Immunoproliferative Effects of Lymphocytic Choriomeningitis Virus in Germfree Mice[1] (34188)

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Lymphocytic choriomeningitis (LCM) virus causes a unique pattern of disease in mice, in which its effects are attuned to the immunological status of the host. It does not induce visible disease in the immunologically-immature or depressed mouse (1–4); however, the disease which it induces in the mature mouse has been interpreted as an immunological reaction pattern in sensitive organs such as the central nervous system. Persistent LCM virus infections in mice result from congenital passage of virus to progeny from carrier mothers, and from virus inoculation into newborn mice (1, 2, 5). As carrier mice grow older, they develop changes in the lymphoreticular system which reflect hyperactivity of the immunological mechanisms (6, 7): (a) the lymph nodes and spleens enlarge and they contain large germinal zones and numerous plasma cells, (b) the visceral organs become increasingly infiltrated with globulin containing lymphoid and plasma cells in the perivascular areas (c) serum globulin levels (IgG and IgM) rise far above normal levels, and (d) the kidneys become damaged by occlusion of glomerular vessels with fibrinoid material which has been identified as antigen–antibody complexes (8). Two additional tissue changes have been observed in congenitally-infected germfree mice which require clarification, as they relate to immunological functions: the thymus glands become depleted of cortical cells, large cysts appear in the medullary areas, and the swollen Peyer's patches do not contain germinal zones. For a study such as this, the tissues in germfree mice provide a more uniform base line of activity through absence of irrelevant antigenic and infectious agents in the environment. Therefore, changes induced in them by agents, such as LCM virus, have a more distinct causal relationship.

Germfree mice with congenital LCM virus infection manifest continued distortions of their immunological mechanisms, which eventually assume, in significant numbers of them, characteristics of neoplastic disease. The present report describes some of the age-related expanding lesions in the tissues of germfree mice with congenital LCM virus infection.

Methods. A Swiss mouse strain with congenitally-acquired persistent LCM virus infection was provided by Dr. Wallace P. Rowe of Bethesda, Maryland. The colony was initiated with Swiss-Webster mice in 1940 by Haas (9). Baby mice of this strain (designated LCM) were derived from mothers by cesarean section directly into germfree isolators where they were foster nursed by germfree C3H/f mice. The weaned LCM mice have now been propagated through five generations under germfree conditions. They produce large and frequent litters of symptom free mice, all of which carry LCM and leukemia viruses(7). However, they are free of detectable macroparasites, bacteria, and Mycoplasma (10). For convenience, they are here referred to as LCM mice, with the understanding that they carry leukemia virus as well as LCM virus.

Since all LCM mice carry congenitally-acquired LCM virus, it was not possible to provide virus-free control mice of the same strain. Young LCM mice do provide a lesion-free base line of activity. In addition, we have included examinations of germfree Lobund Swiss-Webster (SW) and CFW mice which are disease-free carriers of leukemia

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virus (11), but free of LCM virus. All of the mice were maintained under germfree conditions for the duration of the experiments.

Germfree LCM mice, at specified age levels, were selected for examinations, as follows: each mouse was anesthetized, blood was collected from the heart, and tissue specimens were fixed in Bouin's solution and processed for histological examinations. The tissue sections were stained with hematoxylin and eosin. Serum specimens were examined for proteins by the acrylamide gel electrophoresis technique, and for LCM virus antibodies by the microtiter complement fixation test. Tissue imprints were prepared on coverslips from spleens, lymph nodes, and thymuses. They were fixed in cold acetone, stained with fluorescein tagged antimouse serum globulin from goats, and examined by ultraviolet fluorescence microscopy.

Groups of weanling germfree SW mice were inoculated intraperitoneally with 1000 mouse intracerebral LD_{50} of Haas strain LCM virus. At intervals during the next 8 weeks, serum specimens were collected from groups of them and examined for complement fixing antibodies; and their tissues were processed for histological examinations. Tissues and serums from uninoculated mice served as control specimens.

Results. Among 130 LCM mice up to 12 months of age, none showed symptoms of disease; however, they were all carriers of LCM virus and serologically negative for LCM complement fixing antibodies. Up to age 4 months there were few changes detected in the tissues of 32 LCM mice which could be interpreted as lesions: the enlarged lymph nodes and spleens carried prominent germinal zones, and occasional small areas of perivascular accumulations of lymphocytes were noted in the kidneys and pancreases. Small segments of the thymus were depleted of cortical lymphoid cells. The tissue changes were significant because they were not observed in the same tissues of LCM-free germfree Lobund SW and CFW mice.

In 98 LCM mice, aged 4 months and older, progressive age-related tissue changes were observed in the visceral organs, as well as in the thymuses, lymph nodes, and spleens. The latter two organs continued to enlarge and they contained increasing numbers of plasma cells and expanding germinal zones. The livers were increasingly swollen and friable; the kidneys were swollen, discolored, and distorted; and the lungs contained small white solid focal lesions. The thymus glands were very small and the Peyer's patches were swollen and smooth. The pancreases, kidneys, livers, and lungs contained extensive perivascular accumulations of lymphocytes and small plasma cells, among which were numerous mitotic figures. The parenchymatous cells were displaced by the expanding masses of lymphoid cells (Fig. 1). The glomeruli were hypercellular and, with increasing age, appeared relatively acellular and attached to the capsules. Large hyaline masses appeared in the glomeruli, which by electron microscopy, were actually accumulations of fibrinoid material in the capillaries (7). Significant numbers of glomeruli became hyalinized and fibrotic. The thymus glands were largely depleted of cortical cells; and the medullary zones were swollen with numerous lymphoid cells and many large multilocular cysts (Fig. 2). The cysts were partially lined by cuboidal cells and contained lymphoid cells with prominent cytoplasm and some epithelioid cells; however, some of the cysts were empty. Clearly defined germinal zones were not observed in the thymus glands. Small medullary cysts were observed in the thymus glands of control old germfree CFW and SW mice; and the zones of cortical cells were thin but intact.

Lymphoma-like changes were observed in 28 of 54 LCM mice over 8 months of age. The thymus glands and lymph nodes were swollen and pale, and livers were soft, swollen, and friable. Variable amounts of clear yellow fluid were found in the peritoneal cavities. Remnants of the cortical cell layers were detected in the thymus glands, but most of the glands consisted of swollen medullary tissues with lymphoid and plasma cells. In the lymph nodes and spleens, the follicles were compressed by masses of large lymphoid and plasma cells, and large accumulations of similar cells were observed in the livers, kidneys and lungs. Many of the plasma cells carried large masses of cytoplasm with globoid Russell bodies (Figs. 3 and 4). The

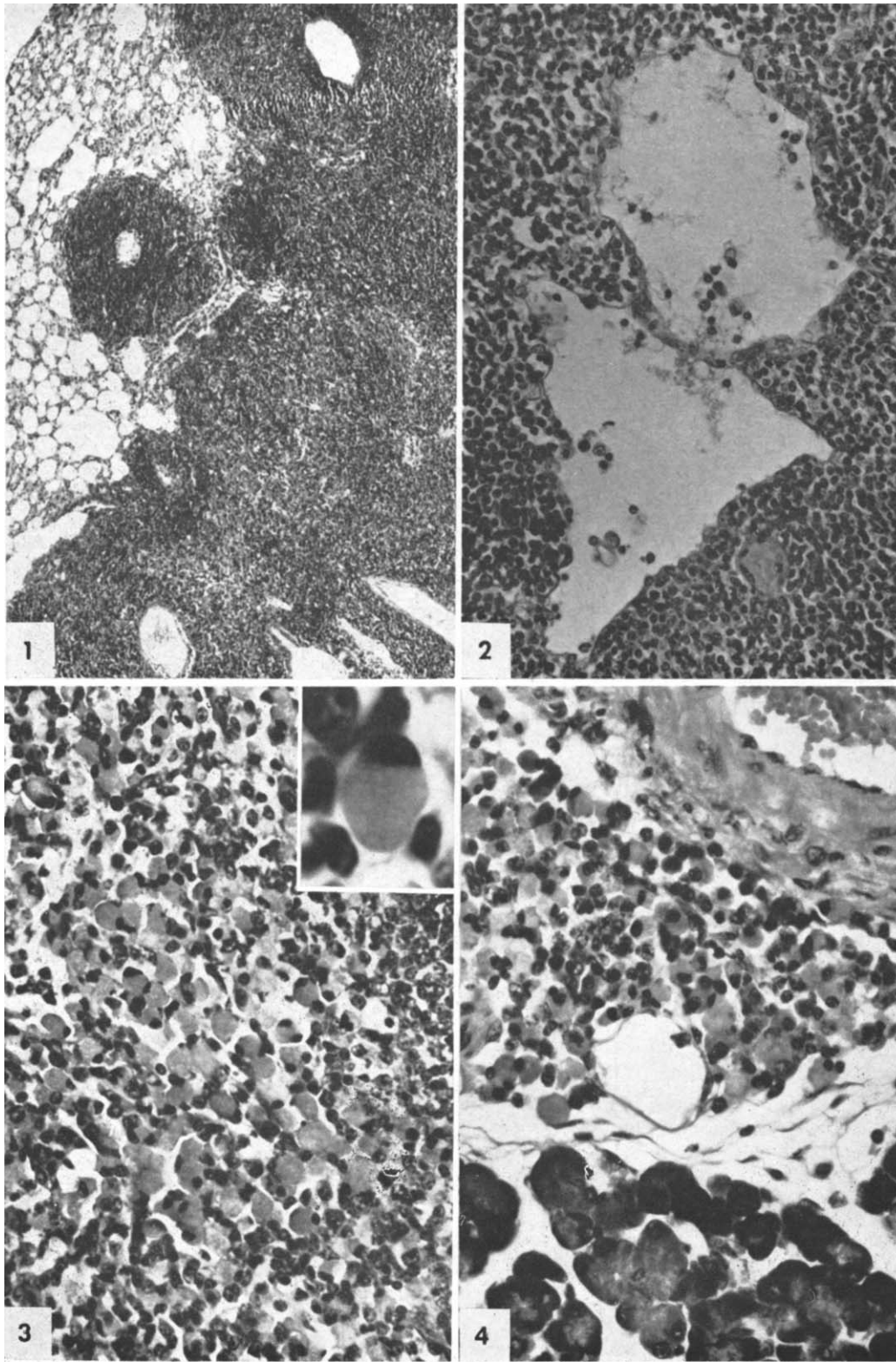
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FIG. 1. Lung tissue from LCM mouse (12 months of age) in which much of the functioning tissues were displaced by expanding masses of lymphoid cells; hematoxylin and eosin stain; ×64.

FIG. 2. Thymic cysts in medulla of LCM mouse (11 months of age). They contain varying numbers of lymphoid cells; H and E stain; ×400.

FIG. 3. Masses of large plasma cells (with Russell bodies) in the lung of LCM mouse (12 months of age); H and E stain; ×400; plasma cell with Russell bodies; ×1200.

FIG. 4. Perivascular aggregation of plasma cells in pancreas of LCM mouse (12 months of age); H and E stain; ×400.

tissues with numerous large plasma cells contained few mitotic cells. The Peyer's patches of LCM mice, at all age levels, were swollen but free of germinal zones; and the intestinal lamina propria were thin and contained few cells.

The Russell bodies in the large plasma cells adsorbed fluorescein-tagged antibody in a peculiar pattern: the margins of the cytoplasmic globules were more heavily stained than the contents. Smaller globulin-containing cells, with eccentric nuclei, were observed in the tissues of LCM mice at all ages. Plasma cells have not been observed in the visceral tissues of the control germfree CFW and SW mice; and rarely in the lymph nodes and spleens. Serum specimens from the old LCM mice contained elevated amounts of gamma globulins, which appeared polyclonal and similar to that described earlier (7).

The germfree adult SW mice which had been inoculated with LCM virus remained disease-free, but they developed evidences of immunohistological and serological responses to the inoculum: within 1 week they developed small germinal zones in the lymph nodes and spleens, which were very large in mice examined from the second week on and, in addition, they contained plasma cells. The tissues of the visceral organs and the thymuses were normal in appearance; however, the Peyer's patches remained free of germinal zones. All of the LCM virus inoculated mice developed detectable levels of complement-fixing antibodies at the second week after virus inoculation, which persisted during the succeeding 6 weeks.

Discussion. The tumors which developed in aged LCM mice were not the leukemic or lymphomatous lesions which have been associated with the effects of Gross-type leukemia virus in germfree mice, because of differences in the histology of the lesions (12). The depletion of cells from the thymuses of preleukemic mice was usually followed by expanding aggregations of large lymphoid cells in the cortex, with many mitotic figures among them; also, cystic structures were rarely observed in the visceral organs of preleukemic, nor in disease-free, germfree mice. When the organs of leukemic mice were infiltrated with lymphoid cells, globulin was not detected in them, the serum globulins remained low, and glomerulonephritis was not detected in them. Because of the large numbers of plasma cells in the swollen thymuses, lymph nodes, spleens, and visceral organs, and because of the increasing levels of serum globulins in such mice, the lesions have been classified as plasmacytomas; however it should be mentioned that these tumors have not yet been transplanted. Similar tissue changes have not been observed in control, LCM-free, germfree mice, nor in germfree mice which had been immunized with LCM virus. The latter mice showed a limited controlled physiological response to the LCM antigens.

The thymus glands seem to be afflicted in all LCM mice beyond 4 months of age. The runting-like syndrome observed by Hotchin (13) has not been observed in LCM mice, which may be a reflection of their axenic environment. The progressive depletion of cortical cells and their replacement by expanding numbers of medullary cells suggests the workings of a disturbed homeostatic mechanism. Perhaps this organ is being depleted or drained of functional cells by an uncontrolled immunogenic response to persistent LCM virus infection. The appearance of plasma cells in all organs suggests that the immunologic system is attempting to fulfill a need for which there is no "feedback" control, as was manifested in the immunized adult SW mice. As yet, the absence of germinal zones in the Peyer's patches of LCM

mice has not been explained.

The immunopathology which develops in mice with peristent LCM virus infection has been interpreted as an "autoimmune" syndrome (13). This can be interpreted in another way: The tissues of LCM mice contained large numbers of plasma cells, their serums had high levels of gamma globulins, and the glomerular capillaries were occluded with antigen–antibody complexes. This syndrome may be exemplary of an immunoproliferative disease process, and causally related to LCM virus. Some characteristics of other diseases resemble the immunoproliferative tissue patterns shown by LCM mice: they include Aleutian mink disease (14), equine infectious anemia (15), and possibly myeloma and rheumatoid arthritis of man. The glomerular and thymic lesions in LCM mice resemble some aspects of systemic lupus erythematosis in man (16); however, the etiology of this latter disease has not yet been identified.

The immunoproliferative lesions which we have observed in LCM mice have not been reported to the same extent in other mouse strains with persistent LCM virus infection (13, 17). Neither runting nor wasting was observed in our LCM virus infected mice. This and other aspects of disease may relate to unique host–parasite interactions among specific mouse strains, or to the congenital nature of infection in LCM mice, or to particular characteristics of the virus carried by LCM mice, or to the more clearly defined tissue changes in germfree LCM mice. Oldstone and Dixon (18) have described a spectrum of susceptibility to LCM virus among several strains of mice among which the levels of viremia varied. Thus, perhaps mice with high levels of viremia may produce proportionately higher levels of antigen–antibody complexes, with the resultant destructive effects on glomerular structure and function. A similar relationship may be responsible for the high incidences of glomerulonephritis among mice of the NZW strain (19); however, we lack supporting evidence for this concept.

Summary. Mice with congenitally-acquired LCM virus infection were maintained under germfree condtions for 12 months. With advancing age, the mice showed histological evidence of uncontrolled immunoproliferative changes: the enlarged lymph nodes and spleens contained large germinal zones and numerous plasma cells. The cortical cells of the thymus glands were progressively depleted of cortical cells, became shrunken, eventually swollen, and infiltrated with large plasma cells. The visceral organs had perivascular infiltrations of lymphoid and plasma cells which displaced significant amounts of parenchyma. The plasmacytomas which developed differed from the lesions associated with murine leukemia.

1. Traub, E., J. Exptl. Med. **68**, 229 (1938).

2. Hotchin, J. and Weigand, H., J. Immunol. **86**, 392 (1961).

3. Rowe, W. P., Naval Med. Res. Inst.-Res. Rept. Proj. NM 005.048.14.01, **12**, 167 (1954).

4. Levey, R. H., Trainin, N., Law, L. W., Black, P. H., and Rowe, W. P., Science **142**, 483 (1963).

5. Lehmann-Grube, F., Arch. Ges. Virusforsch. **14**, 351 (1964).

6. Pollard, M., Sharon, N., and Teah, B. A., Proc. Soc. Exptl. Biol. Med. **127**, 755 (1968).

7. Pollard, M., Kajima, M., and Sharon, N., "Perspectives in Virology," (M. Pollard, ed.), Vol. 6, pp. 193–207. Academic Press, New York (1968).

8. Oldstone, M. B. A. and Dixon, F. J., Science **158**, 1193 (1968).

9. Haas, V. H., Public Health Rept., U.S.P.H.S. **56**, 285 (1941).

10. Wagner, M., Ann. N. Y. Acad. Sci. **78**, 89 (1959).

11. Kajima, M. and Pollard, M., Nature **218**, 188 (1968).

12. Pollard, M., Kajima, M., and Teah, B. A., Proc. Soc. Exptl. Biol. Med. **120**, 72 (1965).

13. Hotchin, J., Public Health Service Publ. No. **1378**, 341 (1965).

14. Leader, R. W., Wagner, B. M., Henson, J. B., and Gorham, J. R., Am. J. Pathol. **43**, 33 (1963).

15. Squire, R. A., Blood **32**, 157 (1968).

16. Miescher, P. A. and Paronetto, F., Textbook of Immunopathology (P. A. Miescher and H. J. Muller-Eberhard, eds.), Vol. 2, p. 675. Grune and Stratton, New York (1969).

17. Hirsch, M. S., Murphy, F. A., and Hicklin, M. D., J. Exptl. Med. **127**, 757 (1968).

18. Oldstone, M. B. A. and Dixon, F. J., J. Immunol. **100**, 355 (1968).

19. East, J. M., DeSousa, A. B., and Parrott, D. M. V., Transplantation **3**, 711 (1965).

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