Transfer Factor: Delayed Hypersensitivity to Schistosoma mansoni and Tuberculin in Macaca mulatta

Abstract. *Delayed hypersensitivity in* Macaca mulatta *infected with either* Schistosoma mansoni *or mycobacteria was demonstrated by biopsies of skin test sites. Both dialyzable and nondialyzable leukocyte extracts from infected donors transferred delayed hypersensitivity to recipient monkeys. In two recipients, skin test conversion was associated with in vitro transformation of the recipients' lymphocytes.*

Nonhuman primates are experimental animals of choice in many studies of immunologic problems in man. Such studies involving cell-mediated immunity (CMI) have, however, been hampered by the inability to transfer delayed hypersensitivity from sensitized nonhuman primates to normal recipients.

The dialyzable, low-molecular-weight transfer factor described by Lawrence (*1, 2*) has been used in man to transfer delayed reactivity to bacteria, fungi, proteins, and skin homografts. Baram *et al.* (*3*) found that not only the dialyzable factor from human peripheral leukocytes was able to transfer delayed hypersensitivity to human recipients, but also that a nondialyzable fraction had this property. Only nondialyzable extracts of peripheral leukocytes of *Macaca mulatta* (rhesus monkey) specifically sensitized both rhesus monkey and human lymphocytes in vitro (*4*). Transfer of delayed hypersensitivity from rhesus monkey to man in vitro has also been carried out with RNA extracts (*5*).

Baram *et al.* (*6*) showed in vitro lymphocyte transformation to be more sensitive than skin tests for detecting tuberculin hypersensitivity in the rhesus monkey. The conventional intrapalpebral skin test used in the monkey limits the number of tests in any one animal. Gross skin reactions (*7, 8*) with erythema (*9*) have been described in rhesus monkeys with delayed hypersensitivity. Contrary to the findings of Mackler *et al.* with ascaris antigen, in our investigations of immediate hypersensitivity in rhesus monkeys infected with *Schistosoma mansoni* (*10*) we never observed erythema when sensitive animals were skin tested on the chest; the allergic nature of these reactions has been confirmed histologically. In this study, delayed skin reactivity was not grossly evident on the chest. By gross observation, delayed hypersensitivity is demonstrable in schistosomiasis in man (*11*).

We report the results of skin biopsy studies of intradermal tests on the chest or back of rhesus monkeys infected with *S. mansoni* or with mycobacteria (*12*). The findings led us to study peripheral leukocyte extracts from man and rhesus monkeys for their ability to transfer delayed hypersensitivity to recipient monkeys. Human donors were PPD [purified protein derivative (tuberculin)] sensitive. Monkey donors were infected either with *S. mansoni* or with mycobacteria [*Mycobacterium kansasii, M. intracellulare, M. scrofulaceum,* or *M. bovis* (BCG strain)]; the mycobacterial infected monkeys had also been repeatedly skin tested with Old Tuberculin.

Skin testing was done with 0.005 mg of tuberculin PPD (Parke, Davis and Co., Detroit) (*13*) and an extract of *S. mansoni* adult worms (*14*) (0.04 mg of nitrogen per dose) in 0.1-ml volume. Biopsies were performed 72 hours later with a 5-mm diameter skin punch. The tissue was immediately fixed in buffered formalin and sections were stained with hematoxylin and eosin. The histopathological examinations were made without knowledge of the histories of the tissues.

Delayed hypersensitivity was characterized by focal or diffuse infiltration of mononuclear cells essentially of a large epithelioid type. The schistosome antigen elicited this reactivity in all of the 11 monkeys infected with *S. mansoni* for 5 months or more but not in 6 monkeys infected with mycobacteria. Conversely, five animals infected with *S. mansoni* did not react to PPD, whereas eight monkeys infected with mycobacteria showed characteristic skin reactivity.

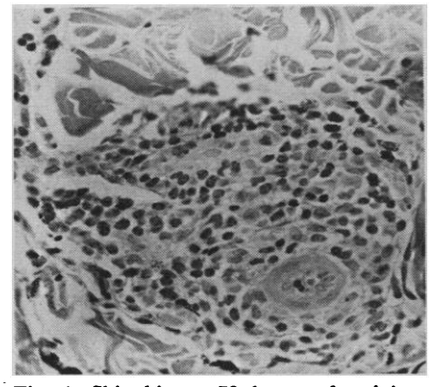

Fig. 1. Skin biopsy 72 hours after injection of *S. mansoni* antigen: recipient of dialyzable schistosome transfer factor showing typical delayed hypersensitivity reaction with large, epithelioid-type mononuclear cells (× 250).

Transfer factor was prepared according to the method of Lawrence (*2*). Peripheral blood leukocytes were obtained from 500 ml of human blood (500×10^6 to 750×10^6 lymphocytes) or 250- to 300-ml pools of monkey blood (about 400×10^6 lymphocytes). These were disrupted by freezing and thawing, treated with deoxyribonuclease, and dialyzed against 100 ml of water. The dialyzate was concentrated by lyophilization and called "dialyzable transfer factor." After dialysis, the remaining portions of the lysates were centrifuged and designated "nondialyzable transfer factor." In addition, some of these lysates were dialyzed further and called "exhaustively dialyzed transfer factor."

All preparations were filtered (Millipore; pore size 0.22 μm) immediately before subcutaneous injection. Skin tests were carried out 48 hours later; skin biopsies were performed 72 hours after the antigen injections. Some skin testing and biopsies were repeated 3 to 4 weeks after the administration of transfer factor.

Results of the transfer factor studies are given in Table 1. Both dialyzable and nondialyzable extracts (provided the latter were not exhaustively dialyzed) transferred delayed hypersensitivity. In positive reactions there is a typical infiltration of large mononuclear cells (see Fig. 1). Both the *S. mansoni* and PPD antigens elicited weak, chronic inflammatory responses characterized by small lymphocytic cells and occasional eosinophils in some of the respective controls. This type of reactivity was readily distinguishable from the delayed hypersensitivity reaction. The exhaustively dialyzed extracts gave weak, atypical reactions with polymorphonuclear or small lymphocytic cells, or both, as well as large monocytic cells in the infiltrates.

Dialyzable and nondialyzable transfer factor from human donors caused conversion of the PPD skin test in all (seven) recipient monkeys when they were tested 48 hours after the injection

Table 1. Skin biopsy results on rhesus monkey recipients of transfer factor. ND, not done.

Donor	Transfer factor	Interval between factor injection and skin test	Positive delayed hypersensitivity reaction	
			PPD	*S. mansoni*
Human tuberculin sensitive	Dialyzable	48 hours	6/6*	0/3*
		3 to 4 weeks	3/3	0/1
		8 weeks	2/2	0/1
		12 weeks	1/1	0/1
Human tuberculin sensitive	Nondialyzable	48 hours	1/1	ND
		3 to 4 weeks	1/1	ND
Human tuberculin sensitive	Exhaustively dialyzed	48 hours	4/5 Atypical	0/5
Monkey tuberculin sensitive	Dialyzable	48 hours	1/2	0/1
		3 to 4 weeks	1/2	0/2
Monkey tuberculin sensitive	Nondialyzable	48 hours	0/2	0/1
		3 to 4 weeks	2/2	0/1
Monkey infected with *S. mansoni*	Dialyzable†	48 hours	0/3	2/4
		3 to 4 weeks	0/3	4/4
Monkey infected with *S. mansoni*	Nondialyzable	48 hours	0/2	1/2
		3 to 4 weeks	0/1	2/2
Monkey nonsensitized	Dialyzable†	48 hours	1/4	0/4
		3 to 4 weeks	3/3	0/3
Monkey nonsensitized	Nondialyzable	48 hours	1/2	0/2
		3 to 4 weeks	0/1	0/1

* Number positive over number tested. † In each of these groups, one recipient received transfer factor prepared from lymph nodes instead of peripheral blood leukocytes. These were extracted from 4.7×10^9 and 3.0×10^9 lymphocytes, respectively.

of the extracts. When monkey donors were used, conversion was not always evident in recipients when skin tested 48 hours later. On retesting 3 to 4 weeks after the injection of the transfer factor, however, the recipients had delayed type reactivity. A time-dependent response was also observed by Baram and Condulis (*4*) when sensitization of monkey or human lymphocyte cultures was carried out with rhesus monkey nondialyzable factor. The requirement of a larger dose of PPD to elicit a tuberculin reaction in nonhuman primates (*15*) compared with that required in man agrees with our evidence that the mechanism of delayed hypersensitivity of nonhuman primates differs quantitatively from that of man. On the basis of body weight, the doses of human transfer factor given to the monkeys exceeded those usually required to convert immunocompetent human recipients to a state of delayed hypersensitivity. When the smaller dose of transfer factor from the monkey donors was given to monkey recipients, the time lag before conversion was demonstrable possibly allowed amplification of the sensitized lymphocyte population. One recipient of dialyzable transfer factor from PPD-sensitive monkey donors did not have demonstrable delayed hypersensitivity 3 weeks after transfer.

The absence of cross reactivity between the PPD and *S. mansoni* systems demonstrates the specificity of the transferred delayed hypersensitivity. The cause of the delayed hypersensitivity to PPD (demonstrable histologically at the cellular level) in four of six monkeys that received leukocyte extracts from nonsensitized monkey donors is not clear. However, these donors had been recently imported. During the screening period prior to use they were skin tested three times with Old Tuberculin (total dose of 2000 to 3000 tuberculin units), and by gross examination they were negative. These repeated intradermal injections may have stimulated delayed hypersensitivity to PPD (*16*).

Further analysis of the transfer of delayed hypersensitivity was attempted by using the in vitro lymphocyte transformation assay. Leukocytes were stimulated with an extract of adult *S. mansoni* worms (40 μg of nitrogen per milliliter) for 120 hours. Stimulation of DNA synthesis was quantitated by addition of 5-iodo-2-deoxyuridine labeled with ^{125}I (Amersham/Searle) and was compared with that of unstimulated control cultures (*17*). Before the animals received the dialyzable transfer factor, their stimulation values were 0.4 and 1.4 times the control values. Three weeks later (at the time of skin test conversion) these values had risen to 6.0 and 4.8, respectively.

Two studies suggest that de novo sensitization by antigen did not occur in our system. Recipients of dialyzable or nondialyzable transfer factor failed to develop antibodies to *S. mansoni*. Extracts of *S. mansoni* readily elicit antibody response in the monkey (*10*). To investigate the question of antigen sensitization further a modification of the migration inhibition technique of Paque *et al.* (*18*) was used to assay transfer factor. A quarter of a dose of dialyzable *S. mansoni* transfer factor was incubated for 48 hours with normal monkey lymphocytes with either *S. mansoni* antigen or PPD. The supernatant culture fluids were tested for migration inhibition properties with normal guinea pig macrophages. Migration in the presence of the *S. mansoni* supernatant fluid was only 57.2 percent of that with the PPD supernatant fluid. Parallel cultures with two batches of transfer factor from normal monkeys showed no inhibition. As a positive control to the system, lymphocytes from a monkey infected with *S. mansoni* were cultured with specific antigen or PPD. Migration in the presence of the *S. mansoni* supernatant fluid was 80 percent of that of the controls. These preliminary experiments clearly indicate the absence of antigenic stimulation in the transfer factor recipients.

These studies at the cellular level of reactivity may open up new avenues for the investigation of CMI. The use of nonhuman primates as experimental models for the transfer of delayed hypersensitivity would greatly facilitate the study of CMI in man. In addition, an animal model for the study of dialyzable, low-molecular-weight transfer factor would offer considerable advantages in the characterization of this extract.

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12. We thank K. D. Stottmeier and R. E. Beam of the Mycobacteriology Unit, Center for Disease Control, for the rhesus monkeys infected with mycobacteria.
13. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.
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Oyster Herpes-Type Virus

Abstract. *A herpes-type virus infection, the first to be found in an invertebrate animal, is reported in the oyster* Crassostrea virginica. *Intranuclear herpes-type viral inclusions were more prevalent in the oyster at elevated water temperatures of 28° to 30°C than at normal ambient temperatures of 18° to 20°C. The inclusions were associated with a lethal disease at the elevated temperatures.*

During a study on the effects of elevated water temperatures on oyster growth and survival, it was discovered that oysters living at an elevated temperature suffered a higher mortality rate than control animals. Histologic examination of the affected oysters revealed a disease in which cells frequently contained intranuclear inclusion bodies comparable to those associated with herpesvirus infections in other animals. Electron microscopy demonstrated typical herpes-type virus particles in the nuclei of cells containing inclusions. This is the first virus disease to be described in oysters (*1*) and, we believe, the first herpes-type virus infection to be recognized in any invertebrate species (*2*).

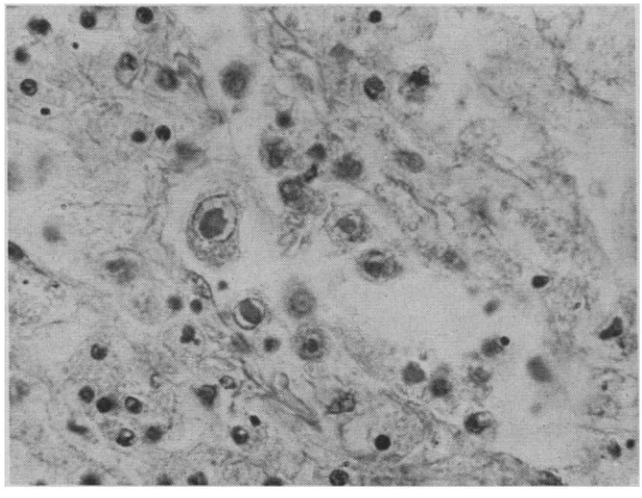
The oysters concerned in this report were all of the species *Crassostrea virginica*. Control and test animals for the initial study of thermal effects were all taken from a single location on the Marsh River, a tributary of the Sheepscot River near Wiscasset, Maine. They had been transplanted from a site in the Piscataqua River near Eliot, Maine, in 1968.

One set of 60 oysters was held in trays receiving water piped directly from the Sheepscot in the area of coolant water discharge from a fossil-fueled generating plant at Wiscasset. The water temperature in these trays was 28° to 30°C, a temperature range which, in the absence of disease, has no adverse effect on oyster survival and growth. A second set of 60 oysters was held under identical conditions except that the water with which the trays were irrigated was taken from the river at a point where there was no temperature elevation from the steam plant's coolant discharge. The water temperature in these trays was 12° to 18°C, and the salinity was the same as for the set held in higher-temperature water. Between June and August 1970, 1 to 2 months after the beginning of the experiment, the mortality in the higher-temperature set was 52 percent (31/60). In the same period, the mortality in the lower-temperature set was 18 percent (11/60).

Ten of the oysters that died in heated water were examined histologically. All were found to have intranuclear inclusions within the cells around the hemolymph sinuses (Fig. 1). The inclusions were consistent with those associated with herpes-type virus infections. The infected oysters had dilated digestive diverticula, cellular infiltrates in the vesicular connective tissue about the hemolymph sinuses, and, in advanced cases, massive cellular aggregates in these sites.

Electron microscopic examination of thin sections demonstrated viral particles within the nuclear inclusions. They were usually hexagonal, 70 to 90 nm in diameter, and had a single coat (Fig. 2). Some particles contained a dense nucleoid, others were empty. Some were seen to have several fine filaments extending through the coat from a dense, eccentrically placed nucleoid; this resulted in a flagellate appearance. Nuclear inclusions sometimes contained tubules with diameters of 45 to 55 nm (Fig. 3). The morphologic features of the virus closely resembled those of the Lucké virus associated with kidney tumors in the frog (*3*), and were characteristic of the structure of herpes-type viruses.

Unfortunately, none of the oysters in the control group grown in unheated water were examined histologically. To remedy this oversight and to deter-



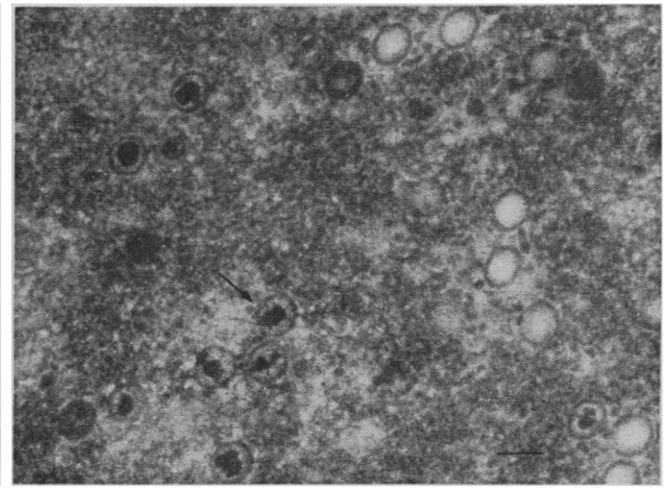


Fig. 1 (left). Intranuclear inclusions. Feulgen-stained section of an oyster infected with herpes-type virus (× 765). Fig. 2 (right). Electron micrograph of a thin section of an infected oyster, showing an intranuclear inclusion with various forms of virus particles, including empty particles and particles that appear flagellate (arrow) (bar 0.5 μm, × 18,600).