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Effects of Solid Tumors on the Resistance of Mice to Viral and Bacterial Infections[1] (41327)

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Abstract. Balb/c mice, athymic mice with a Balb/c genetic background, and their heterozygote littermates were used in experiments to evaluate the consequence of developing solid tumors on resistance to viral and bacterial infections. Mice bearing 15-day tumors were challenged with *Streptococcus pneumoniae, Listeria monocytogenes,* herpes simplex virus type 2, or ectromelia virus. The Balb/c tumors employed were a methylcholanthrene-induced fibrosarcoma (Meth A) and a mammary carcinoma (D2-7041): these were shown experimentally to be immunogenic and nonimmunogenic, respectively. The data show that these rapidly developing tumors do not impair the native resistance of either the immunocompetent or athymic strains when compared to non-tumor-bearing mice. Tumor burden imparted an enhanced resistance of immunocompetent mice to *L. monocytogenes* while exerting no measurable effects on the outcome of pneumococcal or ectromelia and herpes simplex virus infections.

There are ample clinical data emphasizing the high risk and increased incidence of infections among cancer patients (1–4). However, analysis of clinical situations in which infections are associated with malignancy is complicated by surgery, chemotherapy, and other supportive medical procedures. It is therefore difficult to evaluate in a clinical setting, the underlying factors which contribute to the apparent increased susceptibility of the tumor-bearing host.

Although it is evident that the cellular immune functions of experimental animals may be impaired by immunosuppressive factors of tumor origin (5–7) or by suppressor cell populations which arise in response to the presence of a developing tumor (8–10), few experimental data are available on the impact of a growing tumor on native host resistance to bacterial and viral infections.

In this report we made use of a murine tumor model in which mice bearing a pronounced tumor burden were challenged with selected bacterial and viral pathogens so that changes in native host resistance could be detected. A methylcholanthrene-induced fibrosarcoma (Meth A) of Balb/c origin was tested in three syngeneic systems utilizing Balb/c, athymic nude mice of Balb/c genetic background, and their heterozygote littermates. Use of the athymic mouse allowed us to determine if the outcome of an infection in genetically immunocompromised tumor-bearing hosts was significantly different than that of immunocompetent individuals. The data to be presented show that the resistance of immunocompetant mice bearing solid tumors to bacterial and viral agents was not impaired where either antibody or T-cell-mediated immunity (CMI) is a requirement for recovery. Furthermore, resistance did not differ appreciably in tumor-bearing as compared with non-tumor-bearing athymic mice.

Materials and Methods. *Mice.* Initial experiments utilized 18- to 20-g female Balb/c mice obtained from Charles Rivers Breeding Laboratories. Athymic and heterozygote female mice with a Balb/c genetic background were obtained from GL. Bomholtgard Ltd. (Denmark). Subsequently, all mice were bred at the University of Cincinnati using defined-flora Balb/c mice obtained from Charles Rivers Breeding Laboratories and defined-flora athymic mice with a Balb/c genetic background

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from Gibco Animal Resources Laboratories (ARS/Sprague–Dawley), Madison, Wisconsin. Athymic mice were bred and maintained under strict isolator conditions and Balb/c and heterozygotes were bred and maintained in laminar air flow barrier systems. Mice were fed autoclaved laboratory chow (5010c, Ralston Purina) and autoclaved water acidified to pH 2.6 with HCl. Animals were routinely monitored for the presence of bacterial pathogens and screened for selected viruses by Microbiological Associates, Bethesda, Maryland. Mice infected for experimental purposes were housed in isolator facilities removed from the breeding colonies.

Tumors. The Meth A fibrosarcoma was obtained from Alan Kaplan, Medical College of Virginia, Richmond, Virginia, and maintained in ascites form by intraperitoneal passage in Balb/c mice. This tumor was originally described as a methylcholanthrene-induced fibrosarcoma of a Balb/c mouse by Old *et al.* (11). Cells were collected from the peritoneal cavity, washed twice in phosphate-buffered saline (PBS), examined for viability by trypan blue dye exclusion, and suspended at an appropriate concentration in PBS for initiating solid tumors. The D2-7041 tumor which arose spontaneously from a hyperplastic alveolar nodule outgrowth (line D-2), syngeneic for Balb/c mice, was obtained from Daniel Medina, Baylor College of Medicine, Houston, Texas. This tumor was adapted to *in vitro* growth and a large batch of cells was grown in basal medium Eagle (BME) with 10% newborn calf serum, 100 μg penicillin and 100 μg streptomycin/ml. These cells were resuspended in the same medium containing 5% dimethyl sulfoxide and stored under liquid nitrogen. Such cells were thawed, washed in BME, and maintained in culture for the tumor cells utilized in experiments. Cells were removed from the culture flasks by treatment with 0.1% trypsin and 0.2% EDTA in PBS. Harvested cells were washed twice in PBS, examined for viability by trypan blue dye exclusion, and suspended to an appropriate concentration in PBS for initiating solid tumors.

Solid tumors were initiated in mice by subcutaneous injection of 0.1 ml of PBS containing 1×10^7 tumor cells into the shaved dorsal surface. Growth of subcutaneous tumors was determined by calculating tumor volumes according to the method of Attia and Weiss (12). Excision of tumors was done under general anesthesia. Blood vessels were cauterized and wounds were closed with clips.

Infectious agents. Listeria monocytogenes strain CRC was obtained from the Clinical Research Center, Middlesex, United Kingdom. For each experiment a uniform stock culture stored in liquid nitrogen was thawed and inoculated into 50 ml of brain heart infusion broth (BHI, Difco). After 3 hr of growth with aeration at 37° the organisms were washed in PBS, adjusted to a concentration of 8×10^8 bacteria/ml, sonicated briefly to reduce clumping, and diluted in PBS to provide the desired challenge inocula.

Streptococcus pneumoniae type 19 strain M (19M) of intermediate virulence for mice was obtained from Robert Austrian, University of Pennsylvania, Philadelphia, Pennsylvania, and the highly virulent *S. pneumoniae* type 3 strain VIR was obtained from Phillip Baker, NIH, Bethesda, Maryland. Cultures from uniform frozen stock were grown overnight on sheep blood agar under reduced oxygen tension. The bacterial mass was used to inoculate BHI broth supplemented with 10% defibrinated rabbit serum. Three- to five-hour cultures were processed as described for *L. monocytogenes*. Animals were challenged by the intraperitoneal route.

Herpes simplex virus type 2, obtained from Ronald Ash, Richardson Merrill Company, Cincinnati, Ohio, was propagated in Vero cell cultures, pooled and stored in frozen aliquots. Virus titers were determined by plaquing on Vero cells (12). Ectromelia virus strain Hampstead (attenuated) was obtained from R. V. Blanden, NAU, Canberra, Australia. The virus was propagated by chorioallantoic membrane inoculation of 9- to 11-day embryonated chicken eggs and titrated by plaquing on Vero cells (13). Titrated virus stocks were stored at −70° and thawed as needed for intraperitoneal challenge.

LD_{50} determinations. Groups of five mice

per dilution were injected with 10-fold dilutions between 10^{-1} and 10^{-7} of the bacterial or virus suspension. After 21 days, deaths and survivors were recorded and LD_{50} values were calculated by the method of Reed and Meunch (14).

Log resistance. Mice were challenged in the retroorbital venous plexus with 2×10^4 *L. monocytogenes*. After 48 hr the entire spleen and approximately 100 mg of the liver were surgically excised, weighed aseptically, and homogenized as a 10% (w/v) suspension in BHI. Dilutions of the homogenate were plated in BHI agar and bacterial colonies were counted 48 hr after incubation at 37°. The relative resistance or susceptibility to listeria infection is expressed as the difference in log of viable bacteria per gram of tissue between infected non-tumor-bearing and tumor-bearing animals (35). This assay provides on accurate index of resistance to listeria infection since the number of viable bacteria in the liver after 48 hr correlates directly with LD_{50} values (36).

Lactic dehydrogenase virus (LDV). Serum from mice to be tested for the presence of LDV was diluted 1:1000 and 0.1 ml was injected intraperitoneally into normal Balb/c mice known to be free of LDV. Serum lactic dehydrogenase was assayed in these mice 3 days after serum transfer by a quantitative procedure (kit No. 340-uv, Sigma Chemical Co.) based on the spectrophotometric method of Wroblewski and LaDue (15). LDV used to prepare positive control serum was obtained from the American Type Culture Collection, Rockville, Maryland.

Results. *Immunogenicity of Meth A and D2-7041 tumors.* In general, chemically induced tumors possess tumor-specific transplantation antigens which impart various degrees of immunogenicity to the tumors (16, 17). A tumor may be defined as immunogenic if after its removal a second inoculation with the homologous tumor is rejected (11, 16). Utilizing this criterion, spontaneously arising tumors characteristically exhibit low immunogenic activity and are thought to lack tumor-specific transplantation antigens (17, 18). Data in Table I confirm the immunogenicity of the Meth A tumor line under our experimental condition. Thus, animals bearing primary Meth A tumors which had been surgically excised were resistant to a second tumor cell challenge at a contralateral site. In contrast, the D2-7041 primary tumor did not induce an effective antitumor immunity since a secondary implantation resulted in tumor growth.

TABLE I. IMMUNOGENICITY OF BALB/C TUMORS AS DETERMINED BY REJECTION OF A SECONDARY CHALLENGE

Tumor type	No. tumors established/No. mice	
	Primary (immunizing)[a]	Secondary (challenge)
Meth A	6/6	0/6
D2-7041	5/5	5/5

[a] Primary immunizing tumors were initiated in mice by sc injection of 10^7 tumor cells and excised 14 days later. One week after primary tumor excision mice were given a secondary challenge with 10^7 homologous tumor cells.

Kinetics of tumor growth. Figure 1A shows the relative growth rates of the Meth A tumor in Balb/c, heterozygote, and athymic mouse strains after subcutaneous implantation. The heterozygote mice behaved as syngeneic hosts although the average time to death of Balb/c mice after tumor implantation was significantly less. The growth of the Meth A tumor in athymic mice was somewhat more rapid than in Balb/c and heterozygote mice and this may reflect the T-lymphocyte deficit of this mouse strain.

Figure 1B shows that the kinetics of growth of the nonimmunogenic D2-7041 tumor in Balb/c mice was indistinguishable from that of the Meth A tumor.

Native resistance of mice to bacterial and viral pathogens. The natural resistance of athymic and immunocompetent mice to the selected bacterial and viral agents was established by LD_{50} titrations and assays for viable organisms in the spleen and liver. The infectious agents were chosen on the basis of the type of protective immunity necessary for recovery from infection or engendered following effective vaccination. Protective immunity to *L. monocytogenes*

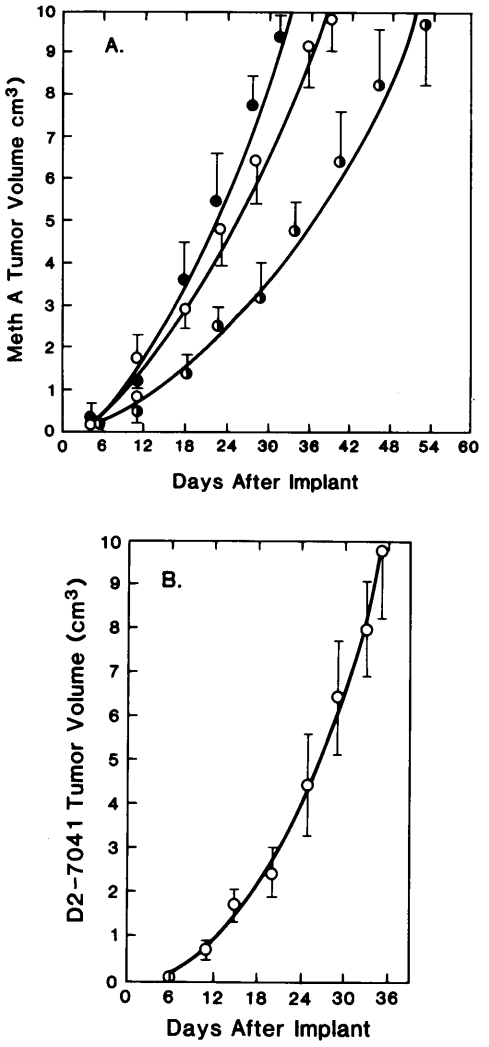

FIG. 1. Development of Meth A (A) and D2-7041 (B) tumors. Subcutaneous tumors were initiated with 1 × 10^7 tumor cells in athymic (●), heterozygote (◑), and Balb/c (○) mice. Eight mice were injected in each group. Vertical lines represent ±standard error of the mean. The mean time to death values for athymic, Balb/c, and heterozygote mice bearing Meth A tumor were 26, 35, and 44 days, respectively. The mean time to death for Balb/c mice bearing the D2-7041 tumor was 37 days.

and ectromelia virus is dependent upon the development of CMI (19). Such immunity is T lymphocyte mediated; the effector cell is the activated macrophage in the case of systemic listeriosis (20) and the cytotoxic lymphocyte for ectromelia virus infection (21, 22). Specific immunoglobulins have no apparent protective function in either of these infections. In contrast, protection against virulent pneumococcal infection is a function of specific anticapsular antibody (23, 24). Resistance to herpes simplex virus infection in mice is primarily dependent upon the development of CMI (25, 26) although antibody-dependent cell-mediated cytotoxicity is effective in destroying HSV-infected cells *in vitro* (27, 28).

Table II provides baseline data on the native resistance of Balb/c, athymic, and heterozygote mice to bacterial and viral agents. Balb/c and heterozygote mice demonstrated essentially the same resistance patterns. The athymic mice, however, responded differently to *L. monocytogenes*. As noted by others, athymic mice possess an anomalous, elevated resistance to some microbial pathogens (29–31) and this is reflected by the significantly higher LD_{50} values for the bacterial pathogen. In the case of ectromelia virus, however, nude mice were significantly more susceptible to lethal infection than were the immunocompetent mice. Comparable resistance of athymic and Balb/c mice to HSV-2 infection was noted. Athymic and heterozygote littermates responded identically to challenge with an unencapsulated strain of *Streptococcus pneumoniae*. Encapsulated pneumococcus type 3 induced lethal infections in both immunocompetent and athymic mice with less than 10 CFU.

Consequence of Meth A tumor burden on resistance to infections. To establish whether or not a rapidly growing solid tumor had a measurable effect on the resistance of mice to these pathogens, LD_{50} titrations were done with mice bearing 15-day Meth A tumors. In this regard, Table III reveals several interesting facts. Rather than impairing the resistance of immunocompetent mice to *L. monocytogenes*, a facultive intracellular bacterium, the Meth A tumor enhanced resistance by 1.0 log unit. This can be attributed to an activated macrophage system elicited by the tumor (32, 33). LD_{50} values in normal and tumor-bearing Balb/c and heterozygote mice challenged with encapsulated or unencap-

TABLE II. NATIVE RESISTANCE OF ATHYMIC AND IMMUNOCOMPETENT Balb/c MICE TO BACTERIAL AND VIRAL AGENTS

Pathogen and route of challenge	LD_{50}[a]		
	Balb/c	Heterozygote	Athymic
S. pneumoniae type 3 (ip)	<10	<10	<10
S. pneumoniae 19M (ip)	—	3.1×10^6	7.2×10^6
L. monocytogenes (iv)	3.9×10^4	1.8×10^4	1.9×10^5
Herpes simplex virus type 2 (ip)	4.2×10^3	3.7×10^3	5.4×10^4
Ectromelia virus (Hampstead strain) (ip)	6.3×10^5	—	$<1.0 \times 10^4$

[a] Groups of five mice received 10-fold dilutions of infectious agents. LD_{50} values were calculated 14 days after infection. Values represent colony-forming units for the bacterial species and plaque-forming units for the viruses.

sulated strains of *Streptococcus pneumoniae* were identical, indicating that the presence of a growing tumor was without effect on native resistance to this bacterial agent. Athymic mice bearing 15-day Meth A tumors failed to demonstrate an increased resistance to *L. monocytogenes*. Athymic and immunocompetent mice responded similarly to pneumococcal challenge whether or not they carried the tumor.

Association of Meth A tumor with lactic dehydrogenase virus (LDV). Since mouse-passaged tumors are readily contaminated with the lactic dehydrogenase elevating agent (LDV) (34), the Meth A tumor used in the initial series of experiments and which had been passaged in Balb/c mice weekly was tested for the presence of LDV. As we described in another study (35) it was ascertained that the tumor had inadvertently become associated with LDV. In view of the immunomodulating effects of this virus (34), it became necessary to determine if its presence in the tumor was of significance in the present experiments. The LDV-contaminated Meth A tumor was cured of virus as previously described (35). Balb/c and heterozygote mice bearing 15-day

TABLE III. EFFECT OF 15-DAY METH A TUMOR ON RESISTANCE TO INFECTIONS

Microbial agent	LD_{50}[a]					
	Balb/c		Heterozygote		Athymic	
	Normal	Tumor bearing[b]	Normal	Tumor bearing	Normal	Tumor bearing
S. pneumoniae type 3	<10	<10	<10	<10	<10	<10
S. pneumoniae 19M	—	—	3.1×10^6	$1.5 \times 10^{6*}$	7.2×10^6	$5.2 \times 10^{6*}$
L. monocytogens	3.9×10^4	1.9×10^5	1.8×10^4	$1.8 \times 10^{5*}$	1.9×10^5	2.8×10^5
Herpes simplex virus type 2	4.2×10^3	—	3.7×10^3	$7.9 \times 10^{3*}$	—	—
Ectromelia virus (Hampstead strain)	6.3×10^5	—	1.5×10^6	1.5×10^6	—	—

[a] Groups of five mice received 10-fold dilutions of infectious agents. LD_{50} values were calculated 14 days after infection and expressed as colony-forming units for the bacterial species and plaque-forming units for the viruses.
[b] Tumors were initiated by subcutaneous implantation of 10^7 Meth A cells 15 days prior to challenge. Values identified by an asterisk were derived with LDV-associated tumor.

tumors were tested for resistance to iv challenge with *L. monocytogenes* by determining the numbers of viable colony-forming units in the livers of mice 48 hr after infection. This assay provides an accurate index of host resistance (36) and we have confirmed that the numbers of viable listeria in the liver 48 hr after challenge are directly correlated with LD_{50} values (35). Figure 2 shows that the resistance of the immunocompetent mice bearing either the virus-free or LDV-associated 15-day tumor was increased to the same extent. Thus, it can be concluded that the presence of LDV virus did not effect the development of augmented host resistance to *L. monocytogenes* (32, 33). This is in contrast to the situation immediately following implant of an LDV-associated Meth A tumor where significant impairment of host resistance to infection is manifested (35). Such an initial depression of resistance to listeria challenge is transient, and mice return to a normal resistance pattern by the third day after injection of the virus-associated tumor (34). Resistance to listeria infection over and above the high native resistance of athymic mice to this pathogen (29, 31) was not observed. Figure 2 shows that non-tumor-bearing athymic mice were marginally more resistant than those bearing 15-day Meth A tumors.

Since the stage of tumor development may be an important factor in detecting alterations in host resistance to infection, an experiment comparing the immune status of Balb/c and athymic mice bearing 1-day, 15-day, and end-stage Meth A tumors was carried out using *L. monocytogens* as the challenge agent. Figure 3 shows that the animals bearing 1-day tumors (free of LDV) were not altered in native resistance to the facultative intracellular bacterium. As already noted, the resistance of Balb/c mice with 15-day tumors can be attributed to a nonspecific activation of the macrophage system by the developing tumor (33). Balb/c mice during the terminal stage of malignancy (25–35 days) exhibit normal levels of resistance to challenge with listeria. The waning activated macrophage system when the tumor reaches a large size is consistent with the observations of Spitalny and North (37).

Consequence of D2-7041 tumor burden on resistance to listeria infection. In efforts to determine if the immunogenicity of the tumor was a factor in altering native host resistance to infection, mice bearing the

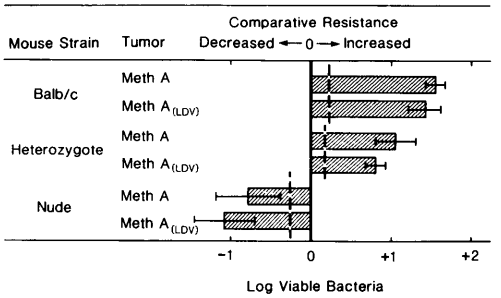

FIG. 2. Resistance of Balb/c, heterozygote, and athymic mice bearing LDV-associated (Meth A_{LDV}) or LDV free (Meth A) tumors. Tumor-bearing and non-tumor-bearing control animals were challenged iv with 2.0×10^4 CFU of *L. monocytogenes* 15 days after tumor implant. Livers of 48-hr-infected animals were excised and assayed for viable organisms. Resistance is expressed as the difference in log viable bacteria per gram between infected non-tumor-bearing and tumor-bearing mice.

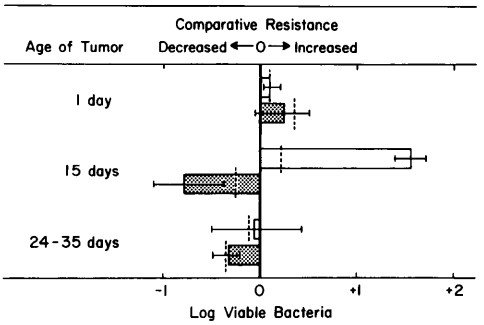

FIG. 3. Resistance of Balb/c and athymic mice bearing Meth A tumor at progressive stages of development. Tumor-bearing and non-tumor-bearing controls were challenged iv with *L. monocytogenes*. Balb/c mice (open bars) were given 2.0×10^4 and athymic mice (stippled bars) given 1×10^5 organisms. Livers of 48-hr-infected animals were excised and assayed for viable organisms. Resistance is expressed as the difference in log viable bacteria per gram between infected non-tumor-bearing and tumor-bearing mice.

nonimmunogenic D2-7041 tumor were also evaluated for resistance to listeria infection. The tumor was free of LDV as determined by serum LDH assays (35). By LD_{50} analysis and quantitative infectivity assays, a 15-day tumor burden in Balb/c and heterozygote mice resulted in enhanced resistance to infection (Table IV). It was therefore concluded that the nonimmunogenic tumor was as effective as the immunogenic Meth A tumor in conferring nonspecific resistance to bacterial infection after 15 days of tumor growth.

Discussion. The concept that neoplastic disease predisposes individuals to infection is generally accepted. While there are ample data to show that cancers involving the lymphoreticular system may significantly modify immune responses (38) the situation with regard to other types of malignant tumors is unclear. Assessing the degree to which individuals bearing solid tumors are compromised by virtue of the malignancy or therapeutic regimen is practically impossible within the context of human medicine. Thus, the experimental model we describe and the data which are presented constitute novel information which may be relevant to the human situation.

TABLE IV. NONSPECIFIC RESISTANCE TO *L. monocytogenes* INFECTION IN MICE BEARING 15-DAY D2-7041 TUMORS

Mouse Strain	Tumor[a]	LD_{50}[b]	48 hr CFU[c]
Balb/c	−	2.5×10^4	5.71 ± 0.25
	+	8.3×10^5	3.29 ± 0.08
Heterozygote	−	5.5×10^4	5.60 ± 0.30
	+	2.3×10^5	3.44 ± 0.11
Athymic	−	—	3.67 ± 0.31
	+	—	3.02 ± 0.30

[a] Tumors were initiated by sc implantation of 10^7 D2-7041 cells 15 days prior to challenge with *L. monocytogenes*.

[b] Groups of five mice received 10-fold dilutions of *L. monocytogenes*. LD_{50} values were calculated 14 days after infection and are expressed as colony-forming units.

[c] Log colony-forming units in the livers of mice 48 hr after iv challenge with 2.0×10^4 CFU *L. monocytogenes*.

The evidence obtained with two murine solid tumors would suggest that the native resistance of mice to bacterial and viral infections is not impaired at an advanced stage of tumor development (15–35 days). The choice of the two bacterial and two viral agents was based on the following rationale. *Streptococcus pneumoniae* infection demands that specific antibody to capsule be present to provide protection. *Listeria monocytogenes* infection is controlled by the development of cell-mediated immunity in which sensitized T lymphocytes and macrophages act as mediator and effector cells, respectively. The two virus agents, ectromelia and herpes simplex are contained primarily by effective cell-mediated immunity although virus-specific immunoglobulins may also play a lesser role in protection. In our experimental model, it would appear that the tumor-bearing individuals are not any more susceptible to systemic infections than are non-tumor-bearing hosts. Furthermore, in situations where resistance is related to an activated macrophage system (19) the presence of a large tumor imparts increased nonspecific resistance to infection (32, 33).

Chemically induced tumors are usually immunogenic while spontaneously arising tumors are not. That the methylcholanthrene-induced tumor (Meth A) and the spontaneous mammary carcinoma (D2-7041) of Balb/c mice tested here fit this pattern was verified experimentally. As the data show, however, the immunogenic and nonimmunogenic tumor did not differ in the way they modulated the native resistance of either immunocompetent (Balb/c and heterozygote) or immunodeficient (athymic Balb/c) mice. The manner in which these Balb/c mouse strains bearing a solid tumor responded to challenge with the selected bacterial and viral pathogens is instructive. The pneumococcal infection, requiring an appropriate antibody response to an antiphagocytic surface component of the organism, was equally severe in tumor-bearing and non-tumor-bearing animals. Since these mice did not possess specific anticapsular antibody against type III pneumococcal

polysaccharide, infected animals invariably succumbed to challenge with the virulent pneumococcal strain. On the other hand, development of a large tumor did not alter host resistance so as to make the relatively avirulent 19M strain more virulent. This was observed with either the immunocompetent or immunodeficient mice challenged with *S. pneumoniae*. In the case of listeriosis where an activated macrophage system is of pivotal importance for the outcome of infection (19) both tumors after 15 days of development enhanced resistance to *L. monocytogenes*. This is consistent with an activated macrophage system arising as a result of considerable antigenic stimulation provided by the growing tumor (32, 33). It is of interest that precisely the same effects were noted whether the developing tumor was immunogenic or nonimmunogenic. Athymic mice did not develop increased resistance to *L. monocytogenes* as did the immunocompetent Balb/c strains bearing 15-day tumors. These data should be viewed within the context that athymic mice possess heightened baseline resistance (as compared to Balb/c) through an activated macrophage system which arises by mechanisms not yet understood (29–31).

The responses of mice to the two viral pathogens were quite different and reflect the unique pathogenesis of ectromelia and herpes simplex virus infections in the murine host. The cell-mediated arm of the immune response is thought to be critical in immunity against both ectromelia and herpes simplex viruses (19). Therefore the extremely low resistance of the athymic mouse to ectromelia virus is probably related to an absence of cytotoxic T lymphocytes required for immunity (19, 21, 22). On the other hand, resistance to herpes simplex virus type 2 is related to the activated macrophage system of athymic mice (29–31). It is well established that macrophages present an effective barrier to dissemination of HSV in the host and represent an important primary defense system (39–41). The presence of tumor did not significantly change the responses to challenge with the viral agents (Table III).

This study shows that two murine solid tumors at progressive stages of development do not impair native resistance to infection of either immunocompetent or immunodeficient strains of Balb/c mice. This failure to impair resistance against bacterial or viral infection was evident in situations where humoral immunity, cell-mediated immunity, or a combination of the two is a requirement for recovery.

It is instructive that the Meth A fibrosarcoma used in these studies produces immunosuppressive factors in tissue culture similar to those reported by several other investigators (5–7). The Meth A tumor products were shown to impair lymphocyte blastogenic responses, adherence of macrophages to plastic surfaces, and ability of macrophages to respond to chemotactic stimuli (42). These inhibitory factors of tumor origin apparently have no deleterious effect on the native resistance of mice to bacterial and viral infections. More suprising is the fact that induction of suppressor T cells by the Met A tumor 12–14 days after implantation into Balb/c mice does not impair native resistance to the diverse microbial agents tested here. Berendt and North (43) showed conclusively that a Meth A tumor of Balb/c mice evoked the generation of suppressor T lymphocytes which prevented the regression of tumors by sensitized spleen cells obtained from tumor regressed mice. We have confirmed that the Meth A tumor employed in our studies produces a similar tumor-specific immunosuppressive population of lymphocytes which prevents tumor regression by sensitized T cells from immune donors (unpublished observations). Thus, mice bearing advanced tumors are not impaired in native resistance in spite of production of immunosuppressive factors (42) and the generation of tumor suppressor T cells which apparently are specific for the Meth A tumor.

In a subsequent report (manuscript submitted) we address the issue of how tumor-bearing Balb/c mice respond to vaccination against bacterial and virus infections. Our data show that the Meth A tumor does not impair the capacity of mice to generate acquired protective immunity against

these same infectious agents even though immunosuppression evoked by the tumor is demonstrable.

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