

## T-CELL-MEDIATED CONCOMITANT IMMUNITY TO SYNGENEIC TUMORS

### I. Activated Macrophages as the Expressors of Nonspecific Immunity to Unrelated Tumors and Bacterial Parasites\*

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It is now generally agreed that most syngeneic tumors possess tumor-specific transplantation antigens and are immunogenic to a larger or lesser degree. Their immunogenicity (reviewed in reference 1) is evidenced by the numerous demonstrations of a state of specific immunity to the growth of a tumor cell challenge in animals that have been immunized with injections of subtumorigenic doses of living tumor cells, with injections of lethally irradiated tumor cells, or that have had their primary tumors removed by surgery or ligation.

Additional evidence for the immunogenicity of transplantable as well as autochthonous tumors is illustrated by examples of concomitant immunity (reviewed in reference 2) in which animals bearing large progressive tumors display a paradoxical state of immunity to the growth of a second implant of the same tumor. That the generation of concomitant immunity may be a common consequence of neoplastic growth is also suggested by the large number of publications (1, 3, 4) which show that tumor-bearing humans as well as tumor-bearing animals can acquire leukocytes that are specifically cytotoxic for tumor cells *in vitro*. Indeed, the large number of examples of this phenomenon is in itself reason for suggesting that concomitant immunity may represent a fairly universal natural response to solid neoplastic growth, and may play a significant part in determining the outcome of certain types of anti-cancer therapy. Again, a good case has been made (5-7) for proposing that concomitant immunity functions to retard the spread and growth of metastases.

There is evidence (6) that concomitant immunity to syngeneic tumors is cell mediated and specific. More recent evidence (8) contradicts this, however, by showing that concomitant immunity to certain murine fibrosarcomas displays a significant element of nonspecificity as judged by the host's capacity to retard the growth of antigenically unrelated tumors. It has been suggested on the basis of this and other evidence (9, 10) that macrophages may participate in the expression of this form of anti-tumor immunity.

This paper provides evidence to support the view that concomitant immunity, although T-cell mediated, is capable nevertheless of suppressing, to a limited

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extent, the growth of apparently unrelated tumors. More importantly, it will show that the generation of concomitant immunity to the syngeneic murine SA1 fibrosarcoma is associated with the concordant development of an activated macrophage system which supplies the host with a greatly enhanced, macrophage-mediated capacity for resisting infection with the bacterial parasite, *Listeria monocytogenes*. The results give credence to the view that macrophages may have evolved to serve the common role of guarding against colonization by neoplastic cells as well as by microorganisms.

### Materials and Methods

*Mice.* AB6F<sub>1</sub> (A/J × C57BL/6J) mice of both sexes were mostly employed. They were produced from parental A/J and C57BL/6J breeding stock obtained from The Jackson Laboratory, Bar Harbor, Maine.

*Tumor.* The SA1 spindle cell sarcoma syngeneic in A/J mice was originally purchased from The Jackson Laboratory. It is passaged weekly intraperitoneally in the ascites form in syngeneic mice. All of the experiments reported here, however, were performed with a single stock of biofrozen tumor cells. They were obtained by injecting a large number of mice intraperitoneally with 10<sup>6</sup> SA1 cells and harvesting tumor cells 7 days later in heparinized phosphate-buffered saline (PBS).<sup>1</sup> They were washed twice in PBS, resuspended to 10<sup>7</sup>/ml in minimal essential medium (MEM) containing 20% fetal calf serum and 20% dimethyl sulfoxide, and biofrozen in small aliquots and stored in liquid nitrogen. For each experiment an aliquot was thawed, washed in PBS, and the cells grown intraperitoneally for 7 days in AB6F<sub>1</sub> mice before being harvested and suspended at an appropriate concentration in PBS for initiating foot pad tumors. Tumor cells were injected in a vol of 0.05 ml with a 30 gauge needle. In most cases primary tumors were grown in the left-hind foot pad, while concomitant immunity against a tumor cell challenge was measured in the contralateral foot pad. The growth of the primary and challenge tumors was monitored against time by measuring increases in the dorsoventral thickness of the foot with dial calipers.

Syngeneic benzpyrene- and methylcholanthrene-induced fibrosarcomas designated BP3 and MC5, respectively, were employed in specificity studies. They had undergone 15 mouse passages at the time of the experiments.

*Irradiation.* Whole-body gamma irradiation was performed in a cesium-137 irradiator that generated a midphantom dose of 35.5 rads/min.

*T-Cell-Deficient Mice.* Mice were made T-cell-deficient (THXB) as adults by thymectomy followed 7 days later by lethal (900 R) whole-body gamma irradiation. They were infused intravenously with 10<sup>6</sup> syngeneic bone marrow cells immediately after irradiation, and employed in experiments 4–6 wk later. Mice treated in the same way except that they were sham-thymectomized (XB) served as controls for the effect of irradiation.

*Tumor Neutralization Test.* The anti-tumor activity of cells from the lymph node (popliteal) draining the site of the primary tumor was investigated with the *in vivo* Winn neutralization test (11). This involved mixing either “immune” or normal lymph node cells with tumor target cells at various lymphocyte to target cell ratios, injecting the mixture in a vol of 0.05 ml into the hind foot pads of normal test recipients, and after tumor growth at this site with dial calipers. Lymph node cells were obtained from normal controls and 10-day tumor-bearing mice. The nodes were finely diced into small pieces which were gently pushed through a 200 mesh stainless steel screen into PBS. They were then passed through six layers of surgical gauze to remove clumps, washed three times in PBS, and suspended at an appropriate concentration in PBS for mixing with tumor cells. For some experiments lymph node cells were depleted of adherent cells. This was done by suspending them at 10<sup>7</sup>/ml in MEM containing 10% fetal calf serum, and incubating them in an atmosphere of 5% CO<sub>2</sub> in air for 2 h at 37°C in large (150-mm diameter) plastic Petri dishes under conditions where there was no competition for space on the substratum. The nonadherent cells were collected, washed, and suspended in PBS for the neutralization test.

<sup>1</sup> *Abbreviations used in this paper:* PBS, phosphate-buffered saline; THXB, T-cell deficient; XB, sham-thymectomized irradiated and bone marrow restored.

*Treatment with Antisera.* AKR anti-C3H $\theta$  serum was obtained from AKR mice which had been given four weekly intravenous injections of  $10^8$  CBA thymocytes. The serum was absorbed with AKR thymocytes ( $5 \times 10^7$ /ml of serum), heat inactivated at  $56^\circ\text{C}$  for 20 min, and stored at  $-20^\circ\text{C}$  until required. The specificity of the antiserum was tested by absorption with brain tissue as previously described (12). Lymph node cells at  $2 \times 10^7$ /ml were incubated at  $37^\circ\text{C}$  for 30 min in a 1:5 dilution of the antiserum in PBS, and then in the same dilution of agarose-absorbed guinea pig serum in PBS for 30 min at  $37^\circ\text{C}$ . The cells were then washed and resuspended in PBS for functional testing.

The Ig fraction of rabbit anti-mouse Ig serum was purchased in a lyophilized form from Miles Laboratories, Inc., Miles Research Div., Kankakee, Illinois. The lyophilized preparation was made up to  $520 \mu\text{g}$  antibody protein/ml in PBS. Lymph node cells were suspended in this at  $5 \times 10^7$ /ml and incubated at  $37^\circ\text{C}$  for 30 min. They were then treated in the same way with guinea pig serum as described above. Normal AKR serum served as a control for anti- $\theta$  serum, and normal rabbit serum as a control for rabbit anti-mouse Ig.

*Antibacterial resistance.* The systemic generation of macrophage-mediated, nonspecific, antibacterial resistance in tumor-bearing mice was measured against time by determining changes in their capacity for inactivating a standard  $10^4$  intravenous inoculum of the intracellular bacterial parasite, *L. monocytogenes*. Changes in anti-bacterial resistance during tumor growth were expressed as changes in  $\log_{10}$  resistance which were obtained by subtracting the 24-h growth of the organism in the livers of tumor bearers from its 24-h growth in the livers of controls. Spleen counts were also obtained but not included in the results. *L. monocytogenes* (strain EGD) was passaged in mice, grown in trypticase-soy broth, and stored in small aliquots at  $-70^\circ\text{C}$ . The thawed aliquots were prepared for intravenous inoculation as described previously (13). Bacteria were enumerated by plating 10-fold serial dilutions of whole liver and spleen homogenates on trypticase-soy agar.

## Results

*Time-Course of Development.* Fig. 1 shows the growth of a standard  $10^6$  SA1 tumor cell challenge in the right-hind foot pad given on days 3, 6, 9, or 12 of growth of a primary tumor initiated with  $10^5$  tumor cells in the contralateral foot pad. It can be seen that a significant level of anti-tumor resistance against the challenge implant was not expressed until 6 days after initiating the primary tumor, and that the level of concomitant resistance increased thereafter. Thus, whereas growth of the challenge given on day 6 was only partially inhibited, growth of the same sized implant given on day 12 was completely inhibited. It will be noted, in addition, that the progressive increase in the level of immunity occurred during rapid growth of the primary tumor. Challenges were not given beyond day 12 because there was not enough time to follow the growth of the challenge before the mice began dying on day 18 from a massive primary tumor burden and multiple lymph node metastases.

Fig. 2 serves to show that the results of concomitant immunity studies partly depend on the size of the challenge implant. It can be seen that when 10-day tumor bearers were challenged with  $10^5$ ,  $10^6$ , or  $10^7$  tumor cells, it was only the  $10^5$  challenge that was completely inhibited from growing, although significant immunity was expressed against the larger implants. Concomitant immunity, therefore, is not absolute, and its apparent strength and rate of development are a reflection of the size of the challenge implant.

Because most of the experiments reported in this paper were performed in semisyngeneic AB6F $_1$  mice, it was necessary to show that comparable levels of concomitant immunity were generated by syngeneic A/J mice. That this was the case is shown in Fig. 3 where it can be seen that the  $10^6$  tumor cell challenge was almost completely inhibited from growing in A/J mice bearing 10-day primary

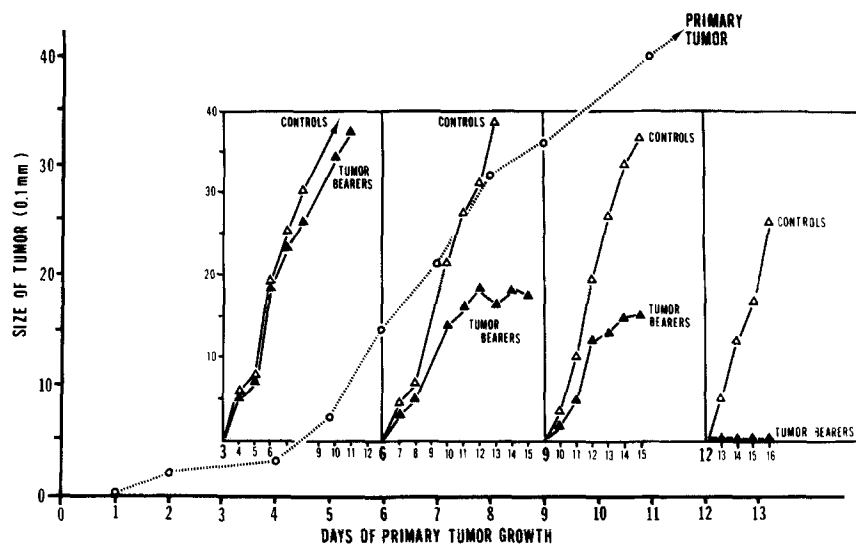


FIG. 1. Development of concomitant immunity during growth of a primary foot pad tumor initiated with  $10^5$  tumor cells. The primary tumor (large graph) showed a latency period of about 4 days before progressive tumor growth became manifest. The growth of a standard  $10^6$  challenge implant given in the opposite foot on days 3, 6, 9, and 12 (enclosed graphs) shows that the capacity to inhibit the growth of the challenge increased with progressive growth of the primary tumor. Means of five mice per time point.

foot pad tumors. Since this result was taken from a time-course study that showed the same rate of development as Fig. 1, it is safe to assume that the results obtained with  $F_1$  hybrids also apply to the syngeneic system.

*Effect of T-Cell Deficiency.* It was found that mice made T-cell-deficient by thymectomy and gamma irradiation, and protected with bone marrow cells developed a much lower level of concomitant resistance than tumor-bearing irradiated control mice. It can be seen in Fig. 4 that in contrast to the strong resistance generated against a  $10^6$  implant by tumor-bearing controls, THXB mice displayed only marginal resistance to the growth of this size implant given on day 9 of primary tumor growth. The results obtained with normal control mice are not shown because they were the same as those obtained with irradiated and bone marrow-restored controls.

*Specific Neutralization of Tumor Growth by Lymph Node T Cells.* The preceding result shows that the generation of concomitant immunity is thymus dependent. It was anticipated, therefore, that the lymph node draining the site of the primary tumor would contain thymus-derived lymphocytes (T cells) capable of causing local neutralization of growth of a tumor cell challenge in normal test recipients. The local Winn assay (11) was employed because of the difficulty experienced in attempting to transfer the immunity systemically.

Fig. 5 shows the growth in the foot pads of test recipients of  $5 \times 10^5$  tumor cells mixed with 10, 25, or 50 times as many normal lymph node cells or "immune" lymph node cells from 10-day tumor-bearing donors. It can be seen that whereas the presence of normal lymph node cells, in all cases, actually caused a slight enhancement of tumor growth, immune lymph node cells caused complete

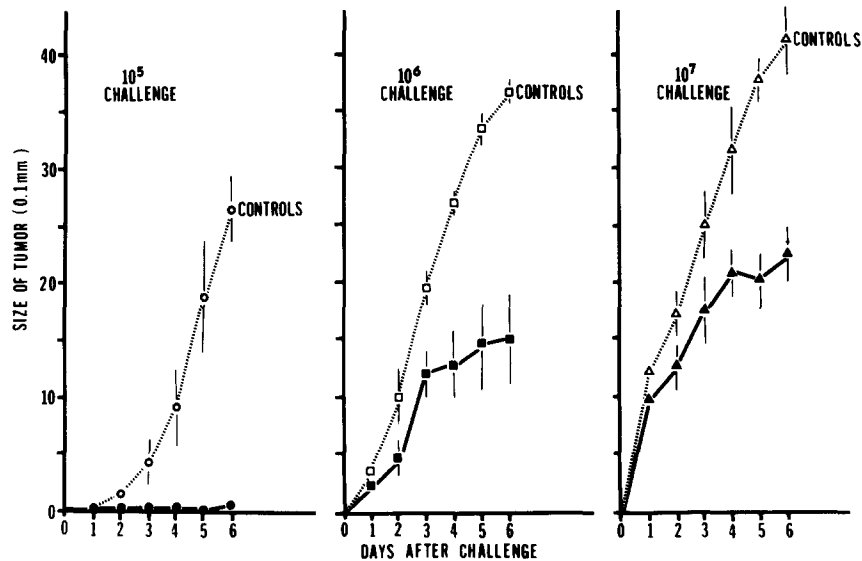


FIG. 2. Concomitant immunity expressed by 10-day tumor bearers against a  $10^5$ ,  $10^6$ , and  $10^7$  challenge implant. It was only the  $10^5$  implant that was completely inhibited from growing, although significant immunity was also expressed against the larger implants. Means  $\pm$  2 SE of five mice per time point.

suppression of tumor growth when present at a 50:1 ratio and partial, though highly significant, suppression when present at a ratio of 25:1. Indeed, even their presence at a 10:1 ratio caused some suppression of growth.

Evidence that the lymph node cells of tumor bearers responsible for tumor neutralization were T cells is supplied in Fig. 6 where it can be seen that their capacity for neutralizing tumor growth when present at a 50:1 ratio was completely ablated by incubating them with anti- $\theta$  serum and complement. Incubation with a high concentration of anti-Ig antibodies, in contrast, had no detectable effect on their anti-tumor activity. The reason why lymphocytes treated with anti- $\theta$  serum caused an actual measurable enhancement of tumor growth is not known, but could be explained on the basis of published reports (14, 15) which show that under certain conditions lymphocytes can stimulate tumor growth. Indeed, since the preceding results revealed that normal lymphocytes stimulated growth of the tumor implant, it seems reasonable to suggest that the same type of lymph node cells were present in the immune population, survived treatment with anti- $\theta$  serum, and were free to cause enhancement.

The specificity of the expression of the tumor-neutralizing capacity of lymph node T cells from 10-day tumor bearers was investigated by determining whether these cells would also neutralize the growth of the apparently unrelated syngeneic BP3 fibrosarcoma when present at a lymphocyte to tumor cell ratio of 50:1. The results in Fig. 7 show that while a high level of anti-tumor activity was expressed against  $5 \times 10^5$  cells of the homologous SA1 tumor, there was no significant suppression of growth of the same number of BP3 cells. To this extent, then, the expression of concomitant immunity to the SA1 sarcoma is specific.

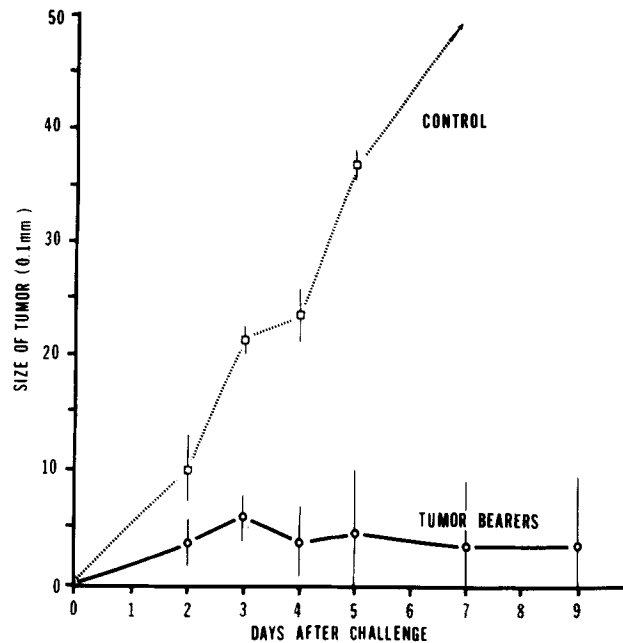


FIG. 3. Evidence that syngeneic A/J mice generate levels of immunity comparable with those generated by AB6F<sub>1</sub> mice. 10-day A/J tumor bearers caused almost complete inhibition of growth of a  $10^6$  challenge implant. Means  $\pm$  2 SE of five mice per time point.

*Neutralization Does Not Require the Presence of Adherent Accessory Cells.* It was shown in a recent publication (10) that neutralization of tumor growth by lymphocytes from concomitantly immune mice is greatly reduced if the neutralization test is performed in lethally irradiated recipients, or if the immune lymphocytes are depleted of glass adherent cells. This led to the suggestion (10) that the expression of concomitant immunity requires the participation of a radiosensitive adherent accessory cell, probably the monocyte-derived macrophage. Attempts to confirm these findings with the SA1 sarcoma were unsuccessful.

Thus it can be seen in Fig. 8 that the removal of adherent cells by allowing them to stick to plastic Petri dishes caused no reduction in the capacity of 50 times as many "immune" lymph node cells from 10-day tumor-bearing donors to neutralize the growth of  $10^6$  SA1 cells in normal test recipients. Likewise, Fig. 9 shows that lethal gamma irradiation given to test recipients 48 h before employing them in the neutralization test, in order to deplete them of blood monocytes (16), caused no reduction in the capacity of donor lymph node cells to locally inhibit the growth of the tumor cell challenge. It seems fairly certain, therefore, that neither mature adherent macrophages nor a mobile pool of monocyte-derived macrophages is essential for the expression of the local tumor-neutralizing capacity of sensitized T cells in this tumor model.

*The Expression of Immunity in the Tumor-Bearing Host is Nonspecific.* The foregoing results indicate that the lymph node draining the site of the primary tumor in concomitantly immune donors contains T lymphocytes that by themselves can inhibit the growth of an implant of cells of the primary tumor in a

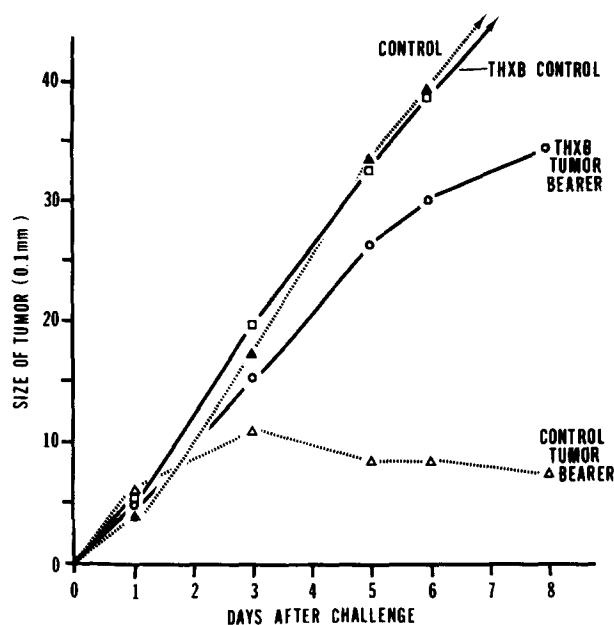


FIG. 4. Evidence that THXB mice developed a much lower level of concomitant immunity to a  $10^6$  tumor cell challenge than control tumor bearers over 9 days of primary tumor growth. The control animals were lethally irradiated and bone marrow restored. They gave the same results as normal controls (not included). Means of five mice.

normal recipient, and that the neutralization is specific for the homologous tumor. In apparent contradiction to this, the results in this section show that when tested in the tumor-bearing host itself, concomitant immunity was non-specific.

It can be seen in Fig. 10, for instance, that besides being capable of inhibiting the growth of a foot pad challenge of  $5 \times 10^5$  SA1 cells, mice bearing a 10 day primary SA1 tumor were also capable of significantly inhibiting the growth of a  $5 \times 10^5$  challenge of cells of the MC5 or BP3 syngeneic fibrosarcomas. It can be seen in addition, however, that when the size of the tumor challenge was increased to  $2 \times 10^6$  tumor cells, the expression of concomitant immunity appeared to be highly specific for the SA1 tumor. These results show, therefore, that concomitant immunity to the SA1 sarcoma possesses a nonspecific element, but that it can only be expressed against relatively small numbers of tumor cells. It is obvious, therefore, that care should be taken in designing experiments for testing for the specificity of anti-tumor immunity.

*Dependence on Progressive Tumor Growth.* The implantation of syngeneic tumor cells, more often than not, is followed by a period of latency before tumor growth becomes manifest. There is evidence to show (17), moreover, that the length of the latency period is inversely proportional to the number of tumor cells implanted. This knowledge was taken advantage of in the following experiments to determine whether the presence of a deposit of immunogenic tumor cells during a long period of latency is in itself enough to evoke the generation of concomitant immunity, or whether concomitant immunity is not generated until after the tumor begins to grow progressively.

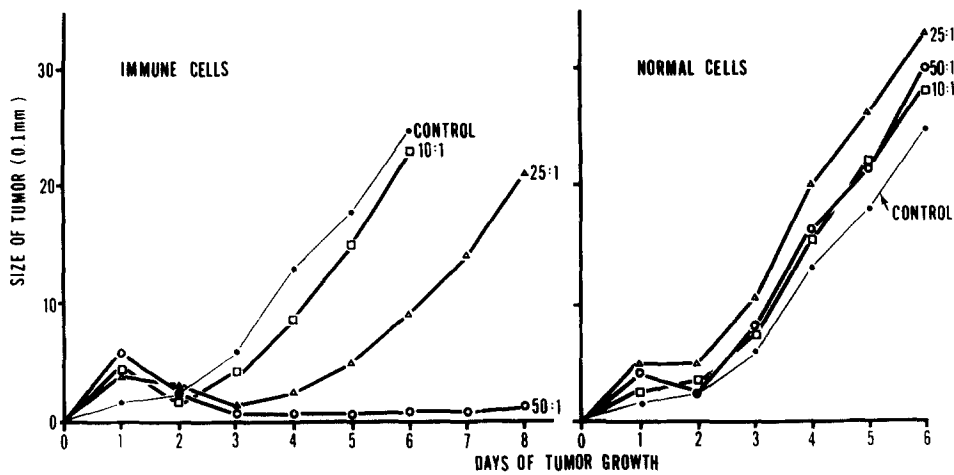


FIG. 5. The effect of mixing tumor cells with 50, 25, or 10 times as many lymph node cells from 10-day concomitantly immune or normal mice on the growth of the tumor cells implanted in a normal test recipient (Winn assay). "Immune" lymph node cells caused substantial neutralization of tumor growth, in contrast to normal lymph node cells which caused a slight enhancement of growth. Means of five mice.

The results in Fig. 11 indicate that the host did not generate concomitant immunity after implantation of SA1 cells until after the period of latency had ended. They also show that immunity to a standard tumor cell challenge increased as the size of the primary tumor increased. It can be seen that although 10-fold reductions in the number of tumor cells used to initiate tumors resulted in corresponding increases in the length of the period of latency, this had little effect on the rate of growth of the tumor after it eventually emerged. Changes in the level of concomitant immunity to a standard sized ( $10^6$ ) secondary implant (bar graphs) are expressed as the difference between the 5-day growth of the implant in tumor bearers and controls. The meaning of this assay can be appreciated from an examination of Fig. 1. It is obvious, however, that since the challenge implant always grew at the same rate in control mice, any increase in the 5-day difference represented an increase in the capacity of the tumor-bearing host to inhibit growth of the tumor challenge. The 5-day differences were taken from complete growth curves of the challenge implants and were used in order to avoid a confusing presentation.

*The Concordant Generation of Anti-Bacterial Resistance.* It was shown in a previous publication (18) that the growth of any one of three transplantable murine tumors resulted in the acquisition of a systemically enhanced capacity for resisting experimental infection with the bacterial parasite, *L. monocytogenes*. It was shown in addition, however, that the generation of anti-bacterial resistance was preceded by a tumor-induced state of greatly suppressed anti-bacterial resistance, the possible biological meaning of which was adequately discussed (18, 19). The purpose of the experiments reported in this section was to obtain additional evidence for the proposition that the acquisition of enhanced macrophage-mediated anti-microbial resistance is a consequence of the generation of concomitant immunity.



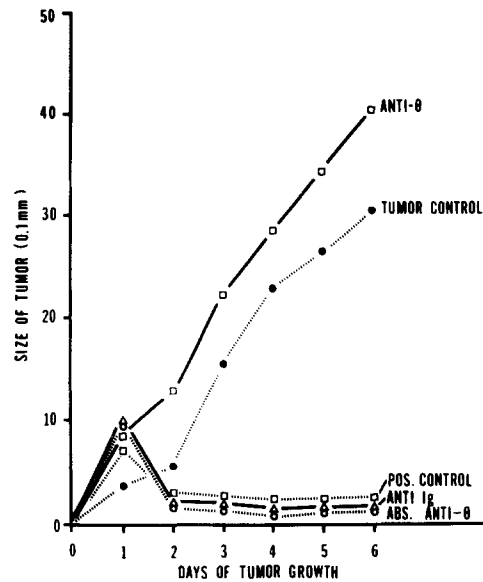


FIG. 6. Evidence that the lymph node cells that neutralize the growth of tumor cells are  $\theta$ -positive T cells. The tumor suppressive action of 50 times as many immune lymph node cells on the growth of  $10^6$  tumor cells implanted in a normal recipient was completely abolished by treating the lymph node cells with anti- $\theta$  serum and complement. In fact, anti- $\theta$  serum-treated lymphocytes caused enhanced growth of the tumor implant. Treatment with rabbit anti-Ig or with anti- $\theta$  serum absorbed with C3H brain (ABS ANTI- $\theta$ ) had no effect on the capacity of lymph node cells to neutralize tumor growth. Means of 5 mice.

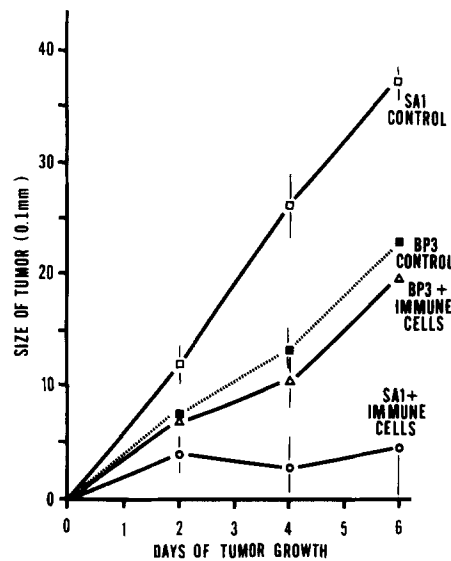


FIG. 7. The tumor-neutralizing capacity of immune lymph node cells was specific for cells of the primary tumor. While lymph node cells from 10-day SA1 bearers were resistant to implant of  $5 \times 10^5$  SA1 cells (50:1 ratio) they had no significant effect on the growth of the same number of BP3 cells. Means of five mice.

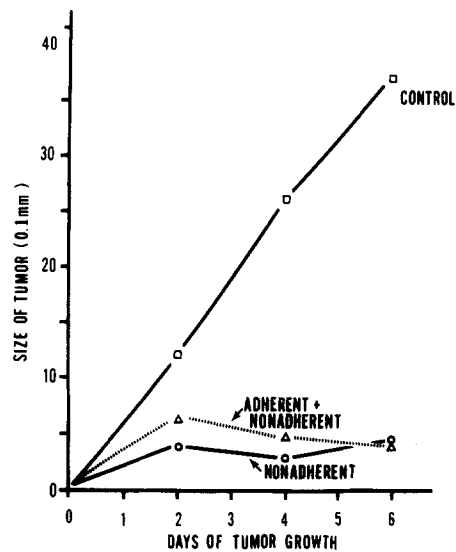


FIG. 8. Removal of adherent lymph node cells by allowing them to react with a plastic surface had no effect on their capacity to cause inhibition of growth of tumor cells in a test recipient. Means of five mice.

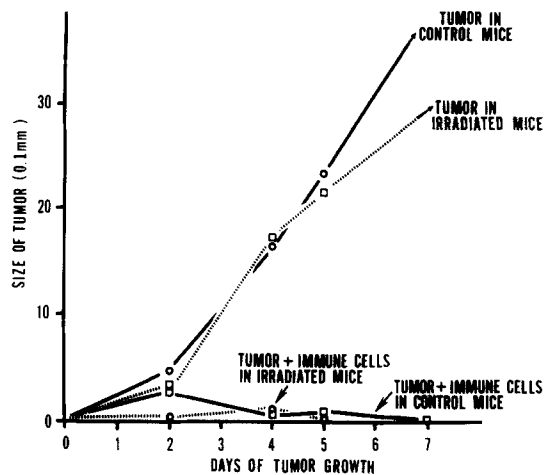


FIG. 9. Lethal gamma irradiation of normal test recipients had no effect on the outcome of the tumor neutralization test performed in them 48 h later. Immune lymph node cells were just as effective in inhibiting tumor growth in irradiated mice as they were in controls. Means of five mice.

Fig. 12 shows changes in resistance (24 h  $\log_{10}$  resistance) to a standard intravenous *Listeria* challenge inoculum against time after initiating tumors with different numbers of tumor cells. As expected from a previous study (18), subcutaneous implantation of tumor cells first resulted in a state of greatly suppressed anti-bacterial resistance, the development of which was faster with larger doses of tumor cells. It can be seen, however, that this was followed by conversion from a state of suppressed, to a state of significantly enhanced,

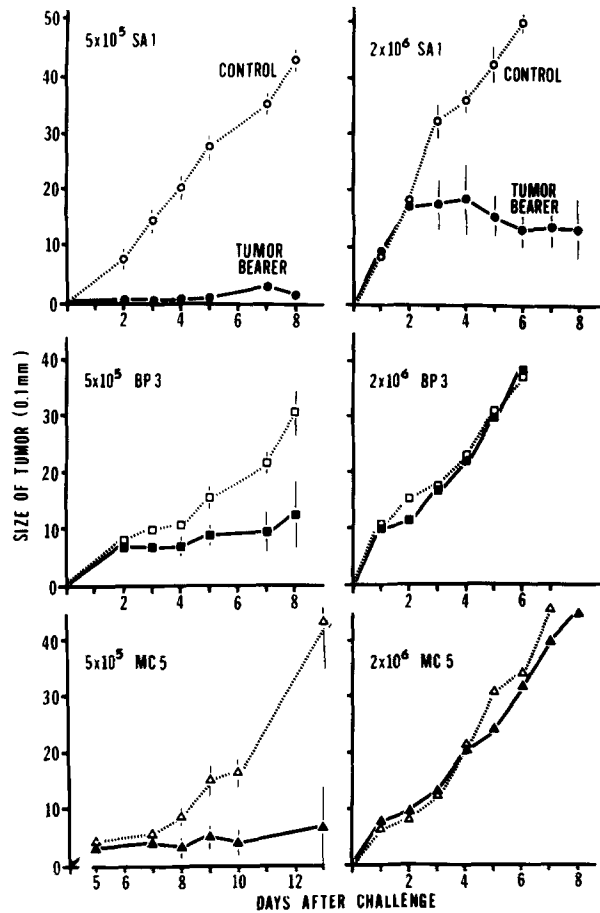


FIG. 10. Evidence that mice concomitantly immune to the SA1 possessed a limited capacity to restrict the growth of unrelated tumors. Mice with a 10 day SA1 primary tumor showed a significant capacity to inhibit the growth of  $5 \times 10^5$  BP3 or MC5 cells as well as  $5 \times 10^5$  SA1 cells, but did not express resistance to the heterologous tumors when the challenge dose was increased to  $2 \times 10^6$ . Means of five mice  $\pm$  2 SE.

resistance and that the speed of this conversion depended on the emergence and progressive growth of the tumor. Thus the shorter the period of latency, the shorter the period of suppressed resistance and the faster the acquisition of anti-bacterial resistance. When these results are compared with those in Fig. 11, it seems obvious that the acquisition of anti-bacterial resistance was a consequence of the generation of concomitant immunity. It also seems certain that the generation of both mechanisms depended on a progressively growing tumor.

Fig. 13 is included to show the meaning of the 24-h differences in bacterial growth that were used to compose Fig. 12. It shows the 3-day growth of the standard bacterial inoculum in the livers of 10-day tumor bearers and controls. It can be seen that the relatively small 24-h differences shown in Fig. 12 were indicative of much larger differences at later times of infection. Thus, while bacterial growth was completely suppressed for 3 days in tumor bearers, it increased log linearly in controls.

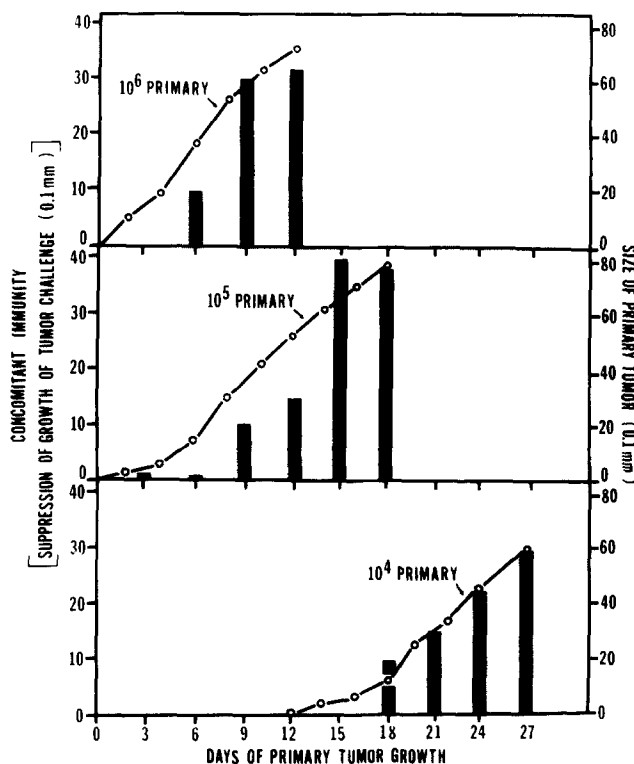


FIG. 11. Rate of development of concomitant immunity (bar graphs) to a  $10^6$  tumor challenge in mice whose primary tumors were initiated with  $10^4$ ,  $10^5$ , or  $10^6$  tumor cells (line graphs). Concomitant immunity is expressed as the 5-day difference between growth of the challenge in tumor bearers and controls when the challenge was given at the times indicated. Concomitant immunity was not generated during the period of latency. It was generated during rapid tumor growth and increased with increasing size of the primary tumor. Means of five mice.

### Discussion

This paper shows that progressive growth of the SA1 sarcoma in syngeneic and semisyngeneic mice results in the systemic generation of a powerful mechanism of concomitant resistance to growth of a second implant of the same tumor. Employment of the Winn (11) neutralization assay showed, in addition, that the generation of concomitant resistance was associated with the production of  $\theta$ -positive T cells in the draining lymph nodes which were capable of inhibiting the growth of tumor cells implanted in a normal test recipient. The neutralization of tumor cells was specific, was accomplished by lymph node cells depleted of adherent cells, and was expressed in test recipients that were lethally irradiated 48 h before testing. These results, together with those which showed that only marginal levels of concomitant immunity were generated in mice made T-cell deficient by thymectomy and gamma irradiation, represent firm evidence for proposing that concomitant immunity to SA1 sarcoma is T-cell mediated and can be expressed by specifically sensitized T cells. The results are in agreement, therefore, with the general conclusion by Kearney et al. (9) that concomitant

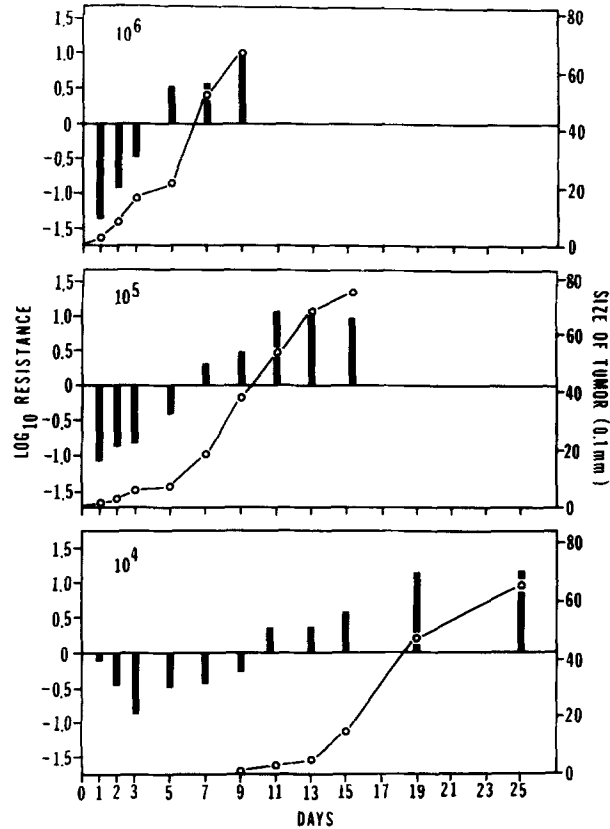


FIG. 12. The generation of resistance (bar graphs) to a  $10^4$  intravenous challenge with *L. monocytogenes* during the growth of primary tumors initiated with  $10^4$ ,  $10^5$ , or  $10^6$  SA1 cells (line graphs). Shown are changes in anti-bacterial resistance ( $\log_{10}$  resistance) as expressed as the difference between the 24 h growth of the parasite in the livers of tumor bearers and controls when the bacteria were given at the times indicated. Implantation of tumor cells first caused a striking suppression of anti-bacterial resistance. Subsequent conversion from a state of suppressed to a state of enhanced anti-bacterial resistance, however, depended on the emergence and progressive growth of the tumor. Means of five mice.

immunity to murine fibrosarcomas is T-cell dependent. It does not support the other findings of these authors, however, that later stages of immunity are not expressed by T cells, but are expressed by other cells including B cells. It is well to realize, however, that their conclusions were based on results obtained with the in vitro microcytotoxicity assay: an assay that requires an incubation period of long enough duration to allow the induction of an in vitro immune response, and which has been shown to give results that conflict with another in vitro assay (20), as well as with in vivo assays (21). For these and other reasons the microcytotoxicity assay has recently come under criticism (22-24).

Our results do agree, however, with those which show (8) that concomitant immunity possesses a nonspecific element when tested in the tumor-bearing host itself. The finding that this nonspecificity was only expressed against a relatively small number of unrelated tumor cells, and that it was apparently not

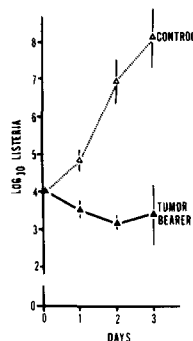


FIG. 13. Evidence that  $\log_{10}$  24 h anti-bacterial resistance shown in Fig. 12 was indicative of a high level of anti-bacterial resistance. Shown is the 3-day growth of a  $2 \times 10^4$  intravenous *Listeria* challenge in the livers of 10-day tumor bearers and controls. It can be seen that a 1.3 log increase in resistance at 24 h was indicative of a 5 log difference at 72 h. Means of five mice  $\pm$  2 SE.

expressed at all against larger implants, indicates that care should be taken when testing the specificity of anti-tumor immunity. The nonspecificity may have been the result of the sharing of common antigens between the tumor lines tested (25–27) while its limited strength of expression could result from the possibility (28) that some tumor cells are more susceptible to cell-mediated lysis than others. It seems more reasonable to propose from the results of this study, however, that the nonspecific expression of resistance to the growth of heterologous tumor cells was the result of the possession by the concomitantly immune host of an activated macrophage system. Since there is convincing evidence (29) that enhanced destruction of *L. monocytogenes* depends on the possession by the host of activated macrophages, there seems little doubt that the generation of high levels of nonspecific anti-*Listeria* resistance during progressive tumor growth was the result of the generation of activated macrophages. Furthermore, the striking temporal correlation between the generation of concomitant immunity and the generation of macrophage-mediated anti-bacterial resistance is strong evidence for hypothesizing that macrophage activation is a T-cell-mediated consequence of the specific immune response to a progressively growing tumor. Hence, the published report (30) that mice bearing the syngeneic Lewis lung carcinoma display enhanced resistance to *Candida albicans* infection, and publications that show that tumor-bearing humans (31–33) as well as animals (34, 35) can display an activated reticuloendothelial system are evidenced by an enhanced capacity for clearing intravenously infused colloids. There is now adequate evidence for proposing that the possession of activated macrophages gives the host the capacity to nonspecifically inhibit the growth of syngeneic tumor cells as well as microbial parasites. Thus, it has been shown (36, 37) that animals with macrophages activated as a result of infection with microorganisms can retard the growth of a tumor cell implant. More convincingly, macrophages harvested from such animals have been shown on many occasions (36, 38–40) to possess nonspecific, potent anti-tumor activity *in vitro*. The present study, then, supplies the reciprocal demonstration that macrophage-mediated, enhanced anti-microbial resistance is generated via an im-

mune response to a tumor itself. It is apparent, therefore, that the macrophage has evolved to serve the dual role of protecting the host from colonization both by neoplastic cells and microorganisms.

Even though the possession of activated macrophages and cytotoxic T cells appears to have no restrictive influence on the growth of the primary tumor, there is ample evidence for proposing that the presence of these components of concomitant immunity plays a large part in determining the rate of establishment and the growth of metastases. It has been shown, for instance, that the rapid decay of concomitant immunity that occurs after surgical removal of the primary tumor is followed by the rapid emergence and growth of multiple metastases (5, 6). Again, experiments performed in this laboratory (to be published), as well as those published by others (9), have revealed that metastases emerge much sooner and grow much faster in tumor-bearing animals that fail to develop concomitant immunity because of a deficiency of T cells. Again, animals with concomitant immunity are more resistant than normal to the establishment of experimental metastases caused by the intravenous infusion of tumor cells (7, 41, 42). It seems highly likely, therefore, that the results of certain types of anti-cancer therapy may depend on whether or not the agents employed for therapy either partially or completely ablate an existing state of concomitant immunity.

It is obvious that the contradiction suggested by the specificity of the neutralization test and the nonspecificity displayed by the tumor-bearing donor is more apparent than real. The neutralization test was specific because it was performed in normal test recipients without activated macrophages. It shows, in agreement with numerous publications (43), that sensitized T cells can act alone as the specific effectors of anti-tissue immunity. It is almost certain, however, that these cells act in concert with activated macrophages in expressing immunity in the concomitantly immune host. On the other hand, no evidence was found for a role for humoral antibody, as evidenced by the failure of either a single or multiple infusions of tumor bearer's serum to retard the growth of a tumor cell challenge. If anything, serum caused a slight enhancement of growth. On the basis of the present level of analysis, therefore, it seems fair to say that T-cell-mediated concomitant immunity generated against a progressive SA1 sarcoma shows striking similarities to T-cell mediated anti-bacterial immunity (28). In both cases, the generation of sensitized T cells in the presence of replicating antigen results in systemic activation of macrophages that consequently results in a high level of nonspecific resistance to microbial parasites and neoplastic cells.

### Summary

Progressive growth of the SA1 sarcoma was shown to result in the generation of a state of concomitant resistance to growth of a second implant of the same tumor. The responding lymph nodes of concomitantly immune mice were shown to contain  $\theta$ -positive T cells that could specifically neutralize the growth of tumor cells in a normal test recipient. Nevertheless, the concomitantly immune host itself was capable to a limited extent of suppressing the growth of unrelated tumors. The generation of immunity, moreover, was associated with the genera-

tion of a powerful state of macrophage-mediated, nonspecific resistance to the bacterial parasite, *Listeria monocytogenes*. It was concluded that systemic macrophage activation was the consequence of the generation of T-cell-mediated immunity to the progressively growing tumor, and that this not only gave the host the capacity to inhibit the growth of unrelated tumors, but also to protect itself against microbial infection. The results give credence to the view that macrophages play a central role in defense against microbial and neoplastic growth.

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