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Cellular changes in the lymphoreticular tissues of C57L/J mice infected with *Echinococcus multilocularis* cysts

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Summary. The pathology of the spleen, lymph nodes and thymus of C57L/J mice, infected intraperitoneally with 20 and 100 cysts of *Echinococcus multilocularis* is described at 2, 4, 8 and 12 weeks postinfection (p.i.). For the first 8 weeks, growth of the larval cyst mass (LCM) was slow and blastogenesis in T-dependent areas of both spleen and lymph nodes was moderate whereas in B-cell compartments it was intense. A rapid growth of the LCM between 8 and 12 weeks p.i., 15–20 times greater than for the first 8 weeks, was associated with depletion of lymphocytes in thymus dependent area (TDA) of both spleen and lymph nodes, gross expansion of the red pulp with extramedullary haemopoiesis, partial atrophy of spleen follicles, but not those of the lymph node, and involution of the thymus. At 12 weeks p.i. the TDA had mainly plasma cells and histiocytes among occasional lymphocytes and blast cells; germinal centre activity and plasmacytosis persisted in B-cell areas. Morphological aspects of these changes are discussed in relation to the invasiveness and proliferation of the LCM during the course of infection.

INTRODUCTION

It has been shown previously that C57L/J mice are hypersusceptible to infection with *Echinococcus multilocularis* cysts (Ali-Khan, 1974a). The invasion of the host tissues by the proliferating cyst mass eventually overcomes the host. Infected mice show a progressive increase in antibody response, lymphopenia, neutrophilia, a generalized hypertrophy of the lymphoid tissues and atrophy of the thymus (Ali-Khan, 1974b).

The reasons for the lack of resistance and control in the regulation of cyst proliferation in these mice are not known. Possibly the large growing mass of larval echinococcus and the prolonged release of potentially antigenic metabolites may alter the distribution of immunocompetent cells or their properties. In a protracted infection one or both of these factors might severely reduce the immunological competence of the infected mice (Ptak, Gaugas, Ress & Allison, 1970; Turk & Waters, 1971). Alternatively, target areas for the cytotoxic action of the sensitized lymphocytes on the surface of the proliferating cysts may be masked by blocking antibodies, a phenomenon analogous to that seen in solid tumours (Feldman, 1972). The laminated layer of both subcutaneous and intraperitoneal cysts of *E. multilocularis* from mice has been found to contain various host immunoglobulins (Ali-Khan in preparation).

We have initiated a series of experiments to investigate the immunological status of C57L/J mice during intraperitoneal proliferation of alveolar hydatid cysts. In this communication, the morphological response of the lymphoreticular tissues, especially the changes in the TDA and B-cell

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compartments is described in mice infected with low and high doses of *E. multilocularis* cysts over a period of 12 weeks.

MATERIALS AND METHODS

The parasite

Echinococcus multilocularis sibiricensis (Alaska strain) originally obtained from Dr R. Rausch, Arctic Health Research Center, Anchorage, Alaska, and established in rodents by Dr G. Lubinsky (Lubinsky, 1960), was used in this study. A laboratory reared cotton rat infected with this strain of parasite was acquired in 1970 from the Institute of Parasitology, MacDonald College, of McGill University, P.Q., Canada. The parasite has since been maintained in our laboratory by intraperitoneal (i.p.) transfer of 20–30 isolated cysts (size, under 2 mm diameter), 3 months postinfection (p.i.) in C57L/J mice (Ali-Khan, 1974a). Brood capsules with protoscolices have rarely been seen and then only in a few 5–6 months post-infected C57L/J mice, which have survived this infection. The source of infection in both stock and experimental mice was cysts from 3 months p.i. mice.

Inocula and tissue samples

Forty-one inbred C57L/J male mice purchased from Jackson Laboratories, Bar Harbour, Maine, U.S.A., were divided into three groups. All mice were 10–12 weeks old. Two groups of sixteen mice were injected i.p. with either 20–25 (low dose) or 100–110 (high dose) cysts of *E. multilocularis*, and nine control mice were injected with Medium 199 used for preparing the inoculum (Ali-Khan, 1974a). At 2, 4, 8 and 12 weeks p.i., four mice from each experimental group were killed, weights of spleen and larval cyst mass (LCM) determined and immediately either the whole or one half of the spleen, lymph nodes (mesenteric, mediastinal, and axillary) and thymus fixed in Carnoy's fixative. Three control mice were killed at each of the above time intervals, except at 4 weeks, and their tissues were fixed similarly. Also, two i.p. (i.p. LCM) and two subcutaneous (s.c. LCM) (from the site of inoculation) larval cyst masses, for the 20 cysts group only, were sliced in half and fixed in 10% formalin.

Histology

Tissues from each mouse were embedded in one paraffin block, sectioned at 4 μm and stained with haematoxylin and eosin (H & E), Giemsa, methyl green pyronin (MGP) and by the reticular staining technique of Gordon & Sweet (Culling, 1963). Five sections stained with H & E and three sections with each of the other stains, from both superficial and deeper areas of each tissue were examined. Sections from the LCM were stained with H & E and haematoxylin-pholoxine-Saffron stains.

All follicles (both complete or cut at an angle) in an area between 30·4 and 31·4 mm^2 (150–155 eye piece disc squares, each 0·2025 mm^2) from each H & E spleen section were counted under a 3·5× objective. On the same slides, 20–35 randomly chosen bipolar follicles (Congdon, 1962) were measured along their longitudinal and transverse axes with an eye piece micrometer disc under ×10 objective. The sizes of follicles in Fig. 1 represent the mean of the two axes. Branched follicles or sections cut at an angle to the axis were excluded from measurement.

Thymus dependent and thymus independent areas

The specific localization of lymphoid cells, differentiated on the basis of surface antigens and functional activity as T and B cells have been well documented for various compartments of the peripheral lymphoid tissue of mice and other mammals (Parrott & de Sousa, 1971).

RESULTS

LCM and spleen weights

Table 1 shows the mean LCM and spleen weights in the experimental and control groups of mice at given time intervals. Mice in both the 20 and 100 cyst groups were positive for cysts at 2 weeks p.i., but their cyst weight could not be determined because the cysts were 1–2 mm in diameter and scattered in the abdominal cavity. Infected mice killed subsequently contained variable LCM growing mainly in the abdominal cavity attached to the omentum, the surface and parenchyma of the liver, and to the underside of the diaphragm, but often also as small subcutaneous masses at the site of inoculation. Weights given in the table include LCM of both origins. Mean LCM and spleen weight inincreased with the progression of infection. At 12 weeks p.i. the weight of cyst isolated from the 100 cyst group was more than double that isolated

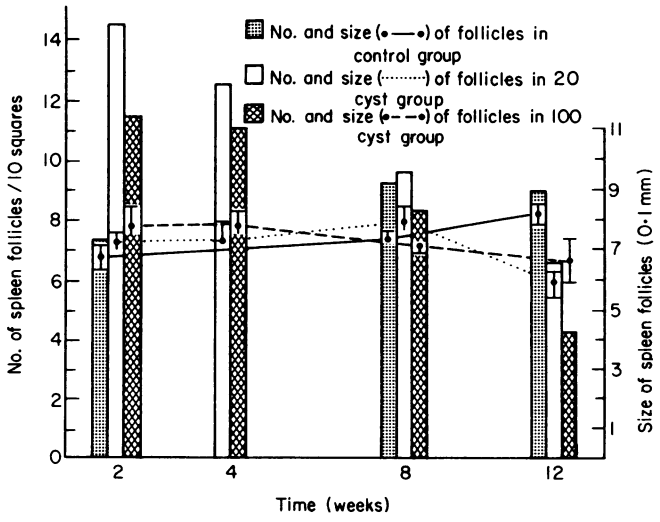


Figure 1. Dose–response relationship between the number of spleen follicles per ten squares (area of one square = 0·2025 mm²) and size in control and two groups of C57L/J mice, infected with 20 or 100 cysts respectively, of *Echinococcus multilocularis*, at 2, 4, 8 and 12 weeks postinfection (p.i.).

Table 1. Mean and (range) of spleen and cyst weights of thirty-two C57L/J male mice (four mice at each time interval) infected intraperitoneally with 20 or 100 cysts of *Echinococcus multilocularis*, at 2, 4, 8 and 12 weeks postinfection and mean and range of spleen weights of nine control mice (three at each time interval)

Weeks postinfection	Control	20 Cyst group		100 Cyst group	
	Spleen (mg)	Spleen (mg)	Cyst (g)	Spleen (mg)	Cyst (g)
2	77 (72–82)	89 (88–98)	+	97 (91–113)	+
4	ND	110 (79–139)	0·11 (0·05–0·21)	141 (107–194)	0·22 (0·05–0·46)
8	92 (76–107)	196 (204–263)	0·6 (0·35–0·83)	225 (136–284)	1·07 (1·04–1·45)
12	96 (93–101)	507 (350–630)	4·5 (1·8–8·2)	387 (327–408)	10·4 (8·9–12·3)

ND, not done. + = positive for *E. multilocularis* cysts.

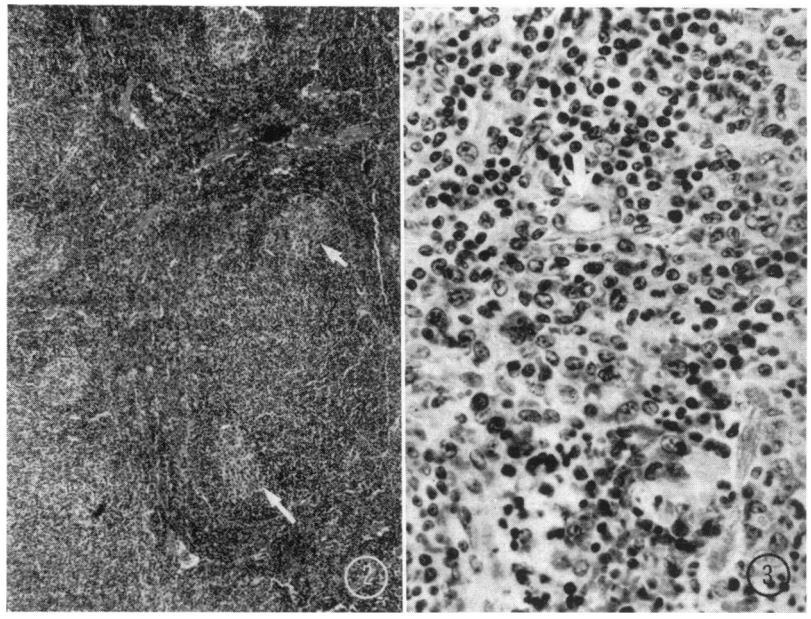
from the 20 cyst group of mice. In contrast, the splenomegaly was more apparent in the 20 cyst group. The rate of LCM growth/week between 8 and 12 weeks p.i. was 15–20 times greater than for the first 8 weeks of infection in these two groups of mice.

Size and number of spleen follicles

Fig. 1 shows the follicular response of infected mice at 2, 4, 8 and 12 weeks p.i. versus the controls at the corresponding periods. The size and number of follicles/unit area increased initially (up to 4 weeks p.i.) in both the 20 and 100 cyst groups of mice, but was greater in the former. Thereafter, both parameters decreased. The number of follicles/unit area with 100 cyst group at 12 weeks p.i. was less than half that of the controls. The difference in the size of the follicles between the control and the two infected groups of mice was not significant ($P > 0·05$).

Spleen

The 20 cyst group. At 2 weeks p.i. most follicles contained germinal centres. The lymphocytes in the peri-arterial area (PAA) appeared unchanged. At 4 and 8 weeks p.i., almost all follicles contained at



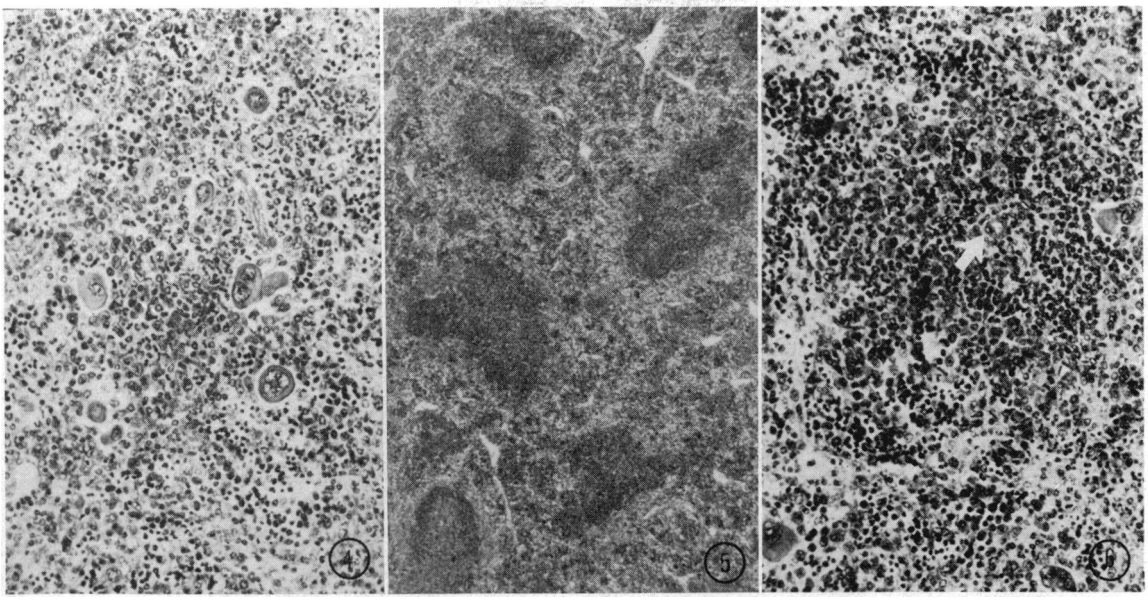
Figures 2 and 3. Sections of spleens from the 20 cyst group of mice. Fig. 2: 4 weeks p.i.; one follicle with two germinal centres (arrows); note germinal centres of the neighbouring follicles. H & E. ×45. Fig. 3: 12 weeks p.i.; central artery (arrow) and periarterial area depleted of lymphocytes; note immature erythrocytes, neutrophils, megakaryocytes and plasma cells. H & E. ×280.

least one and often two germinal centres (Fig. 2). Small lymphocytes tightly packed the PAA in most follicles, while in others, cells were sparse with occasional blast cells. The red pulp had expanded considerably and contained foci of erythroblasts, scattered histiocytes and a few megakaryocytes. The spleen from 12 weeks p.i. mice appeared highly erythropoietic and myelopoietic with considerable reduction of lymphocytes in the sinuses of the expanded red pulp. A few of the sinuses were empty. Megakaryocytes were eight to ten per low power field. Approximately 80% of the follicles were intact. The remaining follicles were distorted and fused with each other and their PAA was infiltrated with plasma cells, histiocytes and myeloblasts (Fig. 3). The reticular pattern of both the follicles and red pulp was disrupted.

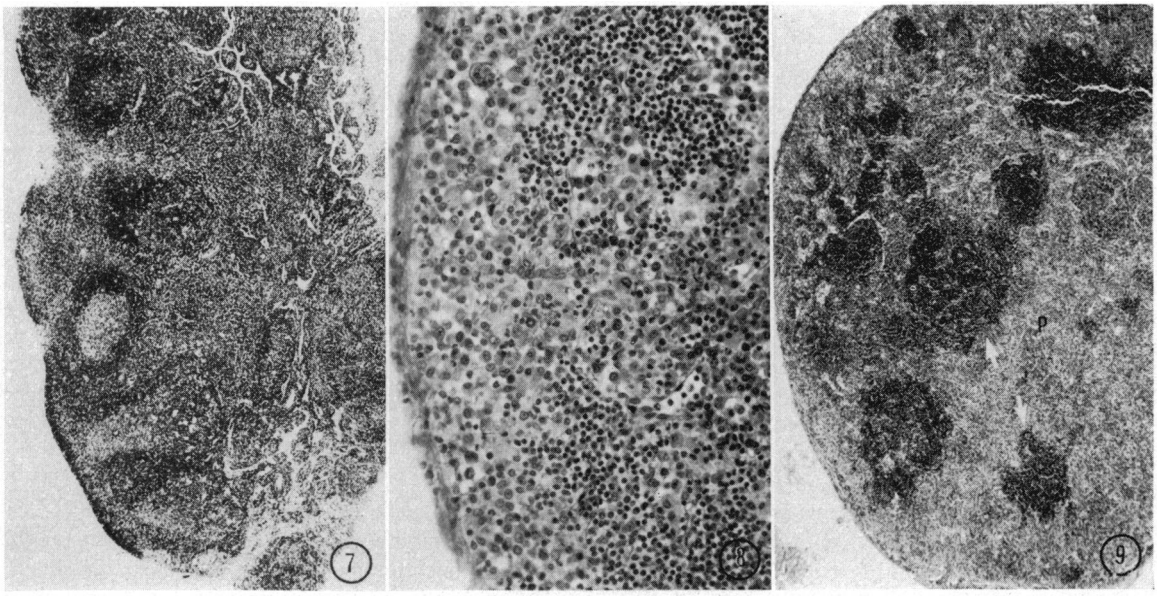
The 100 *cyst group*. Spleens of mice 2, 4, and 8 weeks p.i. were essentially similar to those of the 20 cyst group. At 12 weeks p.i. the red pulp was grossly myeloid and expanded (Fig. 4); an increased number of sinuses were empty. The follicles, relatively fewer in number (Fig. 1), were distorted, but still contained an active germinal centre (Fig. 5). Others were either partially lymphoid (Fig. 6) or completely non-lymphoid, recognizable only by the outline of the marginal sinuses enclosing a branch of the central artery. The PAA of almost all the follicles was altered; a few contained lymphocytes but most only plasma and non-lymphoid cells.

Lymph node

The 20 *cyst group*. No histological changes were observed in the lymph nodes (mesenteric, mediastinal, and axillary) of the 2 weeks p.i. mice. At 4 weeks p.i., the nodes were hyperplastic. The follicles had increased both in size and number and a few with an enlarged germinal centre had extended into the paracortical area (PCA) which contained occasional blast cells. At 8 weeks p.i. follicular response and blastogenesis in the PCA was increased (Fig. 7). Both plasma cells and lymphoblasts were present in the majority of medullary cords. All lymph nodes examined were enlarged and their fibrous capsule thickened at 12 weeks p.i. The subcapsular sinuses were packed with histiocytes (Fig.



Figures 4–6. Sections of spleens from the 100 cyst group of mice; 12 weeks p.i. Fig. 4: Note the myeloid nature of the expanded red pulp; mostly immature neutrophils, erythrocytes and a few megakaryocytes in the field. H & E. ×110. Fig. 5: Note the distorted follicles with germinal centres, expanded red pulp and empty sinuses. H & E. ×16. Fig. 6: A partially lymphoid follicle showing a central artery (arrow) and marginal sinuses. Note immature neutrophils and erythrocytes, plasma cells and megakaryocytes in the follicle. H & E. ×110.



Figures 7–9. Sections of lymph nodes from the 20 cyst group of mice. Fig. 7: 8 weeks p.i.; axillary lymph node showing follicles with pale germinal centres extending into the paracortex, H & E. ×16. Fig. 8: 12 weeks p.i.; mediastinal lymph node showing subcapsular sinuses packed with histiocytes, H & E. ×160. Fig. 9: 12 weeks p.i.; mediastinal lymph node showing primary and secondary follicles (large arrows); note the paracortical area (P) practically devoid of lymphocytes except for isolated patches (small arrows). H & E. ×16.

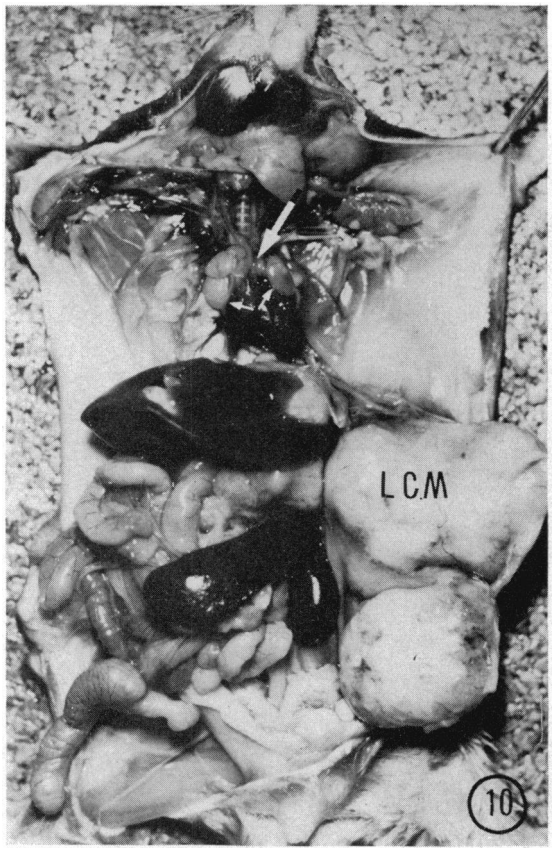


Figure 10. A 12 weeks p.i. mouse infected with 100 cysts. Note two intraperitoneal larval cyst masses (LCM), enlarged spleen and mediastinal lymph nodes (small arrows) and thymus (large arrow) barely visible.

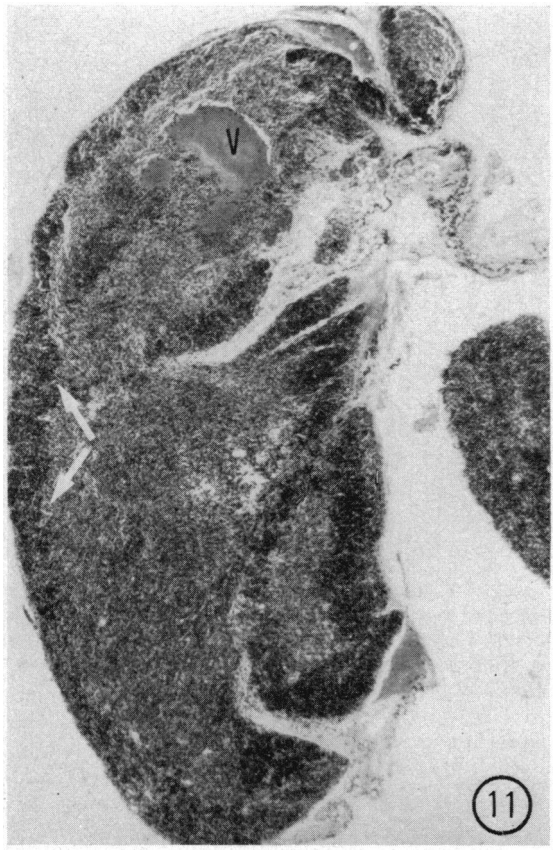


Figure 11. Section of thymus from a 20 cyst group mouse. 12 weeks p.i. Note vacuoles (V) filled with eosinophilic material and cortical thymocytes present as a narrow rim (arrows); cortico-medullary junction indistinct. H & E. ×23.

8) but the follicular response was unchanged. Mesenteric lymph nodes showed a slightly hypocellular, but intact, PCA and a moderate population of lymphoblasts. The PCA in axillary and mediastinal nodes was essentially devoid of lymphocytes except in isolated patches containing a few blast cells (Fig. 9). Plasma cells, and to a lesser degree histiocytes, occupied the PCA and the medullary cords. The post-capillary venule (PCV) appeared normal only in areas of the PCA retaining the lymphocytes, otherwise the endothelial cells lining the PCV appeared flat.

The 100 *cyst group.* The sequence of histological alterations in the lymph nodes (mesenteric, mediastinal, and axillary) of mice at 2, 4, 8 and 12 weeks p.i. corresponded with similar periods described in the 20 cyst group of mice in all essential features except that histiocytosis and neutrophilia were more marked at both 8 and 12 weeks p.i.

Thymus

No discernible changes occurred in the thymus of control mice for the duration of the experiment. In infected mice the thymus progressively decreased in size between 8 and 12 weeks. At 12 weeks the gland was either partially involuted or barely visible (Fig. 10). As a result, the enlarged bilateral mediastinal lymph nodes dominated the thymic area. Two

features, the relative thickness of cortex to medulla and the mitotic activity of the cortical thymocytes, will be described to determine the degenerative changes in the thymus of mice at 8 and 12 weeks p.i.

The 20 *cyst group.* At 8 weeks p.i. the combined thickness of the cortex, determined in a transverse plane on either side of the longitudinal axis of the central medulla, was roughly equal to the medulla. The mean number of mitotic cells, appraised per oil immersion field (× 100 objective) from a total count of such cells in twenty-five fields was 8·5. Cortical cysts (not *E. multilocularis* cysts) were present in two of the four thymuses. They contained eosinophilic material and were lined with ciliated epithelium and foamy cells. Empty vacuoles and histiocytes, the latter with phagocytosed thymocytes, were occasionally seen in the medulla. At 12 weeks p.i. the thymus was approximately half the size of that in the normal mice, and the cortex was moderately to severely depleted of thymocytes (Fig. 11). The cortico-medullary junction was indistinct. Mitotic cells were significantly reduced in number (two cells per field), but pyrinophilic lymphoblast-like cells were still present in the thymic cortex.

The 100 *cyst group.* The thymus was involuted in all mice examined at 8 weeks p.i. After 12 weeks p.i., it was absent from one and barely discernible in the other three mice (Fig. 10). Pathological changes in the thymus were similar to those described earlier.

Larval cyst mass

Both the i.p. LCM and s.c. LCM had peripherally a solid multiple epithelioid cell layer mixed with fibrous tissue with diffusely scattered neutrophils, arterial and venous capillaries and mainly intact cysts. The central portion of the s.c. LCM was liquified. It contained degenerated inflammatory cells and cysts while the peripheral area showed intact cells (Fig. 12). Among them, neutrophils were in a majority; histiocytes, monocytes and other unidentified mononuclear cells were in moderate numbers. A few giant cells and lymphocytes were also present. In the i.p. LCM the central core consisted of fibrous tissue mixed with structureless eosinophilic strands enclosing round spaces representing probably empty shells of degenerated cysts. Neutrophils and lymphocytes present in the interstices were fewer than in the s.c. LCM. Intact cysts were invariably circumscribed by epithelioid cells only or mixed with intact and degenerated neutrophils.

DISCUSSION

We have described pronounced changes in nearby and distantly located central and peripheral lymphoid tissues of mice during the proliferation of i.p. LCM. In both the spleen and lymph nodes, the B-cell compartment remained relatively intact while the T-cell compartment was depleted of lymphocytes.

Splenomegaly in response to parasitic and bacterial infections, and passive immunization has been demonstrated in various experimental models. Hyperplasia of the recticular cells and follicles occurs. Reticular cells sequester the parasite, parasitized and unparasitized anti-erythrocyte antibody coated or haemolysed erythrocytes (Taliaferro, 1965; Woodruff, 1973; Jenkin & Rowley, 1973) and possibly immune complexes (Lambert & Houba, 1974). Follicles generate antibody synthesizing cells (Congdon & Makinodan, 1961; Hanna, 1965; Davies, Carter, Leuchars, Wallis & Dietrich, 1970). Throughout the examination period, follicular and germinal centre hyperplasia followed by plasmacytosis in the lymph nodes, and persistence of active germinal centres in both the intact and partially altered follicles of the spleen of our mice were suggestive of humoral activity (Figs 2, 5, 7 and 9). Also an increasing antibody titre has been shown previously (Ali-Khan, 1974a). The spleen follicles, however, after an initial proliferative activity (up to 4 weeks p.i.), decreased in number between 8 and 12 weeks p.i. (Fig 1) and the follicular pattern was disrupted. The apparent reduction in number might result from both atrophy of follicles (Fig. 6) and gross expansion of the red pulp (Figs. 4 and 5), the latter reducing the number of follicles per unit area. However, the major factors (megakaryocytosis, erythropoiesis and myelopoiesis in the red pulp) contributing to splenomegaly, between 8 and 12 weeks p.i. (Fig. 4), were indicative of extramedullary myelopoiesis. This compensatory mechanism apparently results from pathologic injury to host tissues (Rausch, 1954; Mankau, 1956), and a massive cellular infiltration of the cyst (Fig. 12).

Blastogenesis in the TDA, one of the parameters

of cellular immunity, occurs in animals infected with parasites (Preston, Carter, Leuchars, Davies & Dumonde, 1972; Rogers, Denham, Nelson, Guy & Ponnudurai, 1975) and bacteria (North, Mackaness & Elliot, 1972; Ptak *et al.*, 1970) and in animals grafted with allogeneic skin (Parrott, 1967). It was moderate in our mice up to 8 weeks p.i. At 12 weeks only occasional lymphoblasts were seen among the plasma and non-lymphoid cells which had repopulated the PAA and PCA. The progressive disorganization of the lymphoid tissues of our mice coincided with the growth pattern of the LCM/week (Table 1), which was 15–20 times greater between 8 and 12 weeks p.i., compared with the first 8 weeks. Possibly prolonged and rapid cystic proliferation produces an effect of this type on the lymphoid tissues, either by sustained antigenic stimulation or by causing granulomatous and necrotic changes in the host tissues (Figs 10 and 12; also Ali-Khan, 1974b; Mankau, 1956; Rausch, 1954). Rogers *et al.* (1975) have related morphologically nonfunctional lymph nodes in cats with clearance of microfilariae from the peripheral circulation while Murray *et al.* (1974) have correlated immunosuppression with intense nonspecific stimulation of B cells in the hyperplastic lymphoid tissues of *Trypanosoma brucei* infected mice. In other studies disorganization and functional inactivation of lymphoid tissues in animals have been related to excessively virulent organisms, dose of antigen (Olson, Hunt & Verrier-Jones, 1971; Mackaness, Auclair & Lagrange, 1973) and chronic infection (Ptak *et al.*, 1970; Turk & Waters, 1971).

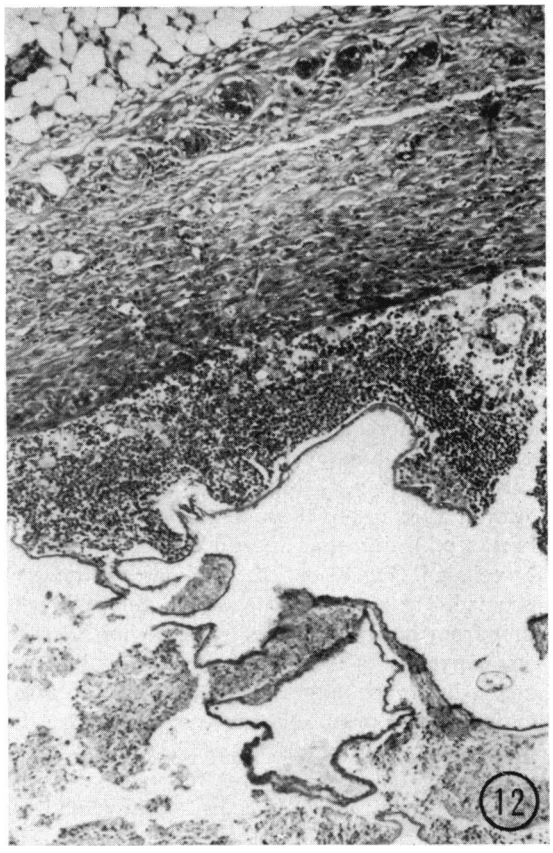


Figure 12. Section of a 12-week-old subcutaneous larval cyst mass; periphery shows fibro-histiocytic reaction and blood capillaries; central area contains free cysts surrounded by inflammatory cells, H & E. × 55.

Regression of the thymic cortex and involution of the thymus (Figs 10 and 11) and depletion of lymphocytes from the TDA (Figs 3 and 9) coincided with rapid proliferation of the LCM. Neither the underlying mechanism nor the relationship between these events is clear.

The effect of depletion of T cells by infection on the immunological status of the host needs to be defined in order to understand more fully the alveolar cyst–host relationship in this model. On the basis of the histological appearance of the lymphoid tissues and their known functional relationship to cellular immunity, a parallelism can be drawn between this experimental model and others where T cells have been depleted either due to protracted infection (Ptak *et al.*, 1970; Turk & Waters, 1971) or experimental manipulations (Preston *et al.*, 1972). In subsequent papers we will describe the response of infected mice to heterologous antigen challenge, and define their population of T and B lymphocytes and the sequence of effector cell infiltration at the site of cyst growth.

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