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Pathogenesis of Herpesvirus sylvilagus *Infection in Cottontail Rabbits*

R. M. HESSELTON, DVM,*‡
W. C. YANG, DVM, PhD,*
P. MEDVECZKY, MD,† and J. L. SULLIVAN, MD*

From the University of Massachusetts Medical School Departments of Pediatrics and Pharmacology,† Worcester, and New England Regional Primate Research Center,‡ Harvard Medical School, Southborough, Massachusetts*

Experimental infection with *Herpesvirus sylvilagus* produces clinical and histopathologic changes in its natural host, the cottontail rabbit (*Sylvilagus floridanus*), similar to those observed in humans acutely infected with Epstein-Barr virus (EBV). Twenty-seven seronegative cottontail rabbits were infected with *Herpesvirus sylvilagus* and all developed antibodies within 10 days. Neutralizing antibody was detected as early as 7 days after infection. Virus was isolated from blood mononuclear cells, spleen, bone marrow, thymus, lymph nodes, kidneys, lung, and liver as early as 3 days after infection. Infected animals showed leucocytosis, monocytosis, and lymphocytosis with the appearance of atypical lymphocytes. Peripheral blood abnormalities peaked at 10–14 days after infection, and returned to normal by 28 days after infection, with the exception of atypical lymphocytosis that persisted in some animals for more than 2 years after experimental infection. More severe histopathologic changes were seen in virus-infected juvenile rabbits than adult rabbits; these changes included viral myocarditis, interstitial pneumonia, and lymphocytic myositis. Reactive hyperplasia and subsequent lymphocytic depletion of spleen and lymph nodes were reminiscent of that seen in virus-associated hemophagocytosis syndrome. Prominent lymphoid hyperplasia of many nonlymphoid organs, most notably the kidney and lungs, was observed.

The development of these lymphoproliferative lesions and other lymphoid changes during *H. sylvilagus* infection suggest that this system may be a model to study similar lesions induced by EBV infection in humans. (Am J Pathol 1988, 133:639–647)

HERPESVIRUS SYLVILAGUS was first isolated in 1968 from the kidneys of a weanling cottontail rabbit (*Sylvilagus floridanus*) that was apparently healthy when trapped in the wild in southern Wisconsin.[1] The virus was identified as a Subgroup B Herpes virus because of its chemical, physical, and biologic properties and because it is strongly cell-associated.[2,3] More recent clarification of nomenclature has classified *H. sylvilagus* in the gamma group of herpesviruses.[4] The virus has a narrow host range. New Zealand White Rabbits (*Oryctolagus cuniculus*), mice, guinea pigs, and hamsters are resistant to experimental infection.[1] The natural host, the cottontail rabbit, can be readily infected by oral and parenteral inoculation. *In vitro* infection can be established in New Zealand White Rabbit kidney cells and adult cottontail rabbit kidney cells[1,5] but the highest titers of virus are obtained by the use of cell lines established from juvenile (JRK) or newborn cottontail rabbit kidneys (NCRK)[6,7] where cytopathic effects (CPE) can be observed as early as 12 hours after infection.

Earlier studies indicated that virus could be consistently isolated from saliva up to 18 weeks after natural infection but that no virus could be isolated from urine, feces, semen, or milk.[8] Placental transmission of the virus could not be demonstrated, although natural infection was observed in newborn or weanling age rabbits.[9] One serologic survey of rabbits that were wild when caught in Wisconsin showed a prevalence rate of the virus of 6%.[10]

Hinze and others[11,12,13] reported the production of disease in cottontail rabbits experimentally infected with the virus. Experimental infection was character-

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Address reprint requests to John L. Sullivan, MD, Department of Pediatrics, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

ized by the induction of a persistent low-grade viremia, infectious mononucleosislike symptoms, stimulation of lymphoid organs that varied from mild to apparently malignant with loss of normal lymph node architecture in 27% of juveniles and 10% of adults, and the lymphocytic infiltration of parenchymal organs. Because of the striking similarities between these findings and those observed in humans naturally infected with Epstein-Barr virus (EBV), we have investigated *H. sylvilagus* infection in rabbits as a model of EBV-induced lymphoproliferation in humans. This report characterizes the clinical and histopathologic changes seen in juvenile and adult cottontail rabbits experimentally infected with *H. sylvilagus.*

Materials and Methods

Experimental Animals

Cottontail rabbits used in this study were trapped in the wild or born in captivity to mothers that were trapped in the wild. Trapping was conducted in early spring in Massachusetts under State and Federal permits. At the time of removal from traps, all rabbits were carefully examined for evidence of disease or trauma, dusted for flea control, ear-notched, and bled for initial evaluation for *H. sylvilagus* antibodies. Rabbits were then placed in cages in an isolation area. Adult rabbits (N = 20) weighed approximately 1 kg. Rabbits of both sexes were used for this study. Juvenile animals (N = 7) were either born in the animal facility to mothers pregnant at the time of capture or in an outdoor rabbit-breeding facility from which they were removed at approximately 4 weeks of age. After isolation all rabbits were kept in standard laboratory caging and fed a pelleted laboratory diet *ad libitum.* Infected and uninfected rabbits were kept in separate rooms. Rabbits were experimentally infected with *H. sylvilagus* by intraperitoneal injection of 5×10^6 pfu of virus. Blood was drawn by cardiac puncture 3, 7, 10, 14, 21, 28, 42, 56, 70, 84, and 112 days after infection and later if possible. All rabbits were anesthetized with a combination of ketamine and xylazine given intramuscularly before any handling. Uninfected control animals were handled in the same way. All handling procedures and protocols were reviewed and approved by this Institution's Animal Welfare Committee before implementation and were in accordance with the National Research Council's Guide on the Care and Use of Laboratory Animals.

At varying time points after infection, rabbits were killed and tissues were harvested for virus isolation and histopathological examination.

Virus and Cell Culture

Virus from an original *H. sylvilagus* stock was provided by Dr. H. Hinze, University of Wisconsin. Virus was grown in one of two cell lines derived from newborn cottontail rabbit kidneys (NCRK-1/NCRK-2).[7] Virus isolation was done on NCRK cells. Virus isolates were identified by specific indirect fluorescent antibody assays.

The spleen, thymus, mesenteric lymph node, popliteal lymph node, bone marrow, peripheral blood mononuclear cells (PBMC), kidney, lung, liver, adrenal gland, and salivary gland were collected aseptically for virus isolation. PBMC were separated from whole heparinized blood by density gradient centrifugation on Ficoll-Hypaque with a density of 1.082–1.085. Identical tissue samples were placed immediately into buffer A (15% Ficoll in Tris borate-EDTA with 0.01% bromophenol blue) for analysis of linear and circular forms of viral genome by gel electrophoresis.[14]

Serology

Serology was performed using indirect fluorescent antibody (IFA) technique on *H. sylvilagus*-infected NCRK cells; all rabbits were negative for antibodies to *H. sylvilagus* before infection. Seroconversion was determined by IFA at 1:20 titer and a standard plaque reduction neutralization assay.

Clinical Pathology

Hematologic Studies

White blood cell count, differential leukocyte counts, total red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were determined by routine methods.

Clinical Chemistry Studies

Blood urea nitrogen, serum creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and blood glucose were determined by automated procedures (Baker Series 7000 Cell Counter, Gemini Series 803 Chemistry Analyzer).

Histopathology

Samples of tissues collected for virus isolation and all other organs were placed into 10% neutral buffered formalin for routine histologic processing. Five to six-micron thick sections of paraffin-embedded tissue

were stained with hematoxylin and eosin (H & E) and permanently mounted on glass slides for microscopic examination.

Results

Virus and Serology Studies

After infection with *H. sylvilagus* none of the infected animals manifested any overt symptoms of disease and no animals died as a result of infection.

Table 1 summarizes results of virus isolation attempts, type of viral genome identified by gel electrophoresis, and gross changes seen at the time of death. *H. sylvilagus* could be isolated from cottontail rabbit PBMC, bone marrow, kidney, lung, and liver as early as 3 days after infection by cocultivation of tissues with NCRK cells. At 4 weeks after infection, virus was recovered from spleen, thymus, and lymph nodes as well. Virus could be isolated from all organs examined from the one animal killed more than 2 years after experimental infection.

Circular and linear forms of viral DNA were separately identified by gel electrophoresis with the modifications of Gardella et al.[14]

Grossly observable lesions were limited to spleen and lymph node enlargement, pulmonary nodules, and occasional renal nodules.

Although virus could be isolated from bone marrow and thymus and viral DNA was isolated from thymocytes (isolation of DNA from bone marrow cells was not attempted), no substantial morphologic changes were noted in the bone marrow or thymus of any of the rabbits examined. Examination and differential enumeration of cells in bone marrow smears revealed no significant difference between infected and control rabbits.

Table 1—Summary of Virological and Gross Pathologic Data Obtained from Analysis of 27 Cottontail Rabbits Infected with *Herpesvirus Sylvilagus*

Tissue	Type of viral DNA identified	Interval after infection when virus recovered	Tissue and organ abnormalities observed
PBMC	Circular	3 days–2 years	Atypical lymphocytes
Bone marrow	None	3 days–2 years	NSF
Spleen	Linear	4 weeks–2 years	Splenomegaly between 6 and 21 days Pi
Lymph nodes	ND	4 weeks–2 years	Enlarged between 6 and 28 days Pi
Thymus	Circular and linear	4 weeks–2 years	NSF
Lung	ND	3 days–2 years	Disseminated gray-white nodules in juveniles between 8 and 16 weeks Pi
Liver	ND	3 days–2 years	NSF
Kidney	ND	3 days–2 years	Small gray-white nodules on capsular surface of 2 juvenile rabbits at 8 weeks Pi
Adrenal gland	ND	8 weeks–2 years	NSF
Salivary gland	ND	8 weeks–2 years	NSF

PBMC, Peripheral blood mononuclear cells; NSF, No significant findings; ND, Not done; Pi, postinfection.

Serology

Antibodies to *H. sylvilagus* were detected by the IFA test as early as 5 days after experimental infection. All animals seroconverted to *H. sylvilagus* by 10 days after infection. Neutralizing antibodies as detected by 50% reduction in a standard plaque reduction assay developed as early as 7 days after infection.

Clinical Pathology

Clinical monitoring during experimental infection showed leukocytosis consisting of monocytosis and lymphocytosis. Figure 1 illustrates values for mononuclear cells over the period of infection with *H. sylvilagus*. Atypical lymphocytes appeared in the peripheral blood in substantial numbers by day 3 after infection and by day 14 constituted as much as 45% of total lymphocytes. Although all other peripheral blood abnormalities peaked at 10–14 days and returned to baseline by about 28 days, atypical lymphocytes persisted in varying numbers (up to 1500/cu mm) in some animals up to 2 years after infection. Kidney and liver function tests performed on infected adult rabbits showed no abnormalities except for a transient rise in SGOT and in SGPT when an animal (infected or control) had been anesthetized more than once in 1 week.

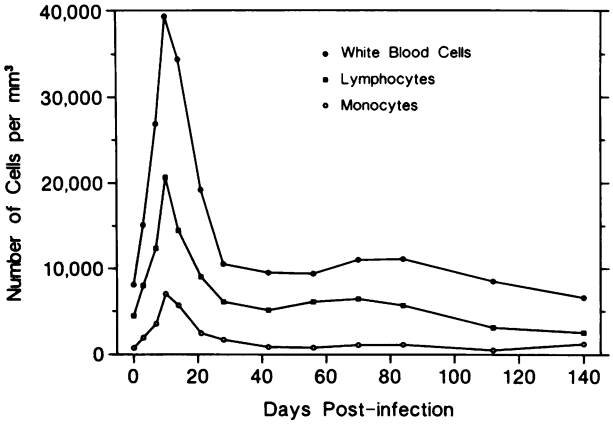

Figure 1—Leukocyte changes during *H. sylvilagus* infection.

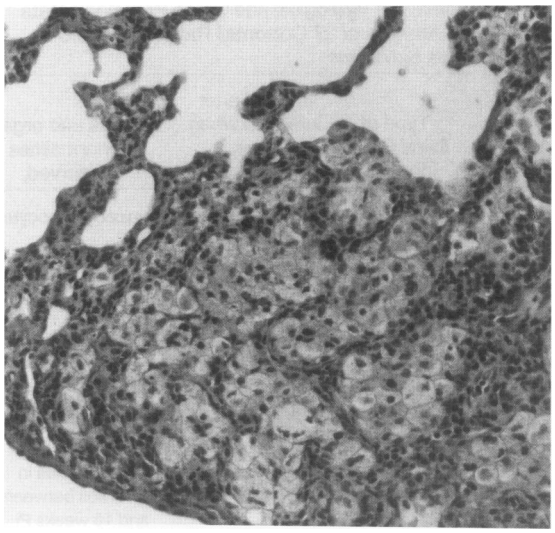

Figure 2—A nodule on the pleural surface of the lung of a juvenile rabbit infected with *H. sylvilagus* consists of interstitial pneumonia and alveolar macrophages. Other nodules in the lung are lymphocytic hyperplasia. H & E, ×100

Histopathology

Inflammatory Changes and Lymphoid Hyperplasia

Early in the course of infection (first 4 weeks) young rabbits developed inflammatory lesions of the heart and lungs. Myocardial infiltrates composed predominantly of lymphocytes with some heterophils (rabbit neutrophils are termed heterophils because of the presence of both basophilic and eosinophilic granules) and histiocytes appeared by 4 weeks after infection. Infiltrates were accompanied by varying degrees of myonecrosis. In some cases there was lymphoid infiltration of the myocardium with little necrosis. Myocardial fibers were replaced locally with the typical mixed lymphocyte populations seen in other areas of lymphoid hyperplasia. Other sections of myocardium from these same animals had a more pronounced inflammatory component to the lesion. These lesions were resolving with fibrosis by 16 weeks after infection. Regional myocardial fibrosis was seen in some infected adults but could not be definitively attributed to viral causes because these animals had been bled by cardiac puncture on multiple occasions. Control adult rabbits were also bled regularly by cardiac puncture and occasionally showed foci of myocardial fibrosis. Juvenile rabbits had not been bled before the time of death.

Table 2—Inflammatory Changes in *H. sylvilagus*-Infected Cottontail Rabbits

Organ	Time after infection	Number affected
Lungs	3 days–16 weeks	7/7 juveniles, 7/9 adults
Myocardium	4 weeks–11 weeks	5/6 juveniles, 3/6 adults
Skeletal muscle	4 weeks–8 weeks	5/6 juveniles, 0/5 adults
Optic nerve	4 weeks–8 weeks	2/6 juveniles, 0/5 adults
Trigeminal ganglion	4 weeks	1/2 juveniles, 0/0 adults
Anterior uvea	4 weeks	1/1 juveniles, 0/1 adults
Adrenal	3 days–54 weeks	3/7 juveniles, 4/11 adults
Liver	6 days–4 weeks	1/2 juveniles, 1/2 adults
Kidney	6 days–30 weeks	0/7 juveniles, 4/9 adults

Myositis involving skeletal muscle was noted in infected juveniles 4 and 8 weeks after infection. Myositis varied from widespread to that involving primarily the extra-ocular muscles. Extensive myositis was not seen in infected adult rabbits.

Pulmonary changes were seen in all adult and juvenile-infected animals examined at 8 weeks or less. A moderate-to-severe, regional-to-diffuse, necrotizing, hemorrhagic, and proliferative lymphocytic, histiocytic, and occasionally heterophilic pneumonia was first noted 6 days after infection though regions of necrosis and hemorrhage were apparent at 3 days after infection. Early lesions were peribronchial with increased amounts of bronchial-associated lymphoid tissue (BALT), moderate necrosis, and histiocytes. Regions of consolidating pneumonia radiated from these centers of necrosis as the lesions became more severe. Lesions were most severe in juvenile animals infected 8 weeks before histologic examination. A juvenile animal examined 16 weeks after infection and an adult examined 8 weeks after infection had resolving lung lesions typified by histiocytic infiltration, mild hyperplasia of Type II pneumocytes, and early fibrosis. Figure 2 is a photomicrograph of the pulmonary lesion in the juvenile rabbit infected with *H. sylvilagus* 16 weeks before death.

In addition to the lesions already described, inflammation characterized by a heterophilic or lymphocytic infiltrate and necrosis with some disruption in normal architecture occurred in the eye, nerves, adrenal gland, and kidney. Periportal lymphocytic hepatitis was seen in two rabbits. All inflammatory changes seen in infected animals, but not in control rabbits are listed in Table 2 along with the time after infection when the lesions were seen.

In the context of this study, we have used the term lymphoid hyperplasia to describe the infiltration of any otherwise normal-appearing organ or tissue by small to very large aggregates of a mixed lymphocytic cell population without accompanying necrosis. Lymphoid hyperplasia is seen in the salivary gland in Figure 3. Cells making up this infiltrate varied from small mature-appearing lymphocytes to lymphoblastoid and plasmacytic lymphocytes to very large vesicular cells with a transformed appearance (Figure 4). Smaller aggregates generally had a perivascular loca-

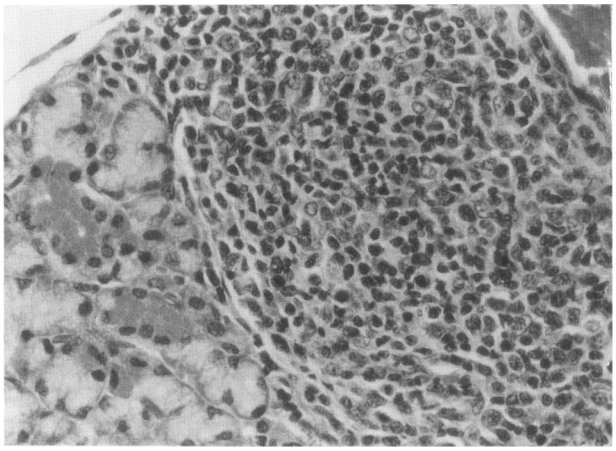

Figure 3—Perivascular lymphoid hyperplasia in the salivary gland of a juvenile cottontail rabbit infected with *H. sylvilagus* 8 weeks before death. H & E, ×200

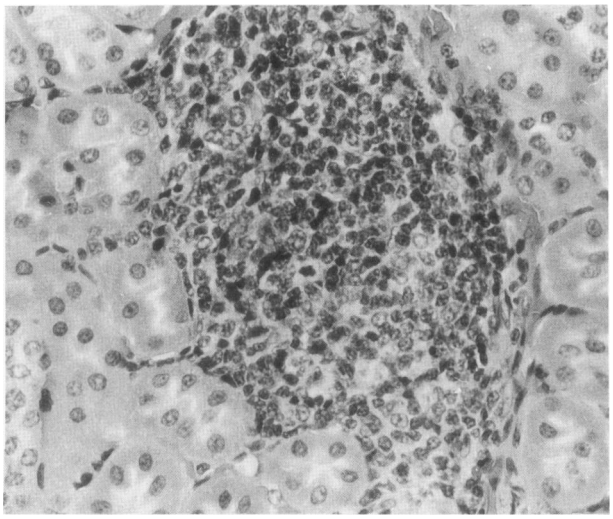

Figure 5—Nodular lymphoid hyperplasia is seen here in the kidney of a juvenile cottontail rabbit infected with *H. sylvilagus* 4 weeks before death. H & E, ×200

tion, larger nodules often had no such identifiable focus.

Renal lymphoid aggregates were confined to the cortical regions and frequently involved blood vessels. Glomeruli and tubules disappeared from the area of infiltration but no necrotic structures were seen (Figure 5, 6). Renal involvement in lymphoid hyperplasia in juvenile animals was often extensive, involving up to 40% of the renal cortex in any given histologic section.

Lung nodules were larger and more numerous in juveniles 8 weeks after infection than 4 weeks after infection, and were, in fact, grossly apparent as tiny raised white to tan foci on the natural and cut surfaces of the lungs. These pulmonary nodules were set in a

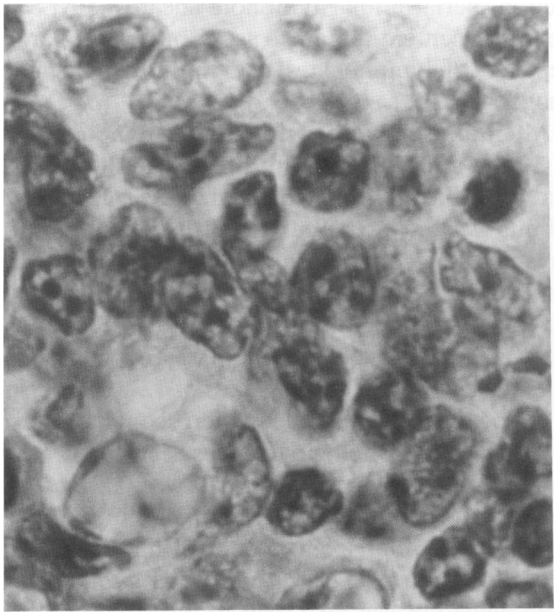

Figure 4—The mixed cellular character of the lymphocytic infiltrate can be seen here in the kidney of a juvenile rabbit infected with *H. sylvilagus* 8 weeks before death. H & E, ×1000, oil immersion

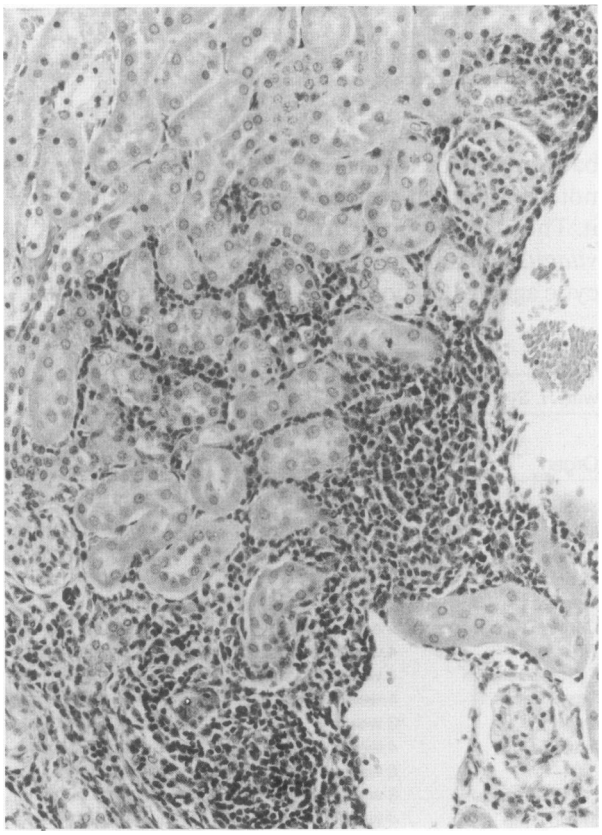

Figure 6—Kidney of a juvenile rabbit infected 8 weeks previously with *H. sylvilagus*. Renal cortical lymphocytic infiltrate expands to separate tubules. H & E, ×100

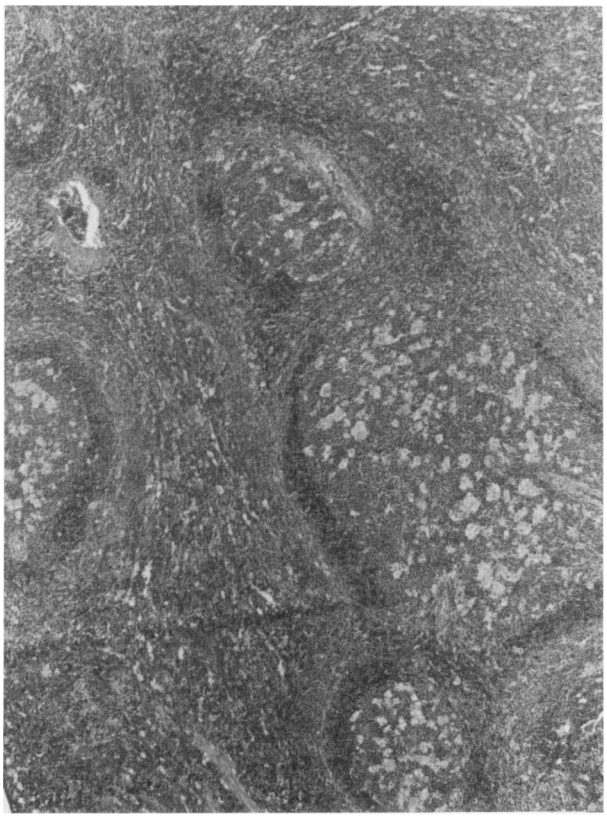

Figure 7—Enlarged germinal centers and periarteriolar lymphoid sheaths are seen in the spleen of a juvenile rabbit experimentally infected with *H. sylvilagus* 4 weeks before death. H & E, ×25

background of the severe consolidating pneumonia noted earlier but seemed to be a separate entity from it. The juvenile rabbit examined 16 weeks after *H. sylvilagus* infection had fairly extensive nodular lymphocytic aggregates along with a resolving pneumonia.

Table 3—Lymphoid Hyperplasia in Cottontail Rabbits Infected with *H. Sylvilagus*

Organ	Time after infection	Number affected
Myocardium	4 weeks–54 weeks	5/7 juveniles, 4/9 adults
Lungs	6 days–>2 years	7/7 juveniles, 8/11 adults
Kidney	3 days–>2 years	7/7 juveniles, 12/12 adults
Salivary gland	6 days–54 weeks	5/7 juveniles, 5/10 adults
Pancreas	4 weeks–16 weeks	5/7 juveniles, 1/7 adults
Ceruminous gland	4 weeks–16 weeks	5/7 juveniles, 0/7 adults
Thyroid	4 weeks–8 weeks	3/6 juveniles, 0/5 adults
Fat/mediastinum	4 weeks–16 weeks	5/7 juveniles, 1/7 adults
Stomach	4 weeks–8 weeks	2/6 juveniles, 1/5 adults
Pylorus	8 weeks	1/5 juveniles, 1/1 adults
Intestine	4 weeks–8 weeks	2/6 juveniles, 0/5 adults
Skin	8 weeks–16 weeks	2/6 juveniles, 0/3 adults
Skeletal muscle	8 weeks–16 weeks	5/6 juveniles, 0/3 adults
Meninges	4 weeks	1/1 juveniles, 0/1 adults
Adrenal	6 days–11 weeks	0/6 juveniles, 3/7 adults
Mucous membranes	8 weeks–54 weeks	0/6 juveniles, 2/5 adults
Lacrimal gland	4 weeks	1/1 juveniles, 0/1 adults

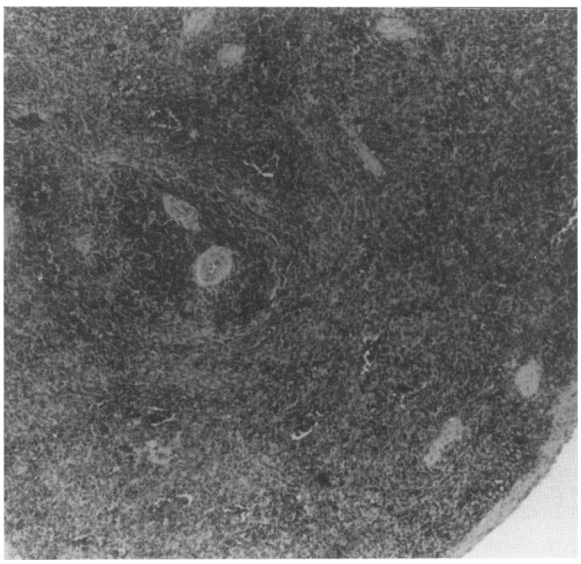

Figure 8—Spleen from an uninfected rabbit. H & E, ×25

Table 3 summarizes the findings of lymphoid hyperplasia in various organs after infection with *H. sylvilagus*. In general, the changes of lymphoid hyperplasia in infected adult rabbits are less pronounced than in juvenile animals infected for a comparable period of time.

Changes in Lymphoid Organs

The spleen of the juvenile rabbit examined 4 weeks after infection displayed large germinal centers, distinct marginal zones and reticuloendothelial cell (RE) hyperplasia (Figure 7, 8). At 8 and 16 weeks after infection, periarteriolar lymphoid sheaths (PALS) and germinal centers remained large but were markedly depleted of lymphocytes and infiltrated by macrophages.

Alterations in lymphoid organs were noted as early as 3 days after infection in infected adult rabbits. At that time, the only change noted in the spleen of adult rabbits was the increased size of the white pulp. By 6 days after infection, plasmacytosis, RE cell hyperplasia, and individual cell necrosis could be appreciated. By 4 weeks and continuing through 12 weeks after infection, lymphocytic depletion was apparent in the spleen. At time periods in excess of 12 weeks, spleens of infected adult rabbits were microscopically normal.

The first morphologic changes seen in the lymph nodes of infected juvenile animals were an increase in the size and number of active follicles in all infected animals in comparison with control animals. Paracortical expansion with or without subsequent lymphocytic depletion was also seen in juveniles at all time periods (Figure 9). Also noted in all infected animals beyond 3 days after infection was hyperplasia of folli-

cle center cells. This change was most pronounced in the nodes of young animals and was coupled with an increasingly apparent lymphocytic depletion as time after infection increased. Lymph nodes of infected rabbits showed dilated sinuses and increased numbers of lymphocytes within the medullary and subcapsular sinuses and lymphocytic traffic through the nodes. Loss of lymph node architecture with effacement of follicular-paracortical boundaries and loss of sinus/medullary cord distinction was noted in a single node of a single adult rabbit 4 weeks after infection. In general, changes in the lymph nodes of infected adult rabbits were comparable with those seen in juvenile rabbits.

Four of five juvenile rabbits infected for 8 weeks showed increased numbers of lymphocytes in follicles and at the antigen-presenting surface of the sacculus rotundus. A slight to moderate increase in number of lymphocytes at the antigen-presenting surface along with RE cell hyperplasia was also seen in four adult infected animals at various times after infection. Cell necrosis within the sacculus follicles was noted in the one adult rabbit that was examined 6 days after infection. Ileal confluence of Peyer's patches by hyperplasia was seen in one of five juvenile animals 8 weeks after infection.

Tonsils were examined in only a few animals; one adult rabbit examined 12 weeks after infection had RE cell hyperplasia and an increased number of follicles in the tonsillar tissue.

Small amounts of thymic hemorrhage and individual cell necrosis were seen in one of five juvenile rabbits examined 8 weeks after infection and small foci of heterophilic infiltrates were noted in the thymus of two of these five animals. The thymic tissue examined from infected adults was within normal limits with marked atrophy and involution seen in those animals that were known to be in excess of 2 years of age at the time of examination. Bone marrow from all animals was examined and no abnormalities were detected in any of the samples.

Discussion

H. sylvilagus was isolated from a variety of tissues as early as 3 days after infection and from virtually all tissues from which virus isolation was attempted after infection from 4 weeks through more than 2 years. This was true for animals showing only minor histopathologic changes (lymphocytic depletion of lymph nodes with maintenance of normal node architecture, aggregates of lymphoid hyperplasia in kidneys, salivary glands) as well as for animals with more severe histopathologic changes. Our studies used the same

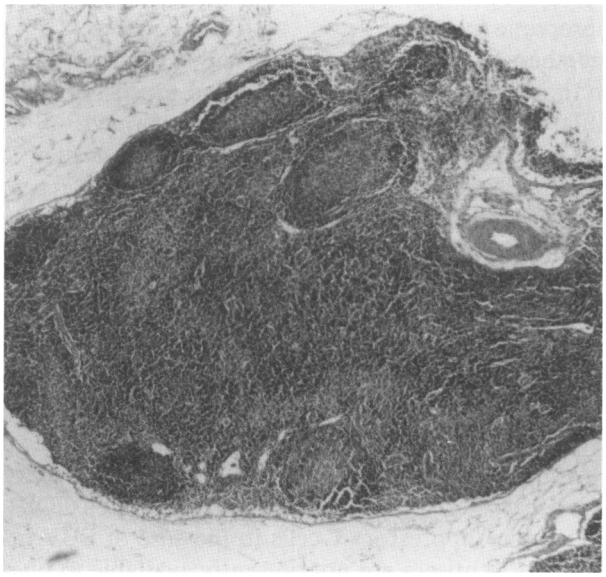

Figure 9—This lymph node from a rabbit infected with *H. sylvilagus* 16 weeks previously shows hyperplasia of both follicles and interfollicular areas. H & E, ×25

experimental design (dose, route of administration) as earlier studies that reported that virus could be isolated only from peripheral blood 1–2 weeks after infection, and from selected organs at later dates in those animals that showed obliteration of normal lymph node architecture.[11]

Because most of the tissues from which virus isolation was attempted were of lymphoid nature, and the nonlymphoid tissues were those that demonstrated lymphoid infiltration, infected lymphocytes may have been the source of virus in all cases. Isolation of virus from peripheral blood mononuclear cells as early as 3 days after infection strongly suggests that PBMC serve as the initial target cell. Studies with EBV, Herpes saimiri, and Marek's disease virus in chickens have indicated linear virus can be isolated only from cells undergoing active viral infection whereas cells with latent infection yield only circularized forms of the viral genome.[14–16] The identification of linear viral DNA in spleen and thymus cells supports the presence of an active replicating viral infection in lymphocytes. It is impossible to tell whether a few cells are infected lytically and are yielding a high number of DNA copies or whether a significant portion of cells are each producing a few copies of linear genomes. The simultaneous finding of circular viral DNA in thymocytes and peripheral blood lymphocytes in these animals indicated that some cells have been latently infected.

In this study, most infected animals at all stages showed some morphologic involvement of the salivary glands and virus could be readily isolated from salivary glands. Shedding of *H. sylvilagus* in the saliva

and oral secretions initially and throughout a 16-week observation period was reported earlier,[8,9] but whether the source virus is from infected lymphocytes in the area of the salivary glands and tonsils, or glandular epithelial cells remains to be determined.

Total leukocyte, monocyte, and lymphocyte counts were increased to 7.5–10 times normal values (Figure 1). Similar but less pronounced changes had been seen previously with *H. sylvilagus* infection.[11] Similar hematologic changes are also seen in EBV-induced infectious mononucleosis.[17] These changes include the appearance of atypical lymphocytes. Such atypical lymphocytes (up to 12%) are seen in small numbers in normal rabbit blood smears just as they may be seen in blood smears from normal humans.[18] Atypical lymphocytes observed during EBV infection have been characterized as predominantly activated CD8+ (cytotoxic/suppressor) T cells in the case of EBV infection.[19] Antisera are not presently available to phenotypically define the atypical lymphocytes seen in *H. sylvilagus* infection of cottontail rabbits.

Inflammatory lesions were prominent in early infection in these animals, especially in juvenile animals. Inflammatory lesions have not been previously reported as a part of *H. sylvilagus* infection, although Hinze reported the death of some infected rabbits with secondary bacterial infections.[11] We believe that the consistent observation of these inflammatory lesions in our experimentally infected juvenile rabbits without evidence of bacterial infection strongly suggests that they are a result of the viral infection. There were no deaths in our study that could be attributed to infection either with the virus or secondary infection.

Lymphoid infiltration in nonlymphoid organs was the predominant histologic finding in experimentally infected animals in this study as well as previous studies. Earlier reports described the lymphocyte-predominant infiltrate of the kidneys, myocardium, and liver.[12] We have seen the same severe infiltration with replacement of functional tissue of the kidneys as well as the myocardium, where it is accompanied by myocardial fiber necrosis and inflammation; however, we did not observe significant morphologic changes in the livers of infected animals. This differs from serious EBV infection in humans, where hepatitis is a prominent lesion. Rather, we saw substantial lymphoid hyperplasia of lung parenchyma not described in earlier studies. Other organs, detailed elsewhere, were affected with lymphoid hyperplasia to varying degrees. Often the aggregated lymphocytes were found in a perivascular or periductal distribution, suggesting that they are aggregates of migrating lymphocytes. It is possible that virus-infected parenchymal cells or phagocytes act as stimuli to attract the mixed population of lymphocytes and studies are currently underway using *in situ* hybridization and immunohistochemical markers to pinpoint the cells that are infected with the virus and to identify the cells that compose the infiltrate.

Although some cells in the infiltrates observed in *H. sylvilagus* infection have a transformed appearance, these lesions develop, peak, and wane. Resolving lesions are seen in animals infected for longer periods of time and minimal infiltrates are present after a year of infection. Lesions may still be present but one can speculate that, like other infected rabbits, these have passed through a more severe stage at earlier points in the infection. We saw no evidence of malignant lymphomalike lesions as reported earlier.[11]

Owl monkeys that have been experimentally infected with EBV develop a lymphoproliferative disease characterized by RE cell and lymphocytic hyperplasia of the lymph nodes, splenic hyperplasia, and lymphoid hyperplasia of the myocardium, liver, meninges, and kidneys with the infiltrate being composed of a variety of lymphocyte types.[20] These lesions are identical to those described herein.

Naturally-occurring cases of fatal lymphoproliferation have been linked to infection with EBV in humans. The disease in this case is believed to be the direct result of proliferation of the virus-infected cells themselves as well as the response of the immune system to that proliferation.[21] Histologic findings in some of these human cases of sporadic fatal infectious mononucleosis include pleomorphic cellular infiltrates of lymphoid organs, liver, and lungs.[22] In cases of X-linked lymphoproliferative syndrome (XLP) additional organs that are regularly affected by the same type of cellular infiltrate include the gastrointestinal tract, brain, kidneys, adrenal, and heart.[22] The histologic picture in parenchymatous organs in these XLP cases is very similar to that seen in the rabbits in this study.

Changes in lymphoid organs were variable in degree in both juvenile and adult rabbits in this study. The most consistent change was reticuloendothelial cell hyperplasia in most lymphoid organs. Hyperplasia of these structural cells was diffuse but most apparent in follicle centers of lymph nodes, sacculus rotundus, and tonsils, and in the white pulp of the spleen. Also pronounced were the changes in lymphocyte populations with marked hyperplasia of both follicular and paracortical area lymphocytes early in infection followed by severe depletion of lymphocytes in both areas and subsequent macrophage infiltration of the follicle centers. Although the macrophage infiltration is unique, the picture of reactive hyperplasia followed by depletion and eventual repopulation of

lymph nodes and other lymph organs is similar to the Virus-Associated Hemophagocytic Syndrome seen in humans infected with a variety of lymphotropic viruses including EBV and Human Immunodeficiency Virus.

H. sylvilagus is a lymphotropic herpesvirus of cottontail rabbits that induces lymphoproliferative lesions similar to those observed in EBV infection in humans. Study of this model may result in a better understanding of the immunopathogenesis of human EBV infection.

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