PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 354–357 (1974)

Intraerythrocytically Administered Tubercidin (7-Deazaadenosine) and Immune Protection of Mice[1] (37809)

DIETER W. GUMP,[2] JULIAN J. JAFFE, SARAH N. KREBS, EUPHRONIA MEYMARIAN, AND ELIZABETH L. HORTON
(Introduced by J. H. Gans)

Departments of Medicine and Pharmacology, University of Vermont College of Medicine, Burlington, Vermont 05401

Tubercidin (7-deazaadenosine; Tu), a cytotoxic antibiotic obtained from *Streptomyces tubercidicus* (1, 2) was found to be schistosomicidal *in vitro* at a concentration of 10^{-7} *M*; however, when administered to schistosome-infected mice either orally or by conventional parenteral routes, schistosomicidal dosage regimens of Tu invariably caused the death of 20–30% of the treated animals (3). The selective toxicity of Tu against schistosomes was subsequently increased by taking advantage of the following facts: (a) Tu is efficiently absorbed and sequestered by mammalian red cells *in vitro* (4); (b) the life-span and functionality of such Tu-laden red cells are not adversely affected when they are returned to the bloodstream of the donor (4); (c) schistosomes feed on red cells, beginning about 2 wk after cercarial penetration of the host (5). When Tu was first absorbed *in vitro* into 20–30% of the total red cells previously removed from mice and monkeys infected with either *Schistosoma mansoni* or *S. japonicum* and then these drug-laden red cells were transfused back into each infected donor following plasmapheresis, marked reductions in both the viability and egg-laying capacity of the schistosomes were observed with no obvious signs of host toxicity (3, 6).

In view of its ability to arrest schistosomiasis mansoni and schistosomiasis japonica in primates with no obvious signs of host toxicity when administered in this manner, Tu could be considered as a candidate for trial in human schistosomiases. However, it was found that after Tu-laden red cells were returned to the bloodstream of the dog or rabbit, Tu was released into the general circulation over a relatively long period of time (4). It seemed desirable, therefore, to determine whether the relatively small but steadily released increments of this cytotoxic purine nucleoside analog (7, 8) might cause less obvious but important detrimental effects in a mammal, such as the inability to respond to an antigenic stimulus. The present study was designed to determine whether intraerythrocytically administered Tu, in a schistosomicidal dosage regimen, would adversely affect the ability of mice to survive a lethal challenge with pneumococci after previous immunization with the appropriate polysaccharide antigen.

Materials and Methods. Swiss Webster female mice weighing approximately 20 g were immunized with a single ip injection of Type I pneumococcal vaccine.[3] The initial concentration of the vaccine (100 μg/ml) was diluted with saline to yield a final concentration of 1.0 μg/ml, and each mouse received 0.5 ml or 0.5 μg of pneumococcal polysaccharide. Preliminary experiments had

[1] This research was supported in part by the United States–Japan Cooperative Medical Science Program, administered by the National Institute of Allergy and Infectious Diseases, and Training Grant 1 TOI-AI-00365, National Institute of Allergy and Infectious Diseases.

[2] Address requests for reprints to D. W. G., University of Vermont College of Medicine, Burlington, VT 05401.

[3] Eli Lilly, Indianapolis, IN.

indicated that this quantity of vaccine was fully protective against ip challenge with 30 fully encapsulated Type I pneumococci administered 8 days after immunization.

The pneumococcal challenge was prepared by diluting 0.5 ml of an overnight culture of a Type I pneumococcus[4] grown in brain heart infusion broth (BHIB), in 4.5 ml of BHIB and incubating the broth at 37° for 4.5 hr. The BHIB was then serially diluted in BHIB so that the final dilution contained the desired number of viable pneumococci. All challenges were administered ip as a suspension of pneumococci in 0.5 ml of BHIB and each mouse received approximately 50 viable organisms. After challenge, the mice were observed for 2 wk, and from each group of 10 which experienced any deaths, one mouse was autopsied and its heart blood was cultured. In every instance, pneumococci were recovered in pure culture.

Mice were treated with Tu by a previously published technique (3). Tubercidin[5] was first solubilized in dimethylsulfoxide (DMSO)[6] and then diluted with saline to yield a concentration of 1 mg Tu/ml of 10% DMSO. Twenty to 30% of the estimated total blood volume was removed by retroorbital puncture and incubated *in vitro* for 1 hr at 37° in the presence of 0.3 mg Tu/ml whole blood; following plasmapheresis, the resuspended drug-laden red cells were returned to each donor iv into a tail vein. In one group of controls, the blood was incubated in the presence of the appropriate volume of 10% DMSO in saline; and in another the blood was incubated in saline only.

Results. The immunization procedures completely protected mice from doses of pneumococci which were lethal to 90–100% of nonimmunized mice (Tables I and II). When mice were immunized with Type I pneumococcal polysaccharide 1 day after receiving Tu and were challenged with viable Type I pneumococci 8 days later, no interference with the protective efficacy of immunization was detected (Table I). It was also evident that Tu, administered after its prior absorption into red cells, was not by itself lethal to mice, whether or not the mice were immunized. Furthermore, removal of 20–30% of the total blood volume and its exposure to either 10% DMSO or saline before replacement of the red cells did not interfere with the protective efficacy of immunization against subsequent pneumococcal challenge.

Identical results were obtained when the mice were immunized with Type I pneumococcal polysaccharide 7 days after receiving Tu and subsequently challenged with pneumococci 8 days after immunization (Table II). Thus, the daily increments of Tu which were released into the general circulation primarily by degeneration of drug-laden senescent red cells, and to a lesser extent by diffusion out of healthy drug-laden red cells (4), for up to 7 days before and 8 days after injection of the polysaccharide antigen, did not interfere with the ability of mice to produce protective antibodies. It was also evident that Tu, which was found previously to be a weak inhibitor of the *in vitro* growth of many species of gram-positive and gram-negative bacteria (1), did not by itself prevent the death of nonimmunized mice which were challenged with pneumococci (Tables I and II, group 3).

Discussion. The results of this study indicate that the intraerythrocytic administration of Tu to mice, in amounts which are known to be schistosomicidal (3), did not affect their ability to survive a lethal challenge with pneumococci after their previous immunization with pneumococcal polysaccharide antigen. It has previously been shown that the protective capacity of pneumococcal polysaccharide immunization is due to the elaboration of type-specific antibodies (9). Apparently the amount of Tu released into the general circulation for periods up to 1 wk before injection of the antigen, and thereafter, did not interfere with the subsequent ability of mice to produce protective antibodies.

[4] Lederle Laboratories, Pearl River, NY.

[5] Reference No. 8458-THP-65.5, kindly provided by Dr. G. B. Whitfield, Jr., Upjohn Co., Kalamazoo, MI.

[6] Spectrograde, Eastman Kodak Co., Rochester, NY.

TABLE I. Tubercidin Treatment Administered 1 Day Before Immunization and 9 Days Before Pneumococcal Challenge.

Treatment	Group no.	Immunization with Type I polysaccharide	Challenge with Type I *S. pneumoniae*	No. survivors/ 10 mice
Tubercidin	1	Yes	Yes	10
	2	Yes	No	10
	3	No	Yes	1
	4	No	No	10
Dimethylsulfoxide	5	Yes	Yes	10
	6	No	Yes	0
Saline	7	Yes	Yes	10
	8	No	Yes	1
None	9	Yes	Yes	9
	10	No	Yes	0

We decided to measure the immune response of mice by challenging them with viable pneumococci since this procedure would not only involve their ability to elaborate antibody against pneumococcal polysaccharide but also their ability to phagocytize and destroy engulfed pneumococci. This procedure had the further advantage of representing a primary immune response, which is more susceptible to immunosuppression than is a secondary immune response (10). The lack of any interference by Tu with the protective efficacy of pneumococcal vaccine can therefore be interpreted as evidence that Tu lacks significant immunosuppressive and cytotoxic activity when administered to mice in the manner described.

Nonetheless, it should be pointed out that had a 50% protective endpoint been used in these studies, rather than a 100% protective endpoint, some interference of antibody synthesis by tubercidin might have been noted. The immunization procedure we employed may have resulted in an amount of antibody considerably in excess of that required to protect the mouse. Therefore, a reduction in amount of antibody produced in the experimental group, might not have been detected.

Close association has been found in man between chronic septicemic salmonellosis and schistosomiasis mansoni (11) and japonica (12) on the one hand, and between *Salmonella* urinary tract infections with intermittent bacteremia and schistosomiasis haematobia (13) on the other. In this context, it would seem important to determine whether an antischistosomal drug to be used in man might also suppress antibody formation, since such a side effect could presumably compromise host defenses and result in enhancement of bacterial infection.

TABLE II. Tubercidin Treatment Administered 7 Days Before Pneumococcal Immunization and 15 Days Before Pneumococcal Challenge.

Treatment	Group no.	Immunization with Type I polysaccharide	Challenge with Type I *S. pneumoniae*	No. survivors/ 10 mice
Tubercidin	1	Yes	Yes	10
	2	Yes	No	10
	3	No	Yes	1
Dimethylsulfoxide	4	Yes	Yes	10
	5	No	Yes	1
None	6	Yes	Yes	10
	7	No	Yes	0

This would be especially significant in the case of Tu since this potent cytotoxic agent would, if sequestered in red cells and administered to man, be continuously released into the general circulation over a relatively long period of time.

The results of this study permit the conclusion that it is unlikely that Tu, administered intraerythrocytically to man, would seriously compromise immune defenses. Nonetheless, proof that Tu administration in this manner to man would lack immunosuppressive activity must await clinical confirmation.

Summary. Tubercidin (Tu) when administered to mice after its prior absorption into 20–30% of their red cells, did not interfere with the ability of Type I pneumococcal polysaccharide to confer full protection upon the mice against challenge with pneumococci of the same serotype. This finding indicates that the amount of Tu released daily into the general circulation for periods up to 1 wk before injection of the pneumococcal antigen, and thereafter, did not inhibit the production of protective antibodies by mice.

1. Anzai, K., Nakamura, F., and Suzuki, S., J. Antibiot., Ser. **10**, 201 (1957).

2. Suzuki, A., and Marumo, S., J. Antibiot., Ser. **14**, 34 (1961).

3. Jaffe, J. J., Meymarian, E., and Doremus, H. M., Nature (London) **230**, 408 (1971).

4. Smith, D. G., Reineke, L. M., Burch, M. R., Shefner, A. M., and Muirhead, E. E., Cancer Res. **30**, 69 (1970).

5. Clegg, J. A., Exp. Parasitol. **16**, 133 (1965).

6. Jaffe, J. J., Doremus, H. M., Dunsford, H. A., Kammerer, W. S., and Meymarian, E., Amer. J. Trop. Med. Hyg. **22**, 62 (1973).

7. Owen, S. P., and Smith, C. G., Cancer Chemother. Rep. **36**, 19 (1964).

8. Acs, G., Reich, E., and Mori, M., Proc. Nat. Acad. Sci. USA **52**, 493 (1964).

9. MacLeod, C. M., Hodges, R. G., Heidelberger, M., and Bernhard, W. G., J. Exp. Med. **821**, 445 (1945).

10. Gabrielsen, A. E., and Good, R. A., *in* "Advances in Immunology" (F. J. Dixon and J. H. Humphrey, eds.), Vol. 6, p. 91. Academic Press, New York (1967).

11. Rocha, H., Kirk, J. W., and Hearey, C. D., Jr., Arch. Intern. Med. **128**, 254 (1971).

12. Tai, T.-Y., Shu, C.-Y., Chang, H.-C., and Liu, Y.-U., Clin. Med. J. **76**, 426 (1958).

13. Farid, A., Higashi, G. I., Bassily, S., Young, S. W., and Sparks, H. A., Amer. J. Trop. Med. Hyg. **21**, 578 (1972).

Received July 18, 1973. P.S.E.B.M., 1974, Vol. 145.