Further Correlations of Cell Metabolism and Resistance to Tuberculosis: Studies on Mononuclear Peritoneal Exudate Cells from Mice and Guinea Pigs*

JOHN M. SMITH, JR.†, MARVIN J. ALLISON, AND BRENDA PAYNE

Division of Clinical Pathology, Department of Pathology, Medical College of Virginia, Richmond

The work of Lurie (1944) and Lurie, Zappasodi, and Tickner (1955) suggested that resistance to tuberculosis resides in certain mononuclear phagocytic cells of the reticuloendothelial system. On the premise that the physical activities of these cells might rest on metabolic activity, one of us (M.J.A.) began a series of experiments aimed at correlating resistance to tuberculosis and the level of enzyme activity in the system of intermediate metabolism of these cells.

Initial studies (Allison, Zappasodi, and Lurie, 1961) gave a close correlation between the level of metabolism of mononuclear cells from the peritoneal cavity and the level of native resistance of various inbred rabbit families to tuberculosis. Families of high resistance had a higher rate of metabolism for these cells than did families of low resistance; families of intermediate position in resistance were also intermediate in their cell metabolism. Later, it was shown that BCG vaccination produced a biphasic pattern of resistance associated with a biphasic pattern of metabolism (Allison, Zappasodi, and Lurie, 1962a and b). Shortly following administration of BCG, there was a depression in host resistance that rose considerably above normal about 4 to 5 weeks later. The mononuclear cell metabolism fluctuated with these changes in resistance. Alterations in resistance to tuberculosis, due to administration of triodothyronine, cortisone, and thyroidectomy, were all associated with alterations in cellular metabolism (Allison and Gerszten, 1962 and 1963). When the host's resistance was depressed, the metabolism of mononuclear exudate cells also decreased.

The aim of this study was to determine if species other than the rabbit also show a correlation in their resistance to tuberculosis with the metabolism of mononuclear cells from the peritoneal cavity. For this purpose, the mouse was chosen as a species that is more resistant than the rabbit and the guinea pig as more susceptible.

Materials and Methods

Three sources of mice were used; a Swiss albino variety, a market albino variety, and the grasshopper mouse. The mice were housed at a constant temperature of 70 F and were fed Purina dog chow checkers and water *ad libitum*. Market guinea pigs were used. Their aver-

* Supported by U. S. Public Health Service Research Grant A1-04686.

† Lederle Research Fellow, Summer, 1963.

age weight was 400 g, and they were housed under similar conditions as the mice, and fed Purina rabbit chow checkers and water. The mononuclear peritoneal exudate cells from the mice and guinea pigs were studied for metabolic activity. The procedure for obtaining these exudates was as follows. In the mouse, 1 cc of sterile mineral oil was injected intraperitoneally five days before the animals were killed by crushing the vertebral column at the base of the neck. The peritoneum was opened and washed with 5 cc of 0.90% citrated saline. The cells were removed from the peritoneum with a 10 cc syringe and then centrifuged for 10 minutes at 1,500 rpm. The supernatant was drawn off and the cells resuspended in 0.90% saline. The guinea pigs were killed by a blow on the head, having been injected four days previously with 5 cc of sterile mineral oil. Guinea pig cells were counted and adjusted in a similar manner, each animal being studied individually. In removing the peritoneal exudate cells, a 50 cc syringe was used to wash the peritoneum with 60 cc of 0.85% citrated saline. Total cell counts were done immediately, using a hemocytometer, and the saline volumes were adjusted to give a standard number of cells per cc. The mouse cells were pooled using ten animals per group. Slides were prepared for differential counts, and these were stained with Wright's stain and counted at a later date.

In studies used to measure inhibition of enzyme activity by histamine, the quadriceps femoris muscle from mice, guinea pigs, and rabbits was used. The muscle was ground in a mortar with a small amount of sea sand and distilled water. This triturated muscle was utilized in the studies discussed below. Ten million peritoneal exudate cell aliquots were used in the modified Thunberg technique (described by Allison *et al.*, 1961) to study the dehydrogenase activity. The following substrates were used: lactic acid, sodium succinate, sodium glycerophosphate, L-malic acid, glycerol, α-keto glutaric acid, β-hydroxybutyric acid, and sodium glycerophosphate for the acid phosphatase determination. The lactic dehydrogenase (LDH) activity of the muscle tissue was also studied, using the Macalaster Bicknell coenzometer. The substrate for this LDH test is prepared by dissolving 7 g of sodium pyrophosphate in 250 ml of hot distilled water. The solution is cooled to room temperature and lactic acid (1.5 ml) and diphosphopyridine nucleotide (1 g) are dissolved into this solution. The pH is adjusted to 8.8 with hydrochloric acid (1 N), and the final volume is adjusted to 280 ml with distilled water; 2.8 ml aliquots are placed in individual test tubes, capped, and stored frozen. This preparation will keep for several weeks. The coenzometer's light source is a 4-watt fluorescent lamp that emits a narrow band of ultraviolet light at 3,400 Å units. The change in concentration of reduced diphosphopyridine nucleotide during the enzyme reaction was measured by noting the change in ultraviolet absorption over a 3-minute period. Total nitrogen analyses were done using the Microkjeldahl method adapted from Hawk, Oser, and Summerson (1954). Acid phosphatases were measured with the Barringer and Woodard Harleco phosphate substrate (pH 4.5) and incubated for three hours. The released phosphate was then determined by the method of Fiske and Subbarow (1925).

Results

Table 1 shows the peritoneal exudate cell counts. Strains I and II mice yielded approximately 5 million cells per animal, with 85% mononuclears. Strain III mouse yielded approximately 6 million cells, 90% mononuclear cells. A five-day exudate following the induction of the chemical peritonitis was found to contain the highest percentage of mononuclears. The guinea pig yielded, on the average, 100 million cells per animal after a four-day

TABLE 1

Peritoneal Exudate Cell Counts

Species or Strain	Total Cells	Mono-nuclears	Poly-mor-phonu-clears	RBC	Mast Cells
Mouse Strain I	$54 \times 10^6 \pm 6.5$	85	3	7	5
Mouse Strain II	$54 \times 10^6 \pm 6.5$	90	3	7	0
Mouse Strain III	$62 \times 10^6 \pm 8.5$	90	1	4	5
Guinea Pig	$102 \times 10^6 \pm 23$	92	5	3	0

TABLE 2

Comparative Metabolic Activity of Peritoneal Exudate Cells from Mice and Guinea Pigs. Values Corrected for Cell Size*

Substrates	Ob-serva-tions	Guinea Pig†	Ob-serva-tions	Mouse Strain I†	Ob-serva-tions	Mouse Strain III†
Lactate	9	.027 ± .004	38	.028 ± .002	9	.039 ± .006
Succinate	10	.015 ± .003	57	.027 ± .002	9	.015 ± .003
Glycerophos-phate	10	.013 ± .004	35	.023 ± .004	20	.037 ± 0.00
Malate	11	.007 ± .002	30	.017 ± .004	20	.007 ± .006
Glycerol	11	.006 ± .003	50	.018 ± .004		
α-Ketoglutarate	8	.004 ± .004	30	.018 ± .004	20	.007 ± .001
β-Hydroxybuty-rate	10	.014 ± .003	55	.022 ± .004		
Acid phospho-tase	6	2.21 ± .44	16	.15 ± .02	40	.60 ± .26
Endogenous	9	.034 ± .004	45	.010 ± .001	9	.020 ± .004

* Values are for 10 million exudate cells.

† Endogenous activity subtracted before correction.

chemical peritonitis. The differential counts of these exudates were not statistically different, except that 5% mast cells were found in the peritoneal exudates from Strains I and III mice. These cells were absent in the exudates of Strain II mice. On the average, the total protein values for all three strains of mice were 0.79 mg per 10 million cells, and for the guinea pig, 1.66 mg per 10 million cells. To correct for differences in cell size, the dehydrogenase activity values were adjusted to the rabbit's cell protein (1.12 mg per 10 million cells); all values then are expressed in terms of rabbit protein. Table 2 shows these metabolic values with the adjustment for protein. The guinea pig showed the highest acid prosphatase activity, having a value of 4.60 mg ± 0.96 mg of phosphorus per 10 million cells per hour. The rabbit was next, with a value of 2.4 mg ± 0.33 mg of phosphorus per 10 million per hour. Acid phosphatases were run only on mouse Strains I and III; the values were 0.11 ± .02 mg and 0.54 ± 0.10 mg of phosphorus per 10 million cells per hour, respectively. Strain I mouse had peritoneal exudate cells lower in dehydrogenation activity than those of the guinea pig or the rabbit; Strain II and, in general, Strain III also "metabolized higher than the rabbit."

Since the activity of the mononuclears from Strains I and III mice metabolized at a lower level than anticipated, it seemed appropriate to study muscle tissue to determine comparative metabolism in the three species. The coenzometer was used to compare the lactic dehydrogenase activity in the three species. The quadriceps femoris was used as the enzyme source. The normal value for the rabbit was 258,500 LDH units; for the mouse, 625,562 LDH units; for the guinea pig, 100,000 LDH units per gram wet weight. The rabbit muscle LDH activity was intermediate between the guinea pig, which was lowest, and the mouse, which was highest.

Because of the possibility that

TABLE 3

The Effect of Histamine Hydrochloride on the Lactic Dehydrogenase Activity of Quadriceps Femoris Muscle of Mice

Group	Observations	Muscle Normal Activity	Observations	Muscle Plus ±0.033 mg. Histamine	Observations	Muscle Plus 0.00013 mg. Histamine	Observations	Muscle Plus 0.000093 mg. Histamine
I	6	1365 ± 240	6	0	—	—	—	—
II	3	1287 ± 159	—	—	3	711 ± 185	—	—
III	3	1246 ± 108	—	—	—	—	3	446 ± 2

histamine released from mast cells could alter the metabolic picture of cells (Graham *et al.*, 1955), we decided to repeat the muscle LDH tests after the addition of small amounts of histamine, to see if this altered the LDH activity. Histamine hydrochloride (Sigma Chemical Company) was used to study the effects on mouse muscle LDH activity. Different doses of histamine were incubated for 1 hour with the mouse muscle tissue. The LDH activity was then measured. Table 3 shows the effects of histamine on muscle LDH activity of mice. With a dose of 0.033 mg, there was no LDH activity displayed by the mouse muscle. At a dose of 0.00013 mg, there was a 45% reduction in activity; with a 0.000093 mg dose, there was a 64% reduction in activity.

Discussion

The metabolic activity of mononuclear cells from mice of different strains and from the guinea pig have been studied and compared to those of the rabbit. It was thought that, since the guinea pig is highly susceptible to tuberculosis, the activity of cells from its reticuloendothelial system would be lower than that of the rabbit. Because of its relative resistance, the mouse was expected to show high metabolic activity. The peritoneal exudate cells of the guinea pig followed the expected pattern by being lower than the rabbit in metabolic activity. Results from two of the three strains of mice used were equivocal, but one strain supported our hypothesis linking metabolic level of the mononuclear cells with resistance to tuberculosis. It was noted that the two "equivocal" strains of mice had in their exudates 5% mast cells. These cells were not found in the rabbit, guinea pig, or mouse Strain III. The different values for metabolism in the strains of mice whose exudates contained these cells suggest a possible interfering substance derived from these cells. Among the substances produced by these cells are histamine, serotonin, and heparin (Padawer, 1963). Motta, Da-Silva, and Fernandes (1960) showed that there is a simple exchange reaction between free histamine and the nicotinamide portion of the DPN molecule. This exchange might interrupt the electron transport system (Alivisatos *et al.*, 1960) and could be the means whereby the dehydrogenase activity of the peritoneal exudate cells of the Strains I and III mice were depressed. Graham demonstrated that dog mast cells produce, on the average, 7 $\mu\mu$g histamine per cell. A dose

similar to this was incubated for 1 hour with mouse muscle tissue, which is relatively low in mast cells. A definite depression in lactic dehydrogenase activity with the muscle-histamine mixture was noted. The exact mechanisms involved here remain to be investigated.

Our findings regarding acid phosphatase are similar to the findings of Colwell, Hess, and Tavaststjerna (1963) and Colwell and Hess (1963), who found a higher acid phosphatase activity in the susceptible guinea pig than in the resistant rat. The present work showed that the guinea pig has about 40 times as much acid phosphatase activity as the mouse. This suggests that this enzyme cannot be correlated with resistance to tuberculosis. It is clear from this work that there is a great species variation in the metabolism of the substrates used. However, the guinea pig clearly demonstrates that a depressed metabolism of the reticuloendothelial system can be correlated with low resistance to tuberculosis. The findings in the mouse also confirm this idea, if allowance is made for the possible depressing effects of histamine from the mast cells on the electron transport system. It is also interesting that different families of mice have different levels of metabolism, as in Lurie's inbred races of rabbits.

Summary

The metabolic activity of mononuclear exudate cells from mice of different strains, and from the guinea pig, have been compared to the rabbit with the aim of relating metabolic activity of these cells to resistance of these species to tuberculosis. The presence of mast cells in the peritoneal exudates of mice was thought to interfere with the dehydrogenation of certain substrates due to the release of histamine. Some experimental evidence presented by the authors seems to support this thesis.

References

ALIVISATOS, S. G. A., F. UNGAR, L. LUKACS, AND L. LAMANTIA. Imidasolytic processes. III. Enzymmatic conversion of coenzyme I to a histamine dinucleotide. *J. Biol. Chem.* 235: 1742–1750, 1960.

ALLISON, M. J. AND E. GERSZTEN. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. III. Studies on variation of thyroid function. *Am. Rev. Respirat. Diseases* 86: 513–517, 1962.

ALLISON, M. J. AND GERSZTEN. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. IV. Studies on cortisone-treated animals. *Am. Rev. Respirat. Diseases* 87: 384–388, 1963.

ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. The correlation of a biphasic metabolic response with a biphasic response in resistance to tuberculosis in rabbits. *J. Exp. Med.* 115: 881–889, 1962b.

ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. I. Studies on cells of normal non-infected animals. *Am. Rev. Respirat. Diseases* 84: 364–370, 1961.

ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. II. Studies on cells from BCG-vaccinated animals. *Am. Rev. Respirat. Diseases* 85: 364–372, 1962a.

COLWELL, C. A. AND A. R. HESS. Mononuclear cells from animals of divergent susceptibility to tuberculosis. II. Antimycobacterial properties. *Am. Rev. Respirat. Diseases* 88: 47–54, 1963.

COLWELL, C. A., A. R. HESS, AND M. TAVASTSTJERNA. Mononuclear cells from animals of divergent susceptibility to tuberculosis. I. Enzyme studies. *Am. Rev. Respirat. Diseases* 88: 37–46, 1963.

FISKE, C. H., AND Y. SUBBAROW. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375–400, 1925.

GRAHAM, H. T., O. H. LOWRY, N. WAHL, AND M. K. PRIEBAT. Mast cells as sources of tissue histamine. *J. Exp. Med.* 102: 307–318, 1955.

HAWK, P., B. L. OSER, AND W. H. SUMMERSON. *Practical Physiological Chemistry*. 13th ed. Philadelphia: Blakiston Company, Inc., 1954, p. 1439.

LURIE, M. B. Heredity, constitution and tuberculosis. An experimental study. *Am. Rev. Tuberc.* 44: (Supplement) 1–125, 1941.

LURIE, M. B., ZAPPASODI AND C. TICKNER. On the nature of genetic resistance to tuberculosis in the light of the host-parasite relationships in natively resistant and susceptible rabbits. *Am. Rev. Tuberc.* 72: 297–329, 1955.

MOTA, I., W. D. DASILVA, AND J. F. FERNANDES. The inhibition of mast cell damage and histamine release in anaphylaxis by pyridine and diphosphopyridine nucleotidase inhibitors. Comparison with compound 48/80. *Brit. J. Pharmacol.* 15: 405–409, 1960.

PADAWER, J. (ed) Mast cells and basophils. *Ann. N. Y. Acad. Sci.* 103: 1–492, 1963.