

INTERLEUKIN 1-INDUCED, T CELL-MEDIATED
REGRESSION OF IMMUNOGENIC MURINE TUMORS
Requirement for an Adequate Level of Already Acquired Host
Concomitant Immunity

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IL-1, originally called lymphocyte activating factor, is a cytokine synthesized and secreted by macrophages in response to stimulation by endotoxin and certain other agents (reviewed in reference 1). IL-1 can also be produced by other cell types (1, 2), including vascular endothelial cells (3, 4), and is thought to play a central role in host defenses by virtue of its ability to augment the replication of activated T cells and to mediate the humoral and cellular events of inflammation. Because the genes for IL-1 α and IL-1 β have been cloned and expressed (5-7), rIL-1 is now available in sufficient quantity to investigate its ability to augment host-immune defense mechanisms in vitro and in vivo, including those that might protect against neoplastic diseases. The claim that IL-1 has antitumor action is based, however, on the demonstrations that it is directly cytostatic for certain tumor cells in vitro (8) and is cytotoxic for others (9, 10). More recently, it was demonstrated (11) that IL-1 can cause complete regression of a small murine fibrosarcoma when injected directly into the tumor, but only partial regression when injected intravenously. There remains a need to determine, therefore, whether IL-1 has the capacity to cause tumor regression indirectly through its ability to systemically augment host antitumor-immune defense mechanisms.

The purpose of this paper is to show that intraperitoneal injection of human rIL-1 can cause complete regression of a relatively large, immunogenic murine sarcoma growing subcutaneously, by augmenting the generation or expression of an already ongoing, T cell-mediated, concomitant immune response.

Materials and Methods

Mice. A/J, DBA/2, AB6F1 (A \times C57BL/6), and B6D2F1 (C57BL/6 \times DBA/2) mice (10-12 wk of age) were obtained from the Trudeau Institute Animal Breeding Facility, Saranac Lake, NY. The mice were known to be free of viral pathogens according to the results of tests routinely performed by the Diagnostic Testing Service of Microbiological Associates, Bethesda, MD. The average weight of the mice was \sim 20 g when they were used in experiments.

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Tumors. The SA1 sarcoma and YAC-1 lymphoma (A/J), and L5178Y lymphoma and P815 mastocytoma (DBA/2) were passaged as ascites, harvested, cryopreserved, and prepared for implantation as described previously (12, 13). The origins of these tumors were also described previously (12, 13). For experiments, tumors were initiated intradermally in the belly region by injecting 10^6 tumor cells in a volume of 0.05 ml of PBS. Tumor growth and regression was monitored by measuring changes against time in the mean of two diameters measured at right angles.

T Cell-deficient (TXB) Mice. T cell deficiency was achieved by thymectomy at 6 wk of age followed 1 wk later by exposure to 1,000 rad of γ radiation delivered from a ^{137}Cs source at 30 rad/min. Immediately after irradiation the mice were infused with 2×10^6 bone marrow cells, and were used in experiments no sooner than 4 wk later.

Adoptive Immunization. Mice bearing the SA1 sarcoma (14) and other tumors (15, 16) are known to possess peak levels of T cell-mediated concomitant immunity on day 9 of tumor growth. Spleen cells from such mice were used to passively transfer concomitant immunity to TXB recipients bearing a smaller (6 d) tumor. The spleen cells were obtained by gently pushing finely diced pieces of spleen through a 60-mesh stainless screen into PBS containing 1% FCS. The resulting cell suspension was triturated with a pasteur pipette to break up clumps, passed through surgical gauze to remove debris, washed in PBS, and resuspended in PBS for intravenous infusion. Recipients received one spleen equivalent (1.8×10^8) of cells from tumor-bearing donors, or two spleen equivalents (2×10^8) of cells from normal donors.

Depletion of T Cell Subsets. To selectively remove Thy-1.2⁺, L3T4⁺, or Ly-2.2⁺ T cells, the spleen cells were incubated for 30 min at 10°C at 5×10^7 /ml in the appropriate mAb solution, and then in a 1:10 dilution of rabbit serum at 37°C for 30 min. Monoclonal anti-Thy-1.2, and anti-Ly-2.2 were secreted by clone 30-H12 and clone TIB-150, respectively (American Type Culture Collection, Rockville, MD). Monoclonal anti-L3T4 was secreted by clone GK-1.5 obtained from Dr. Frank Fitch, Department of Pathology, University of Chicago. All hybridomas were grown to 5×10^5 /ml in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) containing 10% FCS and 100 U/ml each of penicillin and streptomycin. The cultures were centrifuged and the supernatants (containing 10–20 μg of Ab/ml) were dispensed and stored at -20°C until required. They were used at a 1:5 dilution in RPMI 1640 medium.

Interleukin 1. The IL-1 β used in these studies (derived from a clone designated DP516) was produced in *Escherichia coli* from a plasmid containing the DNA sequence for the mature 1.7kD form of human IL-1 β (17). The soluble protein was purified to >96% purity from the supernatant fluids of bacterial extracts by ion exchange and molecular sieving column chromatography. The rIL-1 β differed from the native sequence IL-1 β by the addition of threonine and asparagine residues on the NH₂-terminal end of the molecule before amino acids 117–269 of the IL-1 β precursor molecule. DP516 had a specific activity comparable with that of purified monocyte-derived IL-1 β (10^7 U/mg), as measured by the thymocyte proliferation assay, and had low levels (<10 ng/mg protein) of endotoxin (17). IL-1 was stored at 4°C, diluted in PBS, and injected intraperitoneally in the doses indicated in Results.

Results

Several Small Doses of IL-1 Are Capable of Causing Complete Regression of the SA1 Sarcoma.

It is known (14) that underlying host, T cell-mediated immunity to the SA1 sarcoma is not generated until day 6 of tumor growth and peaks on day 9. Therefore, in the first experiment we tested the effect of giving IL-1 intraperitoneally in a dose of 1 μg daily for 1, 2, 3, or 4 d beginning on day 6 of tumor growth. Fig. 1 shows that 1 μg of IL-1 daily for either 3 or 4 d resulted in complete regression of the tumor in five of five mice, whereas 1 μg daily for 2 d resulted in complete tumor regression in three of five mice. A single 1- μg injection of IL-1 on day 6 of tumor growth failed to cause regression of the tumor in any mice, although the rate of tumor growth temporarily decreased. In all cases, complete regression of the primary tumor resulted in long-term host survival.

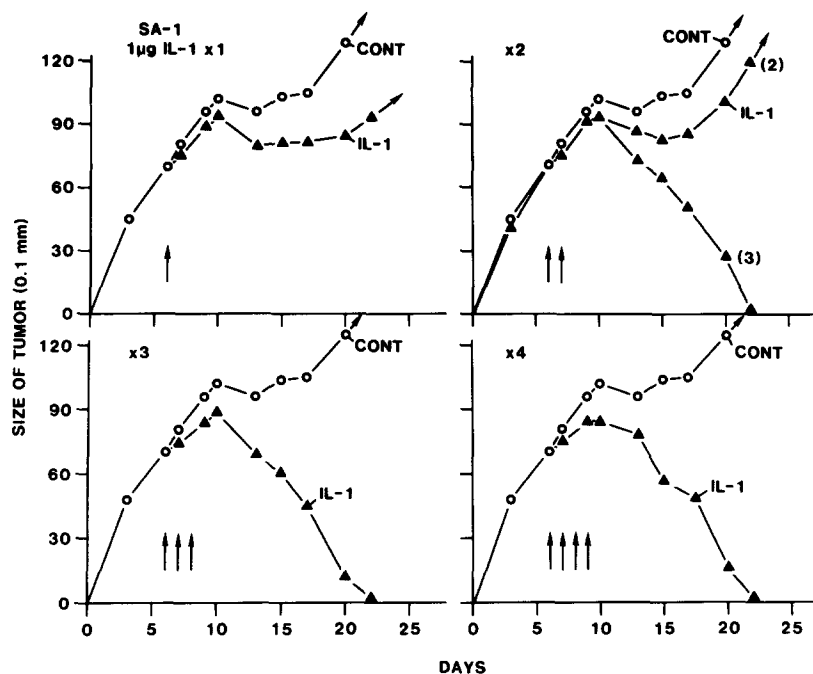


FIGURE 1. Effect on the SA1 sarcoma growing subcutaneously in AB6F1 mice of injecting 1 μ g of IL-1 intraperitoneally on day 6, days 6 and 7, days 6-8, or days 6-9 of tumor growth. Three injections of IL-1 were needed to reproducibly cause complete regression of the tumor in all mice. Means of five mice per group.

It was also found that 1 μ g of IL-1 injected daily between days 6 and 10 of tumor growth resulted in complete regression of the SA1 sarcoma in syngeneic A/J mice. Therefore, since there was no difference between the results obtained with A/J and AB6F1 mice, the latter mice were used in all subsequent experiments, because they were more plentiful and less expensive.

Effect of a Single Dose of IL-1. The possibility that a single large dose of IL-1 was just as therapeutic as several small doses was next investigated by determining the effect of giving a single 10- μ g dose intraperitoneally on day 6 or 9 of tumor growth. As seen in Fig. 2, a 10- μ g dose given on day 9 of tumor growth had no significant therapeutic effect, whereas the same dose given on day 6 caused complete tumor regression in two mice and partial regression in three. Therefore, small doses of IL-1 between days 6 and 9 of tumor growth were more therapeutic than a large dose on day 6 or 9.

In the same vein, Fig. 3 shows that multiple large doses of IL-1 were no more therapeutic than multiple small doses given during the same period of tumor growth. Thus, giving 10 μ g daily between days 6 and 10 of tumor growth was no more therapeutic than giving 5, 1, or 0.5 μ g on the same days, in that the time of onset and the rate of tumor regression were the same in all cases. However, injections of 0.1 μ g daily were without therapeutic effect (result not shown).

IL-1 Was Less Therapeutic Against Two Other Tumors. The foregoing results show

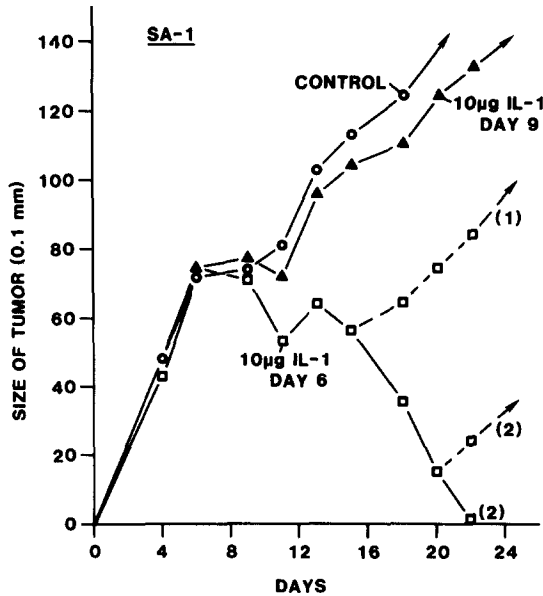


FIGURE 2. Evidence that a single large dose of IL-1 is not as therapeutic as several much smaller doses. A single 10- μ g dose of IL-1 given on day 6 or 9 of tumor growth was less therapeutic than 1 μ g given on days 6-8, as shown in Fig. 1. Giving 10 μ g of IL-1 on day 6 caused complete regression of the tumor in two mice and its partial regression in three. However, giving 10 μ g of IL-1 on day 9 failed to result in a significant therapeutic effect. Means of five mice per group.

that IL-1 given in relatively small doses intraperitoneally is capable of reproducibly causing complete regression of an established SA1 sarcoma growing subcutaneously. It was important to determine next whether IL-1 was capable of causing regression of other immunogenic murine tumors. Therefore, the effect of multiple injections of IL-1 on growth of the L5178Y lymphoma and P815 mastocytoma was determined. Fig. 4 shows that 10 μ g of IL-1 daily for 5 d beginning on day 6 of tumor growth

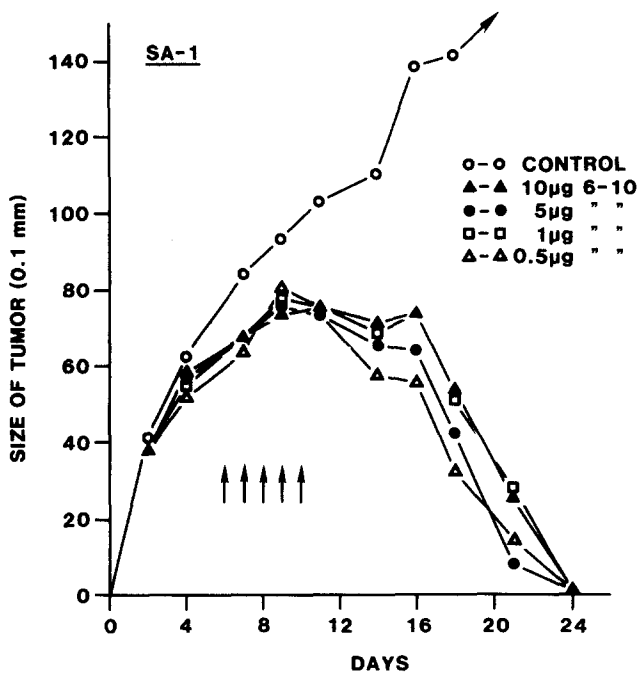


FIGURE 3. Evidence that substantially increasing the dose of IL-1 given daily for 5 d did not result in earlier onset of tumor regression. Injection of 10 μ g of IL-1 on days 6-10 of tumor growth was no more therapeutic than 5, 1, or 0.05 μ g given on the same days. Means of five mice per group.

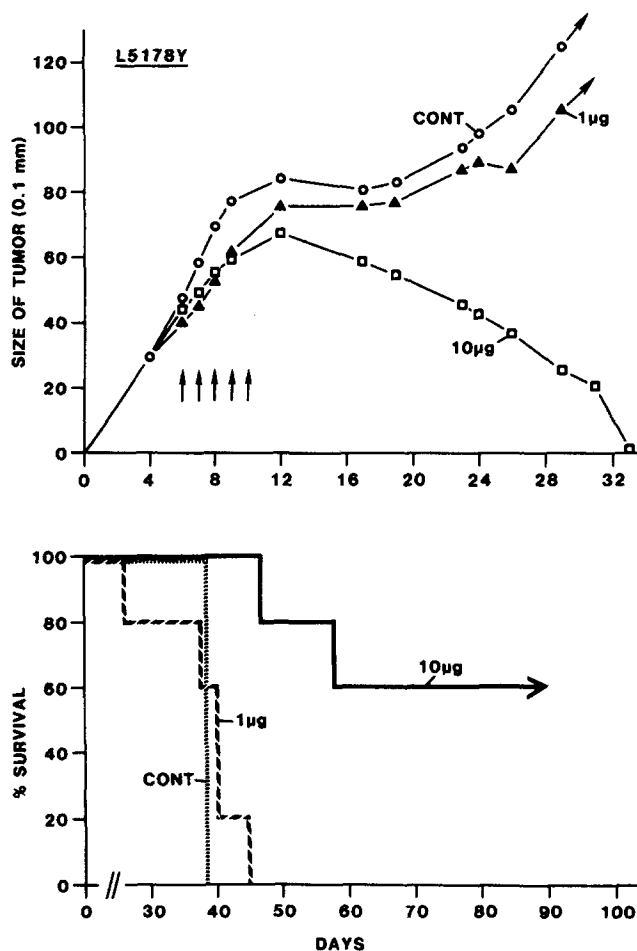


FIGURE 4. Evidence that IL-1 was less therapeutic against the L5178Y lymphoma than against the SA1 sarcoma. Injecting 1 μ g of IL-1 on days 6-10 of growth of the L5178Y failed to cause its regression. However, injecting 10 μ g of IL-1 on the same days caused its complete regression in all mice, and resulted in long-term survival of most mice. Means of five mice per group.

resulted in complete regression of the L5178Y lymphoma in five of five mice, and in long-term survival of three of five mice. In contrast, 1 μ g of IL-1 daily during the same period had no significant effect on tumor growth or on host survival.

IL-1 was less therapeutic against the P815 mastocytoma (Fig. 5), in that not even 10 μ g daily for 5 d caused complete regression of this tumor, although it did cause a temporary decrease in the rate of tumor growth, and an increase in host survival time. Injecting 1 μ g daily was less therapeutic.

IL-1 Is not Therapeutic if Given Too Early During Tumor Growth. The rationale for giving IL-1 between days 6 and 9 of tumor growth was based on the knowledge that underlying host, T cell-mediated immunity to the SA1 sarcoma (14) and to other tumors (15, 16) is not generated until day 6 of tumor growth and peaks on about day 9. If IL-1 requires this ongoing underlying antitumor immune response to be therapeutic, then, IL-1 should not be therapeutic if given during the first few days of tumor growth, before the generation of concomitant immunity has commenced. Fig. 6 shows the result of an experiment that compared the therapeutic effect of giving

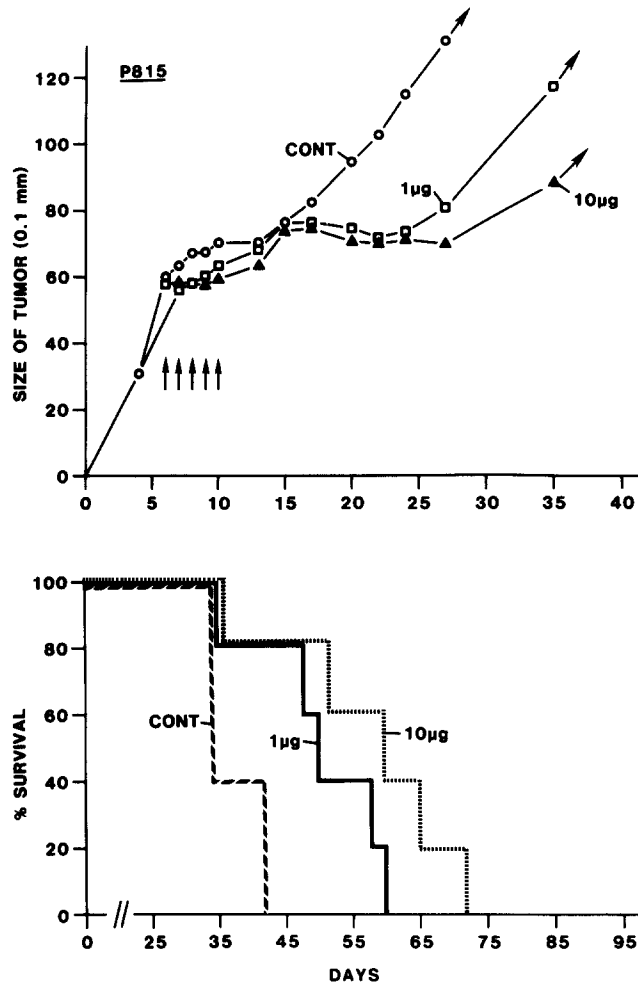


FIGURE 5. Evidence that the P815 mastocytoma was the least responsive to IL-1 therapy of the three tumors tested. A dose of 10 μ g of IL-1 on days 6-10 of tumor growth caused only a temporary halt in tumor progression, although host survival time was significantly increased. Means of five mice per group.

IL-1 on days 1-3 of tumor growth with its therapeutic effect when given on days 6-8. Whereas injection of 10 μ g of IL-1 on day 6-8 of tumor growth resulted in complete regression of the tumor in all mice, injecting the same relatively large dose on days 1-3 had no effect on tumor growth.

IL-1-induced Tumor Regression Is Dependent on Underlying T Cell-mediated Immunity.

The foregoing results suggest that IL-1 treatment cannot exert an antitumor effect until the tumor is large enough to evoke the generation of host concomitant immunity. If so, then IL-1 should not be capable of causing regression of the SA1 sarcoma growing in TXB mice. As shown in Fig. 7, intravenous injection of 1 μ g of IL-1 on day 6-9 of tumor growth caused complete regression of the SA1 in immunocompetent mice, but failed to cause regression of the tumor growing in TXB mice.

Direct evidence that the therapeutic action of IL-1 is dependent on T cell-mediated immunity came from experiments that determined whether IL-1 is capable of

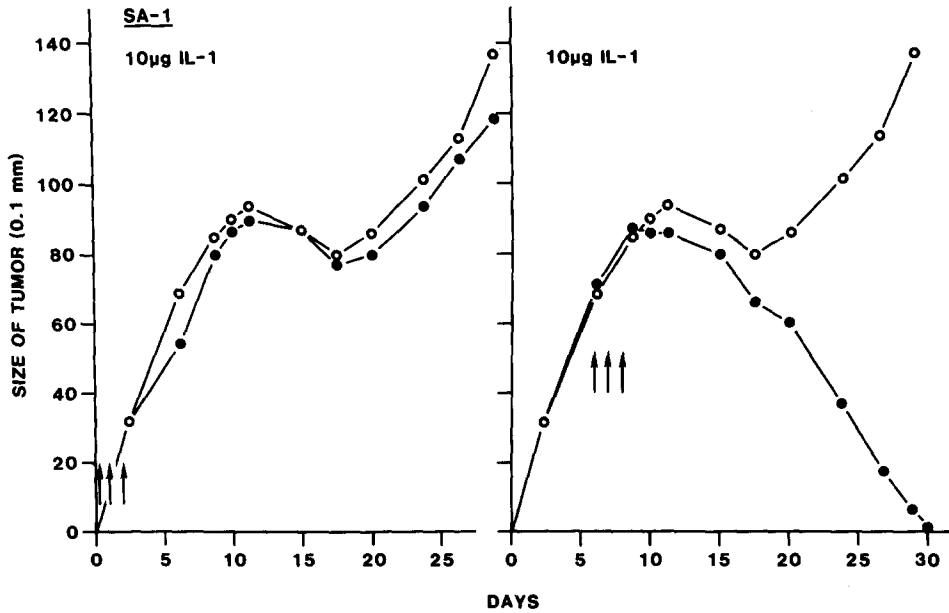


FIGURE 6. Evidence that IL-1 was not therapeutic if given too early during growth of the SA1 sarcoma. A dose of 10 µg of IL-1 given on days 1-3 of tumor growth gave no therapeutic effect, whereas the same dose given on days 6-8 caused complete tumor regression in all mice. Means of five mice per group.

causing regression of a tumor in TXB mice, provided they are infused with T cells from immunocompetent tumor-bearing donors. In the first experiment TXB mice bearing either a 6-d SA1 sarcoma, or a 6-d syngeneic YAC-1 lymphoma, were infused with one organ equivalent (1.8×10^8) of spleen cells from donor mice bearing

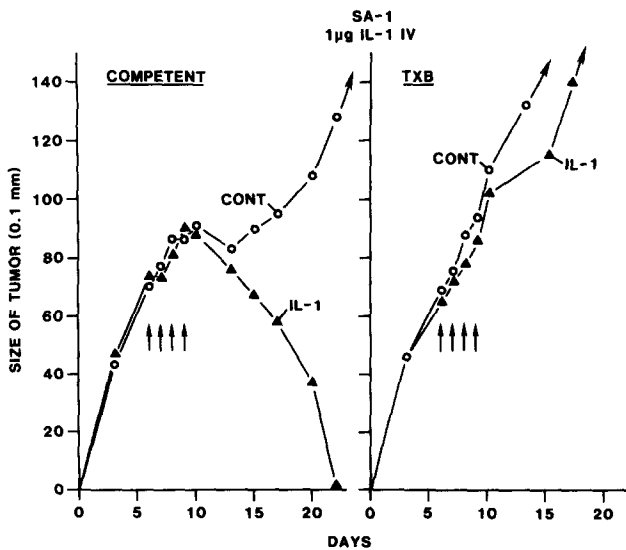


FIGURE 7. Evidence that the therapeutic effect of IL-1 against the SA1 sarcoma is T cell dependent. IL-1 in a dose of 1 µg given intravenously on days 6-9 of tumor growth caused complete regression of the tumor in immunocompetent mice (left), but not in TXB mice (right). Means of five mice per group.

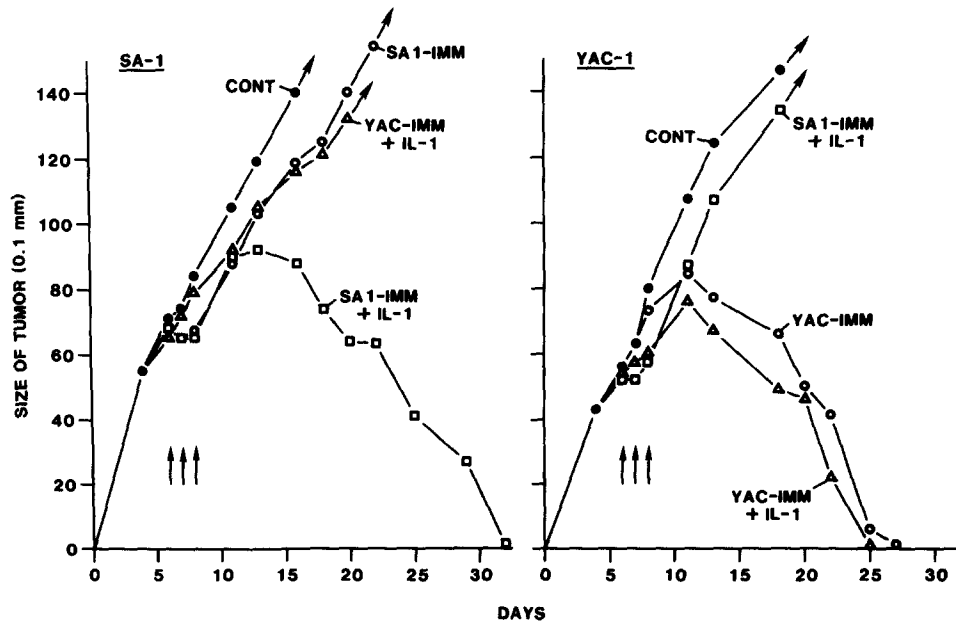


FIGURE 8. Restoration of the ability of TXB mice to cause regression of their tumor in response to IL-1 therapy by infusing them with spleen cells from donor mice bearing a 9-d tumor. SA1-bearing TXB recipients (*left*) were able to cause regression of their tumor in response to injection of 1 μ g of IL-1 on days 6-8, provided they were infused on day 6 with spleen cells from SA1-bearing donors (SA1-IMM + IL-1), but not with spleen cells from YAC-1 lymphoma-bearing donors (YAC-IMM + IL-1). YAC-1 lymphoma-bearing TXB recipients (*right*) failed to cause regression of their tumors in response to IL-1 therapy if they were infused with spleen cells from SA1-bearing donors (SA1-IMM + IL-1). However, spleen cells from YAC-1 lymphoma-bearing donors were capable of causing regression of this tumor without (YAC-IMM), as well as with IL-1 (YAC-IMM + IL-1). Means of five mice per group.

one or the other of these tumors, and injected on day 6-9 with 1 μ g of IL-1. It can be seen in Fig. 8, that combined treatment with immune spleen cells and IL-1 resulted in complete regression of the SA1 sarcoma in all mice. In contrast, treatment with IL-1 alone, or in combination with normal spleen cells, failed to give a significant therapeutic effect (Fig. 9). Moreover, in order for tumor regression to occur, it was necessary to infuse spleen cells from donors bearing the homologous tumor, in that IL-1 caused regression of the SA1 sarcoma in TXB mice infused with spleen cells from SA1-bearing donors, but not in TXB mice infused with spleen cells from YAC-1-bearing donors (Fig. 8). However, in this experiment infusion of spleen cells from YAC-1 lymphoma-bearing donors resulted in regression of the YAC-1 lymphoma in TXB recipients without the need for IL-1 treatment. This undoubtedly was because the YAC-1 lymphoma, being highly immunogenic, evoked the generation of high levels of immunity in the donors. This immunity was sufficient to cause regression of the donors tumor at the time that spleen cells were harvested for passive transfer. Regardless of this, it is apparent from results with the SA1 that injection of IL-1 served to convert a subtherapeutic level of adoptive anti-SA1 immunity to a therapeutic level.

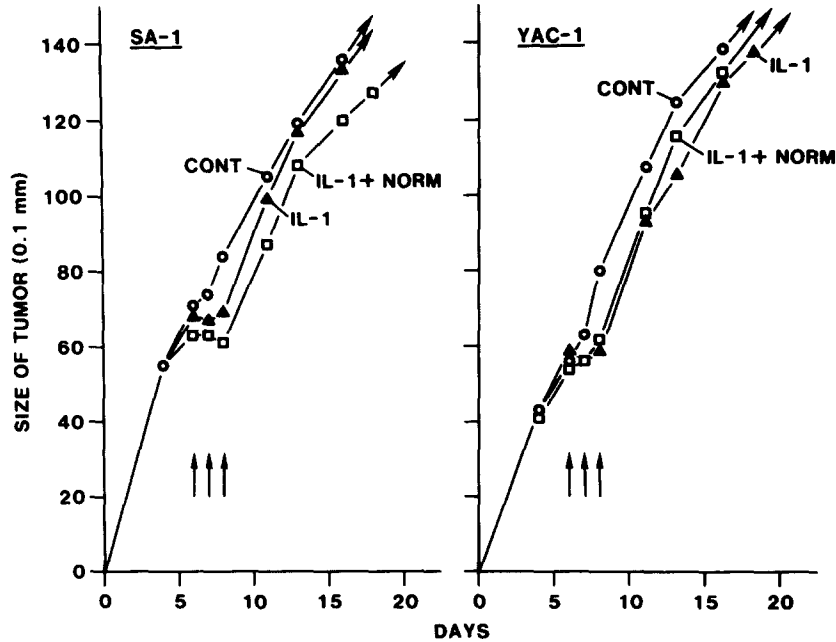


FIGURE 9. Additional controls for showing that normal spleen cells did not restore the ability of TXB mice to cause regression of the SA1 sarcoma (*left*) or YAC-1 lymphoma (*right*) in response to IL-1 therapy (IL-1 + NORM). Means of five mice per group.

Evidence that the spleen cells from SA1-bearing donors that imparted to TXB recipients the ability to cause regression of the SA1 sarcoma in response to IL-1 therapy are T cells is provided in Fig. 10. It can be seen that IL-1 failed to cause regression of the SA1 sarcoma in TXB mice infused with donor spleen cells treated with anti-

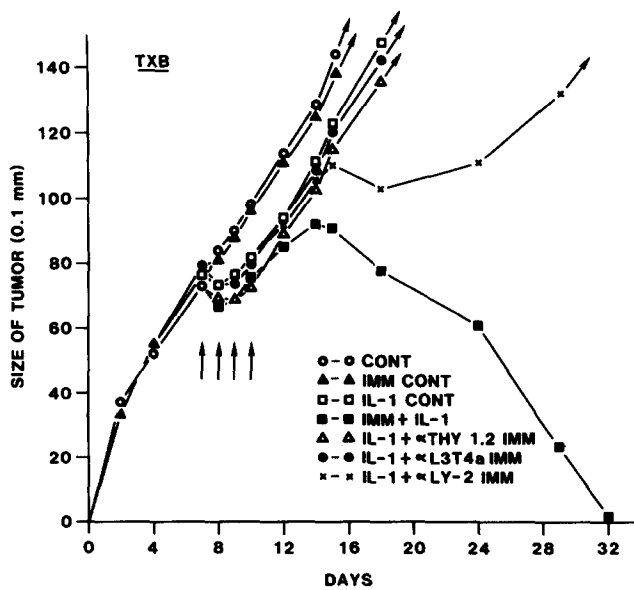


FIGURE 10. Evidence that the spleen cells from immunocompetent tumor-bearing donors that restore the ability of TXB recipients to cause regression of their tumor in response to IL-1 therapy were Ly-2⁺ and L3T4⁺ T cells. Infusion of donor spleen cells on day 6 of tumor growth enabled TXB recipients to cause regression of their tumor in response to injection of 1 μ g of IL-1 on day 6-10 (IMM + IL-1), but not if the spleen cells were treated with anti-Thy-1.2 antibody (IL-1 + α Thy-1.2 IMM), anti-L3T4 antibody (IL-1 + α L3T4 IMM), or anti-Ly-2 antibody (IL-1 + α Ly-2 IMM) and complement. Means of five mice per group.

Thy-1.2 antibody and complement, anti-Ly-2.2 antibody and complement, or anti-L3T4 antibody and complement. Therefore, both L3T4⁺ and Ly-2⁺ tumor-sensitized T cells were required for the therapeutic action of IL-1.

Discussion

The results of this study show that intraperitoneal injection of human rIL-1 β can cause regression of the immunogenic SA1 sarcoma and L5178Y lymphoma growing subcutaneously in syngeneic or semisyngeneic mice. The SA1 sarcoma was the more responsive of these two tumors to IL-1 therapy and underwent complete regression after injection of 0.5 μ g of IL-1 daily between days 6 and 10 of tumor growth. The L5178Y lymphoma failed to undergo regression in response to injection of 1 μ g of IL-1 on the same days, but did so after injections of 10 μ g. On the other hand, the P815 mastocytoma did not undergo complete regression, even in response to injections of the 10- μ g dose, although host survival was significantly prolonged. Therefore, although it remains to be determined whether higher doses of IL-1 can cause regression of the P815 mastocytoma, the results obtained thus far leave no doubt that some immunogenic tumors will prove to be much more susceptible to IL-1 therapy than others, and that nonimmunogenic tumors are likely to prove quite refractory.

Evidence that IL-1 therapy depends on host antitumor immunity is supplied by results showing that IL-1 failed to cause regression of the SA1 sarcoma growing in TXB mice that were incapable of generating a T cell-mediated concomitant immune response. More direct evidence is seen in the additional demonstration that IL-1 therapy could cause regression of the SA1 sarcoma growing in TXB mice, provided these mice were first infused with splenic T cells from immunocompetent donor mice bearing a 9-d SA1 sarcoma. On the other hand, IL-1 failed to cause regression of the SA1 sarcoma in TXB mice that were infused with normal splenic T cells, or with splenic T cells from donor mice bearing a different immunogenic tumor. Furthermore, the T cells that imparted to TXB recipients the ability to reject the SA1 sarcoma in response to IL-1 therapy were susceptible to anti-Ly-2 antibody and complement, and anti-L3T4 antibody and complement.

It is known that concomitant immunity to the SA1 sarcoma (14) and other tumors (15, 16), as measured by the presence in the host of T cells capable of passively transferring immunity to appropriate recipients, is first evident on day 6 of tumor growth, peaks on day 9, and progressively decays thereafter. This was the reason why IL-1 was given between days 6 and 9 of tumor growth. Indeed, because IL-1 was therapeutic when given daily between days 6 and 9 of tumor growth, but not when given between days 0 and 3, it can be suggested that the therapeutic action of the molecule requires that the host concomitant immune response be already induced and ongoing. This would be in keeping with the interpretation (18) that IL-1 stimulates replication of T cells only after they have been activated by mitogens or specific antigens. Results relevant to the findings presented here were recently published by Mannie et al. (19), who showed that rat T cells sensitized to myelin basic protein and capable of causing allergic encephalomyelitis in recipient rats can be expanded in number by incubating them with IL-1 *in vitro*, but only in the presence of myelin basic protein. Thus, although the role of IL-1 in T cell replication remains a controversial topic (2, 18), it is reasonable to suggest on the basis of the results presented that IL-1 causes regression of the SA1 sarcoma by stimulating an increase in the

production of SA1-sensitized T cells, thereby converting a subtherapeutic number of sensitized T cells to a therapeutic number. A study still in progress (North, Dye, and Dunn) shows that SA1-bearing mice treated with IL-1 between days 6 and 9 of tumor growth contain more SA1-sensitized T cells than control tumor bearers. This is evidenced by the finding that it required far fewer spleen cells from these mice on day 9 of tumor growth than from control tumor bearers to cause regression of a 5-d tumor in irradiated recipient mice. Whether this involves an increase in the replication of T cells already in cycle, or the recruitment into cycle of T cells that are suboptimally activated is not yet known, but is amenable to analysis. Also not known at this time is why a single 10- μ g dose of IL-1 given on day 6 or 9 of tumor growth is not as therapeutic as a much smaller dose given on days 6-9.

The results of this study as a whole are not in keeping with the view that the therapeutic action of IL-1 is based on its capacities to directly inhibit the growth of, or to destroy, tumor cells *in vivo*. IL-1 given intraperitoneally did not cause a tumor hemorrhagic reaction of the type that occurs rapidly after intravenous injection of TNF (20). Instead, the antitumor effect of IL-1 was not obvious until long after it would have been expected to have disappeared from the circulation. Moreover, the appearance of the tumor during IL-1-induced regression was similar to its appearance while undergoing regression in response to adoptive T cell-mediated immunity, as studied previously (21), in that the ring of living tumor tissue external to the tumor's necrotic core lost its pink color and slowly resorbed. Therefore, the relevance of the results presented here to descriptions of a direct cytostatic (8) or cytotoxic (9, 10) action of IL-1 for certain tumor cells *in vitro* is not obvious.

Exogenous IL-1, then, appears to be an *in vivo* immunoaugmenting agent capable of causing the regression of tumors of sufficient immunogenicity by augmenting an already ongoing host concomitant immune response. The results presented serve to illustrate the importance of having a knowledge of the magnitude and kinetics of generation of this host immune response, before attempts are made to cause tumor regression with IL-1, or other suspected immunomodulators. It was shown recently, in this regard, that tumor regression caused by administration of exogenous TNF (20), or by induction of endogenous TNF by endotoxin (21), is dependent on the possession by the host of an adequate level of concomitant antitumor immunity.

Summary

Intraperitoneal injection of human rIL-1 in a dose of 0.5 μ g daily for 5 d, or 1 μ g daily for 3 d, was capable of causing complete regression of immunogenic SA1 sarcoma growing subcutaneously in syngeneic or semisyngeneic mice. Higher doses of IL-1 were not more therapeutic against the SA1 sarcoma, but needed to be given to cause complete regression of the immunogenic L5178Y lymphoma. On the other hand, the P815 mastocytoma was much less responsive to IL-1 therapy, in that it failed to undergo complete regression in response to doses of IL-1 capable of causing regression of the L5178Y lymphoma. IL-1 caused regression of the SA1 sarcoma when given on days 6-8 of tumor growth, but not when given on days 1-3. This refractoriness of a small tumor to IL-1 therapy suggests that the antitumor action of IL-1 is based on an underlying host-immune response that is not generated until after day 3 of tumor growth. Direct evidence for the participation of host immunity in IL-1-induced tumor regression was supplied by results showing that IL-1 was not

therapeutic against the SA1 sarcoma growing in T cell-deficient (TXB) mice, unless these mice were first infused with Ly-2⁺ and L3T4⁺ T cells from donor mice bearing an established SA1 sarcoma. In contrast, normal T cells, or T cells from donor mice bearing a YAC-1 lymphoma, failed to provide TXB recipients with the ability to cause regression of their SA-1 sarcoma in response to IL-1 treatment. The results are in keeping with the interpretation that exogenous IL-1, by augmenting the production of tumor-sensitized T cells, converts a subtherapeutic level of host immunity to a therapeutic level. The results suggest, in addition, that IL-1 only stimulates the replication of T cells that are already engaged in the antitumor immune response.

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