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Passive Transfer of Resistance to Tuberculosis Through Use of Monocytes.* (25506)

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Numerous attempts have been made to elucidate the mechanism of acquired resistance to tuberculosis. In particular, the possible role of cellular changes in acquired resistance has received considerable attention. Initial experiments by Lurie(1) indicated that rate of multiplication of tubercle bacilli was reduced in monocytes obtained from immunized animals when compared to monocytes obtained from nonimmunized animals. These early experiments were performed with a technic which employed the anterior chamber of eyes of nonimmunized rabbits as culture medium. Rich(2) however, using tissue culture technics, was unable to demonstrate inhibition of multiplication of tubercle bacilli in leukocytes obtained from immunized animals. Recent experiments utilizing improved tissue culture methods also resulted in divergent findings. Suter(3) reported significant inhibition of multiplication of tubercle bacilli in monocytes obtained from immunized guinea pigs when compared to multiplication in monocytes obtained from normal guinea pigs. He also found that serum obtained from immunized animals had no influence on results. Mackaness(4) on the other hand, reported no difference in this respect between monocytes from normal and immunized rabbits. Fong *et al.* (5) indicated that effective resistance to virulent tubercle bacilli by immune monocytes required continuous presence of immune serum. Lastly, Berthrong and Hamilton(6) recently reported that monocytes from immune guinea pigs limited intracellular multiplication of virulent tubercle bacilli. A direct resolution of the role of cells in acquired resistance to tuberculosis would involve the demonstration of transfer of resistance to tuberculosis to normal animals through use of monocytes from immunized animals. The present paper deals with experiments demonstrating this transfer.

Materials and methods. Mice of Strong A strain were used. Animals weighing initially 18-22 g were housed in separate metal cages in groups of 10 and were fed food and water *ad lib.* Mice were challenged by administration of 1 mg wet weight of H37Rv strain of *Mycobacterium tuberculosis* var. *hominis,* For this purpose, 3 week old surface pellicle cultures were harvested from modified Proskauer and Beck medium and standardized, using methods previously described(7). Immunized animals employed as donors for passive transfer experiments were given 1 mg wet weight of BCG‡ intravenously 3 weeks before sacrifice. These mice were immunized at appropriate consecutive intervals so that in each instance of passive transfer, donor preparations were available of the same age and as nearly alike as possible. A summary of ma-

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‡ BCG strain 4 was furnished through courtesy of Dr. Sol Roy Rosenthal, Tice Clinic, Univ. of Ill., Chicago.

TABLE I. Effect of Passive Transfer of Material from Normal Mice on Survival of Normal Mice.

	Material transferred	No. of mice	Median survival time in days and 95% confidence limits
1.	Plasma	35	14.8 (12-17)
	None	30	14.6 (12-17)
2.	Macrophages	25	16.2 (14-18)
	None	30	14.8 (12-17)
3.	Plasma & macrophages	50	16.0 (14-18)
	None	45	15.2 (13-18)
4.	Spleen homogenate	35	15.2 (14-18)
	None	30	15.0 (13-18)
5.	Spleen filtrate	20	15.6 (14-18)
	None	20	15.2 (14-18)

terials transferred and number of animals employed is given in Table I and II. Materials were obtained from either normal or BCG immunized mice and in each instance, were transferred the day before and 2 and 5 days after challenge by intravenous administration of 0.4 ml of appropriate preparation. All donor animals were sacrificed by hyperextension of the neck. Blood was obtained by thoracotomy and opening the beating heart. Blood from sacrificed mice was pooled, and serum removed for administration. Macrophages were obtained from peritoneal cavity by stimulation with 1 ml Klearol† given 5 days before transfer. Immediately after sacrificing, peritoneal cavities were opened and in each instance washed with 5 ml of chilled Tyrode's solution. Pooled washings were centrifuged 5 minutes at 250 g in refrigerated centrifuge (4°C). The supernatant was discarded and cells resuspended and centrifuged in chilled Tyrode's solution for additional 5 minutes at 250 g in refrigerated centrifuge. The supernatant was again discarded and the cells suspended in sufficient chilled Tyrode's solution to give a concentration of 400,000 to 600,000 monocytes/0.2 ml. Number of white cells in blood and peritoneal exudate was determined by counting in hemocytometer. Differential white cell counts were made from smears of blood and peritoneal exudates stained with Wright's stain. Spleen preparations were made by homogenization of spleens using size "A" Teflon grinders and matching ignition tubes. For each spleen, 5 ml of chilled Tyrode's solution was added. After 10 minutes homogenization, the preparation was diluted to contain 100 mg tissue in 0.2 ml. Equivalent filtrates were prepared from similar preparations by passing homogenized material through large pore sintered glass filter. In no instance did more than 2 hours elapse between time of sacrifice of donor animals and injection of cells, serum, or tissue into recipient animals. All materials transferred were given by intravenous route. The following preparations were employed in transfers: 1. For serum: 0.2 ml serum + 0.2 ml Tyrode's sol. 2. For monocytes: 0.2 ml monocytes in Tyrode's sol. + 0.2 ml Tyrode's sol. 3. For serum and monocytes: 0.2 ml serum + 0.2 ml monocytes in Tyrode's sol. 4. For spleen homogenate: 0.2 ml of homogenate + 0.2 ml of Tyrode's sol. 5. For spleen filtrate: 0.2 ml of filtrate + 0.2 ml of Tyrode's sol. The methods of Youmans and Youmans(8) were used for producing standardized tuberculous infection in mice and for evaluation of immune response.

Results. Table I gives results with material transferred from normal mice, while Table II gives results with material transferred from BCG immunized mice. Separate controls were employed in each experiment. Control animals were selected at random and received no materials at time passive transfers were made with test animals. Analysis of mouse white

† Klearol, a highly refined mineral oil.

TABLE II. Effect of Passive Transfer of Materials from BCG Immunized Mice on Survival of Normal Mice.

	Material transferred	No. of mice	Median survival time in days and 95% confidence limits
1.	Plasma	65	15.0 (13-17)
	None	60	14.8 (12-17)
2.	Macrophages	25	18.8 (17-21)
	None	20	14.6 (12-17)*
3.	Plasma & macrophages	70	19.2 (17-21)
	None	65	14.2 (12-16)†
4.	Spleen homogenate	35	14.8 (12-17)
	None	30	14.6 (13-17)
5.	Spleen filtrate	30	15.0 (13-17)
	None	20	15.2 (13-17)

* Significant prolongation of median survival time ($P = 0.05$).

† *Idem* ($P = 0.01$).

blood cell counts and differential counts revealed that the number of white cells was between 3,000 and 5,000 cells/ml^3, both before and after passive transfer. Distribution of types of cells was: Polys 10-18%; Lymphocytes 64-73%; Monocytes 6-11%; and Eosinophiles 0.2-0.5%. No white cell determinations were made after fifth day of challenge.

A comparison of white blood cell determinations before and after passive transfers indicated that no significant change occurred in total number of white cells after transfers. However, after each of the first 2 transfers of monocytes, the differential white cell count showed elevation of monocyte level to 12-18% of total count. This elevated level fell to near normal within 3 days after each of the first 2 passive transfers. No determinations of this type were made after third transfer.

Passive transfer experiments conducted with various preparations indicate that transfers involving macrophages from BCG immunized mice resulted in significant prolongation of survival. Prolongation of survival was small but significant at a level at which $P = 0.05$. Simultaneous transfer of macrophages and plasma from immunized mice resulted in prolongation of survival of recipient animals, statistically signifiacnt at a level at which $P = 0.01$. No other materials from immunized mice nor any materials from normal mice affected survival time.

Discussion. Passive transfer of resistance to tuberculosis through use of monocytes from immunized animals has been demonstrated by our experiments. In no instance was the effect as great as that seen with active immunization with BCG(8), nor did any of the other materials tested have any effect on survival. Simultaneous transfer of monocytes and plasma from immunized animals further demonstrated the transfer of resistance to tuberculosis, however the effect was not significantly different from that found with monocytes alone.

Transfer of monocytes resulted in 50-100% increase in peripheral monocyte level each time they were given. This is in agreement with the theoretical level expected in animals of this size. If it is assumed also that all transferred cells were viable and became fixed in tissues, each animal received a total of approximately 1.5 million additional functioning tissue monocytes. Since the infecting dose of tubercle bacilli contained approximately 40,000,000 viable particles(9) there would be one monocyte transferred for every 26-27 tubercle bacilli or clumps of tubercle bacilli. This number of tubercle bacilli/monocyte is well within the limits observed capable of being ingested by monocytes *in vitro*(3-6). However, normal monocytes of recipient animals would be expected to engulf many infecting organisms, thus making the effect of transferred cells less pronounced.

A similar increase in monocyte level has been noted in animals injected with BCG(10) and in human beings with active tuberculosis. Based on these observations it appears likely that a greater prolongation of survival would occur with transfer of larger numbers of monocytes from immunized animals.

While the existence of active cellular mechanisms resulting in acquired resistance to tuberculosis, appears to be established, the degree to which these mechanisms function in the infected animal remains to be elucidated. The question raised by investigations of Sever and Youmans(9,10) as to whether a quantitative change in monocyte response or a qualitative change in monocytes is involved in acquired resistance to tuberculosis, would appear to be partially resolved. Since passive transfer of monocytes obtained from normal animals produced no response while use of the same number of cells obtained from immunized animals increased resistance, it appears that a qualitative change in monocytes themselves was necessary. It is still possible that a larger number of normal monocytes than was used, might also produce some increase in survival time of mice.

The nature of the qualitative change in monocytes remains to be established. Some mechanisms available to the altered monocytes have been suggested by investigations of Sever and Youmans(9,10). Based on their observations, it appears that acquired resistance to tuberculosis may be due, at least in part, to rapid migration of macrophages into tuberculous areas which would result in local anoxia sufficient to inhibit the tuberculous

process. Also, Howard *et al.*(11) observed that increased metabolic activity of cells of the reticulo-endothelial system of BCG immunized animals may bring about development of intracellular environment unfavorable for bacterial multiplication. Should these cellular alterations function to their fullest extent, availability of immune monocytes in localized areas of infection could be sufficient to confine and inhibit the tuberculous process.

Summary. Experiments are reported which demonstrate passive transfer of a limited degree of increased resistance to tuberculosis through use of monocytes from immunized animals. Use of monocytes from normal animals had no effect on resistance. No increased resistance was seen in animals receiving plasma, spleen homogenate, or spleen filtrate obtained from either normal or immunized animals.

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Chromatography of Proteins of Squamous Cell Carcinomas and Normal Epithelium of Mice.* (25507)

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Electrophoresis has been used to fractionate proteins of extracts of various tumors and normal tissues(1,2). The method of chromatography of proteins on substituted cellulose ion exchange columns has been used in this laboratory to fractionate soluble proteins of liver and several enzyme activities localized in various fractions(3). The present paper is concerned with chromatographic fractionation of proteins of extracts from squamous cell carcinomas and normal epidermis of mice. Three enzymes have been localized in the fractions.

Methods. The normal epidermis of mice was removed from dermis mechanically as reported previously(4). Tumors used were: (a) primary squamous cell carcinomas induced in Swiss mice by painting the skin with methylcholanthrene; and (b) 3 lines of transplanted squamous cell carcinomas carried for several generations in this laboratory. Extracts were prepared by homogenizing at 0°C at high speed with a "Virtis" homogenizer in .005 *M* tris (hydroxymethyl)aminomethane-phosphate buffer, pH 8.00 ± 0.03. The homogenates were then spun 30 to 45 minutes at 13,500 rpm in Sorvall SS-1 centrifuge; the clear supernatants were dialyzed overnight against the same buffer, then stored frozen at −15°C. Chromatography on diethylaminoethylcellulose anion-exchange columns was carried out as reported previously(3). These methods will be reported later. Proteins were determined by the Lowry method(5); in addition absorbances at 280, 260 and 413 mμ were measured in each fraction. For β-glucuronidase assay, the Fishman method was used(6); for lactic-dehydrogenase (LDH),

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