CELLULAR MEDIATORS OF ANTI-LISTERIA IMMUNITY AS AN ENLARGED POPULATION OF SHORT-LIVED, REPLICATING T CELLS

KINETICS OF THEIR PRODUCTION*

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It is now established that acquired immunity in the mouse (1) and the rat (2) to infection with Listeria monocytogenes is cell-mediated. It has been shown (1) for example that normal mice can be adoptively immunized against lethal Listeria challenged with splenic lymphoid cells, but not with serum from convalescing donors. It has also been shown (3-5) that the responsible lymphoid cells are thymus-derived lymphocytes (T cells) as evidenced by their susceptibility to anti-θ serum and complement.

The appearance of these cells in the spleen is associated with a striking increase in cell division which peaks on about the 6th day of infection (6). It is also at this time that the host displays maximum delayed sensitivity to Listeria antigens, and its splenic lymphoid cells are most efficient at adoptively immunizing normal recipients. After the 6th day these parameters of the anti-Listeria response decay.

This paper will show that by the 6th day of infection the spleen acquires a greatly enlarged population of replicating T cells, and that the cellular mediators of anti-Listeria immunity reside within this population. In addition, it will provide evidence concerning the longevity of these T cell mediators of antimicrobial immunity and the features they have in common with other effector cells of this class.

Materials and Methods

Mice.—Adult B6AF1 (C57BL/6 × A/J) mice of both sexes were employed. Parental C57BL/6 and A/J strains were obtained from Jackson Laboratories, Bar Harbor, Maine; as were the CSH and AKR strains used for producing anti-θ serum.

Bacteria.—Listeria monocytogenes (strains EGD) was maintained in a virulent state by repeated passage in CD-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.). Before each experiment it was isolated from a 3 day infected spleen, grown in Trypticase soy broth (Baltimore Biological Laboratories, Baltimore, Md.) for 12-15 h, and diluted appropriately in 0.9% sodium chloride solution for intravenous injection. The infecting dose varied between 9 × 10⁵ and 1.5 × 10⁶.

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Delayed Sensitivity.—Delayed skin reactivity was determined at stated times by injecting 20 μg of Listeria antigens in 0.04 ml of phosphate-buffered saline (PBS) into the right-hind footpad. Increase in footpad thickness was measured 24 h later with dial calipers, and compared with the thickness of the left-hind footpad injected with the same volume of PBS. Listeria protein antigens were purified from dialysed culture medium by column chromatography (Ishibashi and Mackaness, unpublished observation).

Antisera.—The production of anti-θ C3H serum was elicited in AKR mice by injecting them intravenously and intraperitoneally at 7-day intervals over 5 wk with 10^7 C3H thymocytes. Mice were bled from the heart 7 days after the last injection. The antiserum was diluted 1:1 in Dulbecco’s PBS and absorbed for 30 min at 37°C with AKR thymocytes (10^5/ml). It was then stored in aliquots at −20°C. Evidence that the cytotoxicity of this antiserum can be removed by absorption with C3H brain has been published elsewhere (4).

Rabbit antimouse immunoglobulin serum (anti-Ig) or the Ig fraction of this antiserum, was purchased from Miles Laboratories, Inc., Kankakee, Ill., and from Nutritional Biochemicals Corp., Cleveland, Ohio. Antiserum from the first mentioned supplier was accompanied by immunoelectrophoretic data which attested its specificity for mouse Ig. Antiserum from the latter supplier was shown to be specific for mouse Ig by immunoelectrophoresis against whole mouse serum.

The IgG fraction of fluorescein isothyocyanate-conjugated rabbit antimouse immunoglobulin serum (FIC anti-Ig) was purchased from Miles Laboratories. It was diluted in PBS to 2 mg protein/ml (520 μg antibody protein/milliliter), absorbed for 30 min at 4°C with 10^7 B6AF1 thymocytes/milliliter, and stored in aliquots at −20°C.

Immunofluorescence.—The method of Raft (7) was used to determine the numbers of θ-positive and Ig-bearing lymphocytes in normal and 6 day-infected spleens. Individual spleens were diced finely with a scalpel blade and forced through a 200 mesh stainless steel sieve into PBS containing 5% fetal calf serum (PBS-FCS). The cells were dissociated from clumps by repeated pipetting, and were passed through six layers of surgical gauze. They were then washed three times by repeated centrifugation (350 g) and resuspension in PBS-FCS and diluted appropriately for counting in a hemacytometer and for testing viability by dye exclusion. All of the above procedures were performed at 4°C. Viability varied between 87% and 95%.

The immunofluorescent technique relied on the knowledge (7) that FIC anti-Ig alone stains Ig-bearing B lymphocytes, whereas after incubation with anti-θ serum it also stains θ-positive T lymphocytes. The former display mainly “capped fluorescence” and the latter diffuse “ringed fluorescence”. 2 × 10^7 spleen cells were suspended in 0.5 ml of either 1:1 anti-θ serum, or 1:1 normal AKR serum at 4°C for 20 min. They were then washed twice with 5 ml of PBS-FCS and resuspended in 0.5 ml of 1:5 FIC anti-Ig for 15 min at 4°C and for 5 min at 23°C. This was followed by two washes in PBS-FCS at 4°C. The cells were then resuspended in 0.25 ml of PBS-FCS and examined under ultraviolet light with a Zeiss fluorescence microscope (Carl Zeiss, Inc., New York) fitted with a dark field condenser and an Osram HBO-200 watt high pressure mercury lamp. Zeiss excitation filter BG12, and barrier filters nos. 53 and 44 were employed. The percent fluorescence cells per 300 cells was determined for each sample.

The specificity of the above reagents was established by the findings that FIC anti-Ig alone, or after incubation with normal AKR serum did not stain either C3H or B6AF1 thymocytes, but stained 100% C3H and B6AF1 thymocytes after incubation in anti-θ C3H serum.

Transfer of Anti-Listeria Immunity.—Spleen cell suspensions from normal and 6 day-infected donors were prepared as described previously (1). Donors were injected subcutaneously with 5,000 U of potassium penicillin G, and given drinking water containing 0.5 mg of ampi-
cillin/milliliter 24 h before harvesting their spleen cells. This reduced the number of Listeria in donor spleens by 100-fold at the time of cell transfer. Thus, after processing, the number of Listeria infused with “immune” spleen cells was never more than $2 - 3 \times 10^5$. Normal recipient mice were challenged intravenously with 10 LD$_{50}$ Listeria $(4.5 \times 10^4)$ and infused 2 h later with either $1.5 \times 10^8$ normal or immune spleen cells. The organism was enumerated at stated times in recipients’ livers and spleens by plating 10-fold serial dilutions of organ homogenates on Trypticase soy agar.

The susceptibility of the cellular mediators of anti-Listeria immunity to anti-O serum was tested by suspending spleen cells at $10^8$/ml in 1:6 anti-O serum for 20 min at either 37°C or 4°C for 20 min. This was followed by a 20 min incubation at 37°C in the same volume of 1:5 agarose-absorbed (8) guinea pig serum. After two washes in 50 ml of PBS the cells were suspended at an appropriate concentration for intravenous injection. Control cells were treated the same way except that they were incubated in normal AKR serum instead of anti-O serum.

The effect of a 1:5 dilution (520 μg antibody protein/milliliter) of anti-Ig serum on the cellular mediators of immunity was tested in the same way. Control cells were incubated in the same volume of heat-inactivated normal rabbit serum. There is convincing evidence (9) that anti-Ig serum plus complement is cytotoxic for Ig-bearing B cells in vitro.

Estimating Spleen Cell Proliferation.—In vivo spleen cell proliferation was estimated by pulsing mice intravenously with $20 \mu$Ci tritiated thymidine ($[^3H]TdR$) of specific activity 3 Ci/mM (New England Nuclear, Boston, Mass.). 30 min later the spleens were weighed and known weights extracted with trichloroacetic acid (TCA) (10). $[^3H]DNA$ was counted by liquid scintillation spectrometry as described previously (10).

DNA synthesis by spleen cells was measured in vitro by incubating $20 \times 10^6$ cells for 30 min at 37°C in 2.5 ml of Eagle’s minimal essential medium containing 5% FCS and 0.5 μCi $[^3H]TdR$ per milliliter. After two washes in 10 ml PBS they were resuspended in 5 ml of cold (4°C) TCA for 1 h, washed in a further 5 ml cold TCA and then extracted for 1 h in hot (90°C) TCA. $[^3H]DNA$ in the extract was counted as above.

The effect of anti-O serum and anti-Ig serum on $[^3H]TdR$ incorporation in vitro was tested by suspending $20 \times 10^6$ spleen cells in 0.5 ml of a 1:5 dilution of either antiserum for 15 min at 4°C, and then in 0.5 ml of 1:5 agarose absorbed guinea pig serum for 30 min at 37°C. The guinea pig serum contained 200 U/ml of DNAse (Sigma Chemical Company, St. Louis, Mo.). The cells were then incubated for 30 min in $[^3H]TdR$-containing medium as above and extracted for DNA.

Vinblastine Sulphate.—This was obtained from Eli Lilly and Co., Indianapolis, Ind. It was dissolved in PBS and administered in a dose of 100 μg subcutaneously. This dose should have been active in destroying mitosing cells for a period of approximately 15 h (11). Its effect on the mediators of immunity was investigated by injecting it into prospective donors on day 6 of infection and preparing spleen cell suspensions 15 h later.

RESULTS

Kinetics of the Production of the Cellular Mediators of Immunity in the Spleen in Relation to Increases in DNA Synthesis, Cellularity, and Delayed Sensitivity.—Fig. 1 shows the time-course of the production of the cellular mediators of immunity in the spleens of mice given an intravenous immunizing dose of Listeria $(1.3 \times 10^9)$. The production of these cells is shown in relation to changes in total DNA synthesis, spleen weight, and numbers of nucleated cells. The production of mediator cells was measured by determining the capacity of one organ equivalent of spleen cells, taken at progressive stages of infection, to protect normal recipients against a standard lethal Listeria challenge (4.5 —
Adoptive immunity (log protection) is expressed as the log difference between the number of *Listeria* in the spleens of recipients of "immune" spleen cells and normal spleen cells after 48 h of the challenge infection. Assuming that the level of protection transferred was a function of the number of cellular mediators in the spleen, rather than to a change in their individual efficiency, the results show that cellular mediators were first produced in significant numbers in the spleen between days 2 and 4 of infection. They rapidly increased in numbers to peak on day 6, were sustained in numbers large enough to give maximum protection until day 10, and then progressively decreased in number until day 20 when the spleen no longer contained enough to transfer significant immunity. The total numbers of spleen cells giving the above levels of adoptive immunity are represented by the curve for spleen cellularity (Fig. 1). The production of the cellular mediators of immunity was obviously associated with a striking increase in spleen cellularity and a corresponding increase in spleen weight. Clearly, the splenomegaly of infection is due in part to an accumulation of immune mediator cells.

That the production of mediator cells depended on increased cell division is...
indicated by the curve for $[^{3}H]Tdr$ incorporation into total spleen DNA (Fig. 1). It can be seen that the increase in the number of mediator cells coincided with a major increase in DNA synthesis. The progressive loss of mediator cells, on the other hand, closely followed a decrease in DNA synthesis. It is apparent, therefore, that the curve for DNA synthesis represents the time-course of the production of the mediators of immunity.

The above results indicate that maximum production of cellular mediators was sustained between days 6 and 10 of infection. It seemed possible, however,
indicates that the number of mediator cells present in blood was determined by the level of their production in the spleen. Again, it can be seen that *Listeria* grew unrestrictedly in the spleen for 48 h, and was not subjected to any inhibitory influence until the production of the cellular mediators of immunity was underway, and the host had developed a capacity to express a delayed-type sensitivity reaction against the organism.

**Cellular Composition of the Spleen at Peak Response.**—A comparison between the cellular composition of normal and 6 day-infected spleens is shown in

| Group* | Spleen wt (mg) | No. cells per spleen × 10^6 | In vivo [%H]Tdr incorp. cpm × 10^4 | Cells stained anti-Ig anti-Ig | Cells stained anti-Ig anti-theta | Cells stained anti-Ig anti-Ig | No. Ig-bearing cells × 10^6 | No. Theta-positive cells × 10^6 |
|--------|----------------|----------------------------|-----------------------------------|-------------------------------|--------------------------------|--------------------------------|-----------------------------|-----------------------------|
| 6 day-infected‡ | 316.67 ± 23.9 | 267 ± 17 | 318.3 ± 43 | 61.5 ± 2.5 | 28.4 ± 0.9 | 33.1 ± 1.7 | 75.82 ± 4.5 | 88.37 ± 8.4 |
| Controls | 91.25 ± 3.64 | 129 ± 15 | 92.7 ± 9 | 63.4 ± 3.5 | 65.1 ± 6.2 | 46.57 ± 8.2 | 35.21 ± 6.4 |
| 15 day-infected§ | 162 ± 6.07 | 169 ± 12 | 120 ± 15.3 | 55.64 ± 5.67 | 25.5 ± 1.55 | 30.13 ± 5.74 | 43.3 ± 4.6 | 54.6 ± 9.4 |

* Means ±SE of four mice per group.
‡ Infection resulted in 6 days in (a) 3.47-fold increase in spleen wt; (b) 3.4-fold increase in [%H]Tdr incorporation; (c) 2.07 times more nucleated cells; (d) 1.62 times more Ig-bearing cells; (e) 2.51 times more theta-positive cells.
§ By day 15 of infection the cellular composition of the spleen was returning to normal.

Table I. An infection-induced increase in cellularity was evidenced both by an increase in spleen weight and an increase in the yield of nucleated cells. Immunofluorescence showed that increased cellularity was associated with a major increase in the total numbers of Ig-bearing plus theta-positive cells. Thus, the total number of cells which stained with FIC anti-Ig after treatment with normal AKR serum (Ig-bearing B cells) increased 1.5 times. Most of these (80-90%) showed capped fluorescence while the remainder showed either sharp ringed or speckled fluorescence. Incubation with anti-theta serum greatly increased the total number of cells which stained with FIC anti-Ig. This was caused by the presence of theta-positive T cells, which showed diffuse ringed fluorescence of the type displayed by B6AF1 thymocytes exposed to the same reagents. By subtraction, it can be seen that *Listeria* infection resulted in a 2.5-fold increase in
the total number of \( \theta \)-positive cells in the spleen; in this experiment an increase of \( 53 \times 10^6 \) T cells.

The percentages of Ig-bearing and \( \theta \)-positive cells found in normal spleens in this study are in general agreement with the published findings of Raff (7); so were the staining characteristics of these cells. It will be noted, however, that an increase in their total numbers in response to \textit{Listeria} infection was not necessarily reflected by an increase in their proportions. It will also be noted that only about 60% of the total spleen cell population shown in Table I was Ig-bearing and \( \theta \)-positive according to immunofluorescence. This was signifi-

### TABLE II

Changes in the Cellular Composition of the Spleen Associated with Peak Production of \textit{Listeria}-Committed Lymphocytes*

| Group       | Spleen wt | No. cells per spleen \( \times 10^6 \) | In vivo \( \text{[H]TdR} \) incorp. \( \times 10^3 \) | Cells stained anti-\( \theta \) + anti-Ig | Cells stained anti-Ig | \( \theta \)-positive cells | No. Ig-bearing cells \( \times 10^3 \) | No. \( \theta \)-positive cells \( \times 10^3 \) |
|-------------|-----------|--------------------------------------|---------------------------------|---------------------------------|-----------------------|---------------------------|-------------------------------|-------------------------------|
| Exp. 1*     | 6 day-infected | 300 215 286.5 90 33 57 | 71 123 |                     |                       |                          |                             |                             |
| Controls    | 99 115 82.7 88.2 38.9 49.3 | 44.7 56.7 |                     |                       |                       |                          |                             |                             |
| Exp. 2*     | 6 day-infected | 378 319 298.3 93.68 55.11 38.57 | 175.8 123 |                     |                       |                          |                             |                             |
| Controls    | 106 101 81.2 82.69 48.0 34.69 | 48.5 35.0 |                     |                       |                       |                          |                             |                             |

* Results obtained with pooled spleen cells of five mice per group.

These results essentially confirm those shown by Table I.

The increased numbers of \( \theta \)-positive and Ig-bearing cells in the spleen was short-lived after peak response. This is shown by Table I where it can be seen that by day 15 the numbers of \( \theta \)-positive and Ig-bearing cells were approaching control values. By this time the numbers of cellular mediators in the spleen were also greatly reduced (compare Figs. 1 and 2).

Evidence that a Large Proportion of \( \theta \)-Positive and Ig-Bearing Cells in Immune
Spleens were Synthesizing DNA.—The foregoing experiments showed that the infection-induced increase in the numbers of θ-positive and Ig-bearing cells in the spleen was associated with a major increase in [3H]TdR incorporation into total spleen DNA. The following results indicate that part of this increase in [3H]TdR incorporation can be accounted for by an increase in the total numbers of θ-positive and Ig-bearing cells in DNA synthesis. Table III shows the effect of anti-θ serum and anti-Ig serum on the capacity of spleen cells from normal

|                   | [3H]TdR incorporation mean cpm/20 × 10^6 cells ±SE | Mean percent inhibition ±SE |
|-------------------|-----------------------------------------------|-----------------------------|
| Normal AKR serum  |                                               |                             |
| 6 day-infected    | 7,745 ± 269                                    | 20.44 ± 3.4                 |
| (n = 4)           |                                               | 28.53 ± 2.7                 |
| Controls          | 1,103 ± 397                                    | 25.20 ± 1.95                |
| (n = 4)           |                                               | 23.12 ± 1.05                |

and 6 day-infected mice to incorporate [3H]TdR in vitro. It can be seen that immune spleen cells incorporated over seven times more [3H]TdR into DNA than the same number of normal spleen cells. Furthermore, pretreatment with either anti-θ serum or anti-Ig serum caused a significant suppression of [3H]TdR incorporation both in normal and immune spleen cells, the percent inhibition being roughly the same. In terms of total inhibition, however, anti-θ serum caused over six times, and anti-Ig serum over eight times, more inhibition in immune than in normal spleen cells. By referring to Table I, (results of a concurrent experiment with mice of the same age and sex) it will be seen that the proportion of θ-positive cells was roughly the same in normal and immune spleen cell populations, and that there was even a smaller proportion of Ig-bearing cells in the immune spleen cell population. It follows, therefore, that the higher total inhibition of in vitro [3H]TdR incorporation in immune spleen cells must have resulted from the action of the antisera either on a larger proportion of θ-positive and Ig-bearing cells in DNA synthesis, or on similar proportions of replicating cells with faster rates of DNA synthesis. The former possibility seems more likely, because it is difficult to conceive a six- to eightfold difference in the rate of DNA synthesis by cells of the same lineage, particularly since the lymphocytes which divide in the normal spleen like those in the immune spleen must be considered as blast cells. If equal rates of DNA synthesis can be assumed, and if the anti-θ and anti-Ig serum performed with equal efficiency against replicating lymphocytes from normal and immune
spleens, the results indicate that on a cell for cell basis 6 day-infected spleen cells contained over six times more \( \theta \)-positive and about eight times more Ig-bearing cells in DNA synthesis than normal. Furthermore, because the spleens of 6 day-infected mice contained a total of 2.5 times more \( \theta \)-positive and 1.5 times more Ig-bearing cells than normal spleens, it follows that they must have contained a total of about 14 times more \( \theta \)-positive and 14 times more Ig-bearing cells in DNA synthesis. Similar results were obtained with pooled spleen of five mice in three separate experiments. Even if these results are considered approximations, they leave no doubt that the anti-Listeria response results in a striking increase in the total numbers of replicating \( \theta \)-positive and Ig-bearing lymphocytes in the spleen.

**Evidence that the Mediators of Anti-Listeria Immunity are Dividing T Cells.**—It is the acquired population of replicating T cells, rather than B cells, which contains the cellular mediators of anti-Listeria immunity. This is shown in Fig. 3 where it can be seen that treatment of immune spleen cells with anti-\( \theta \) serum and complement completely eliminated their capacity to adoptively immunize normal recipients against lethal challenge. In contrast, treatment with anti-Ig
serum and complement had no effect on the protective capacity of immune spleen cells. The same result was obtained whether the spleen cells were treated with the antisera at 4°C or at 37°C.

Evidence that the mediators of anti-Listeria immunity are rapidly dividing cells can be seen in Fig. 4 which shows the effect of a 15 h pulse of the antimitotic drug, vinblastine, on the capacity of spleen cells from 6 day-infected donors to protect normal recipients against challenge infection. Even three organ equivalents (4 X 10⁶ cells in two doses given 30 min apart) of spleen cells from vinblastine-treated donors failed to protect normal recipients. Most of the mediator T cells must have entered mitosis and been destroyed during the 15 h pulse of vinblastine.

**DISCUSSION**

Assuming that the immunofluorescence technique employed gives a reasonable approximation of the number of θ-positive and Ig-bearing lymphocytes, this study leaves no doubt that the production of the mediators of anti-Listeria immunity is associated with a striking increase in the production of T cells and B cells in the spleen. Furthermore, the demonstration that the mediators of immunity are eliminated by treatment with anti-θ serum, but not by treat-
ment with anti-Ig serum, means that they are part of the expanded T cell population, not the expanded B cell population. It seems likely from the negative result with anti-Ig serum, that mediator T cells have either very few, or no immunoglobulin determinants exposed on their plasma membranes.

The elimination of mediator T cells from donor spleens at peak response by a 15 h pulse of vinblastine, indicates that the majority of them must enter mitosis within this time span. This agrees with published findings on the vinblastine sensitivity of the mediators of anti-Listeria immunity in the rat (12). The conclusion that they are rapidly dividing cells is reinforced by the additional finding that an increase in their numbers in the spleen is associated with a major increase in DNA synthesis. Furthermore, a significant proportion of the higher than normal rate of DNA synthesis in vitro by spleen cells from immunized animals could be suppressed by anti-θ serum and complement. This allows the calculation that a large increase in the total number of T cells in the spleen at peak response is associated with a much larger increase in the number of them in DNA synthesis.

A progressive loss of mediator cells from the spleen, on the other hand, is closely associated with a progressive decrease in the total number of T cells and also with a progressive decrease in DNA synthesis. These data indicate that an effective population of mediator T cells can only be sustained by rapid self-replication. It is apparent that when the stimulus for their division declines, they have only a relatively short life-span in the spleen. Furthermore, the finding that a progressive loss of cellular mediators from the spleen is associated with a corresponding and progressive decrease in delayed skin reactivity to Listeria antigens indicates that these cells also have a short life-span in blood. This can only mean that their effective existence after their formation is quite a brief one. It seems reasonable to suggest at this point that the progressive decay of the immune response after day 6 of infection probably results from the rapid elimination of the infecting organism from the tissues and the consequent decline in the intensity of antigenic stimulation (Fig. 2).

It should be realized that the adoptive transfer experiments described here represent an attempt to transfer the bulk of an ongoing immune response to normal recipients. The onset of adoptive immunity is rapid, and depends on the activation of recipient macrophages by a large number of infused mediator T cells (1). It is obvious from the results of this study (Figs. 1 and 2) that the level of macrophage activation developed by recipients of immune spleen cells was directly proportional to the number of mediator T cells infused. These experiments did not detect the presence of a significant number of "memory cells" in donor spleens after the conclusion of the immune response. Presumably, if such cells exist, they are not present in sufficient numbers or an appropriate physiological condition to protect recipients against a moderate challenge infection.

The relation of the T cell mediators of anti-Listeria immunity to other types
of T cells deserves comment. It was shown previously (6) that the accumulation of
Listeria-sensitized T cells in the spleen is associated with increased numbers of
rapidly replicating pyroninophilic cells in the red pulp. The white pulp, in
contrast, does not appear to undergo change. It was also shown (6) that treat-
ment with cortisone acetate greatly depletes the spleen of Listeria-committed T
cells, and at the same time greatly reduces the number of replicating cells in
the red pulp, but not the white pulp. These observations indicate that Listeria-
committed T cells are not present in large numbers in the so-called thymus-
dependent regions of the spleen: the arteriolar lymphatic sheaths. It is apparent,
therefore, that most of them do not accompany the recirculating lymphocytes
which include the long-lived, cortisone-resistant, immunocompetent T cells
which initiate allograft immunity (13, 14). This conclusion is supported by the
published finding (2) that the mediators of anti-Listeria immunity in the rat
do not move freely from blood to central lymph.

The T cell mediators of anti-Listeria immunity do appear to resemble, how-
ever, the activated T cells which effect allograft immunity, particularly with
respect to the time-course of their production and their longevity. It has been
shown both in the mouse (15) and the rat (16), for instance, that intraperitoneal
injection of allogeneic cells results in the production in the spleen of lympho-
cytes which are specifically cytotoxic for the allogeneic cells in vitro. Cytotoxic
lymphocytes are first present in significant numbers in the spleen at about day 4
after immunization, reach peak numbers between about days 5 and 8 and then
progressively disappear. They cannot be detected in the spleen in significant
numbers 2–3 wk after peak production. It seems highly likely, therefore, that
the mediators of anti-Listeria immunity and the effectors of allograft immunity
are the same type of activated T cells, i.e., rapidly dividing and short-lived. It
should also be mentioned in this connection that the cellular mediators of anti-
Listeria immunity in the rat (17) and the mouse (North, unpublished observa-
tion) can be concentrated in sterile inflammatory exudates, as can the sensitized
lymphocytes which participate in the effector mechanisms of other examples of
cell-mediated immunity (see ref. 18).

The significance of the increased production and accumulation of rapidly
dividing Ig-bearing B cells in the spleen during Listeria infection is not known.
The published findings that primary infections with Listeria need not result in
the production of detectable circulating agglutinins (19), and the knowledge
(North, unpublished observation) that antibodies to protein antigens of Listeria
could not be detected in serum during primary infection by the passive hemag-
glutination technique is tentative evidence that the increased production of B
cells is not associated with the production of specific humoral antibody. It is
possible that their production results from the action of mitogenic factors re-
leased by replicating T cells, as has been shown to occur in vitro when T cells
are activated by phytomitogens (20). It is also possible that the newly formed
B cell population may represent potential antibody-forming cells which are
suppressed in a response geared to the production of activated T cells.
SUMMARY

An intravenous immunizing infection with the facultative, intracellular parasite, *Listeria monocytogenes* results in the production in the spleen of a population of immunologically-committed lymphocytes which can adoptively immunize normal recipients against a lethal challenge infection. These cellular mediators of immunity are first produced in the spleen between days 2 and 4 of infection and reach peak production on day 6. Their production then progressively decreases until about day 20 when their presence can no longer be detected.

Increased production of cellular mediators is coincident with major increases in cell division, cellularity, and spleen weight. Decreased production of cellular mediators, on the other hand, is associated with decreases in cell division, cellularity, and spleen weight. Again, the level of delayed sensitivity to *Listeria* antigens expressed by the host at any one time is proportional to the number of cellular mediators in the spleen.

Increased production of cellular mediators is also associated with major increases in the total numbers of replicating T cells and B cells in the spleen. That the cellular mediators of immunity are part of the replicating T cell population, rather than the B cell population, is evidenced by their susceptibility to anti-θ serum and by their resistance to anti-Ig serum. Furthermore, they can be completely eliminated from the spleen by a brief pulse of the antimitotic drug, vinblastine. This study allows the conclusion that the cellular mediators of anti-*Listeria* immunity belong to an expanded population of rapidly dividing, short-lived T cells. It is suggested that they have the same properties as the T cell effectors of allograft immunity.

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