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Experimental Listeria Monocytogenes-Lymphadenitis

Pathohistological Observations

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Summary. The auricular lymph nodes of SPF guinea pigs were examined light microscopically at various stages (30 min to 14 days) after subcutaneous injection of Listeria monocytogenes. The bacteria entered the lymph node parenchyma through the marginal sinuses and were phagocytosed by polymorphonuclear leukocytes and monocytes at early stages of the infection (30–60 min). Whereas retothelial cells of the sinuses did not phagocytose bacteria, submarginal reticulum cells showed a high bacterium-phagocytosing activity. Starting at 12 hours after inoculation lymphocyte activation occurred in the interfollicular diffuse lymphatic tissue of the cortex and in the paracortex. The activated lymphocytes were released into the sinuses. There was also paracortical distension, which reached its peak on the 6th day of infection. The submarginal area at the junction of the afferent lymphatic with the cortex and the perivascular regions of the lymph node paracortex were the preferred sites of granuloma formation. On the 8th day with a dose of 10^6 bacteria the macrophages of the granulomas revealed only unidentifiable debris in the cytoplasm, whereas in the earlier lesions a large number of mononuclear phagocytes, especially in the submarginal granulomas, contained many intracytoplasmic listeria. The granulomas gradually became smaller and smaller. With larger infectual doses the process did not subside within the experimental period; the granulomas developed the appearance of "reticulocytäre abszedierende Lymphadenitis".

Introduction

Acquired resistance to Listeria is one of the most exhaustively studied examples of cell-mediated immunity to a facultative intracellular parasite. However, to our knowledge, there have been no detailed studies of histological changes in the regional lymph nodes following injection of Listeria monocytogenes. Conway (1938) described in detail the changes in the lymph nodes of rabbits and guinea pigs infected i.v. with L. monocytogenes, but her report was concerned primarily with the reaction of lymphocytes. Important discoveries made since then, i.e. the significance of cellular immunity in infections produced by facultative intracellular parasitic bacteria, the role of the mononuclear phagocytic system in the defense of the organism, lymphocyte activation, etc., may bring a better understanding of the pathology of this infection.

This study dealt with the reaction of the regional lymph nodes after subcutaneous injection of L. monocytogenes into guinea pig ears.

Material and Methods

Culture Preparation. L. monocytogenes (Paterson 7973, Serotype 1/2a) was used[1]. The strain was maintained on tripticasesoy agar (Difco). It was able to induce keratoconjunctivitis in guinea pigs. Before the organism was injected it was cultured in Mueller-Hinton bouillon at 37° C for 18 h, centrifuged, washed in 0.9% NaCl, and recentrifuged. The centrifugate was finally brought to the original volume with 0.9% NaCl.

Technique of Infection. We used male and female specific pathogen-free (SPF) guinea pigs weighing 250–300 g each (Centraal Proefdierenbedrif TNO Zeist, Holland).

26 animals were infected through intradermal injection of 0.1 ml bacterial suspension (5×10^6 bacteria) into the dorsal surfaces of both ears. Groups of 2 animals were killed through intracardial injection of Methitural (Thiogenal®, Merck, Germany).

In pilot experiments 54 guinea pigs of our own breed were used. 18 animals of this series were infected with 10^6, 10^7, or 10^8 doses of Listeria.

The regional auricular lymph nodes were removed for histological examination (at 30 min; 1 and 12 hrs; 1, 2, 3, 4, 6, 8, 10, and 14 days).

There were 2 control groups: 3 untreated animals and 3 killed 24 hours after the inoculation of 0.1 ml 0.9% NaCl.

Light Microscopy. Immediately after the animals were sacrificed their regional lymph nodes were removed and cut into 2 pieces. One was fixed in Zenker-fixative and embedded in paraffin; 5 μ sections were stained with hematoxylin-eosin, Giemsa, and Gram, and silver impregnated with Gomori. The other part of the lymph node was fixed in 5% glutaraldehyde (0.1 M cacodylate buffer pH 7.3), postfixed in 1% OsO_4 (Rhodin buffer), dehydrated in acetone, and embedded in Araldite. Semithin sections were stained with Azure II methylene blue (Richardson *et al.*, 1960). Imprints prepared from the fresh cut surface of the lymph nodes were stained with Pappenheim.

Results

The Normal Auricular Lymph Node of the Guinea Pig

The appearance of normal auricular lymph nodes of the conventional guinea pig has been described by Oort and Turk (1965). They found a wide variation in structure between two extremes: the "highly active" control lymph node and the "less active" one. The histological appearance of auricular lymph nodes of SPF animals was very similar to that of the latter type and showed no considerable microscopic variations. The marginal and medullary sinuses contained many small lymphocytes, several sinus macrophages (Nopajaroonsri *et al.*, 1971), and monocytes, but no polymorphonuclear leukocytes. The submarginal sinus was lined with retothelial cells (Mori and Lennert, 1969). Between these cells and the lymphatic parenchyma there was a more or less continuous layer of reticulum cells. In this study the term submarginal reticulum cell is used to designate these cells. The small rim of the cortex showed mostly small follicles with no active germinal centers. The paracortical areas were not extensive. They consisted mostly of small lymphocytes and contained many high endothelial venules (Herman *et al.*, 1972). Plasma cells were present in the medulla, but not in large numbers. No changes were seen in the histology of the lymph nodes of the 3 animals treated with NaCl.

Pathohistological Findings

30 min after infection the sinuses of the lymph nodes were moderately dilated. The lumen contained both extracellular bacteria and bacteria which had been phagocytosed by polymorphonuclear leukocytes and mononuclear phagocytes with a morphological similarity to blood monocytes. Several medullary sinus macrophages also contained intracellular bacteria (Fig. 1). We found intracellular

1 We wish to thank Professor Dr. H. P. R. Seeliger, Director, Institut für Hygiene und Mikrobiologie der Universität Würzburg, for supplying the Listeria strain.

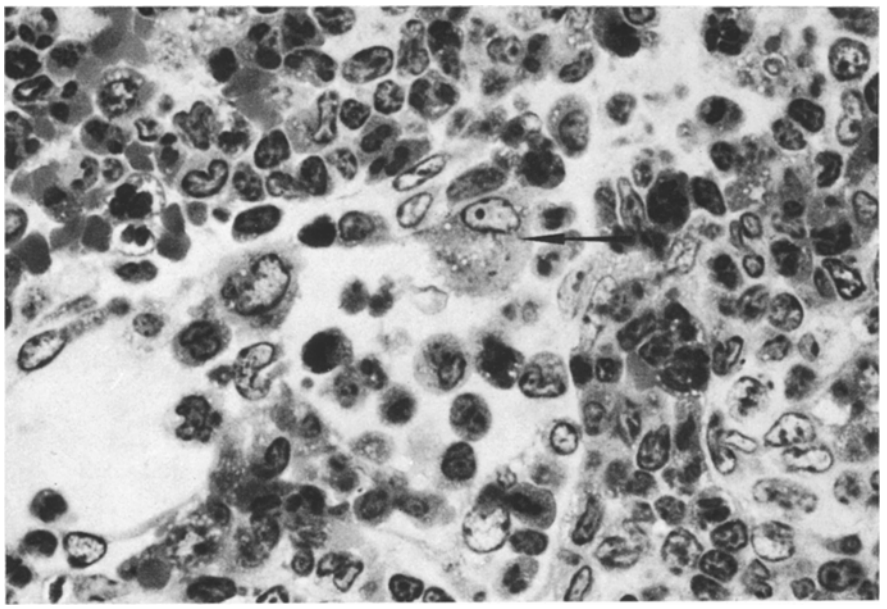


Fig. 1. Sinus macrophage (arrow) containing intracellular L. monocytogenes. Auricular lymph node 30 min after infection. Semithin section. Richardson's stain. × 880

bacteria in several submarginal reticulum cells but not in sinus retothelial cells. Polymorphonuclear leukocytes were seen in diapedesis from postcapillary venules all over the lymph node parenchyma.

1–3 hours after inoculation the cellular reaction was more pronounced. The number of polymorphonuclear leukocytes had increased more rapidly than the number of monocytes. The inflammatory reaction was especially noticeable in a wedge-shaped area at the junction of the afferent lymphatic with the cortex, where intra- and extracellular bacteria were also more numerous. Many submarginal reticulum cells contained phagocytosed Listeria (Fig. 2). Most of the follicles did not reveal active germinal centers. There were also leukocytes in diapedesis from follicular capillaries.

After 12 hours the inflammatory reaction had advanced further. The submarginal area now showed an irregular form. The blood vessels of the lymph node were more prominent. It is worth mentioning that the high endothelial venules of this region were also "paved" with polymorphonuclear leukocytes (Fig. 3).

At 24–48 hours the sinuses contained an increased number of mononuclear phagocytes and the cytoplasm of some of these cells contained phagocytosed leukocytes. Several activated lymphocytes were also found in the sinuses (Fig. 4). The area of submarginal infiltration was enlarged. Most of the inflammatory cells were mononuclear phagocytes (Fig. 5). Many of them were scattered and contained a number (about 8–10) of bacteria in the cytoplasm. The localization of the bacteria was most evident in semithin sections (Fig. 6). Polymorphonuclear leukocytes appeared near them as a rule. Other mononuclear phagocytes showed

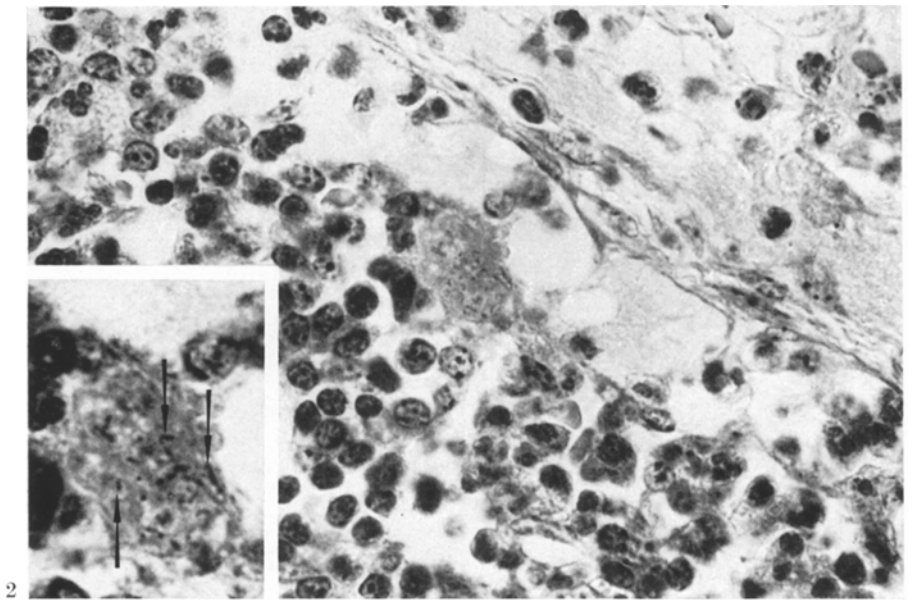


Fig. 2. Submarginal reticulum cell containing many phagocytosed Listeria. (The sinus retothelial cells are not seen on the picture.) Auricular lymph node 1 hour after infection. Arrows indicate bacteria. Gram's stain. × 880

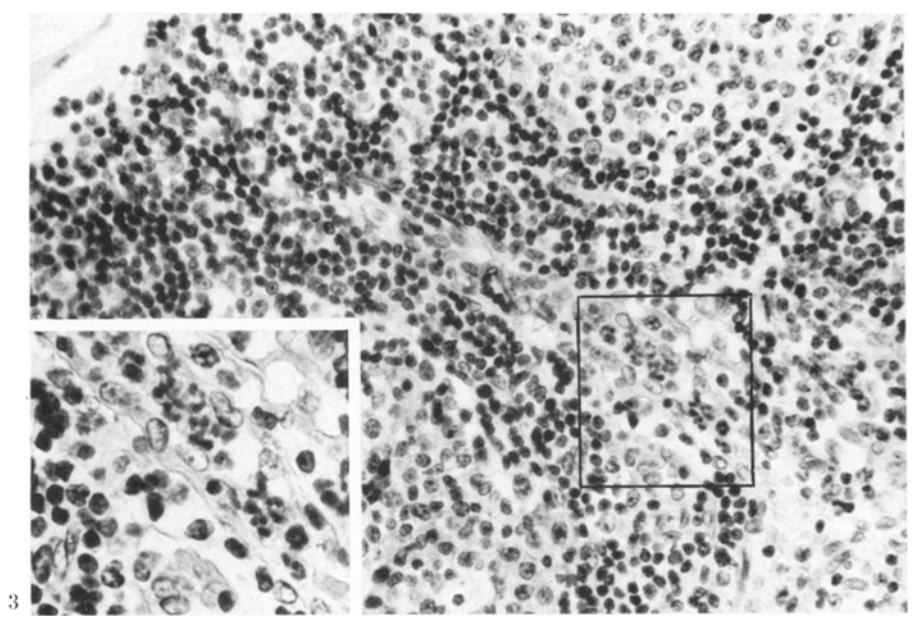
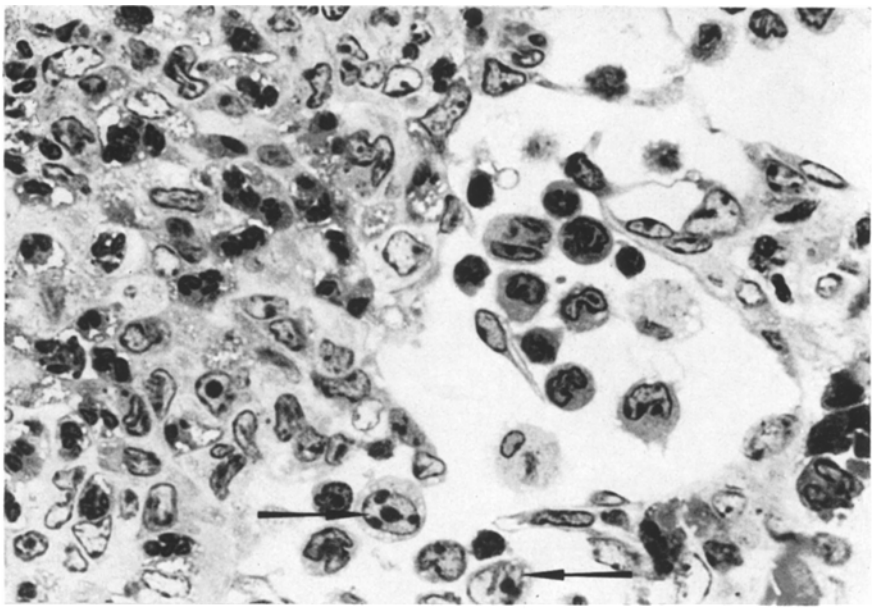


Fig. 3. High endothelial venule (arrows) with several polymorphonuclear leukocytes in the lumen. Auricular lymph node. 12 hours after infection. H & E. × 350, Inset × 560



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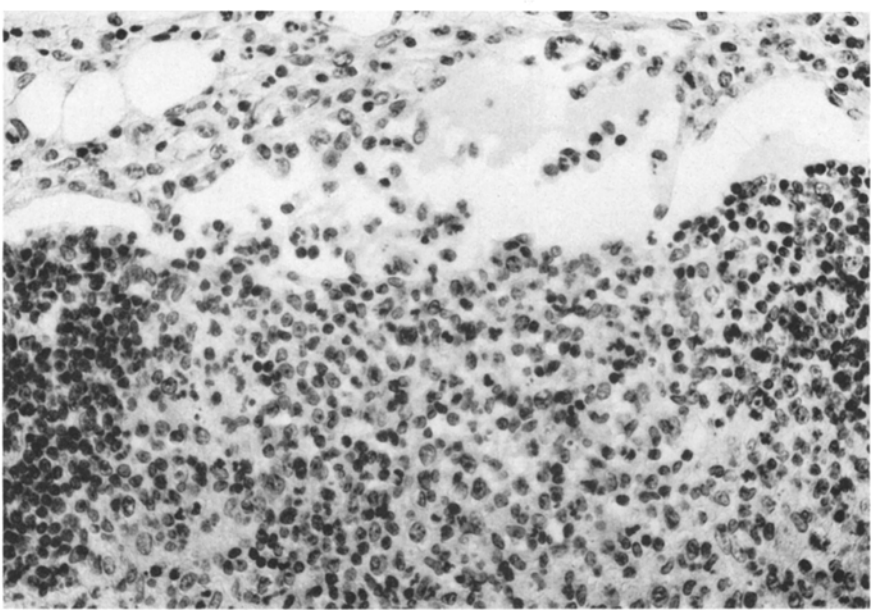

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Fig. 4. Several monocytes and activated lymphocytes (arrows) in the lumen of the submarginal sinus. Many polymorphonuclear leukocytes in the area of submarginal infiltration. 24 hours after infection. Semithin section. Richardson's stain. × 880

Fig. 5. Several monocytes and polymorphonuclear leukocytes in the lumen of the submarginal sinus. The submarginal area is infiltrated with inflammatory cells, mostly mononuclear phagocytes. 48 hours after infection. H & E. × 350

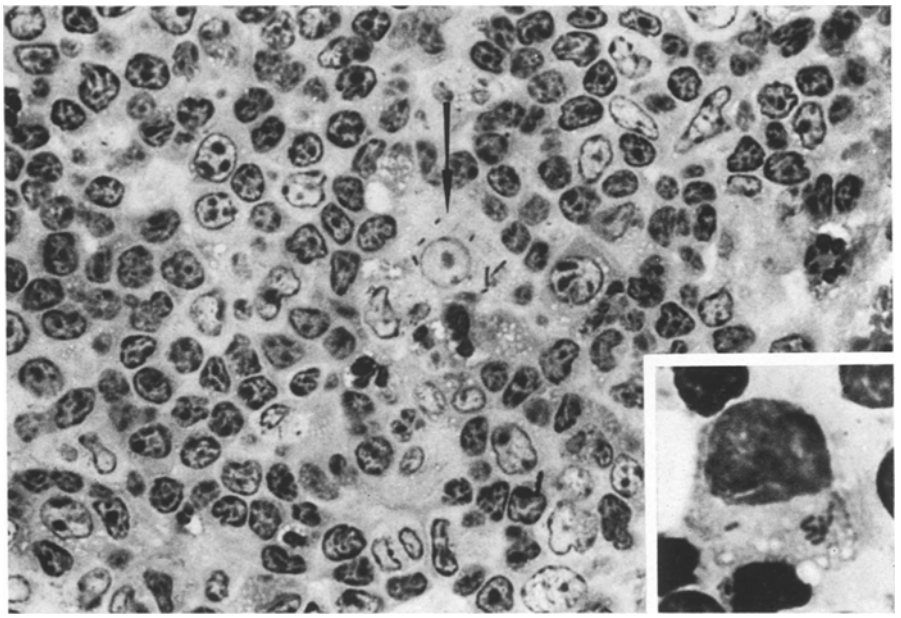

Fig. 6. Mononuclear phagocyte containing intracytoplasmic bacteria (arrow). Several polymorphonuclear leukocytes and activated lymphocytes in the vicinity. Submarginal infiltration area. 24 hours after infection. Semithin section. Richardson's stain. × 880. Inset: Mononuclear phagocyte with a well developed cytoplasm containing many bacteria. Imprint from an auricular lymph node. 24 hours after infection. Giemsa stain. × 1400

no signs of bacterium-phagocytosing activity. In imprints most of these cells were morphologically similar to blood monocytes. Only some of them were large and had a well developed cytoplasm containing many Listeria (Fig. 6 inset). Accumulations of inflammatory cells were also observed in the paracortical area. These infiltrates were frequently localized around the high endothelial venules and were composed mainly of mononuclear phagocytes. Here the number of Listeria-phagocytosing mononuclear cells was smaller than in the submarginal area. Polymorphonuclear leukocytes were seen only rarely. In the pilot experiments with larges doses of bacteria (10^7, 10^8) both the submarginal and perivascular inflammatory areas were larger in size and sometimes fused together. Here the number of polymorphonuclear leukocytes was also larger than that observed in the main experiment.

The diffuse lymphatic tissue of the interfollicular and paracortical areas in the lymph nodes of the control animals and earlier in the experiment was composed of a fairly uniform mass of small lymphocytes and a few scattered, medium-sized and large lymphocytes. However, starting at 12 hours after inoculation it gradually lost this uniformity. The number of both medium-sized and large lymphocytes progressively increased and at 48 hours the diffuse lymphatic tissue of the cortex and paracortex were packed with large lymphoid cells. These cells had large pale nuclei, prominent nucleoli, and a strongly basophilic cytoplasm. Such "activated" lymphocytes were also seen within and around the submarginal and paracortical

infiltration areas. They were often in mitosis. The follicles remained small and compact. The number of plasma cells found in the medulla was within the normal range. The number of "activated" lymphocytes in the marginal sinuses was increased, in line with the cortex. Such cells were also found in the intermediary sinuses, but in a significantly smaller amount.

After 3 or 4–6 days the sinuses contained a large number of cells, mostly mononuclear phagocytes. They were increased in size and usually had a well developed cytoplasm ("activated mononuclear phagocyte"). The difference in the composition of the submarginal and perivascular cell accumulation was more distinct than earlier. In both the large "activated" mononuclear phagocytes were predominant. However, in the submarginal inflammatory foci the number of polymorphonuclear leukocytes and cells with a morphological appearance similar to that of blood monocytes was much larger. Here there were also many more mononuclear phagocytes with intracytoplasmic bacteria. These granulomas were surrounded by scattered large "activated" lymphocytes, some of them in mitosis. On the other hand, the perivascular granulomas contained mostly mononuclear phagocytes. The number of "activated" lymphocytes in the cortex and paracortex was larger than on the second day after infection. The progressive increase in the size of the paracortical areas starting on the second day reached a peak on the sixth day of the inflammatory process. The paracortex replaced one third or more of the whole lymph node. Several follicles contained germinal centers, but most of them remained small and showed no sign of activation. There was no increase in the number of cells of the plasma cell series. The sinuses were dilated and closely packed with lymphocytes, mononuclear phagocytes, and sinus macrophages.

8–10 days after infection the marginal sinuses were packed with small lymphocytes (Fig. 7a). This phenomenon was noticed in several animals in other sinuses too, particularly at the paracortico-medullary border, but also in other sinuses throughout the paracortex. The submarginal reticulum cells sometimes formed a continuous layer. There was no change in the size of the paracortical areas over earlier. The number of "activated" lymphocytes had, however, continuously decreased. Many follicles contained germinal centers. A slightly increased number of plasma cells was found in the medullary cords. The most striking change observed in these lymph nodes was a progressive decrease in the size of the granulomas. By this time they were round in shape (Fig. 7b), in contrast to the irregular, diffuse accumulation seen earlier. There was also a great change in the cellular composition. Whereas earlier the granulomas showed a large variety of cells, after the eighth day of infection they were of uniform appearance and consisted mostly of activated mononuclear phagocytes. We found only a few cells with a morphological appearance similar to that of blood monocytes or polymorphonuclear leukocytes in the granulomas. The mononuclear phagocytes that had increased in size until the eighth day showed no further increase and now contained only cellular debris but no intact bacteria in the cytoplasm (Fig. 7b). The number of extracellular bacteria decreased in line with the number of intracellular Listeria in the granulomas.

In marked contrast, in the pilot experiment with a large dose (10^8) of bacteria there was no change in the cellular composition and size of the granulomas.

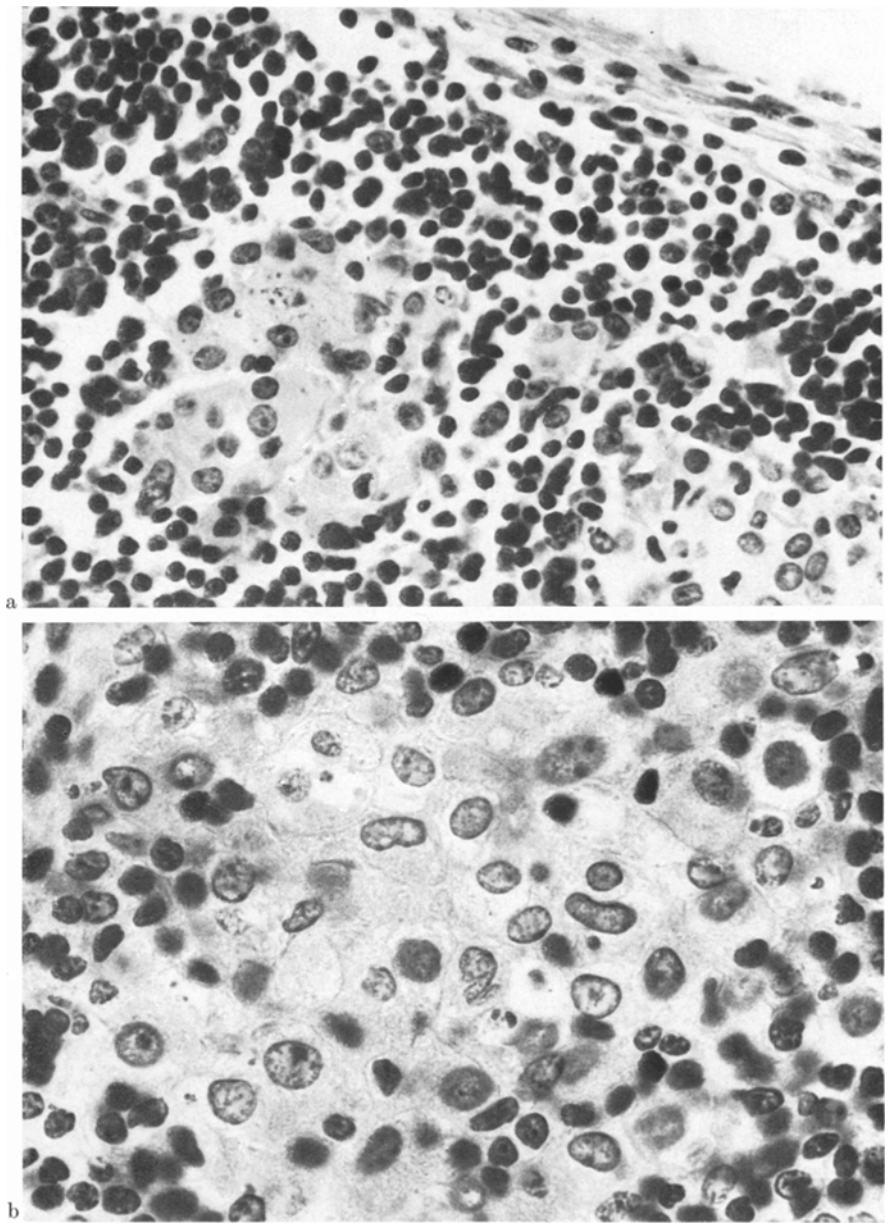

Fig. 7. a A round "micro-granuloma" in the submarginal region. Auricular lymph node 8 days after infection. H & E. × 560. b Submarginal granuloma composed of activated mononuclear phagocytes. Auricular lymph node 8 days after infection. H & E. × 880

The number of Listeria did not greatly decrease, as in the main experiment. At the center of these granulomas there was usually an area of necrosis containing many polymorphonuclear leukocytes (Fig. 8). Such abscess formation was found

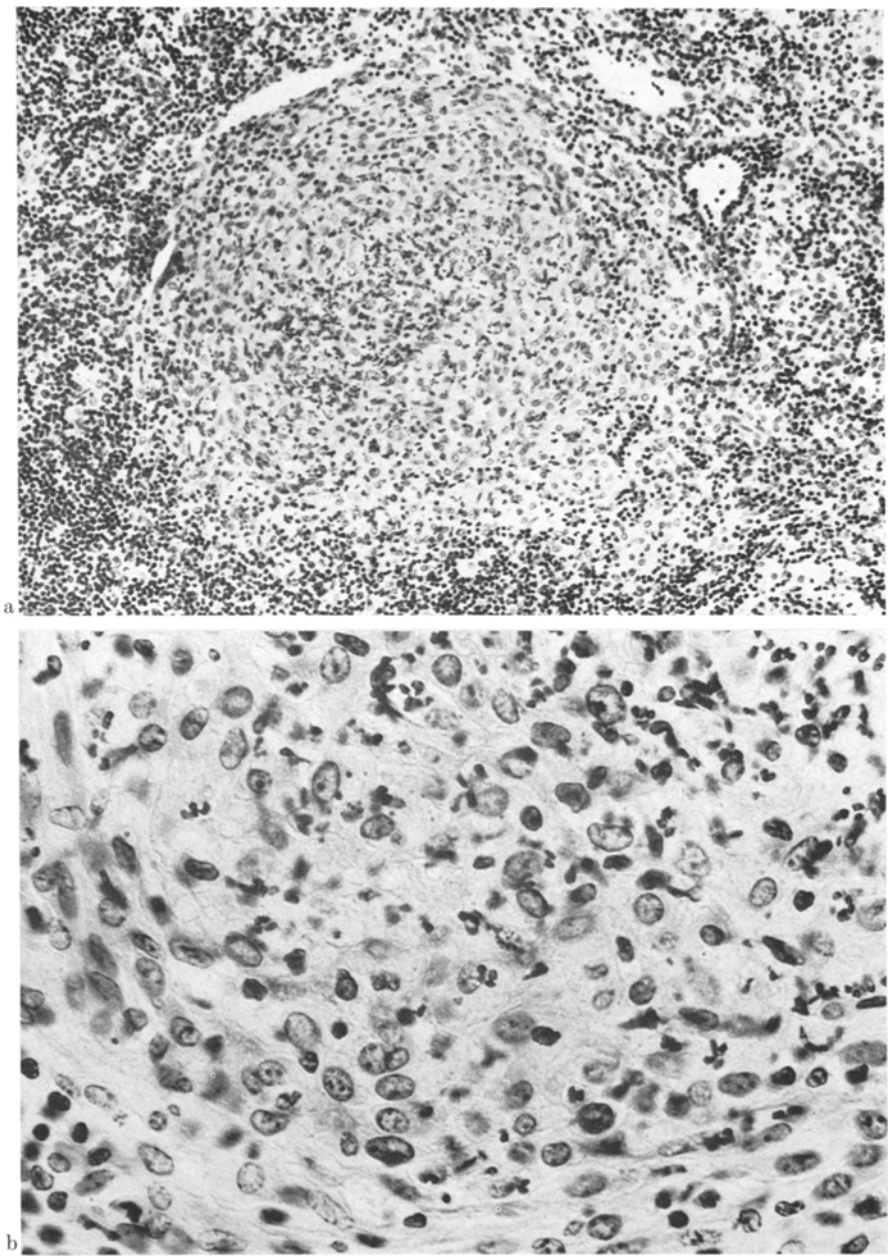

Fig. 8. a Granuloma with a central area of necrosis containing many polymorphonuclear leukocytes. Auricular lymph node 8 days after infection. H & E. × 350. b Part of the same granuloma. H & E. × 560

in only four animals with a dose of 10^7 bacteria and in one animal on the eighth day in the main experiment.

14 days after infection there was no essential change in the histological appearance of the lymph node. The sinuses contained fewer cells compared with the 8–10th days of infection. The number of "activated" lymphocytes in the cortex and paracortex had decreased even further and the granulomas had become even smaller.

Discussion

The observations described show that experimental Listeria lymphadenitis is a suitable model for studying the interaction between pathogenic bacteria and the cellular reactions in the host organism. In the regional lymph node the marginal sinus is the point of entry of L. monocytogenes from the primary focus of infection in the ear. In the first 3 hours of the experiment retothelial cells seldom contained phagocytosed bacteria. On the other hand, the submarginal reticulum cells showed signs of intensive bacterium-phagocytosing activity. Similar findings have been reported by Nopajaroonsri *et al.* (1971) for colloidal carbon phagocytosis in the lymph node. They established that the lymphatic endothelial cells phagocytose only a small amount of carbon after overloading of the macrophages. It has also been reported that subsinus phagocytes are especially strongly labeled one hour after the injection of labeled flagellin (Ada *et al.*, 1964). The role of submarginal reticulum cells in the host-parasite relationship will be discussed in a later paper on the basis of electron microscopic observations.

During the course of infections caused by intracellular parasitic bacteria there is an improvement in the ability of the host's mononuclear phagocytes not only to phagocytose but also to inactivate the bacteria after ingestion (Mackaness, 1964). Ando and Dannenberg (1972) have demonstrated that monocytes are relatively short-lived cells whose bacillary load is ingested several times by a series of new mononuclear phagocytes entering from the blood stream in an immature state. The functional capacity of mononuclear phagocytes is influenced by a variety of stimuli, both non-immunological and immunological in origin. Morphological findings on peritoneal macrophages (Blanden, 1968; North and Mackaness, 1963) have indicated that activated mononuclear phagocytes have a more extensive cytoplasm. These data coincide with our observation that most of the mononuclear phagocytes gradually increased in size until the 6–8th day of infection. The changes in the size and structure of the macrophages were especially evident in imprints. Until the appearance of cellular immunity (Mackaness, 1964) mononuclear phagocytes are thought to not have the capacity to kill bacteria multiplying in the cytoplasm. We also observed that on the eigth day the macrophages of the granulomas revealed only unidentifiable debris in the cytoplasm, whereas a large number of mononuclear phagocytes in the earlier lesions contained intracytoplasmic bacteria.

It is thought that the primary inflammatory focus is the other source besides the blood stream of mononuclear phagocytes in the lymph nodes. Gaafar and Turk (1970) have examined regional lymph nodes from guinea pigs given an intradermal injection of various antigens, including BCG vaccine. They reported that granulomas were mainly formed by mononuclear phagocytes that had come through the afferent lymphatic from primary lesions formed in the skin. We also presume that, at least during the first days of Listeria infection, most of the mononuclear phagocytes of the infiltration area in the subcapsular region immediately

underlying the incoming lymphatic had passed down the afferent lymphatics to the marginal sinus of the lymph node. This would be the major reason why they accumulated at the junction of the afferent lymphatic with the cortex. Our unpublished findings from histological examination of the primary ear lesions support this concept. We have often seen mononuclear phagocytes with intracytoplasmic Listeria in the lymphatic vessels. In the perivascular granulomas lying deep in the paracortex there were presumably fewer mononuclear phagocytes originating from the primary inflammatory focus. The high endothelial venules seem to play an important role as the source of the monocytes in the paracortical area. From the observations of Gowans and Knight (1964) and Marchesi and Gowans (1964) it is known that under physiological conditions the high endothelial venules are the site of penetration of recirculating lymphocytes into lymphoglandular tissue. There is very little known, however, about the role of the high endothelial venules under pathological conditions. Our findings suggest that at earlier stages of the infection mainly polymorphonuclear leukocytes, later chiefly monocytes come from their lumen.

L. monocytogenes situated in the cytoplasm of the host cell are very slightly toxic (Racz *et al.*, 1972; Wilder and Edberg, 1973). The drastic multiplication of the Listeria, however, finally causes the destruction of the mononuclear phagocytes. Bacteria released in this manner will be phagocytosed again by polymorphs or by other mononuclear phagocytes. It seems likely that the number of polymorphs depends mainly on the presence of bacteria. In contrast to mononuclear phagocytes, polymorphonuclear phagocytes kill phagocytosed Listeria at the beginning of the infection; toxic substances are released as a result of their Listeria-phagocytosing activity (Racz *et al.*, 1972). Polymorphs were found dispersed among other cells of the infiltration. However, the polymorphonuclear leukocytes were sometimes concentrated at the center of the granulomas. Many of these cells later died and abscesses were formed in the central part of the granulomas. These granulomas were similar to so-called "reticulocytäre abszedierende Lymphadenitis" (Lennert, 1961). In the pilot experiment with a 10^8 dose of Listeria we found such granulomas in the lymph nodes of 6 animals, with a 10^7 dose of bacteria in 4 animals, and in the main experiment in only one animal. These findings indicate the importance of bacteria in abscess formation. According to Lennert (1961), lesions resembling "reticulocytäre abszedierende Lymphadenitis" also occur in human listeriosis, especialy in cervico-granular type of Listeria lymphadenitis.

Cellular immunity is the outcome of a complex interaction between immunologically committed lymphocytes and mononuclear phagocytes. The exact course and localization of this process is not known, but there are several findings which seem to be connected with it, e.g. lymphocyte activation and paracortical distension. Conway (1938) already reported that the number of large lymphocytes (which are now called activated lymphocytes) increased significantly in the diffuse tissue of the lymph node cortex of guinea pigs 12 hours after the injection of L. monocytogenes. We noticed the same phenomenon in the interfollicular diffuse lymphatic tissue of the cortex and especially in the paracortical areas. We presume that this is fundamentally similar to lymphocyte activation produced by various mitogens in tissue cultures. If this is really the case, it is reasonable to assume that different chemical substances are released by Listeria which induce nonspecific lymphocyte activation and, at the same time, initiate the proliferation and activation of immunocompetent lymphocytes. The activated lymphocyte population probably consists in part of precursors of specifically committed

lymphocytes. It is also conceivable that the appearance of activated lymphocytes in the sinuses partly corresponds to the escape of committed cells into the efferent lymph. This agrees with the findings of McGregor *et al.* (1970). They noticed that in rats the delivery of committed cells to the thoracic duct is preceded by the appearance of blast cells in the paracortex of lymph nodes draining the sites of bacterial implantation. It has been shown (McGregor and Logie, 1973) that large lymphocytes are the principal, although probably not the exclusive, effector cells involved in transmitting immunity to L. monocytogenes, at least in the rat. We assume that besides the paracortical regions the areas immediately surrounding the granulomas are the other source of immunocompetent lymphocytes. Turk and Oort (1970) have pointed out that the crowding of immunoblasts around the granulomas in the paracortical area would indicate that the lymphocytes are in contact with antigen at the edge of the granuloma. The lymphocytes become activated in situ because they are already situated in the right milieu for proliferation to occur. We noticed that such lymphocyte activation takes place not only around but also within the granulomas, especially at early stages of the infection.

The paracortical distension noticed after the second day was analogous to that observed during the development of homograft immunity in the rabbit (Scothorne and McGregor, 1955) and in developing contact sensitivity in guinea pigs (Oort and Turk, 1965). Paracortical distension is thought to be connected with cellular immunity. The immunological significance of the appearance of germinal centers after the eighth day and of the moderate increase in the number of plasma cells in the medullary cords for Listeria immunity is not known. However, Gaafar and Turk (1970) noticed a similar phenomenon in regional lymph nodes after infection with BCG, another intracellular parasite.

Failure of the mononuclear phagocyte system to kill intracellular organisms is the major cause of granulomatous inflammation (Spector *et al.*, 1970). The results of our study are consistent with this finding. Whereas the early lesions showed a large number of bacteria and were composed of many young mononuclear phagocytes morphologically identical with blood monocytes, and while a high rate of division was observed, the granulomas at later stages of the infection consisted of activated macrophages with only cellular debris in the cytoplasm and only a low rate of division could be seen. The other differences between the first inflammatory areas and the later granulomas seen on the 6–8th day of infection were the shape and size of the lesions: the former were larger and irregular in form, the latter were round and became gradually smaller and smaller with time. Inflammatory granulomas are divided into two categories according to the rate at which the cells are replaced by recruits from the circulation or by mitotic division of the existing cells: "high or low turnover" (Spector, 1969). Ando and Dannenberg (1972) have demonstrated that mononuclear phagocytes show a lower rate of turnover, activation, and division in healing tuberculous lesions than they do in active tuberculous lesions. Fewer mononuclear phagocytes entered the healing lesions and those that did lived longer. From the morphological appearance of the lesions in our experiment, we assume that there is a shift from a moderately high to a low rate of turnover during the course of Listeria infections.

The lymph nodes play an important role in the immune response. The aim of this study was to histologically characterize the process and localization of lymphocyte- and monocyte-activation and of granuloma formation. The interaction between bacteria and the host cells at the ultrastructural level will be described in a later report.

References

Ada, G. L., Nossal, G. J. V., Austin, C. M.: Antigens in immunity. V. The ability of cells in lymphoid follicles to recognize foreignness. Aust. J. exp. Biol. med. Sci. **42**, 331–346 (1964)

Ando, M., Dannenberg, A. M., Jr.: Macrophage accumulation, division maturation, and digestive and microbicidal capacities in tuberculous lesions. IV. Macrophage turnover, lysosomal enzymes, and division in healing lesions. Lab. Invest. **27**, 466–472 (1972)

Blanden, R. V.: Modification of macrophage function. J. reticuloendoth. Soc. **5**, 179–202 (1968)

Conway, E. A.: Reaction of lymphatic tissue in early stages of Bacterium monocytogenes infection. Arch. Path. **25**, 200–227 (1938)

Gaafar, S. M., Turk, J. L.: Granuloma formation in lymph-nodes. J. Path. **100**, 9–20 (1970)

Gowans, J. L., Knight, E. J.: The route of re-circulation of lymphocytes in the rat. Proc. roy. Soc. B **159**, 257–282 (1964)

Herman, P. G., Yamamoto, I., Melius, H. Z.: Blood microcirculation in the lymph node during the primary immune response. J. exp. Med. **136**, 697–714 (1972)

Lennert, K.: Handbuch der speziellen pathologischen Anatomie und Histologie. Lymphknotendiagnostik in Schnitt und Ausstrich. Cytologie und Lymphadenitis, Bd. I/3. Berlin-Göttingen-Heidelberg: Springer 1961

Mackaness, G. B.: The immunological basis of aquired cellular resistance. J. exp. Med. **120**, 105–120 (1964)

Mackaness, G. B.: Cellular immunity. Ann. Inst. Pasteur **120**, 428–437 (1971)

Marchesi, V. T., Gowans, E. J.: The migration of lymphocytes through the endothelium of venules in lymph nodes. An electron microscopic study. Proc. roy. Soc. B **159**, 283–290 (1964)

McGregor, D. D., Koster, F. T., Mackaness, G. B.: The short lived small lymphocytes as a mediator of cellular immunity. Nature (Lond.) **228**, 855–856 (1970)

McGregor, D. D., Logie, P. S.: The mediator of cellular immunity. VI. Effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection. J. exp. Med. **137**, 660–674 (1973)

Mori, Y., Lennert, K.: Electron microscopic atlas of lymph node cytology and pathology. Berlin-Heidelberg-New York: Springer 1969

Nopajaroonsri, C., Shing, C. L., Simon, G. T.: Ultrastructure of normal lymph node. Amer. J. Path. **65**, 1–12 (1971)

Nopajaroonsri, C., Simon, G. T.: Phagocytosis of colloidal carbon in a lymph node. Amer. J. Path. **65**, 25–42 (1971)

North, R. J., Mackaness, G. B.: Electron microscopical observations on the peritoneal macrophages of normal mice and mice immunized with Listeria monocytogenes. II. Structure of macrophages from immune mice and early cytoplasmic response to the presence of ingested bacteria. Brit. J. exp. Path. **44**, 608–611 (1963)

Oort, J., Turk, J. L.: A histological and autoradiographic study of lymph nodes during the development of contact sensitivity in the guinea pig. Brit. J. exp. Path. **46**, 147–154 (1965)

Racz, P., Tenner, K., Merö, E.: Experimental Listeria enteritis. I. An electron microscopic study of the epithelial phase in experimental Listeria infection. Lab. Invest. **26**, 694–700 (1972)

Richardson, K. C., Jarett, L., Finke, E. H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. **35**, 313–323 (1960)

Scothorne, R. J., McGregor, I. A.: Cellular changes in lymph nodes and spleen following skin homografting in the rabbit. J. Anat. (Lond.) **89**, 283–292 (1955)

Spector, W. G.: The granulomatous inflammatory exudate. Int. Rev. exp. Path. 8, 1–55 (1969)

Spector, W. G., Reichhold, N., Ryan, G. B.: Degradation of granuloma-inducing micro-organisms by macrophages. J. Path. **101**, 339–354 (1970)

Turk, J. L., Oort, J.: The production of sensitized cells in cell-mediated immunity. In: Handbuch der allgemeinen Pathologie, Bd. VII/3, S. 392–431. Hrsg.: H.-W. Altmann, F. Büchner, H. Cottier, E. Grundmann, G. Holle, E. Letterer, W. Mashoff, H. Meessen, F. Roulet, G. Seifert, G. Siebert, A. Studer. Berlin-Heidelberg-New York: Springer 1970

Wilder, M. S., Edberg, J. C.: Interaction of virulent and avirulent Listeria monocytogenes with cultured mouse peritoneal macrophages. Infection and Immunity **7**, 409–415 (1973)

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