

# Effect of Group A Streptococci on Transplantable Leukemia of Mice\*

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Bacteria or their products have repeatedly been reported to have a beneficial effect on human neoplastic disease (2, 7, 12). The earlier work has been reviewed by Shear (8), Shwartzman (9), and Andervont (1). Since then, many additional observations on tumor inhibition or regression associated with naturally occurring or artificially induced infections have been reported (4). Despite the extensive literature concerning the effects of infectious agents on the course of tumors and leukemias, there is little information concerning the mechanism by which microorganisms exert their beneficial effect. It was believed that the superimposition of a localized streptococcal adenitis upon a localized lymphoma would provide an experimental model to help elucidate the mechanism by which an infectious agent interferes with tumor growth.

The intranasal deposition of ascites-form leukemic cells (L4946) in AKR mice produces tumor growth initially confined to submaxillary and cervical lymph nodes (3). Similarly, the intranasal deposition of Group A streptococci is also followed by a regional adenitis which remains confined to the submaxillary and cervical lymph nodes for several days (3). No foci of tumor or bacterial multiplication occurred between the nasal mucosa and the submaxillary nodes, and thoracic extension with systemic dissemination of the tumor never preceded the local disease. Bacteremia and dissemination of streptococci rarely occurred before necrosis of submaxillary nodes, suggesting that the afferent lymphatics from the nasal mucosa were the route by which tumor cells and streptococci reached the submaxillary nodes. This report describes experiments in which mice received tumor cells intranasally and were challenged 48 and 72 hours later with Group A streptococci.

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## MATERIALS AND METHODS

*Mice.*—Mice were of the AKR/Jax strain obtained from Bar Harbor or from colonies raised from brother-to-sister matings of AKR/Jax breeders. The L4946, line B "ascites" form of lymphatic leukemia<sup>1</sup> used produces a uniformly fatal disease in AKR/Jax mice. The Group A streptococcus<sup>2</sup> used was originally isolated from a spontaneous outbreak of cervical adenitis in mice (5).

Methods for the preparation, quantitation, and nasal instillation of streptococci and tumor cells have been described (3). Evidence for bacterial infection was based on enlarged, palpable, purulent lymph nodes, encrusted blood around the eyes (Fig. 1), and positive bacterial cultures of involved tissues and tail blood.

The diagnosis of leukemia in mice was based on the presence of typically enlarged peripheral lymph nodes, hepatosplenomegaly, and characteristic blood leukocyte pattern. When the diagnosis of leukemia was questioned, confirmation was established by microscopic examination of liver or spleen and transplantation of suspensions of these organs into normal mice. All mice received tumor cells and/or the challenge dose of streptococci in the right nasal cavity, and animals were examined twice daily for evidence of infection or development of tumors.

## RESULTS

*Control of streptococcal adenitis with penicillin.*—To determine a suitable schedule of penicillin therapy for the control of the infection, treatment with penicillin was started in all infected leukemic mice 3–4 days after instillation of streptococci or as they developed typical signs of streptococcal adenitis. The dose of penicillin ranged from  $5 \times 10^2$  to  $5 \times 10^4$  units, depending on the severity of infection and course of the disease. In many instances, the streptococcal lymphadenitis could

<sup>1</sup> Supplied by Dr. Lloyd Law, Bethesda, Md.

<sup>2</sup> Obtained from Dr. J. B. Nelson, Rockefeller Institute, New York, N.Y.

be cured or controlled by manipulating the dose of penicillin administered.

To determine the effectiveness of treating streptococcal adenitis with penicillin, fifteen mice with palpable cervical and submaxillary streptococcal lymphadenitis received intraperitoneal injections of penicillin; an identical group of mice was left untreated (Table 1). Thirteen of the fifteen untreated mice died by the 27th day. The remaining mice developed suppurative lymphadenitis from which Group A streptococci were isolated. These two mice died on the 33d day. Two of the fifteen

TABLE 1  
EFFECTIVENESS OF PENICILLIN IN THE TREATMENT\*  
OF STREPTOCOCCAL ADENITIS OF AKR MICE

EXPERIMENTAL	DAYS OF DEATH									MICE SURV. >30 DAYS
	7	9	10	15	22	23	27	29	30	
Treated (15 mice)	0	1	0	0	0	0	0	1	0	13
Untreated (15 mice)	1	0	1	2	2	3	2	1	1	2

\* Treatment started 4 days after instillation of streptococci.

treated mice died of streptococcal infection despite treatment with penicillin, and thirteen recovered.

*Course of streptococcal infection in tumor-bearing mice.*—Streptococci deposited intranasally in mice bearing submaxillary tumors results in superimposed infection of the tumors. The infected nodes became purulent with drainage through the skin (Fig. 2). In certain instances animals with suppurative lymphadenitis treated with penicillin survived the treatment and remained free of tumors, while in other animals existing tumors regressed. In surviving mice the cervical region eventually stopped draining, with closure and healing of the skin perforations.

*Infection 48 hours after tumor instillation.*—Of the 100 mice which received streptococci 48 hours following tumor transplants and were treated with penicillin, 40 died of tumor, adenitis, or pneumonia by the 21st day (Table 2). All 29 tumor control mice had succumbed at this time. The remaining 54 test animals developed superimposed infection of the tumor-bearing nodes. As the streptococcal infection progressed, these surviving mice developed necrotic submaxillary nodes. Six of these 54 died, while 48 survived and remained tumor-free.

*Infection 72 hours after tumor instillation.*—Of the 100 mice which received streptococci 72 hours following tumor transplants and were treated with penicillin, 68 died by the 21st day (Table 2, Chart 1). The remaining 32 tumor-bearing mice developed superimposed streptococcal infection. As the infection progressed, these mice developed purulent lymph nodes from which pure cultures of gram-positive cocci were recovered. Six died of tumors, while 26 survived and remained tumor-free.

In both the 48- and 72-hour series, some tumor-bearing mice died as early as the 1st day after superimposition of the streptococcal infection (Chart 1). The etiology of these early deaths cannot be fully explained but was attributed to streptococcal pneumonia and septicemia. In general, mice in which typical adenitis did not occur died of septicemia in less than 48 hours.

Surviving mice were observed for several weeks, but reported data were based on a 30-day observation period to obtain minimum variation in statistical analysis. Data used for the determination of mean longevity were based on the assumption that all surviving mice would have died on the 31st day. The mean longevity in ascites control mice was 14.5 days, with an intra-group variance or standard error of  $\pm 0.6$  days (Table 2). In the 72-hour

TABLE 2  
EFFECT OF SUPERIMPOSED STREPTOCOCCAL INFECTION\* ON L4946 LYMPHOMA† OF AKR MICE

EXPERIMENTAL PROCEDURE	No. MICE	CUMULATIVE DEATHS				TOTAL CUMULATIVE		MEAN LONGEVITY (days)	DIFFERENCE	
		Days after nasal instillation of leukemic cells				Deaths	Survivors		In mean longevity ('t' test)	In per cent mortality ( $\chi^2$ test)
		7	14	21	30	(per cent)				
Streptococci 48 hr. after leukemic cells	100	15	25	40	52	52	48	22.6 $\pm$ 1.0	P < 0.001	P < 0.01
Streptococci 72 hr. after leukemic cells	100	28	40	51	74	74	26	18.2 $\pm$ 1.1	0.05 < P < 0.10	P < 0.01
Controls: Ascites leukemic cells only	29		55	100		100	0	14.5 $\pm$ 0.6		

\* Infection by intranasal instillation of Group A streptococci in right nasal cavity.

† Lymphoma by intranasal instillation of ascites form leukemic cells in right nasal cavity.

series the mean longevity was 18.2 days, approximately 4 days longer than that of the ascites control mice, with an intra-group variance of 1.1 days. In the 48-hour series the mean longevity in test mice was 22.6 days, with an intra-group variance of  $\pm 0.1$  day. This represents an increase in longevity of 8 days over the ascites control mice and 4 days over the 72-hour series. When the mean survival time of uninfected ascites control mice was compared with streptococcus-infected tumor-bearing mice by Fisher's "t" test (13), a significant increase in longevity of superinfected animals was found in the 48-hr. series ( $P < 0.001$ ) as well as the 72-hr. ( $P < 0.01$ ) series.

Data on the difference in per cent mortality between experimental and control groups were treated on the assumption that experimental animals surviving 30 days would survive to infinity. Streptococcus-infected tumor-bearing animals in both the 48-hr. ( $P < 0.01$ ) and 72-hr. ( $P < 0.01$ ) series showed a significant increase in absolute survival over the uninfected ascites control mice. A significant increase in the per cent mortality in both the 48-hr. ( $P < 0.01$ ) and 72-hr. ( $P < 0.01$ ) series was found (Table 2).

#### DISCUSSION

These experiments provide further confirmation of previous observations that superimposed infection may modify host response to neoplastic disease. Although these studies provide no definitive evidence for the mechanism of tumor regression or inhibition, the massive degeneration and necrosis of streptococcal-infected tumor-bearing nodes was striking. Among the factors considered important in tumor oncolysis and regression induced by bacteria are (a) the biologic competition of two actively growing systems in the presence of a limited nutritional supply, (b) toxicity of bacterial metabolic end-products on tumor growth, (c) the existence of a direct cytopathogenic effect of the microorganism upon the malignant cell, (d) stress response elicited by the presence of infectious agents, and (e) stimulation of "non-specific" host substances similar to properdin (6). A direct cytopathogenic effect of the streptococci on the tumor cells appears most probable from these studies.

The data in these experiments clearly show that streptococci were more effective in inhibiting tumor growth or producing tumor regression when the streptococci were given 48 rather than 72 hours after the tumor inoculation. After 48 hours, although tumor cells had reached the regional nodes and had begun to multiply, they were still susceptible to the damaging effects of the strepto-

cocci. It is believed that up to 48 hours these tumor cells remained largely confined to the regional submaxillary lymph nodes and were destroyed by the rapidly multiplying bacteria.

Streptococcal (St 9439) adenitis in the mouse after nasal instillation usually remained confined to the involved nodes despite the most fulminating infections (3). Streptococci may be more oncolytic within the confines of the lymph node than for metastatic tumor cells. On the other hand, Group A streptococci are known to liberate at least nine different toxins (10). Among the toxins liberated are powerful leukocidins known to have a destructive action on polymorphonuclear leuko-

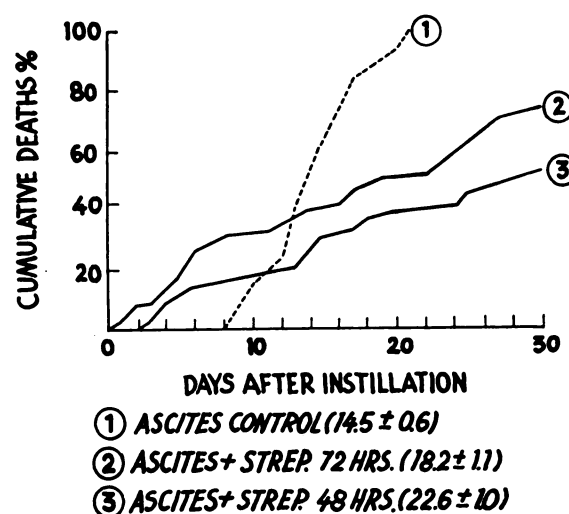


CHART 1.—AKR mice challenged with streptococci 48 and 72 hours after instillation of ascities leukemic cells.

cytes (11). This is of interest in light of clinical reports of tumor regression following Group A streptococcus infections (4).

#### SUMMARY

Two hundred AKR mice with submaxillary tumors produced by the intranasal deposition of ascites-form leukemic cells were superinfected with Group A streptococci at 48- and 72-hour intervals. Of 100 mice which received streptococci 48 hours after instillation of leukemic cells, 54 developed superimposed infection of the tumor-bearing nodes. After treatment with penicillin, 48 mice survived and remained tumor-free. Of the 100 mice which received streptococci 72 hours following tumor transplants, 32 developed superimposed infection of the tumor-bearing nodes. After treatment with penicillin, 26 mice survived and remained tumor-free. Infected tumor-bearing mice survived longer than did uninfected tumor-bearing controls. Mean survival times in days were:

tumor control series,  $14.5 \pm 0.6$ ; 48-hour infected series,  $22.6 \pm 0.1$ ; and 72-hour infected series, 18.2. Infected tumor-bearing animals in both the 48- and 72-hour series showed a significant absolute increase in survival over the uninfected ascites control mice.

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**FIG. 1.**—Photograph showing characteristic blood-encrusted eyes in AKR mouse with lymphadenitis induced by nasal deposition of Group A streptococci.

**FIG. 2.**—Photograph of AKR mouse with streptococcal infected tumor-bearing lymph nodes draining through the skin.