SUPPRESSION OF CELL-MEDIATED IMMUNITY TO INFECTION BY AN ANTIMITOTIC DRUG

Further Evidence That Migrant Macrophages Express Immunity*

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The response to infection with *Listeria monocytogenes* is characterized by substantial host cell division involving lymphoid cells in the spleen and mature resident macrophages in the liver and peritoneal cavity (1, 2). Peak division of these cells is followed immediately by the expression of high levels of antimicrobial immunity; but the macrophages responsible for expressing anti-*Listeria* immunity in infective foci in the liver are derived predominantly from circulating macrophage precursors which are being constantly produced by division (3). It would seem logical to postulate, therefore, that the development of cell-mediated immunity to this infection should be highly vulnerable to the action of an antimitotic drug.

In this study, the Vinca alkaloid vinblastine (Vb) was used to selectively destroy dividing host cells during infection. This compound irreversibly blocks cell division at metaphase (4). It is particularly useful for this type of study because it has a relatively short half-life in vivo (5), and therefore, it can be used to destroy dividing cells over limited periods during the host response.

It will be shown that a 15 hr pulse of Vb can inhibit the development of cellular immunity to a primary *Listeria* infection, provided it is administered to the host before infective foci become populated by activated macrophages.

Materials and Methods

The method for studying the growth of *L. monocytogenes* in the livers and spleens of mice was the same as that used in the preceding paper (3). Infections were initiated by the intravenous injection of approximately 2×10^3 viable organisms.

Vinblastine sulfate (Velban; Eli Lilly & Co., Indianapolis, Ind.) was dissolved in 0.9% sodium chloride solution and administered intravenously. This drug has a half-life in mice of 3.5 ± 1.2 hr (5). Therefore, a 100 μg dose should ensure the destruction of dividing host cells for about 15 hr. It was shown in preliminary experiments that Vb has no effect on the in vitro multiplication of *L. monocytogenes*.

Radiometry and radioautography were performed in the same way as described previously (3). All histological observations were made on plastic-embedded liver.

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RESULTS

Effect of a 15 hr Pulse of Vinblastine Given at Different Times before and during Infection

Pulsing before Infection.—A large number of mice were divided into groups according to whether they received a single 100 μg injection of Vb either 12,

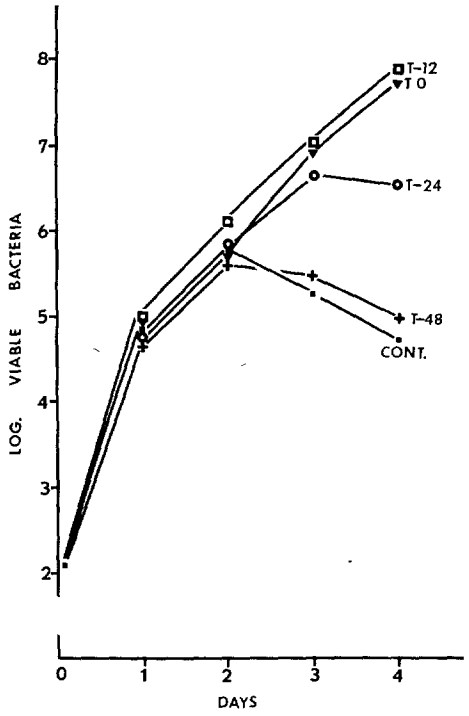


FIG. 1. Effect of pulsing with Vb before infection. Mice received a single 100 μg dose of Vb either 48, 24, or 12 hr before infection or at the time of infection. Mice given Vb 12 hr before or at the time of infection failed to acquire immunity. Note that the effect of the drug was not apparent until between 48 and 72 hr of infection.

24, or 48 hr before infection or at the time of infection. The effect of Vb, given at these times, on the growth curve of *L. monocytogenes* in the spleen is shown in Fig. 1, where it can be seen that the drug had no effect on the development of host resistance when given 48 hr before infection and had only a partial effect when given 24 hr later. In contrast, Vb given either 12 hr before, or at the time of infection, resulted in a complete failure on the part of the host to acquire antimicrobial immunity.

It will be noted that the growth curve of *L. monocytogenes* in Vb-treated mice was almost identical with that in control mice up to the 2nd day of infec-

tion. The sudden increase in bacterial growth in Vb-treated mice between 48 and 72 hr was more obvious in the liver, as will be shown later.

Pulsing with Vinblastine during Infection.—These experiments were performed as above except that the Vb injections were given at the time of infection or at 12, 24, 48, 72, or 96 hr during infection. Fig. 2 shows that the development of antimicrobial immunity could be completely or partially suppressed

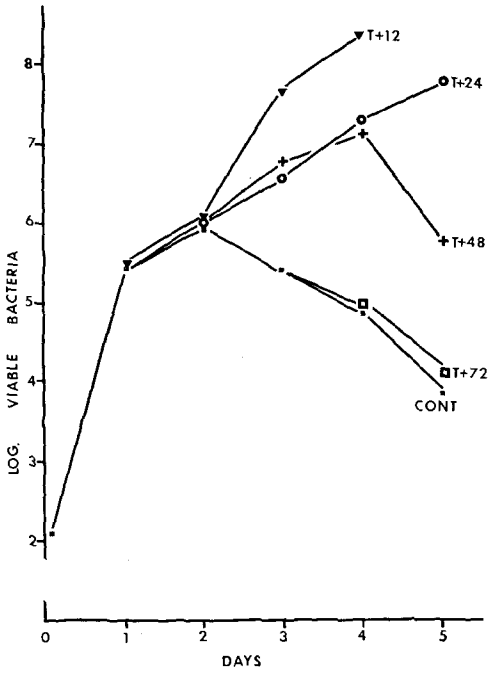


FIG. 2. Effect of pulsing with Vb during infection. Mice injected at 12 or 24 hr during infection failed to acquire cellular resistance. Vb had no effect when given at 72 hr of infection or later (results of pulsing at 96 or 120 hr are not included). Note that effect of drug did not become apparent until 48–72 hr of infection.

only during the first 48–72 hr of infection. After this time Vb had no significant effect on the host-parasite relationship. It is evident, therefore, that Vb has no effect on established immunity. Instead, it interferes with host components which generate this immunity. These components were most sensitive to Vb during the first 24 hr of infection. A pulse of Vb given at 12 or 24 hr resulted in complete failure to acquire control over bacterial multiplication, whereas a pulse given at 48 hr resulted in only a temporary loss of immunity, indicating that the generation of antimicrobial immunity is partly completed by this time. It will be noted again that the growth curve of *L. monocytogenes* in mice treated

with Vb at 12 or 24 hr of infection was almost identical with that in control mice during the first 48 hr of infection.

Effect of Vinblastine on Immunity in the Liver

The foregoing results illustrated the effect of Vb on immunity in the spleen. It was found in all cases that Vb caused the same effect on immunity in the liver. This is partly illustrated in Fig. 3 which shows the growth curve of *L.*

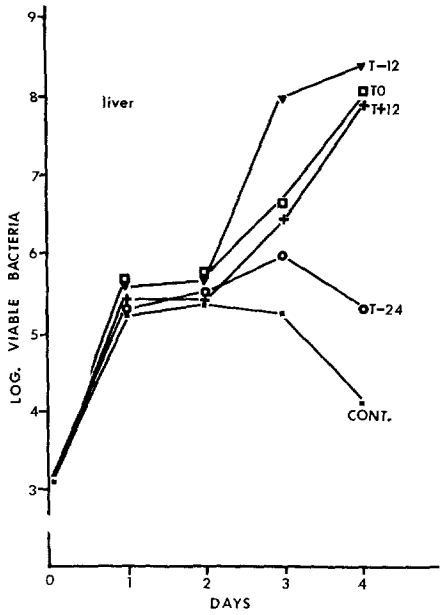


FIG. 3. Growth curves of *L. monocytogenes* in livers of mice treated with Vb either before, at the time of, or early during infection. The liver curves serve to show the sudden increase in bacterial multiplication between 48–72 hr of infection.

monocytogenes in the livers of mice pulsed with Vb either just before or just after initiating infection. It will be noted that the results are the same as those obtained for the spleen, except that the liver served better to show that the growth curve of *L. monocytogenes* was the same in control mice and Vb-treated mice during the first 48 hr of infection.

Effect of Vb on the Division of Kupffer Cells during Infection

It has been suggested (2) that the massive division of Kupffer cells during the 2nd and 3rd days of a *Listeria* infection could be an important element in the development of resistance in this organ. The following experiment was per-

formed in order to determine whether a suppression of Kupffer-cell division is responsible for the failure of mice to develop resistance when Vb is given at the time of infection.

Mice were infected with *L. monocytogenes* and pulsed 30 min later with 100 μg of Vb. The division of Kupffer cells was detected by flash labeling with 20

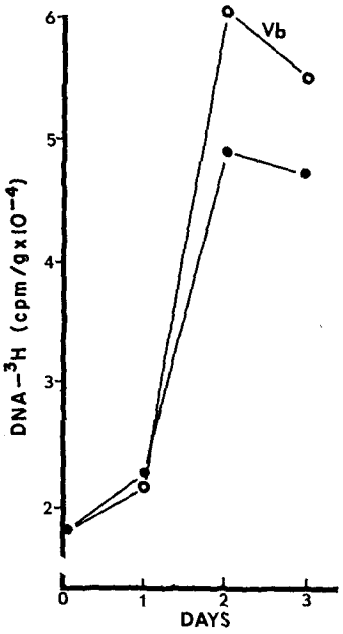


FIG. 4. Effect of Vb on division of Kupffer cells during infection. Mice were pulsed with Vb 30 min after initiating infection. Division of Kupffer cells was detected by flash labeling with TdR-^{3}H at 24-hr intervals and counting liver DNA-^{3}H by radiometry. An early pulse of Vb did not inhibit the division of liver macrophage.

μCi of tritiated thymidine, as described previously (2). Fig. 4 shows that a pulse of Vb given at the time of infection did not suppress the subsequent incorporation of TdR-^{3}H into Kupffer cells which began dividing 2 days later. In fact, it was found consistently that more incorporation occurs in Vb-treated mice. It is apparent, then, that cell division and the resulting increase in the number of Kupffer cells are not enough in themselves for the expression of antimicrobial immunity in the liver. It is evident that some additional host component is required.

The Failure of Monocyte-Derived Macrophages to Populate Infective Foci in the Livers of Vb-Treated Mice

It has been shown previously (3) that acquisition of host immunity against infection with *L. monocytogenes* is coincident with the accumulation of monocyte-derived macrophages in infective foci in the liver. It was thought probable, therefore, that the suppression of immunity by Vb is due to its ability to

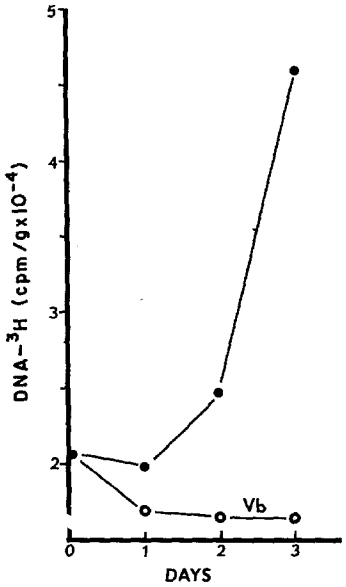
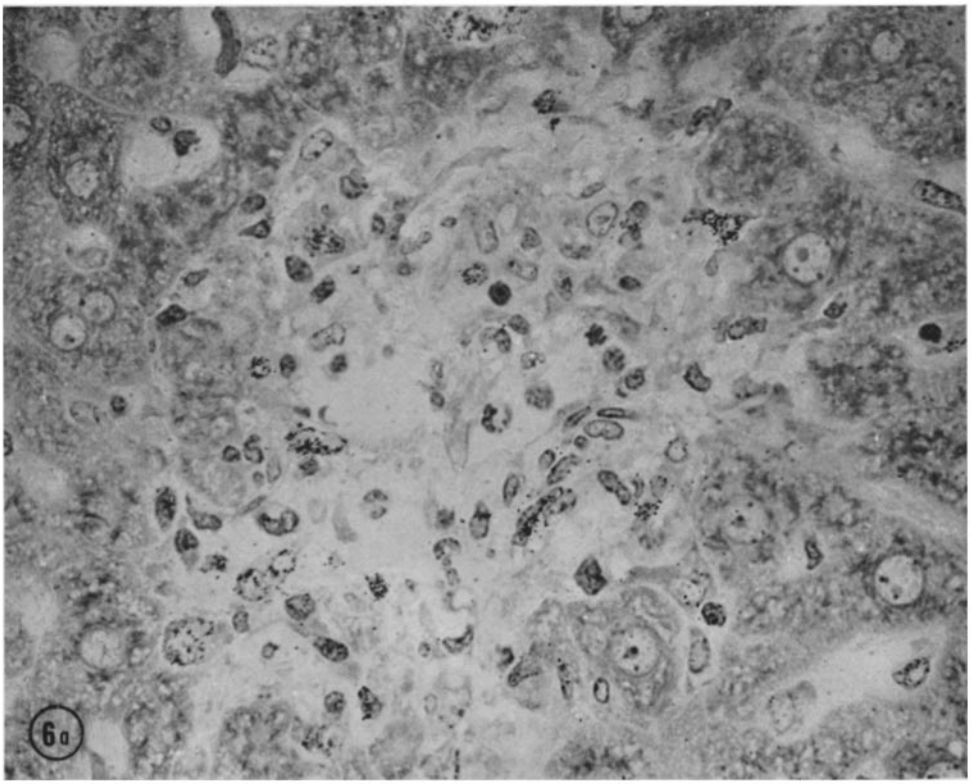


FIG. 5. Effect of Vb on the influx of migrant macrophages into infected liver. Precursors of migrant macrophages were labeled with TdR-^{3}H 2 hr before infection, and the mice were injected 30 min later with Vb. After a further 30 min, they were infected with *L. monocytogenes*. Radiometry showed that labeled cells accumulated in infected livers of control mice during infection. No influx of labeled cells occurred in the livers of Vb-treated mice.

destroy the precursors of monocytes which are known to be constantly dividing in bone marrow (6, 7). Mice were injected intravenously with a single 20 μCi dose of TdR-^{3}H and then injected again 30 min later with 100 μg of Vb. After a further 30 min, they were infected with a standard dose of *L. monocytogenes*.

FIG. 6. Infective foci in the livers of (a) control mice and (b) Vb-treated mice on the 3rd day of infection. The infective foci in control mice are populated by macrophages, some of which were labeled by a pulse of TdR-^{3}H 2 hr before infection. In contrast, the infective foci in Vb-treated mice are acellular, and the parasite is growing unchallenged both extracellularly and in surrounding parenchyma. × 560.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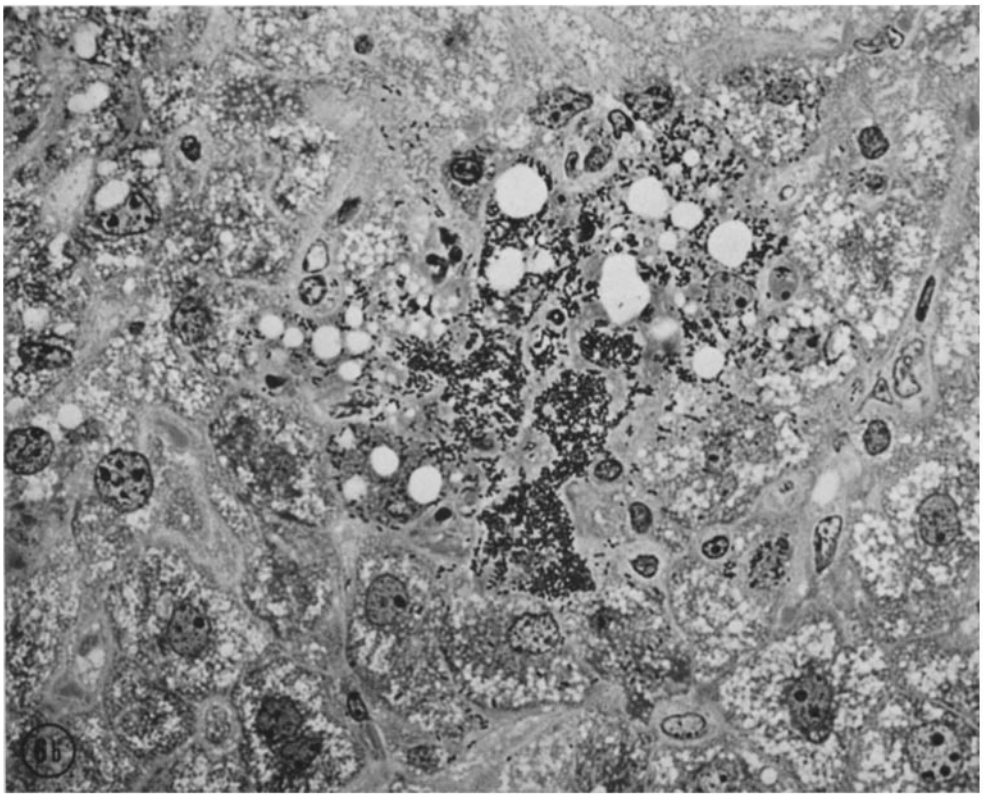
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The livers of randomly selected mice were processed for radiometry and radioautography immediately after initiating infection and on the 2nd and 3rd days of infection. Control mice did not receive Vb. Fig. 5 shows that a pulse of TdR-^{3}H given to control mice 2 hr prior to infection resulted in a significant increase in liver DNA-^{3}H during infection. This increase did not occur in the livers of mice which received Vb just before infection. Radioautography showed that the increase in liver DNA-^{3}H in control mice corresponded to increased numbers of labeled macrophages in sinusoids and in infective foci (Fig. 6a). They were derived from labeled precursors circulating in blood (3). In striking contrast to this, no labeled macrophages were found in the livers of the Vb-treated mice. In fact, the infective foci in Vb-treated mice failed to acquire macrophages (Fig. 6b), so that after 48 and 72 hr the lesions were acellular and contained large numbers of extracellular bacteria which tended to invade parenchymal cells and leak into sinusoids.

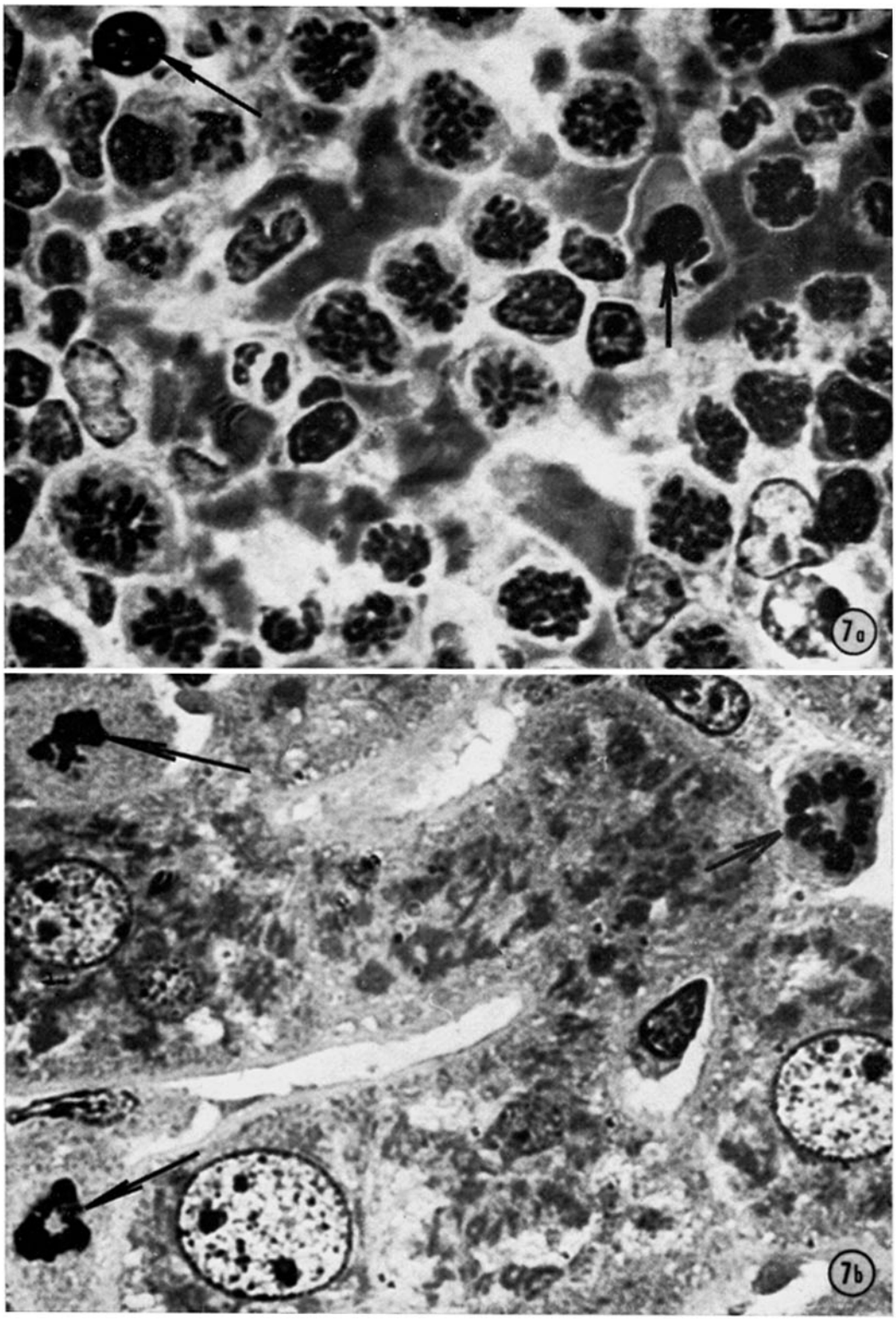
Cytological Observations on Tissues of Infected Mice Treated with Vb

The aim of this part of the study was to find cytological evidence for the antimitotic effect of Vb on those host cells which are known to proliferate in response to infection. Mice were injected with 100 μg of Vb at different stages of infection. 4 hr after the injection of Vb, the livers and spleens of the mice were fixed in neutral formalin and embedded in glycol methacrylate (3). The spleens of all Vb- treated mice contained large numbers of mitotically arrested cells. The numbers were much higher, however, in the spleens of mice treated with Vb at 24 or 48 hr of infection (Fig. 7a). It was not until 48 hr, however, that substantial mitotic activity developed in sinus-lining macrophages of the liver (Fig. 7b). In both organs, mitotic figures were abnormal. It is obvious, therefore, that a 100 μg dose of Vb destroys large numbers of dividing host cells during infection.

DISCUSSION

This study shows that a 15 hr pulse of Vb given 12 hr before infection or up to about 24 hr during infection causes complete failure on the part of the host to generate effective antimicrobial immunity to a *Listeria* infection. The drug has only a partial effect when given 24 hr before infection or 48 hr after initiating infection; and it is entirely without effect when given after 72 hr of infection, i.e., when antimicrobial immunity is already established. The drug, therefore, does not interfere with the functioning of the activated macrophages which are already engaged in destroying bacteria within infective foci. Its effect

FIG. 7 Sections of liver and spleen of mouse which received Vb at 48 hr of infection. Arrested mitotic figures were allowed to accumulate in the liver and spleen for 4 hr before sacrificing the animal. Many mitotic figures in the spleen (a) are abnormal, and in some cases chromosomes have amalgamated into single clumps (arrows). The liver (b) contains Kupffer cells in mitotic arrest with abnormal chromosomes (arrows). × 2200.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must therefore result, directly or indirectly, from its capacity to prevent the generation of these cells.

It has been shown (3) that the onset of immunity to *L. monocytogenes* is coincident with the accumulation of recently formed migrant macrophages in infective foci in the liver. These macrophages are derived mainly from newly formed precursor cells in blood, and it is almost certain that they are blood monocytes. Furthermore, because blood monocytes are the progeny of constantly dividing precursor cells in bone marrow (6, 7), their formation should be highly vulnerable to the action of an antimitotic drug. A recent publication has shown (8) that a similar dose of Vb causes a dramatic fall in the number of circulating macrophage precursors in mice, and that it takes several days for their number to return to normal. This accords with the present finding that monocytes which should have been labeled by thymidine (given 2 hr before infection) failed to reach the lesions of animals which were also given Vb at the time of infection. It seems that Vb must have blocked their production in bone marrow.

According to the above interpretations, a pulse of Vb given as late as 48 hr of infection would not be expected to greatly interfere with the acquisition of immunity, because by this time infective foci already will have acquired some macrophages and will continue to acquire those newly generated monocytes entering the blood. The observed partial suppression of immunity due to an injection of Vb at 48 hr of infection probably resulted from the destruction of a proportion of macrophages dividing in infective foci (2). It seems unlikely, from this and from a previous study (3), that the effect is due to destruction of dividing Kupffer cells. Presumably, the host response is no longer vulnerable to the action of Vb after 72 hr of infection because by this time enough macrophages will have accumulated in infective foci to insure continued bacterial elimination.

Although the results of these experiments can be explained in terms of the direct action of Vb on the generation of host macrophage precursors, nevertheless, why infective foci normally are not populated by macrophages until after 48 hr of infection still needs to be explained. One reason for this may be that macrophages do in fact enter infective foci before this time, but most of them are destroyed because they have not, at this stage of the host response, acquired adequate antimicrobial mechanisms. In other words, the macrophages which persist in infective foci are activated macrophages. There is evidence that the activation of host macrophages begins at 48 hr of a primary *Listeria* infection (2) and that this occurs at a time when specific delayed hypersensitivity to *Listeria* antigens is first detected (2). This evidence, together with that which shows that macrophage activation is immediately preceded by intense lymphoid cell proliferation in the spleen (1) and that immunity to *L. monocytogenes* can be adoptively transferred to normal mice with lymphoid cells from immune mice (9), has led to the suggestion that macrophage activation is mediated

through specifically sensitized lymphoid cells. It is possible, therefore, that besides blocking the generation of circulating monocytes, Vb may have the additional effect of interfering with another component of the host response by destroying proliferating lymphoid cells. If committed lymphocytes are necessary for the activation of host macrophages, their absence would have the same ultimate effect on resistance as would the blocking of macrophage production itself. This possibility can be studied in Vb-treated mice which have been reconstituted with lymphoid cells, bone marrow cells, or both.

SUMMARY

The development of acquired cell-mediated immunity to infection with *Listeria monocytogenes* can be blocked by a 15 hr pulse of the antimitotic drug, vinblastine (Vb). The drug has no effect on the host-parasite relationship after 72 hr of infection when a high level of immunity is being expressed, i.e., when infective foci are populated by activated macrophages. Infective foci in mice treated early during infection with Vb do not acquire migrant macrophages, but they become acellular after 48 hr of infection. The results indicate that Vb destroys the dividing precursors of migrant macrophages. The possibility that Vb prevents the activation of these cells by destroying dividing lymphoid cells engaged in the specific immunological phase of the host response is also considered.

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