Activation of the Cell-Mediated Response in Guinea Pigs Via Subcellular Units of *Listeria Monocytogenes*

by

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ABSTRACT

The purpose of this study was to investigate the cell-mediated activity of the facultative intracellular parasite, Listeria Monocytogenes, in the guinea pig. Special emphasis was placed upon the particular subcellular component responsible for the demonstration of the two lymphokines: Migration Inhibitory Factor and Skin Reactive Factor.

In order to isolate the specific subcellular components it was necessary to fractionate the live organism. This was accomplished using a variety of methods. When these antigenic preparations were injected into guinea pigs immunized with live Listeria Monocytogenes, definite skin test reactions were noted. These same preparation, when employed in the capillary tube technique for assaying macrophage migration, demonstrated a marked inhibition of macrophage migration. These crude preparations evidently contain the specific subcellular component responsible for the demonstration of the cell-mediated response in guinea pigs.

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Thanks are also due Dean Gilliland, Pete Kelleher, and Richard Cowart for their advice and assistance.

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Activation of the Cell-Mediated Response in Guinea Pigs Via Subcellular Units of Listeria Monocytogenes[1]

B.R. Bloom demonstrated in 1971 that specifically sensitized cells in an animal, known as lymphocytes, release a variety of soluble mediators upon secondary antigenic stimulation. (1) These mediators, grouped under the title "lymphokines", affect the cells responsible for cell-mediated immunity. Demonstration of any of these lymphokines is a direct confirmation of the cell-mediated or hypersensitive state of the animal. (2)

Two such lymphokines were chosen as in vitro correlates for delayed-type hypersensitivity in this study: Migration Inhibitory Factor and Skin Reactive Factor.

This test system employed the organism Listeria monocytogenes. This organism was chosen because it provides an excellent model for the demonstration of cell-mediated immunity. It is a facultative intracellular parasite resistant to the homeostatic activity of normal macrophages. Its intracellular location eliminates the role of humoral antibodies in resistance to infection. In addition, it is superior to other facultative intracellular parasites in its favorable growth rate and less exacting media requirements.

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[1]The format of this paper follows that required by the Journal of Immunology.

When a microorganism with its complete set of antigenic determinants is introduced into an animal such as a guinea pig it will most probably be engulfed by macrophages performing their homeostatic or "housekeeping" functions and destroyed via enzyme degradation. If however the organism is a facultative intracellular parasite such as Listeria monocytogenes it will be engulfed by macrophages but remain resistant to enzyme degradation, thus occupying a priveleged position in the guinea pig. This priveleged position renders the action of any antibodies produced in response to the antigenic stimulus ineffective. (4,6) The animal is then susceptible to infection.

The macrophage then transports this organism throughout the host's body to present it to another cell key to the immune response, the T cell or thymus-derived lymphocyte. This is the primary antigenic stimulus which results in a specifically sensitized T cell. This sensitization initiates a clonal proliferation of antigen-specific sensitized lymphocytes. Upon secondary antigenic stimulation with the same organism the aforementioned lymphokines are produced which in turn affect the cells responsible for cell-mediated immunity. At this point, the animal becomes immune to the listeric infection through the action of these lymphokines, principally, that known as MAF.[2]

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[2]Abbreviations used in this paper: MIF, Migration Inhibitory Factor; HBSS, Hanks Buffered Salt Solution; MEM, Minimal Essential Media; BHI, Brain Heart Infusion Broth; PE, Peritoneal Exudate; MAF, Macrophage Activation Factor; LM, Listeria monocytogenes.

This immunity depends upon the activation of normal macrophages to "killer macrophages" via MAF. (3,5) These activated macrophages have undergone changes in morphology and behavior which now enable them to kill the organism. They exhibit greater phagocytic properties and enhanced bactericidal capacity. This was noted by G.B. Mackaness in 1962. (4)

To understand the basic mechanism of macrophage activation, it is necessary to examine what actually works upon the T cell to produce MAF. To date, sensitization has been possible only with the live viable organism. (4) This presents an enormous variety of antigenic determinants which must be separated and classified to determine which component macromolecule is responsible for the sensitization of T cells.

Release of lymphokines from sensitive T lymphocytes has been accomplished only through their exposure to viable and killed Listeria monocytogenes cells. It was the objective of this research to find an antigenic preparation(s) of Listeria monocytogenes that would cause the release of lymphokines. The lymphokines chosen for this study were MIF and Skin Reactive Factor.

MATERIALS AND METHODS

Antigen Preparations.

1. Alumina

Grind 3 grams of previously-frozen LM culture with 3 grams of alumina until mixture is fairly sticky. Grind an additional 5 minutes and add 3 grams of alumina batch-wise. Grind 5 minutes and resuspend.

Centrifuge at 8,000 Xg to separate cellular debris, intact cells, and alumina. Lyophilize.

2. Dialyzed

Dialyze a 10X solution of BHI vs. distilled water for 16-20 hours. Dispense diaysate and sterilize by autoclaving. Inoculate flasks with 3-4 mls of a 16-hour culture of LM. Incubate at 37°C under shake flask. Centrifuge. Pool the supernate and bring to 75% saturation with ammonium sulfate. Harvest the precipitate with repeated centrifugation. Redissolve the precipitate with repeated centrifugation. Redissolve the precipitate in 0.01M PO_4 buffer at ph 7.4.

3. Sonicate

Prepare 6 liters of BHI. Sterilize. Inoculate each with 3-4 mls of a 16-20 hr. culture of LM. Grow at 37°C for 18 hours. Add 5% w/v phenol and incubate an additional 8 hours. Collect cells by centrifugation at 8,000Xg for 20 minutes in a refrigerated centrifuge. Wash once with sterile saline and three times with distilled water. Resuspend in 50mls of distilled water. Disrupt by sonic oscillation and centrifuge at 10,000Xg for 30 minutes at 4°C. Save supernate. Resuspend debris, centrifuge, and save supernate. Pool supernates and dialyze against distilled water. Lyophilize.

Sensitization.

Random bred guinea pigs of both sexes were sensitized using 5×10^6 viable Listeria monocytogenes cells; 0.10 of an LD_{50}.

Skin Test.

Seven days after sensitization, animals were skin tested with approximately 50ug of each antigenic preparation subcutaneously. The degree of induration and erythema was measured at 24 and 48 hours.

Cell Harvest.

Twelve to fourteen days after sensitization peritoneal exudate cells were harvested. Three days before harvest the animals were injected with 30mls of sterile light mineral oil. The PE cells were washed 3X in HBSS and resuspended to 10% in MEM supplemented with 10% fetal calf serum.

Assay for Migration Inhibitory Factor.

Macrophage migration or inhibition was determined by the capillary tube method. Chambers were prepared using 35mm Falcon plates with spots of silicone grease to anchor capillaries containing peritoneal exudate cells. The plates ere filled with Eagle's Minimal Essential Medium (MEM) containing varying concentrations of each antigen preparation. Incubation took place for 48 hours at 5% CO_2, 95% air, water humidified. Migration of inhibition was determined against a dialyzed media control. Migration patterns were photographed and their images projected onto bond paper and traced. The patterns were then cut and weighed. The Percent Migration was determined by the following formula:

$$\% \text{ Migration} = 100 - \frac{\text{wt. of projected migration image of test}}{\text{wt. of projected migration image of control}} \times 100$$

RESULTS

Skin Test.

Seven days after the sensitization of the guinea pig with 5×10^5 live Listeria monocytogenes organisms, the various antigen preparations were injected subcutaneously to determine their efficacy in the release of Skin Reactive Factor as demonstrated by the degree of induration and erythema. These results are seen in Figures 1 and 2.

Migration Inhibitory Factor Assay.

Five days after skin testing, the animals were sacrificed in order to determine the efficacy of each antigen preparation in the release of Migration Inhibitory Factor. Peritoneal exudate cells were harvested, washed, and pelleted via the capillary tube method and incubated in chambers containing varying concentrations of the antigen preparations. These results may be seen in Table 1.

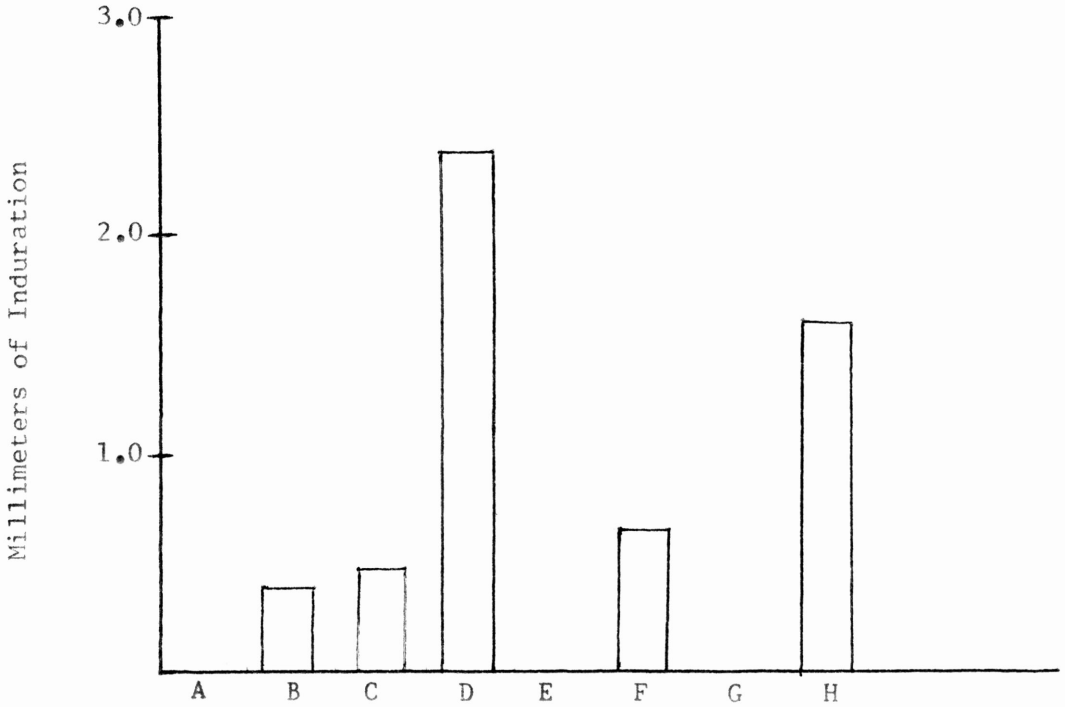

Figure 1. The degree of induration, measured in millimeters, is shown for both a normal and an immunized guinea pig. Measurements were taken at 24 and 48 hours. Group A reflects 24-hour measurements from a normal guinea pig. No induration was apparent. Group B represents 24-hour measurements from an immunized guinea pig. The The antigen used was the dialyzed antigen. Groups C and D reflect 24-hour measurements using the sonically-disrupted antigen. Group C is the 24-hour normal measurement, Group D the 24-hour immunized measurement. Groups E and F represent 48-hour measurements using the dialyzed preparation. Group E is the 48-hour normal measurement, Group F the 48-hour immunized measurement. Groups G and H reflect the 48-hour measurements using the sonically-disrupted antigen. Group G is the 48-hour normal measurement, Group H the 48-hour immunized measurement.

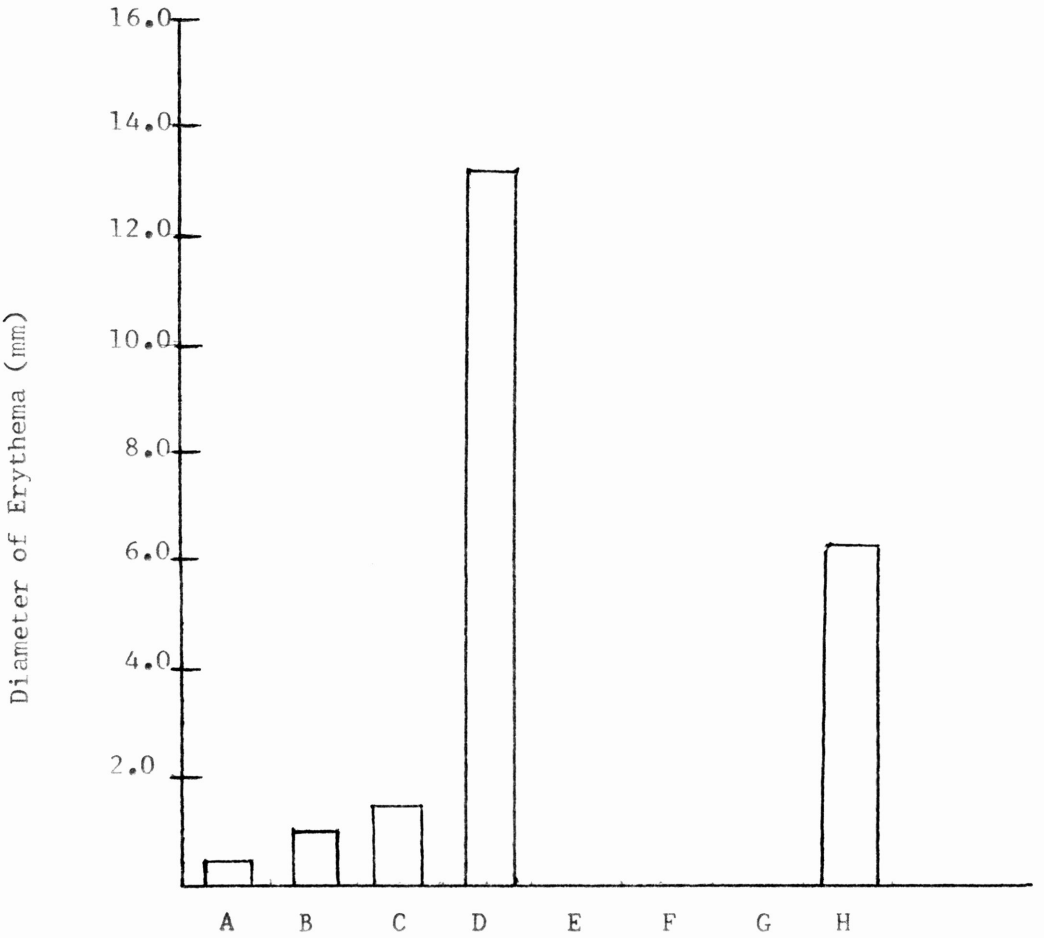

Figure 2. The degree of erythema evident at 24 and 48 hours in both a normal and an immunized guinea pig. Groups A-D reflect 24-hour measurements. Groups E-H reflect 48-hour measurements. Group A represents 24-hour measurements from a normal guinea pig, Group B an immunized guinea pig at 24 hours. The antigen preparation used was the dialyzed antigen. Group C represents 24-hour measurements from a normal guinea pig, Group D an immunized guinea pig at this time. The antigen preparation is the sonically-disrupted antigen. Groups E and F reflect measurements for both the normal and immunized guinea pigs at 48 hours using the dialyzed antigen preparation. No erythema was present at that time. Group G represents measurements from a normal guinea pig at 48 hours using the sonically-disrupted antigen. No erythema was evident. Group H represents 48-hour measurements from an immunized guinea pig using the sonically-disrupted antigen.

TABLE 1.

Percent Migration Inhibition

of varying concentrations of antigen preparations.

ug antigen	Dialyzed Antigen Preparation	Sonic Antigen Preparation
5	99.8	42.9
50	97.3	62.5
500	80.2	44.3

DISCUSSION

Skin test data indicates that the sonically-disrupted preparation of Listeria monocytogenes is very effective in producing high levels of both induration and erythema. This preparation is markedly more effective than the dialyzed antigen at both 24 and 48 hour time intervals. It would seem, therefore, that the sonically-disrupted preparation contains the antigenic determinant necessary to elicit the lymphokine responsible for induration and erythema, Skin Reactive Factor.

The dialyzed antigen preparation, on the other hand, is far more effective than the sonically-disrupted antigen in the production of MIF, as seen in Table 1. This would indicate the presence of the specific antigen responsible for release of MIF in this preparation.

This would indicate that there are two distinct determinants responsible for the release of the lymphokines, MIF and Skin Reactive Factor. Alternatively, the discrepancy may be due to a difference in antigenic determinants present after manipulation of the live organism. The procedures for antigen preparation are not uniformly mild and some determinants may be lost through vigorous sonication.

Certainly, more work should be done in the separation and purification of these crude preparations to determine the precise antigenic fraction(s) responsible for the release of these lymphokines.

It should be noted that this conclusion is based on a single experimental trial, therefore statistical evaluation has been eliminated.

REFERENCES

1. Bloom, B.R. 1971. In vitro approaches to the mechanism of cell mediated immune reactions. Adv. Immunol. 13:101.

2. Yoshida, T., Sonozaki, H. and S. Cohen. 1973. The production of migration inhibition factor by B and T cells of the guinea pig. J. Exp. Med. 138:784.

3. Burrows, W., Textbook of Microbiology, Twentieth Edition, p.379. W.B. Saunders Company, Philadelphia, 1973.

4. Mackaness, G.B. 1962. Cellular resistance to infection. J. Exp. Med. 116:381-406.

5. Simon, H.B. and Sheagren, J.N. 1971. Cellular Immunity in vitro. J. Exp. Med. 133:1377-1389.

6. David, J.R. 1967. Macrophage Migration. From the Intersociety Symposium on "In Vitro Correlates of Delayed Hypersensitivity". Federation Proceedings, Federation of American Societies for Experimental Biology, Volume 27, pp.6-12.