomas

Abstract. *The mechanism by which plasmacytomas (PC) depress the primary immune response to sheep red cells was investigated by determining the ability of normal spleen cells to produce antibody when enclosed in Millipore chambers and implanted in PC-bearing mice. Chamber-enclosed normal spleen cells implanted in PC-bearing mice responded poorly to the sheep red cells when compared to similar cells enclosed in chambers and implanted in normal mice or in mice with other lymphoid and nonlymphoid tumors. The data suggest that PC-induced immune suppression is mediated by a humoral factor.*

The immune response is frequently impaired in animals and man bearing various malignancies. The humoral and cellular immune responses are both suppressed in mice with some transplantable tumors and in animals with tumors induced by several viruses and chemical carcinogens (*1*). A similar observation has been made in patients with leukemias and lymphomas (*2*). However, the humoral immune response seems to be normal in mice bearing several solid tumors (*3, 4*), and only delayed hypersensitivity is significantly impaired in humans with several types of tumors (*5*).

The immunodeficiency syndrome seen in patients with multiple myeloma and in mice with plasmacytomas (PC) appears to differ from the immunodeficiency described in other malignant states. Patients with myeloma and mice bearing PC both exhibit a severely depressed ability to mount a primary antibody response to any of a number of antigens. But their ability to respond to a secondary challenge of antigen is much less severely and less frequently affected, and the delayed skin reactivity of these patients to several commonly encountered antigens indicates that at least this aspect of their cellular immune response is apparently unaffected (*2, 6*).

The immune response in mice with PC has been extensively studied, and it has been shown that various PC differ in the extent to which they suppress the humoral immune responses of their hosts, but that all PC tested significantly depressed their hosts' ability to mount a primary immune response. For each PC, the degree of immunosuppression is proportional to the size of the tumor borne by the hosts. Previous work suggested that PC-induced immunosuppression cannot be explained on the basis of nutritional competition between neoplastic and normal cells, feedback of the myeloma protein on antibody-producing cells, or replacement of normal immunocompetent cells in the lymph nodes and spleens of host animals with neoplastic plasma cells (*3, 6*).

One hypothesis that remains tenable in light of experimental findings suggests that PC, or normal tissues in PC-bearing mice, produce a substance that shuts off cellular processes leading to the development of antibody-producing cells. To investigate the validity of this hypothesis, we studied the ability of normal spleen cells to mount a primary immune response to sheep red cells (SRC) when enclosed in Millipore chambers and implanted in the peritoneal cavities of normal mice and mice bearing PC or other solid tumors.

Millipore chambers were constructed from Lucite rings, Millipore filters (pore size of 0.1 μm), and Millipore MF cement (*7*). The chambers were prepared as described (*8*) with the following modifications. A double layer of cement was applied when gluing the membranes to the rings to ensure against leaks; horse serum in which the complement was inactivated was added to the medium used both for detoxification of the chambers and for suspension of cells; the chambers were detoxified during the 24 hours prior to their use by soaking them in two changes of medium, with special attention to flushing the inside of the chambers as well as the outside. Chambers were filled with a single-cell suspension of 7×10^7 normal spleen cells and 2×10^7 SRC in a total volume of 0.9 ml. Six days after surgical implantation of the chambers into the peritoneal cavities of the anesthetized mice, the chambers were removed, wiped clean, and opened. The contents of each chamber were transferred to a test tube and mixed with 1.1 ml of 0.5 percent Pronase B (Calbiochem) in 0.02*M* tris-buffered minimal essential medium–Hank's base (Gibco), *p*H 7.2. They were kept, with gentle shaking, at room temperature for 60 to 75 minutes. The tubes were then centrifuged at 1000 rev/min at 4°C for 10 minutes. The supernatant was decanted, and the cell pellet was resuspended in 3 ml of the above-mentioned tris-buffered minimal essential medium. Portions were removed and the cells were counted and assayed for the presence of antibody-forming cells by the Jerne plaque technique (*9*).

Female BALB/c and DBA/2 mice were used, and chamber-enclosed spleen cells were always syngeneic to the chamber host. Mice bearing one of three PC were tested: MPC-11, TEPC-183, and SPQC-11; these tumors produce the following immunoglobulins (Ig): $IgG_{2b}\kappa$, $IgM\kappa$, and $IgG_{2b}\kappa$, respectively. In addition, we tested mice bearing the PU-5 lymphoma (*10*), a thymic lymphoma (RLδ1) (*3*), and a pigmented melanoma (S91) (*3*). All tumors grew as subcutaneous nodules. The chambers were inserted 6 days prior to the time when each tumor would reach an optimal size of 5 to 19 percent of the hosts' body weight. Three groups of mice were used in each experiment: normal or tumor-bearing animals with chambers containing SRC only, normal animals with chambers containing SRC and normal spleen cells, and tumor-bearing animals with chambers containing SRC and normal spleen cells. We never detected nucleated cells or an antibody response in the contents of the chamber that only contained SRC; this indicates that there was no migration of nucleated cells from the hosts into the chambers.

The data shown in Table 1 indicate that the ability of lymphoid cells to respond to antigen was adversely affected when placed in Millipore chambers in hosts bearing PC. The response of chamber-enclosed spleen cells that were implanted in mice bearing MPC-11, TEPC-183, or SPQC-11 (two experiments) was 35, 34, 21, and 21 percent of the response given by chamber-enclosed spleen cells implanted in normal mice.

The data in Table 2 show that the number of antibody-forming cells per chamber was significantly lower in mice bearing PC SPQC-11 (animals 1 to 6, Table 2) than in nontumor-bearing controls (animals 7 to 16, Table 2). There is no correlation between tumor size and the decreased responsiveness of the cells in the chambers,

although previous experiments in vivo had shown that the number of antibody-forming cells per spleen in mice bearing PC was inversely related to the size of the tumors.

To determine whether tumors of lymphoid origin, other than PC, could affect chamber-enclosed lymphoid cells, we studied a lymphoma derived from T lymphocytes (RL♂1) and a lymphoma derived from theta-negative lymphocytes (PU-5). The chamber-enclosed cells from animals bearing RL♂1 and PU-5 gave an immune response to SRC that did not differ significantly from the response of chamber-enclosed cells from control animals (Table 1). To determine whether solid nonlymphoid tumors of mice had a similar effect on the immune response of chamber-enclosed lymphoid cells, normal DBA/2 mice and DBA/2 mice bearing S91 melanoma were used as chamber recipients. There was no significant difference in the response of the chamber-enclosed cells from these mice with and without the melanoma (Table 1).

The recovery of cells from chambers was monitored in four experiments. After 6 days of culture in vivo 3.5 to 7.7 percent of the original cell population was recovered from the chambers. In two out of three studies of PC-bearing mice it was found that a small but statistically significant decrease occurred in the number of recovered cells per chamber from PC-bearing mice compared to the number of recovered cells per chamber from normal mice (Table 1).

Previous experiments had shown that the viability of chamber-enclosed spleen cells decreased during the first 2 days after implantation and thereafter increased as a result of proliferation (*11*). Therefore, the lower number of cells recovered from chambers from PC-bearing mice could be due to the failure of cells to proliferate after the initial period in the chamber when a large proportion of cells die or could be due to a cytotoxic factor in PC-bearing mice. The presence of a nonspecific cytotoxic factor seems unlikely. A cell poison would be expected to affect equally all types of cells, and therefore the ratio of antibody-forming cells to chamber-enclosed cells would be the same in normal and PC-bearing mice. This ratio does differ, however, a finding that supports the concept that the immunocompetent cells within the chambers are failing to proliferate. Previously published data also suggested this hypothesis—that is, that there is a block in the proliferative step leading to antibody synthesis in PC-bearing mice (*3*).

Since the immunodepression is not observed in animals bearing S91 melanoma, RL ♂ 1 thymic lymphoma, or PU-5 lymphoma, we conclude that this humoral factor is not a substance associated with all malignant states or with lymphoid tumors other than plasmacytomas. However, it cannot be determined from these studies whether the humoral substance is being produced by the malignant plasma cells or by nonmalignant tissues in the tumor host which are stimulated by this particular tumor. Nevertheless, the data presented here and those obtained by Sullivan and Salmon (*12*) are consistent with the hypothesis that both nor-

Table 1. Response of spleen cells to sheep red cells in Millipore chambers in normal and tumor-bearing mice. BALB/c mice were used in all experiments except in the study of S91 melanoma in which DBA/2 mice were used. The response of chamber-enclosed DBA/2 spleen cells in normal and tumor-bearing DBA/2 mice was consistently lower than the response of BALB/c cells in chambers in BALB/c mice; N.D., no data collected.

Chambers implanted in mice bearing:	Mice tested (No.)	Average % body weight of tumor*	Cells per chamber recovered [(No. ± S.E.) × 10^6]	*P*	Antibody-forming cells per chamber ± S.E.	*P*
MPC-11 plasmacytoma	6	10.7 (6.2–15.2)	3.2 ± 0.4	< .005	3740 ± 1341	< .02
No tumor	7	0	4.9 ± 0.2		10697 ± 2003	
TEPC-183 plasmacytoma	9	13.4 (5.2–19.5)	N.D.		2473 ± 266	< .001
No tumor	10	0	N.D.		7230 ± 1132	
SPQC-11 plasmacytoma	6	12.5 (10.2–15.1)	2.7 ± 0.3	> .5	2092 ± 809	< .005
No tumor	6	0	2.5 ± 0.1		9840 ± 1753	
SPQC-11 plasmacytoma	6	11.8 (5.5–15.8)	3.6 ± 0.3	< .02	3259 ± 1544	< .005
No tumor	10	0	4.5 ± 0.2		15248 ± 2330	
S91 melanoma	10	13.0 (6.2–24.1)	N.D.		2580 ± 603	> .4
No tumor	8	0	N.D.		3015 ± 567	
RL♂1 thymic lymphoma	14	7.8 (6.2–10.7)	4.7 ± 0.2	> .05	7957 ± 1459	> .3
No tumor	9	0	5.4 ± 0.5		10180 ± 1896	
PU-5 lymphoblastic lymphoma	8†	5.6 (2.0–7.4)	N.D. N.D.		6412 ± 1071	> .8
No tumor	9	0			6807 ± 1012	

* (Weight of tumor/weight of mouse) × 100. The range is given in parentheses. † Generalized metastases in addition to nodular, subcutaneous growth.

Table 2. Immune response to sheep red cells by spleen cells in diffusion chambers implanted in normal mice and mice bearing plasmacytoma SPQC-11.

Animal No.	Tumor* (% body weight)	Cells recovered per chamber (No. × 10^6)	Antibody-forming cells per chamber (No.)
1	5.5	4.32	3940
2	9.1	2.52	520
3	12.2	3.06	3080
4	13.5	3.96	1122
5	15.0	3.96	10420
6	15.8	3.60	476
Mean (1 to 6) ± S.E.	11.8 ± 1.6	3.57 ± 0.27†	3259 ± 1544†
7	0	3.84	3960
8	0	4.85	12180
9	0	3.76	21604
10	0	4.80	19160
11	0	4.14	10880
12	0	4.80	29580
13	0	5.88	16560
14	0	4.86	17500
15	0	4.86	7340
16	0	3.48	13720
Mean (7 to 16) ± S.E.	0	4.53 ± 0.23†	15248 ± 2330†
17‡	0	0	0

* (Weight of tumor/weight of mouse) × 100. † Statistical analysis of this experiment is shown in Table 1. ‡ At time of implantation, diffusion chamber contained 2 × 10^7 sheep red cells, but no spleen cells.

mal and malignant plasma cells produce a soluble substance other than immunoglobulin, which regulates the growth of clones of antibody-producing cells. Because of the large number of malignant plasma cells in patients with myeloma and in mice with PC, a larger quantity of this chalone-like substance might be produced, and this might account for the antibody deficiency syndrome that is so characteristic of this malignant state.

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Single Cell Layered Heart: Electromechanical Properties of the Heart of Boltenia ovifera

Abstract. *The heart of* Boltenia ovifera *(the sea potato) is a tubular structure formed by a single layer of myocardial cells. Electron microscopic studies show that each cell contains a single myofibril located adjacent to the luminal surface of the cell. Electrical and mechanical measurement of a cannulated perfused heart demonstrate that only the luminal membrane is excitable and elicits contraction on depolarization. Calcium and magnesium exert antagonistic effects on tension, and potassium depolarizes the myocardium and produces contractures when the luminal membrane is exposed to various concentrations of these ions. The extraluminal membrane does not respond electrically or mechanically to calcium, magnesium, or potassium, and its potential seems to be effectively "clamped" by the luminal membrane. Functionally, therefore, this heart consists of a single active membrane with the adjacent contractile apparatus.*

Boltenia ovifera is a species of tunicate inhabiting the coastal waters of northern Maine at depths of 60 to 90 m. Secured to the ocean floor by a 20- to 30-cm stalk, the sea potato filters seawater for nutrients through two siphons. Tunicates are classified as chordates and are considered the immediate precursors of vertebrates (*1*).

The heart of the sea potato is 0.3 cm in diameter and 5 cm long, and consists of a straight, valveless tube which runs the length of the animal. It lies enclosed in another fluid-filled tube, the pericardium, to which it is attached by a longitudinal raphe. Light microscopy (serial sections) in our laboratory showed that the myocardium of this animal is formed by a single layer of tightly packed cells which are fused together at the raphe by connective tissue. There is no evidence that nonmyocardial cells exist at any site other than the raphe, as has been reported in other species of tunicates (*2*). Electron microscopic studies (Fig. 1b) indicate that each cell contains a single myofibril located near the luminal surface. The luminal membrane of the cells is lined with an electron-opaque material; in contrast, the extraluminal membrane is smooth and devoid of any covering. Adjacent cells are interconnected by specialized junctions located primarily near the extraluminal surface, which appear to seal the luminal from the extraluminal aspects of the cells. In other species of tunicates, junctions similar in appearance have been demonstrated to meet the criteria for tight junctions (*3*).

The beating myocardium is readily visible as the thick outer tunic of the sea potato is dissected away. The heart can be excised intact inside the pericardium after it has been ligated at both ends. To permit continuous perfusion of the inside and outside of the

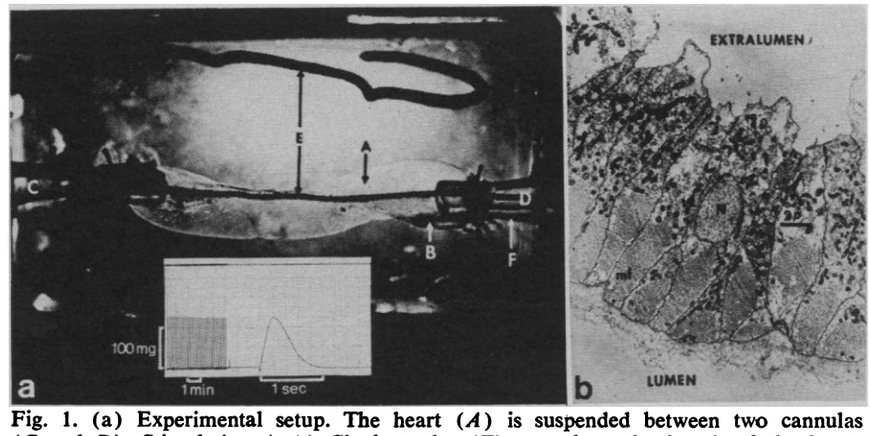

Fig. 1. (a) Experimental setup. The heart (*A*) is suspended between two cannulas (*C* and *D*). Stimulating Ag/AgCl electrodes (*E*) run along the length of the heart, and a tension transducer (*F*) is attached to a pericardial fragment adjacent to the raphe (*B*). (Inset) A typical tension recording at fast and slow time scales. (b) Electron micrograph of the myocardium of the sea potato, which is one cell layer thick. Myofilaments (*mf*) are located along the luminal surface and prominent nuclei (*N*) along the extraluminal surface, with abundant mitochondria scattered throughout.