STUDIES OF TUBERCLE BACILLUS–HISTIOCYTE RELATIONSHIPS

VIII. Comparative Study of Cellular Resistance Induced by Brucella and Mycobacteria*

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, July 8, 1964)

Earlier reports (1, 2) from this laboratory have shown that immunization of animals with the BCG strain of tubercle bacillus resulted in the development of resistant populations of histiocytes in these animals; this form of cellular resistance was manifested by the refractoriness of infected immune histiocytes to necrotization by virulent tubercle bacilli (H37Rv strain). The expression of such resistance was, however, dependent upon the presence of some non-specific factor present in homologous and heterologous immune sera (*e.g.* anti-BCG, antiovalbumin, anti-*Salmonella* sera).

Further studies (3) have shown that a similar type of cellular resistance was demonstrable with the histiocytes of rabbits immunized with the Rev I strain of *Brucella melitensis*. There was, moreover, a state of cross-immunity between the *Brucella*-immune (from animals immunized with Rev I) and the BCG-immune (from animals immunized with BCG) histiocytes such that infection of one or the other of the two histiocytes with either the homologous or heterologous pathogen did not result in destruction of cells by the parasite. An obvious inference which may be drawn from this observation is the probable identity of the mechanism or mechanisms for this type of cellular resistance.

Since the above findings have been reported, additional characterization of the tubercle bacillus–histiocyte model system has been achieved (4–6). It is now known that cellular resistance against mycobacteria can be induced in normal animals by injections of immune histiocytes, recipient histiocytes (histiocytes of normal animals which had been injected with resistant cells), and by immune ribosomes and ribosomal RNA (ribonucleic acid). It is also an established fact that serial transfer of resistance in normal animals against mycobacteria is achievable by injection of animals with resistant histiocytes or subfractions (ribosomes) derived therefrom. Moreover, it has been shown that passage of virulent tubercle bacilli in immune histiocytes resulted in attenuation of bacilli for both mice and normal histiocytes (*i.e.* passaged bacilli lose their inherent ability to necrotize the histiocytes of normal animals).

In view of the above findings, it seemed propitious to investigate the identity or lack of identity of the basic mechanisms which underlie cellular immunity

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

against *Brucella* and mycobacteria. The present paper reports the results of a comparative study of *Brucella*-immune and BCG-immune histiocytes, the findings of which appear to indicate fundamental differences between the mechanisms of resistance for these two microbes.

Materials and Methods

Detailed descriptions of procedures and materials used in these studies have been described in earlier reports (1–6) and are therefore briefly described herein.

Rabbits.—Adult male rabbits were used in all the experiments reported herein; these rabbits served as donors of normal or immune serum and cells and as recipients in transfer experiments.

Immune donors consisted of rabbits immunized with either the Rev I strain of *Brucella melitensis* (7) or the BCG strain of tubercle bacillus (1.0 to 2.0 × 10^8 bacilli were inoculated into each animal to be immunized; the intradermal route was used for BCG and the intravenous route for Rev I).

Histiocytes and Subcellular Fractions.—Rabbit 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; in tests for cellular resistance, additional treatment of histiocytes was needed, as described below.

Immune ribosomes were prepared according to the procedure of Littlefield (8). The method of extraction of RNA from ribosomes was that described by Gierer and Schramm (9). Application of these procedures to histiocytes has been described previously (5).

Transfer Procedure.—Intact histiocytes, ribosomes, or ribosomal RNA was injected intradermally into normal recipient animals. The injections were made into several sites in the skin; the numbers of sites injected depended on the total volume of material to be injected. The total numbers of cells or cell equivalents 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 animals (*Brucella*- or BCG-immunized rabbits) and recipient animals (animals given normal or immune donor cells or their subcellular fractions 13 days earlier). Details concerning the test for cellular resistance have been previously described (4). Briefly, the procedure was as follows: part of the histiocytes which had been collected from donor animals or recipient animals was sedimented by centrifugation and redispersed in a small volume of 0.25 per cent trypsin in Tyrode's solution. After 30 minutes of trypsinization the cells were washed and sedimented and resuspended in a few milliliters of homologous normal or immune serum. 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; this consisted of mixing the H37Rv strain of tubercle bacillus with 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 homologous normal or immune serum medium. The number of histiocytes was determined in a hemocytometer, and the infected suspension was diluted with

additional normal or 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.

Cultivation of Histiocytes.—This was carried out in the culture chambers described by Mackaness (10). Approximately 500 to 1000 uninfected or infected histiocytes were introduced into the space delineated by a plastic ring affixed to the bottom coverslip of the culture chamber. After adherence of histiocytes to the bottom coverslip, the culture chamber was closed by insertion of the top coverslip. Sufficient normal or 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.

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 (1). Cellular resistance was evidenced by absence of cellular degeneration and constancy of cell numbers in infected cell cultures.

Percentage of Infected Histiocytes.—This was determined by counting a total of 200 stained cells. The method of preparing stained specimens has been described earlier (1).

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 tubercle bacilli 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 darkground 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 immunization of animals the bacterial growth from a 2-week-old culture was ground with steel balls, suspended in physiological saline, and diluted to contain the desired number of bacteria per ml.

The *Brucella melitensis* (Rev I strain) used in these experiments was grown on Albimi agar. For use in parasitization, bacteria from 48 hour cultures were removed and washed twice with Tyrode's solution.

Preparation of Passaged Bacteria.—The preparation of virulent tubercle bacilli which had been grown in immune histiocytes has been described in an earlier paper (6). The same methods were used in the present studies except that virulent H37Rv were grown in *Brucella*-immune histiocytes rather than BCG-immune histiocytes.

Nutrient Media.—The media used for cultivation of histiocytes consisted of 40 per cent serum (normal serum or anti-BCG serum) in Tyrode's solution (a modified Tyrode's solution containing no calcium was used). The pH of all nutrient media was adjusted to 7.4 with 5 per cent CO_2 in air before use.

EXPERIMENTAL RESULTS

Inability of Brucella-Immune Histiocytes and Ribosomes to Induce Cellular Resistance in Normal Animals.—Previous investigations (4, 5) have shown that the resistance of histiocytes against virulent tubercle bacilli was transferable to

normal animals by injections of immune histiocytes or of ribosomes and ribosomal RNA derived from the immune histiocytes. It therefore seemed reasonable that if the mechanism of resistance of *Brucella*-immune cells resembled that of the BCG-immune cells, transference to normal animals of immunity against *Brucella* should be similarly achievable. The results of these investigations are given in Table I.

TABLE I

Non-Transferability of Cellular Resistance with Brucella-Immune Histiocytes and Ribosomes

Type of cell tested for resistance	Infected histiocytes*	Average No. bacteria per infected histiocyte*	Serum for cultivation of infected histiocytes‡	Infecting bacteria	Degeneration (hrs. after infection)§	
					24	48
	per cent				*per cent*	*per cent*
Histiocytes of animals injected with immune histiocytes‖	20	3	NRS	Mycobacteria	18	48
	25	3	IRS	Mycobacteria	36	51
	21	3	NRS	*Brucella*	24	48
	23	3	IRS	*Brucella*	36	52
Histiocytes of animals injected with immune ribosomes‖	23	3	NRS	Mycobacteria	28	46
	22	3	IRS	Mycobacteria	25	50
	22	3	NRS	*Brucella*	27	51
	24	3	IRS	*Brucella*	26	48
Donor (*Brucella*-immune) histiocytes	22	4	NRS	Mycobacteria	23	48
	25	4	IRS	Mycobacteria	0	0
	24	5	NRS	*Brucella*	15	49
	19	5	IRS	*Brucella*	0	0

* These values are presented to show that infection of the test histiocytes is fairly uniform and that the average numbers of bacteria per infected histiocyte do not vary greatly.

‡ NRS designates normal serum medium; IRS indicates immune serum medium.

§ Figures represent average per cent degeneration (in two or more chambers) from original histiocyte counts.

‖ Each recipient animal was injected intradermally with immune histiocytes or ribosomes (equivalent to 13.7×10^8 histiocytes). The histiocytes of the recipients were tested 14 days after transfer of cells or ribosomes.

It is apparent that neither the whole histiocytes nor the histiocytic ribosomes of *Brucella*-immunized animals were capable of inducing cellular resistance in normal animals. Thus, while the original immune histiocytes from *Brucella*-immunized rabbits exhibited the usual pattern of resistance (*i.e.* no destruction of cells by bacilli when infected cells were cultivated in immune serum medium; see lower third of table) against both *Brucella* and mycobacteria, recipient histiocytes (from animals inoculated with immune histiocytes or immune ribosomes) were entirely devoid of resistance against either pathogen; as shown in the top

and middle third of Table I, infected recipient histiocytes maintained in immune serum media were as susceptible to destruction by the parasites as were those maintained in normal serum media.

Inability of Brucella-Immune Histiocytes to Modify Virulent Tubercle Bacilli.— An important effect that BCG-immune histiocytes have upon tubercle bacilli is modification of their virulence. This effect is apparent after cultivation of virulent tubercle bacilli in immune histiocytes and is manifested in part by a loss of

TABLE II

Inability of Brucella-Immune Histiocytes to Modify Virulence of Tubercle Bacillus

Type of cell tested for resistance	Type of bacilli used to infect histiocytes*	Infected histiocytes	Average No. bacteria per infected histiocyte	Cultivation medium	Degeneration (hrs. after infection)	
					24	48
		per cent			*per cent*	*per cent*
Brucella-immune histiocytes	Virulent H37Rv	21	5	NRS	31	53
		22	5	IRS	0	0
Normal histiocytes	H37Rv passaged in *Brucella*-immune histiocytes cultured in normal serum medium	20	3	NRS	69	88
		21	3	IRS	36	75
Normal histiocytes	H37Rv passaged in *Brucella*-immune histiocytes cultured in immune serum medium	22	3	NRS	48	81
		21	3	IRS	36	56

See also footnotes in Table I.

* Virulent bacilli were grown *in vitro* in tween-albumin liquid medium; passaged bacilli were grown in *Brucella*-immune histiocytes; the infected immune histiocytes were maintained either in normal or in immune serum medium.

their ability to cause necrotization of normal histiocytes. Therefore, as a second test of the identity or dissimilarity of the mechanisms of histiocytic resistance against *Brucella* and mycobacteria, virulent tubercle bacilli (H37Rv strain) were cultivated in *Brucella*-immune histiocytes and tested for their effect upon the histiocytes of normal rabbits.

The results in Table II clearly show that passage of virulent tubercle bacilli in *Brucella*-immune histiocytes maintained in either normal serum medium (middle part of table) or immune serum medium (lower part of table) failed to effect modification of bacillary virulence; this was evidenced by the marked destruction of infected histiocytes even when cultured in the presence of immune serum. That this inability of *Brucella*-immune histiocytes to cause attenuation of the mycobacteria was not due to use of non-resistant cells for cultivation of

the bacilli is indicated by the ability of the original *Brucella*-immune histiocytes to resist virulent tubercle bacilli (top of Table II).

Capacity of BCG-Immune Ribosomes to Effect Serial Induction of Resistance in Normal Animals against Both Brucella and Mycobacteria.—Contrary to the initial expectation, the results of the preceding two sections have shown a dissimilarity of action for *Brucella*-immune and BCG-immune histiocytes. In view of these findings, it seemed important to determine whether the resistance (induced by immunization with either Rev I or BCG) of histiocytes against *Brucella* and mycobacteria was dissociable. To this end, the ability of BCG-immune ribosomes and BCG-immune ribosomal RNA to effect serial induction of cellular resistance in normal animals against both *Brucella* and mycobacteria was investigated. The rationale of these experiments was that if BCG-immune histiocytes possessed two mechanisms of resistance (*e.g.* a specific component residing in the ribosomes or the ribosomal RNA of the histiocyte and a nonspecific component existing in the cytoplasm of the cell), then dissociation of the two forms of resistance would be achievable by use of specific fractions of the immune histiocytes. The results of these experiments are described in this and the next section.

The experiments shown in Table III were designed to determine the ability of immune ribosomes (from BCG-immune histiocytes) and recipient ribosomes (from histiocytes of animals injected with either immune ribosomes or other recipient ribosomes) to induce cellular resistance in normal rabbits against both *Brucella* and mycobacteria. It is evident that serial induction of resistance against both pathogens occurred when BCG-immune ribosomes were used as the starting material. Thus, the histiocytes of animals inoculated with recipient-1 ribosomes (ribosomes derived from histiocytes of animals injected with immune ribosomes) and recipient-2 ribosomes (ribosomes from histiocytes of animals injected with recipient-1 ribosomes) exhibited patterns of resistance which were identical with the histiocytes from animals injected with immune ribosomes (*i.e.* susceptibility to both pathogens in normal serum media and refractoriness to necrotization in immune serum media).

The results of these experiments therefore appear to suggest a basic dissimilarity in the mechanisms of resistance of *Brucella*-immune histiocytes and BCG-immune histiocytes. Whereas transference of cellular resistance was not possible with the first type of histiocytes, such transference, even when made serially, and with subcellular components (ribosomes), was demonstrable with the second type of histiocytes. Moreover, these results show quite clearly that the two forms of cellular resistance (*i.e.* against *Brucella*, on the one hand, and mycobacteria, on the other) in the BCG-immune histiocyte or its derivatives are intimately bound to each other and not easily separable.

Induction of Resistance against Brucella and Mycobacteria by Ribosomal RNA of BCG-Immune Histiocytes.—Inasmuch as induction of cellular resistance by

use of immune and recipient ribosomes failed to effect dissociation of *Brucella* resistance from resistance to mycobacteria, the preceding experiments were repeated but with immune ribosomal RNA and recipient ribosomal RNA as inducing agents. The decision to test RNA in this respect was based on the assumption that if cytoplasmic components were responsible for induction of

TABLE III

Capacity of Ribosomes of BCG-Immune Histiocytes to Induce Resistance against Brucella and Mycobacteria

Type of cell tested for resistance	Infected histiocytes	Average No. bacteria per infected histiocyte	Cultivation medium	Infecting bacteria	Degeneration (hrs. after infection)	
					24	48
	per cent				*per cent*	*per cent*
Histiocytes of animals injected with immune ribosomes*	19	4	NRS	Mycobacteria	27	53
	21	4	IRS	Mycobacteria	3‡	3‡
	19	2	NRS	*Brucella*	25	56
	20	3	IRS	*Brucella*	0	0
Histiocytes of animals injected with recipient-1 ribosomes*	24	3	NRS	Mycobacteria	36	58
	22	4	IRS	Mycobacteria	0	0
	20	4	NRS	*Brucella*	19	67
	22	3	IRS	*Brucella*	0	0
Histiocytes of animals injected with recipient-2 ribosomes*	22	4	NRS	Mycobacteria	38	62
	23	4	IRS	Mycobacteria	4‡	5‡
	21	3	NRS	*Brucella*	22	46
	21	3	IRS	*Brucella*	0	0

See also footnotes in Table I.

* Immune ribosomes were from animals immunized with BCG; recipient-1 ribosomes were from animals injected with immune ribosomes; recipient-2 ribosomes were from animals injected with recipient-1 ribosomes. Amount of ribosomes injected in these experiments ranged between 1.1 to 1.6 $\times$ 10^9 cell equivalents.

‡ Cell degeneration of 5 per cent or less is not significant, since the limit of errors in counting is about 5 per cent.

resistance against *Brucella*, then RNA might prove to be a more selective inducing agent because of the smaller possibility of its gross contamination with cytoplasmic materials (*i.e.* the additional steps in preparation of RNA from ribosomes would further reduce cytoplasmic contamination).

The results in Table IV reveal that induction of cellular resistance by immune ribosomal RNA resembled that caused by immune ribosomes; thus, the histiocytes of animals injected with RNA proved refractory against the necrotizing action of both *Brucella* and mycobacteria (top of Table IV). In contrast to

the action of ribosomes, however, it was not possible to effect serial transfer of resistance with recipient ribosomal RNA (note marked degeneration of all cultures of infected histiocytes in lower part of Table IV). Whether this resulted from the greater lability of extracted RNA as opposed to intact histiocytic ribosomes or from other causes was not established in these experiments. It is of interest, however, that the inability of recipient ribosomal RNA to induce resistance in normal animals encompassed both types of resistance; this lends further credence to the belief engendered by the findings of the preceding sec-

TABLE IV

Capacity of Ribosomal RNA of Resistant Histiocytes to Induce Resistance against Brucella and Mycobacteria

Type of cell tested for resistance	Infected histiocytes	Average No. bacteria per infected histiocyte	Cultivation medium	Infecting bacteria	Degeneration (hrs. after infection)	
					24	48
	per cent				*per cent*	*per cent*
Histiocytes of animals injected with immune ribosomal RNA	20	4	NRS	Mycobacteria	20	53
	22	4	IRS	Mycobacteria	0	0
	18	3	NRS	*Brucella*	23	51
	19	3	IRS	*Brucella*	0	0
Histiocytes of animals injected with recipient-1 ribosomal RNA	22	4	NRS	Mycobacteria	22	60
	23	4	IRS	Mycobacteria	31	63
	20	3	NRS	*Brucella*	26	51
	19	4	IRS	*Brucella*	35	50

See footnotes in Table III (but substitute "ribosomal RNA" for the word "ribosomes").

tion that resistance against *Brucella* and mycobacteria in BCG-immune histiocytes and their derivatives was not easily dissociable, if at all.

Persistence of Cellular Resistance in Recipient Animals after Induction by Ribosomes.—On the assumption that if the resistance against *Brucella* developed non-specifically during the establishment of a specific immunity against mycobacteria, it seemed possible that separation of the two kinds of resistance might occur naturally with passing of time. To test this point, the histiocytes of animals injected with immune ribosomes were tested for resistance against the two pathogens at 4 and 6 months after exposure to active ribosomes.

The results of Table V indicate that the resistance of recipient histiocytes against both *Brucella* and mycobacteria was still demonstrable even 4 and 6 months after the initial, inducing injection of immune ribosomes. Although row 4 shows a slight amount of degeneration (7 to 9 per cent at 24 and 48 hours postinfection) for infected, immune histiocytes from animals treated with im-

mune ribosomes 4 months earlier, such degeneration was not observed in histiocytes from animals injected with ribosomes 6 months previously (row 8). This low level of degeneration in row 4 is therefore probably of no significance, particularly when compared with the 57 per cent degeneration of *Brucella*-infected histiocytes cultured in normal serum medium (row 3).

TABLE V

Persistence of Cellular Resistance against Brucella and Mycobacteria after Induction by Immune Ribosomes

Type of cell tested for resistance	Time of testing of histiocytes*	Infected histiocytes	Average No. bacteria per infected histiocyte	Cultivation medium	Infecting bacteria	Degeneration (hrs. after infection)	
						24	48
	mos.	*per cent*				*per cent*	*per cent*
Histiocytes of animals	4	21	4	NRS	Mycobacteria	25	49
injected with ribo-	4	25	4	IRS	Mycobacteria	0	0
somes from BCG-	4	24	5	NRS	*Brucella*	19	57
immunized rabbits‡	4	25	5	IRS	*Brucella*	7	9
	6	19	3	NRS	Mycobacteria	18	56
	6	18	3	IRS	Mycobacteria	0	0
	6	17	3	NRS	*Brucella*	24	60
	6	17	3	IRS	*Brucella*	0	0

See also footnotes in Table I.

* Indicates number of months after injection of animals with ribosomes.

‡ Animals were injected with immune ribosomes which were equivalent to 11.0×10^8 histiocytes.

DISCUSSION

An important question in connection with the phenomenon of cellular resistance is concerned with the immunological specificity of such resistance. Our own studies of this phenomenon have shown that the histiocytes of animals immunized with the BCG strain of tubercle bacillus were resistant not only to challenge with the H37Rv strain of tubercle bacillus but also to infection with *Brucella melitensis* (3). When considered in itself, this observation would appear to indicate that the cellular resistance induced in rabbits by immunization with BCG was non-specific in nature. At the same time, however, there seemed to be some element of specificity in this type of resistance (2), for the histiocytes of BCG-immunized animals failed to resist necrotization by other facultative intracellular parasites such as salmonellae. Since our earlier investigations (3) have shown that *Brucella*-immune histiocytes (from rabbits immunized with *Brucella melitensis*) also exhibited resistance against both *Brucella* and myco-

bacteria, it seemed reasonable to analyze more closely the question of specificity of cellular resistance by comparing the behavior of *Brucella*-immune histiocytes with that of BCG-immune histiocytes. The rationale of such an approach was that if cellular resistance is in part of non-specific nature, the behavior of the *Brucella*-immune histiocyte should in many respects parallel that of the BCG-immune histiocyte; conversely, if cellular resistance is immunologically specific, it should most likely be reflected by differences in the resistance patterns of these two types of histiocytes.

Investigations along the lines indicated above have shown a marked dissimilarity in the behavior of histiocytes from *Brucella*-immunized animals and BCG-immunized animals. One major difference between these two histiocytes pertains to their capacity for induction of cellular resistance in normal animals. Thus, while *Brucella*-immune histiocytes failed to induce cellular resistance against either *Brucella* or mycobacteria, the histiocytes and histiocytic ribosomes and ribosomal RNA of BCG-immunized rabbits proved fully effective in eliciting resistance in normal animals against both parasites. A second important difference between BCG-immune histiocytes and *Brucella*-immune histiocytes is the ability of the former but not the latter to effect modification of virulent tubercle bacilli with consequent loss of bacillary capacity for necrotization of normal histiocytes.

It is apparent that no definitive conclusions can be drawn concerning the nature of cellular resistance in the *Brucella*-immune histiocyte, in view of the negative observations recorded herein for this cell. The findings presented for the BCG-immune histiocyte, however, do permit tentative conclusions about the immunological specificity of resistance in this cell. The observation that, of the two types of histiocytes studied, only those from BCG-immune animals proved capable of modifying virulent tubercle bacilli is certainly suggestive of a specific cellular immunity; the fact that the BCG-immune histiocyte also proved resistant to *Brucella* is not necessarily incompatible with this concept, for the resistance against *Brucella* may be attributable to various epiphenomena arising from a primary and immunologically specific change (*e.g.* the deep-seated and durable changes which occur in the BCG-immune histiocyte might in part be reflected in metabolic alterations of the cell, some of which coincidentally renders the cell incapable of providing *Brucella* with those factors which are essential to production of their virulence components). Moreover, the inducibility of cellular resistance in normal animals against, albeit, both *Brucella* and mycobacteria not only by histiocytes but also by subcellular components (ribosomes) and macromolecules of fundamental importance (ribonucleic acid) is difficult to visualize in terms of non-specific mechanisms.

SUMMARY

Comparison of the *Brucella*-immune and the BCG-immune histiocyte, each of which is resistant to necrotization by either *Brucella* or mycobacteria, has

revealed a number of dissimilarities in their behavior. The *Brucella*-immune histiocyte was found to be incapable of transferring its resistance to the cells of normal animals; it was also unable to achieve attenuation of virulent tubercle bacilli. In contrast, the BCG-immune histiocyte and certain of its subcellular components (ribosomes and ribosomal RNA) were effective in inducing cellular resistance in normal animals against both *Brucella* and mycobacteria. When RNA was used, only immune ribosomal RNA was effective; when intact ribosomes were used, both immune and recipient ribosomes proved active.

These investigations have also shown that the resistance of the BCG-immune histiocyte against *Brucella* and mycobacteria was of long duration and not readily dissociable.

BIBLIOGRAPHY

1. 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.
2. Fong, J., Schneider, P., and Elberg, S. S., Studies on tubercle bacillus–monocyte relationship. II. Induction of monocyte degeneration by bacteria and culture filtrate: Specificity of serum and monocyte effects on resistance to degeneration, *J. Exp. Med.*, 1957, **105,** 25.
3. 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.
4. Fong, J., Chin, D., and Elberg, S. S., Studies on tubercle bacillus–histiocyte relationship. V. Passive transfer of cellular resistance, *J. Exp. Med.*, 1962, **115,** 475.
5. Fong, J., Chin, D., and Elberg, S. S., Studies of tubercle bacillus–histiocyte relationships. VI. Induction of cellular resistance by ribosomes and ribosomal RNA, *J. Exp. Med.*, 1963, **118,** 371.
6. Fong, J., Chin, D., and Elberg, S. S., Studies on tubercle bacillus–histiocyte relationship. IV. Effects of passage in normal and immune systems upon virulent bacilli, *J. Exp. Med.*, 1961, **114,** 75.
7. Elberg, S. S., and Faunce, K., Immunization against *Brucella* infection. IV. Immunity conferred on goats by a non-dependent mutant from a streptomycin-dependent mutant strain of *B. melitensis*, *J. Bact.*, 1957, **73,** 211.
8. Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnik, P. C., Studies on cytoplasmic ribonucleoprotein particles from the liver of the rat, *J. Biol. Chem.*, 1955, **217,** 111.
9. Gierer, A., and Schramm, G., Infectivity of ribonucleic acid from tobacco mosaic virus, *Nature*, 1956, **177,** 702.
10. Mackaness, G. B., The action of drugs on intracellular tubercle bacilli, *J. Path. and Bact.*, 1952, **64,** 429.
11. 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.