STUDIES ON TUBERCLE BACILLUS–HISTIOCYTE RELATIONSHIP*

V. Passive Transfer of Cellular Resistance

By JACOB FONG, Ph.D., DENNIS CHIN, and SANFORD S. ELBERG, Ph.D.

(From the Department of Bacteriology, University of California, Berkeley)

(Received for publication, November 15, 1961)

Cumulative knowledge concerning immunity against microbial infections permits categorization of resistance factors into humoral and cellular components. In acute bacterial infections, acquired resistance includes the participation of both mechanisms, but specific protective antibodies are generally of overriding importance because in their presence the efficiency of cellular mechanisms is greatly enhanced. In a chronic disease such as tuberculosis, it has as yet not been possible to adduce unequivocal proof of the existence of protective antibody; faced with this dilemma, various investigators have postulated a form of acquired resistance mediated largely, if not entirely, by cells.

Studies of acquired cellular resistance in tuberculosis have centered on histiocytes, and certain of these investigations suggested a prominent role for these cells in defense of the host. Thus, the histological and cultural studies of Lurie (1) on the fate of tubercle bacilli contained in normal and immune histiocytes cultivated in the chambers of the eyes of normal rabbits indicated a greater capacity of the cells of the tuberculous animal to inhibit multiplication of tubercle bacilli. The use of tissue culture methods (2, 3) has yielded more controversial findings, but one of these earlier studies (2) appeared to confirm Lurie's conclusions concerning acquired cellular resistance. Our own investigations involving *in vitro* cultures of infected histiocytes have shown that peritoneal exudate cells of normal and immunized rabbits could modify virulent tubercle bacilli, thereby reducing bacillary virulence for mice and inducing changes in certain other bacterial attributes (4). Although these modifications of virulent tubercle bacilli occurred after interaction of bacilli with either normal or immune histiocytes, only immune histiocytes were able to alter virulent tubercle bacilli to the extent where they lost the ability to necrotize the histiocytes themselves (5); this latter observation may be indicative of a greater capacity of immune histiocytes to modify bacillary virulence. Additional evidence for the concept of acquired cellular resistance in tuberculosis may be found in the recent observations of Sever (6) that immune histiocytes will passively transfer resistance to tuberculosis in the whole animal.

While the findings reported above afforded circumstantial evidence for the hy-

* This work was supported by a grant from the United States Public Health Service (Grant E-1372).

pothesis of cell-mediated resistance and indicated some of the end results of cellular action upon tubercle bacilli, they provided little information concerning the more fundamental aspects of cellular resistance. In the course of our studies of tubercle bacillus–histiocyte interaction, it was found that virulent tubercle bacilli killed the histiocytes of normal animals but had no effect upon the histiocytes of immunized animals (7, 8). This refractoriness is in essence a type of acquired cellular resistance and is uniquely suited to studies on the basic nature of cellular resistance.

The present paper reports one aspect of such a study, namely, passive transfer of cellular resistance by histiocytes and by lymph node cells; it also reports on the relative efficiency of transfer by these two cell types and on the successful transfer of resistance by histiocytic lysates.

Materials and Methods

Preparation of Cells for Transfer Experiments.–The three cell types used in passive transfer experiments included those cells which were generally found in varying numbers in the peritoneal cavities of rabbits which had been injected with klearol; these cell types were histiocytes, lymphocytes, and polymorphonuclear leucocytes. In the present studies histiocytes and polymorphonuclear cells were obtained from peritoneal exudates following injection of klearol, but lymphocytes were obtained from the mesenteric lymph nodes since these represented more readily available sources of lymphocytes. Details concerning the preparation of these cell types are described below; these procedures consistently yielded populations of cells in which the desired cell type comprised at least 90–95 per cent of the total cell population.

(*a*) *Histiocytes.*–In the earlier experiments normal adult rabbits and rabbits injected intradermally with the BCG strain of tubercle bacillus were used as histiocyte donors; animals given BCG were not used as donors until 30 or more days after injection of bacilli at which time the animals were regularly tuberculin-positive. In later studies only BCG-immunized animals were used as donors.

In experiments involving serial transfer of cells, histiocytes for the first transfer were obtained from rabbits immunized with BCG; in all subsequent transfers, histiocytes were obtained from recipient animals of the immediately preceding transfer series.

The histiocytes were obtained by washing the peritoneal cavity of each donor rabbit with 200 ml of chilled Tyrode's solution 5 days after intraperitoneal injection of 50 ml of klearol. The suspension of cells was centrifuged at 250 *g* for 3 to 4 minutes and the sedimented cells were washed 3 times with 150 to 200 ml of fresh Tyrode's solution at each washing. After the final washing, the histiocytes were suspended in a small volume of Tyrode's solution and the numbers of cells present were determined by counting in a hemocytometer. Cells prepared in this way were used in transfer experiments within 5 hours of harvesting.

Stained preparations of these cell suspensions showed 90–95 per cent histiocytes; the remainder consisted largely of polymorphonuclear leucocytes and lymphocytes.

(*b*) *Lymph Node Cells.*–In experiments not involving serial transfer of cells, the mesenteric lymph nodes of animals used as histiocyte donors were removed, freed of adhering tissue, washed, and the contained cells squeezed out with a pair of forceps after first cutting open the lymph nodes with a scalpel. The cells released from the lymph nodes were used as a source of lymphocytes in transfer experiments; these lymphocytes were handled and prepared in the same manner as outlined above for histiocytes.

In serial transfer experiments with lymphocytes, cells for the first transfer were extracted from the mesenteric lymph nodes of the same animals which served as histiocyte donors. All

subsequent transfers utilized lymph nodes obtained from recipient animals of the immediately preceding transfer series; to simulate the conditions existing in animals serving as histiocyte donors, the recipient animals which were to be used as lymphocyte donors were also given 50 ml of klearol 5 days prior to the collection of the lymph node cells.

Examination of stained smears of lymph node cells showed approximately 81 per cent small- and medium-sized lymphocytes. The remainder consisted of large lymphocytes (18 per cent) and cells which seemed to be lymphoblasts (1 per cent). Cells with the typical morphology of histiocytes were either absent or present in extremely small numbers, for none were found in the stained smears of lymph node cells.

(*c*) *Polymorphonuclear Leucocytes.*–These cells were obtained from the peritoneal cavities of normal or BCG-immunized rabbits which had been inoculated intraperitoneally 24 hours earlier with 50 ml of klearol. The method of collection and preparation of these cells followed the procedure described above for histiocytes.

Microscopic examination of stained smears showed 92–95 per cent polymorphonuclear leucocytes; the remaining 5 to 8 per cent of cells resembled histiocytes. Little or no lymphocytes were found.

Serum Used in Transfer Experiments.–The sera of normal and BCG-immunized rabbits which served as histiocyte donors were collected and used immediately in transfer experiments.

Passive Transfer Procedure.–The sera and the various cell types were injected intradermally into normal adult rabbits. The injections were made into several sites in the skin, each site receiving 0.5 ml of serum or cell suspension. The numbers of sites injected depended on the total number of cells or quantity of serum to be injected. In these experiments 4.5 ml of undiluted serum were inoculated intradermally. The total numbers of cells injected varied in different experiments and the values are indicated in the appropriate Tables.

Tests for Cellular Resistance.–These tests were made with the histiocytes of donor rabbits (normal rabbits and BCG-immunized rabbits) and recipient rabbits (rabbits given normal or immune serum, normal or immune donor cells, or cells derived from other recipient animals when serial transfers were made). In performing these tests, part of the histiocytes which had been collected from donor animals or recipient animals was centrifuged at 250 *g* for 3 to 4 minutes; the sedimented cells were redispersed in 6 to 8 ml of freshly prepared 0.25 per cent trypsin in Tyrode's solution. After 30 minutes at 24–26°C, the trypsinized cells were washed 3 times with Tyrode's solution. The packed cells from the last washing were resuspended in a few milliliters of immune serum (from BCG-immunized rabbits). The numbers of cells present were determined by counting in a hemocytometer. Part of these histiocytes were used as cell controls to determine that the uninfected cells used in the experiments did not undergo spontaneous degeneration. The rest of the histiocytes were tested for resistance against virulent tubercle bacilli according to the following procedure:

(*a*) *Parasitization of Histiocytes.*–The H37Rv strain of tubercle bacillus was mixed with the histiocytes in a ratio of approximately 10 bacteria per cell; 0.5 ml of the mixture was placed in a paraffin-lined bottle, centrifuged for 10 minutes at 850 *g*, and refrigerated 1 hour at 4°C. After refrigeration the supernatant fluid was discarded and the sedimented cells resuspended in a small volume of immune serum medium. The number of histiocytes was determined in a hemocytometer, and the infected suspension was diluted with additional immune serum medium to yield approximately 15 cells per mm^3; the diluted suspension of infected cells was used for *in vitro* cultures as described below.

(*b*) *Cultivation of Histiocytes.*–This was carried out in the culture chambers described by Mackaness (9). Approximately 0.05 ml of diluted infected histiocyte suspension was introduced into the space delineated by a plastic ring affixed to the bottom coverslip of the culture chamber. This yielded about 500 to 1000 cells in the culture chamber. After adherence of histiocytes to the bottom coverslip, the culture chamber was closed by insertion of the top coverslip.

Sufficient immune serum medium was introduced *via* lateral drill holes in the chamber to fill approximately two-thirds of the remaining space within the culture chamber. The chambers were incubated at 37°C.

(*c*) *Examination of Cultures.*–The number of histiocytes in the central area of the culture chamber was determined at the start of the experiment and at certain intervals thereafter. Counts were made with a phase contrast microscope and a 10 × objective. The full details concerning enumeration were described previously (7). Cellular resistance was evidenced by absence of cellular degeneration and constancy of cell numbers in infected cell cultures.

Staining of Preparation on Coverslips.–When it was desired to establish the proportion of infected histiocytes or the numbers of ingested bacteria, a coverslip with a centrally affixed plastic ring was prepared, and a small amount of the parasitized cell suspension was introduced into the central area. After adherence of the cells to the coverslip, the fluid was removed and the specimen allowed to air-dry. The specimen was fixed with heat, passed through successive changes of xylol and alcohol, and stained by the Ziehl-Neelsen method.

Percentage of Infected Histiocytes.–This was determined by counting a total of 200 stained cells.

Average Number of Bacteria per Infected Histiocyte.–This was obtained by examining 200 stained cells, counting the total number of intracellular bacteria, and dividing this total by the number of infected histiocytes.

Bacteria.–The bacteria used in these studies were the H37Rv and BCG strains of *Mycobacterium tuberculosis.*

For use in parasitization of histiocytes, the H37Rv strain was grown in tween-albumin liquid medium for 7 days at 37°C. The week-old culture was washed several times in tween-albumin medium; after the last washing the sedimented bacteria were resuspended in a small volume of medium and centrifuged at 250 *g* for 3 minutes to remove larger aggregates. The supernatant fluid obtained in this manner was found to consist mainly of bacteria occurring singly; after determination of bacterial cell numbers in a Petroff-Hausser chamber under dark-ground illumination, the supernatant fluid was used as a source of bacteria in parasitization of histiocytes.

The BCG strain of tubercle bacillus was cultivated on Calmette's potato medium. For use in injection of animals which were employed as cell and serum donors, the bacterial growth from a 2-week-old culture was ground with steel balls, suspended in physiological saline, and diluted to contain 1.0 to 2.0 × 10^8 bacteria per ml. This bacterial suspension was injected intradermally into rabbits in 0.1 to 0.2 ml amounts.

Nutrient Media.–The media used for cultivation of histiocytes consisted of 40 per cent rabbit serum (normal serum, anti-BCG serum, or serum from recipients in transfer experiments in Tyrode's solution (a modified Tyrode's solution containing no calcium was used). The particular type of serum used in an experiment is indicated in Table which summarizes the results of the experiment.

The pH of all nutrient media was adjusted to 7.4 with 5 per cent CO_2 in air before use.

Histiocyte and Lymphocyte Lysates.–Lysates of histiocytes were made by mixing large numbers of washed and packed histiocytes (the total numbers of cells used in different experiments for preparing these lysates are shown in the appropriate Table) with 5.0 ml of distilled water; after dispersion of the cells, the cell-water mixture was kept in a 37°C water bath for 3 hours with occasional shaking of the sample by hand. At the end of 3 hours, the sample was centrifuged for 10 minutes at 250 *g* to remove cellular debris and any unlysed cells; the 5.0 ml of relatively clear supernatant fluid was removed and the entire amount inoculated intradermally into a single rabbit in each transfer experiment.

Lysates of lymphocytes were prepared in a similar manner. The supernatant material following centrifugation of the lysed lymphocytes exhibited a gel-like appearance and it was

necessary to disperse this material as thoroughly as possible by repeated pipetting before injection into rabbits. The entire 5.0 ml of lysate was inoculated as in the case of histiocytic lysate.

TABLE I

Passive Transfer of Cellular Resistance by Histiocytes and Lymphocytes

Type of cell or serum used in transfer*	Number cells ($\times 10^7$) or ml serum transferred	Cell resistance tested post-transfer‡	Infected histiocytes	Average No. bacilli per histiocyte	Average degeneration 48 hours post-infection§
		days	*per cent*		*per cent*
Normal histiocytes and lymphocytes‖	129	7	16	3	40
Immune histiocytes and lymphocytes‖	127	7	19	4	44
Normal histiocytes and lymphocytes‖	355	13	24	3	47
Immune histiocytes and lymphocytes‖	328	13	21	3	0
Normal histiocytes	174	13	18	4	49
Normal lymphocytes	516	13	18	4	47
Immune histiocytes	315	13	20	3	4
Immune lymphocytes	230	13	18	4	0
Normal polymorph	30	13	22	4	56
Immune polymorph	409	13	20	4	62
Normal serum	4.5	13	17	3	49
Immune serum	4.5	13	18	3	55

* Cells or serum were inoculated intradermally into normal adult rabbits.

‡ Cellular resistance was tested by removing histiocytes from the peritoneal cavities of recipient rabbits, infecting them with virulent H37Rv, culturing them *in vitro* in presence of immune serum, and observing them for signs of degeneration. Cellular resistance was evidenced by absence of degeneration in infected cultures. All the necessary uninfected cell controls in serum media were included in each experiment, but only the results obtained with the infected experimental samples are shown in Table I.

§ Represents average per cent degeneration (from initial count of approximately 500 to 1000 histiocytes per culture chamber) in 2 to 3 replicate cultures.

‖ Ratio of histiocytes to lymphocytes was approximately 1:2. Normal indicates unimmunized rabbit and immune indicates BCG-immunized rabbit.

EXPERIMENTAL RESULTS

Passive Transfer of Cellular Resistance by Histiocytes and Lymphocytes.—The capacity of histiocytes (derived from the peritoneal exudates of BCG-immunized rabbits) and lymph node cells (from mesenteric lymph nodes of rabbits used as histiocyte donors) to passively transfer cellular resistance to normal rabbits is shown in Table I. It may be seen that after intradermal injection of a mixture of immune histiocytes and lymphocytes, histiocytes removed 13 days

later from the peritoneal cavities of recipient rabbits resisted necrotization by virulent tubercle bacilli; this was evidenced by the absence of cellular degeneration in *in vitro* cultures of these histiocytes which were infected 48 hours earlier with the H37Rv strain of tubercle bacillus. In contrast, infection of the histiocytes of recipient animals given a mixture of normal histiocytes and lymphocytes resulted in a 47 per cent loss of cells over the same time span. A similar refractoriness to necrotization was absent in the histiocytes of animals which had been given a mixture of immune histiocytes and lymphocytes 7 days earlier, for 44 per cent of these histiocytes had degenerated 48 hours after infection. Although recipients in the 7-day experiments received a smaller dose of cells than recipients in the 13-day experiments, the greater than 1×10^9 cells given animals in the 7-day experiments far exceeded the minimum numbers of cells needed for passive transfer of cellular resistance (see Results presented in the next section). It thus seems more reasonable to attribute the difference in cellular resistance noted between the histiocytes of the 7-day and 13-day animals to a difference in time of testing.

Individual testing of immune histiocytes and immune lymphocytes revealed that either cell could effect passive transfer of cellular resistance; thus, when approximately 2 to 3×10^9 immune histiocytes or lymphocytes were inoculated, the histiocytes of recipients were undamaged after 48 hours of infection (the 4 per cent degeneration noted for cells from recipients given immune histiocytes was within the limits of errors in the method of counting cells). On the other hand, infection of histiocytes from recipients inoculated with approximately 2 to 5×10^9 normal histiocytes or lymphocytes resulted in 47 to 49 per cent degeneration of cells over the 48 hour period.

When polymorphonuclear leucocytes of rabbits immunized with BCG were used in transfer experiments, the histiocytes obtained from recipient animals 13 days after intradermal injection of 4×10^9 leucocytes failed to exhibit any resistance; the 62 per cent degeneration noted for these histiocytes 48 hours after infection was as pronounced as that for histiocytes from rabbits injected with normal polymorphonuclear leucocytes.

The intradermal injection of 4.5 ml of undiluted normal or immune serum (from BCG-immunized rabbits) likewise failed to confer resistance upon the histiocytes of recipient animals and 49–55 per cent of these cells were destroyed 48 hours after infection.

As shown in Table I, exposure of histiocytes to tubercle bacilli resulted in a fairly uniform percentage of cells becoming infected in the different experiments (16–24 per cent). The average numbers of bacilli per infected histiocyte were also quite similar in these experiments (3 to 4). The differences in the resistance of histiocytes obtained from the various animals used in these studies were therefore not attributable to variations in degrees of infection of histiocytes.

Relative Efficiency of Histiocytes and Lymphocytes in Passive Transfer of Cellu-

lar Resistance.—The above findings have shown that intradermal injection of the histiocytes and lymphocytes of BCG-immunized rabbits rendered the histiocytes of recipient animals resistant to virulent tubercle bacilli and that similar cells from normal animals lacked this capacity. The contamination of the suspensions of histiocytes with small numbers of lymphocytes, as revealed by microscopic examination, necessitated studies of the relative efficiency of these two cell types to effect passive transfer. This was done by quantitative determination of the numbers of immune histiocytes and immune lymphocytes required for passive transfer of cellular resistance.

As shown in Table II, immune histiocytes were more effective in passive transfer of cellular resistance than immune lymphocytes. While either histiocytes or lymphocytes proved effective at a concentration of 1×10^8 cells, reduc-

TABLE II

Relative Efficiency of Histiocytes and Lymphocytes in Passive Transfer of Cellular Resistance

Type of cell used in transfer	Number cells ($\times 10^6$) transferred	Cell resistance tested post-transfer	Infected histiocytes	Average No. bacilli per histiocyte	Average degeneration 48 hours post-infection
		days	*per cent*		*per cent*
Immune histiocytes	100	13	19	3	0
Immune histiocytes	10	13	17	4	0
Immune histiocytes	1	13	17	4	57
Immune lymphocytes	100	13	19	3	0
Immune lymphocytes	10	13	18	4	59

See explanatory footnotes in Table I.

tion of cell numbers to 1×10^7 showed that only immune histiocytes permitted development of cellular resistance in recipients (0 per cent degeneration of histiocytes from recipients given immune histiocytes *versus* 59 per cent degeneration of histiocytes from recipients given immune lymphocytes at 48 hours postinfection). There is apparently a minimal number of cells needed for passive transfer of cellular resistance even with immune histiocytes; thus, injection of 1×10^6 histiocytes yielded no development of cellular resistance in recipient animals, for infection of their histiocytes resulted in a 57 per cent degeneration of cells over a 48 hour period.

These quantitative comparisons of the numbers of histiocytes and lymphocytes needed for passive transfer made it evident that successful transfers with histiocytes were not dependent upon the presence of contaminating lymphocytes. This was clearly the case in the transfer experiment with 1×10^7 histiocytes. Microscopic examination of the suspension of histiocytes used in this experiment showed at least 90 per cent histiocytes; if the remaining 10 per cent

consisted entirely of lymphocytes, there should be at most 1×10^6 lymphocytes. This amount of lymphocytes is obviously inadequate for passive transfer of cellular resistance.

Since 1×10^7 immune histiocytes approximated a minimal dose of cells needed for passive transfer, it is also unlikely that the successful transfer using 1×10^8 immune lymphocytes was referable to contaminating histiocytes. A concentration of 1×10^8 lymphocytes would have to contain 10 per cent histiocytes to be effective, and this level of histiocytic contamination, which should be detectable upon microscopic examination of stained cells, was not found. It is therefore apparent that even though immune lymphocytes were less efficient

TABLE III

Duration of Cellular Resistance After Passive Transfer of Histiocytes and Lymphocytes

Type of cell used in transfer	Number cells ($\times 10^6$) transferred	Cell resistance tested post-transfer	Infected histiocytes	Average No. bacilli per histiocyte	Average degeneration 48 hours post-infection
		days	*per cent*		*per cent*
Immune histiocytes	268	11	22	3	0
Immune histiocytes	268	13	20	4	0
Immune histiocytes	268	26	23	4	0
Immune histiocytes	268	35	20	3	0
Immune histiocytes	268	42	19	3	0
Immune lymphocytes	263	11	18	3	2
Immune lymphocytes	263	13	20	4	0
Immune lymphocytes	263	26	19	3	65
Immune lymphocytes	263	42	22	4	60

See explanatory footnotes in Table I.

than immune histiocytes, they nevertheless exhibited an independent capacity for passive transfer of cellular resistance.

Duration of Cellular Resistance in Recipients Following Injections of Immune Histiocytes and Immune Lymphocytes.—A dissimilarity in time of duration of cellular resistance in recipient animals given immune histiocytes and immune lymphocytes was also found, and this is shown in Table III. The histiocytes of recipients given immune lymphocytes resisted necrotization with virulent tubercle bacilli when tested at 11 and 13 days after transfer; as evidenced by the 60–65 per cent degeneration of histiocytes, resistance was not apparent at 26 to 42 days after injection of lymphocytes. In contrast, the histiocytes of recipients given immune histiocytes resisted necrotization by virulent tubercle bacilli over the entire period of 42 days.

This difference in duration of cellular resistance in the two groups of recipients did not result from variations in the numbers of cells transferred, per-

centage of cells infected by virulent bacilli, or average numbers of bacilli per infected histiocyte (Table III).

Passive Transfer of Cellular Resistance by Histiocytic Lysates.—The successful passive transfer of cellular resistance by appropriate concentrations of immune histiocytes and immune lymphocytes prompted experiments to determine whether lysates of these cells possessed a similar capacity. The results of these tests are shown in Table IV.

Injection of immune lymphocyte lysates into rabbits (one rabbit was given lysate equivalent to 36×10^9 lymphocytes and another rabbit was given lysate equivalent to 3.1×10^9 cells; the two lysates were prepared from separate

TABLE IV

Passive Transfer with Histiocytic and Lymphocytic Lysates

Type of lysate used in transfer	Number cell equivalent ($\times 10^8$) in lysate*	Cell resistance tested at post-transfer	Infected histiocytes	Average No. bacilli per histiocyte	Average degeneration 48 hours post-infection
		days	*per cent*		*per cent*
Immune histiocytic lysate	330	13	15	4	0
Immune histiocytic lysate	88	13	16	4	7
Immune lymphocytic lysate	360	13	14	4	47
Immune lymphocytic lysate	31	13	15	3	56

See footnotes in Table I.

* Cell lysate was prepared by addition of distilled water to cells and incubation of mixture at 37°C for 3 hours; after centrifugation of sample, the supernatant fluid was used as cell lysate. The various cell concentrations indicated in Table IV were lysed with 5.0 ml water; after centrifugation to remove cellular debris and any unlysed cells, the entire 5.0 ml of supernate was injected intradermally into a normal adult rabbit.

batches of cells) failed to confer cellular resistance upon the histiocytes of recipient animals; this was shown by the 47–56 per cent degeneration of their histiocytes 48 hours after infection. Similar tests made with two lysates prepared from immune histiocytes (recipients received the equivalent of 33×10^9 and 8.8×10^9 immune histiocytes) indicated successful passive transfer of cellular resistance. The histiocytes of recipient rabbits receiving the equivalent of 33×10^9 immune histiocytes showed no degeneration 48 hours after infection; the 7 per cent degeneration of histiocytes from recipients given the smaller dose of lysate, while exceeding the limits of errors (5 per cent) in enumeration of cell numbers, was still considerably less than the 47–56 per cent degeneration observed for recipients of the lymphocytic lysates.

Behavior of Recipient Serum in Tests for Cellular Resistance.—Since histiocytes are highly efficient phagocytes, the passive transfers of cellular resistance noted above may constitute mere transfer of bacilli and bacillary antigens with conse-

quent active induction of cellular resistance in recipients. The successful transfer of cellular resistance by phagocytically inefficient lymphocytes would in part negate this contention. The results of experiments reported in this and the following section offer further evidence against the idea of active induction of cellular resistance.

Previous studies have shown that immunization of rabbits with the BCG strain of tubercle bacillus may result in development of cellular resistance and the appearance of a protective serum factor (this serum factor protected immune histiocytes against necrotization by virulent tubercle bacilli). These two

TABLE V

Behavior of Recipient Serum in Tests for Cellular Resistance

Type of cell tested for resistance*	Cultivation serum for infected histiocyte‡	Infected histiocytes	Average No. bacilli per histiocyte	Average degeneration 48 hours post-infection
		per cent		*per cent*
Immune histiocytes.....	Normal serum	0	0	0
Immune histiocytes.....	Immune serum	0	0	2
Immune histiocytes.....	Recipient serum	0	0	0
Immune histiocytes.....	Normal serum	17	4	42
Immune histiocytes.....	Immune serum	18	4	0
Immune histiocytes.....	Recipient serum	18	3	44

See footnotes in Table I.

* The immune histiocytes used in these cellular resistance tests were obtained from BCG-immunized rabbits. The procedure for testing cellular resistance was as described under Materials and Methods except that during parasitization, the histiocytes were suspended in the same type of serum as that used for subsequent cultivation of the infected histiocytes.

‡ Normal serum was from tuberculin-negative rabbits; immune serum was from BCG-immunized, tuberculin-positive rabbits; recipient serum was from rabbits given immune histiocytes 13 days earlier.

events were dissociable, and production of protective serum factor was more readily achieved than development of cellular resistance (5). Consequently, if the present successful transfers depended on active induction of cellular resistance in recipients by bacilli or bacillary antigens, there should be simultaneous appearance of protective serum factor in recipients. As shown in the lower half of Table V, the sera of recipient animals failed to protect immune histiocytes against necrotization by virulent bacilli. In fact, the identical losses in cell numbers (42 and 44 per cent) observed for infected immune histiocytes maintained in recipient serum and in normal serum would indicate that recipient serum was completely devoid of protective factor. When the same infected immune histiocytes were cultivated in the presence of an established immune serum, no degeneration of histiocytes occurred.

The possibility that recipient serum was cytotoxic (10) because of the injec-

tion of histiocytes in passive transfer was ruled out by the data shown in the upper half of Table V. There was no degeneration in cultures of uninfected immune histiocytes maintained in the same sera as those used in experimental cultures.

With due consideration to the earlier observations on the greater ease of production of protective serum factor after injection of bacilli, the present inability to demonstrate protective factor in the sera of recipients exhibiting

TABLE VI

Serial Passive Transfer of Cellular Resistance by Histiocytes and Lymphocytes

Type of cell used in transfer*	Number cells ($\times 10^7$) transferred	Type of cell tested for resistance*	Average degeneration 48 hours post-infection
			per cent
Immune histiocytes	86	Histiocytes of first recipients	0
Histiocytes of first recipients	64	Histiocytes of second recipients	2
Histiocytes of second recipients	55	Histiocytes of third recipients	0
Histiocytes of third recipients	49	Histiocytes of fourth recipients	0
Immune lymphocytes	75	Histiocytes of first recipients	0
Lymphocytes of first recipients	64	Histiocytes of second recipients	3
Lymphocytes of second recipients	55	Histiocytes of third recipients	45
Lymphocytes of third recipients	44	Histiocytes of fourth recipients	41

See footnotes in Table I.

* Immune histiocytes were from BCG-immunized rabbits; histiocytes of first recipients represented cells of rabbits given immune histiocytes; histiocytes of second recipients represented cells from rabbits injected 13 days earlier with histiocytes of the first recipients; histiocytes of third recipients represented cells from rabbits injected 13 days previously with histiocytes of the second recipients; histiocytes of fourth recipients represented cells from rabbits previously injected with histiocytes of the third recipients. (Histiocytes from similar groups of rabbits were pooled before use). The designation for lymphocytes follows the same general scheme as for histiocytes.

cellular resistance points against active induction of cellular resistance by bacilli as a mechanism of passive transfer.

Serial Passive Transfer of Cellular Resistance by Immune Histiocytes.—A second test for possible transfer of bacillary antigens by immune histiocytes involved serial transfers of histiocytes. There can be no question that the methodology of serial transfer will result in dilution and elimination of non-replicating bacillary antigens; this procedure should be equally effective if surviving but non-proliferating bacilli occur. Since in the experiments to be described, microscopic examination of the cell suspensions used for transfer failed to disclose presence of acid-fast bacilli, it does not seem unreasonable to assume that actively proliferating bacilli were absent.

In these experiments, the first transfer was made with the histiocytes of

rabbits immunized with the BCG strain of tubercle bacillus; after 13 days, the recipient animals were sacrificed and their pooled histiocytes were used in tests of cellular resistance and for injection into another group of normal rabbits; this procedure was repeated at 13 day intervals until four successive transfers had been made (*i.e.*, one transfer with cells of BCG-immunized animals and three with recipient cells in which each transfer of recipient cells constituted one further step in removal of transfer cells from the original immune histiocytes).

The upper half of Table VI shows that the histiocytes of all four groups of recipient animals given injections of histiocytes from other animals in the series became refractory to necrotization by virulent tubercle bacilli. This was evidenced by absence of degeneration in all experiments shown in the upper half of the Table. It is therefore apparent that the histiocytes of the last animals in the transfer series were as capable of passive transfer of cellular resistance as the original histiocytes derived from animals directly immunized with BCG.

A similar serial transfer experiment using lymphocytes is shown in the lower half of Table VI. There was apparent loss of the capacity for passive transfer of cellular resistance by lymphocytes after the second transfer. Thus, while no significant degeneration occurred after infection of histiocytes from the first and second groups of animals, 41–45 per cent degeneration was found for histiocytes from the third and fourth groups of animals.

Determinations of percentage infection of histiocytes and of average numbers of bacilli per infected histiocyte were also made in these experiments. The results (not shown in Table VI) indicated that the general magnitude of variability of these two sets of controls resembled that shown in preceding Tables.

DISCUSSION

A general and primary defensive mechanism of vertebrate animals against infectious microbes is embodied in host cells of mesenchymal derivation. Intensive studies of these cells in recent times have allowed partial delineation of their functions and assignment of antibody production chiefly to plasma cells and probably lymphocytes. The function of histiocytes is much less clearly defined, and speculations concerning their role in antibody production must necessarily be related to the particular hypothesis of antibody formation toward which the investigator is inclined. The role of histiocytes in resistance unrelated to antibody production is perhaps only slightly better characterized. The existence of immune histiocytes has been suggested by various investigators studying tubercle bacilli (1, 2, 7, 8) and brucellae (11, 12); the observations of Good (13) on the response of congenital agammaglobulinemics to viral infection might be similarly interpreted.

While it is probably true that general acceptance of the existence of cellular resistance is increasing, the real significance of this form of resistance remains enigmatic. Questions concerning the mechanism of induction of cellular im-

munity, the particular cell types involved, the heritability of this state of cellular activity, and other pertinent aspects of the problem stand unanswered.

The present studies have attempted to analyze one facet of the phenomenon, namely, passive transfer of acquired cellular resistance. The results obtained have shown that suspensions of immune histiocytes possessed the capacity for passive transfer of cellular resistance. While it cannot be stated categorically that immune histiocytes were the prime initiators of cellular resistance in recipients, there is good reason to suspect this. Thus, of the three cell types (histiocytes, lymphocytes, polymorphonuclear leucocytes) found in suspensions of histiocytes used in these experiments, polymorphonuclear leucocytes were shown to be definitely lacking in ability to effect passive transfer of cellular resistance. Although immune lymph node cells were able to confer cellular resistance upon the histiocytes of recipient animals, they were much less efficient than immune histiocytes; this was evidenced by the smaller numbers of immune histiocytes needed for passive transfer, the greater capacity of histiocytes in serial passive transfer, the longer duration of cellular resistance in recipients given histiocytes, and the ability of histiocytic but not lymphocytic lysates to effect passive transfer. Consequently, unless there was an active, undetected contaminating cell type present in minute numbers, it may be concluded that histiocytes played a primary role in this phenomenon.

Investigations of the mechanism of passive transfer of cellular resistance by histiocytes seemed to rule out the possibility of active induction of cellular resistance through transfer of bacillary antigens by histiocytes. This conclusion was based on the inability to demonstrate protective factor in the sera of recipients and the successful serial passive transfer of cellular resistance. The latter observation in which histiocytes from recipient animals at the end of the transfer series proved as effective as the original immune histiocytes (from BCG-immunized rabbits) would imply active replication of some substance responsible for these successful passive transfers. Whether this "cell resistance factor" is the genetic material of the cell itself or whether it replicates in or is elaborated by cellular cytoplasmic components remains for future study. It is apparent, however, that irrespective of the nature of the "cell resistance factor," it is extractable from immune histiocytes by simple lysis of the cells.

While various explanations may be advanced for the observation that immune lymphocytes also effected passive transfer of cellular resistance, these cells are perhaps best considered as inadvertent carriers of resistance factor. This interpretation seems reasonable since in serial transfer experiments there was only brief perpetuation of "cell resistance factor" in lymphocytes.

The passive transfer of cellular resistance by immune cells and not immune serum reported herein calls to mind the studies of cellular transfer of the delayed type of hypersensitivity first reported by Chase (14). The passive transfer of cellular resistance by histiocytic lysates finds a parallel in the studies of transfer

factor in the delayed type of hypersensitivity (15, 16). A basic difference in the passive transfers of these phenomena appears to be the initiating cell type; thus, in transfer of cellular resistance, histiocytes were of prime importance whereas in transfer of the delayed type of hypersensitivity, lymphocytes were essential. Determination of the relationship of these phenomena to one another is of obvious major importance for true comprehension of the entire pattern of vertebrate response to infectious microbes. Active investigation of these relationships is in progress.

SUMMARY

Studies of passive transfer of cellular resistance, as manifested by refractoriness to necrotization with virulent tubercle bacilli, have shown that immune histiocytes or immune lymphocytes were effective transferring agents; immune polymorphonuclear leucocytes and immune serum as well as comparable cells from normal animals lacked this capacity.

Comparisons of immune histiocytes and immune lymphocytes showed that the former cells were more efficient; this was indicated by (*a*) the smaller numbers of immune histiocytes needed for passive transfer, (*b*) the longer duration of cellular resistance in recipients given histiocytes than in those given lymphocytes, (*c*) the greater capacity of histiocytes to effect serial passive transfer, and (*d*) the ability of histiocytic but not lymphocytic lysates to transfer cellular resistance.

Experiments to establish the mechanism of passive transfer of cellular resistance showed that there was no active induction of resistance in recipients through transfer of bacillary antigens contained in immune histiocytes; in fact, the results of serial passive transfers with immune histiocytes suggested an active replication of the "cell resistance factor."

BIBLIOGRAPHY

1. Lurie, M. B., The fate of tubercle bacilli ingested by mononuclear phagocytes derived from normal and immunized animals, *J. Exp. Med.*, 1942, **75,** 247.
2. Suter, E., Multiplication of tubercle bacilli within mononuclear phagocytes in tissue cultures derived from normal animals and animals vaccinated with BCG, *J. Exp. Med.*, 1953, **97,** 235.
3. Mackaness, G. B., The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits, *Am. Rev. Tuberc.*, 1954, **69,** 495.
4. Fong, J., Chin, D., and Elberg, S. S., Studies on tubercle bacillus–monocyte relationship. IV. Effects of passage in normal and immune systems upon virulent bacilli, *J. Exp. Med.*, 1961, **114,** 75.
5. Fong, J., Chin, D., Akiyama, H. J., and Elberg, S. S., Studies on tubercle bacillus–monocyte relationship. III. Conditions affecting the action of serum and cells: modification of bacilli in an immune system, *J. Exp. Med.*, 1959, **109,** 523.
6. Sever, J. L., Passive transfer of resistance to tuberculosis through use of monocytes, *Proc. Soc. Exp. Biol. and Med.*, 1960, **103,** 326.

7. Fong, J., Schneider, P., and Elberg, S. S., Studies on tubercle bacillus–monocyte relationship. I. Quantitative analysis of effect of serum of animals vaccinated with BCG upon bacterium-monocyte system, *J. Exp. Med.*, 1956, **104,** 455.
8. Fong, J., Schneider, P., and Elberg, S. S., Studies on tubercle bacillus–monocyte relationship. II. Induction of monocytic degeneration by bacteria and culture filtrate: Specificity of serum and monocyte effects on resistance to degeneration, *J. Exp. Med.*, 1957, **105,** 25.
9. Mackaness, G. B., The action of drugs on intracellular tubercle bacilli, *J. Path. and Bact.*, 1952, **64,** 429.
10. Harvie, N. R., and Elberg, S. S., Observations on cytotoxic effect of antihistiocyte serum, *Proc. Soc. Exp. Biol. and Med.*, 1961, **107,** 8.
11. Holland, J. J., and Pickett, M. J., A cellular basis of immunity in experimental brucella infection, *J. Exp. Med.*, 1958, **108,** 343.
12. Elberg, S. S., Schneider, P., and Fong, J. Cross-immunity between *Brucella melitensis* and *Mycobacterium tuberculosis*: intracellular behavior of *Brucella melitensis* in monocytes from vaccinated animals, *J. Exp. Med.*, 1957, **106,** 545.
13. Good, R. A., Host-parasite relationships in living cells, Springfield, Illinois, Charles C. Thomas, 1957, 78.
14. Chase, M. W., The cellular transfer of cutaneous hypersensitivity to tuberculin, *Proc. Soc. Exp. Biol and Med.*, 1945, **59,** 134.
15. Lawrence, H. S., The transfer of generalized cutaneous hypersensitivity of the delayed tuberculin type in man by means of the constituents of disrupted leucocytes, *J. Clin. Inv.*, 1954, **33,** 951 (abstract).
16. Lawrence, H. S., The transfer in humans of delayed skin sensitivity to streptococcal M-substance and to tuberculin with disrupted leucocytes, *J. Clin. Inv.*, 1955, **34,** 219.