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Induction of Disseminated Virulent Cytomegalovirus Infection by Immunosuppression of Naturally Chronically Infected Wild Mice

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Wild mice immunosuppressed with antithymocytic sera show a high incidence of disseminated virulent cytomegalovirus infection.

The inadvertent dissemination of otherwise latent or chronic herpesvirus, especially cytomegalovirus (CMV), is an important consideration in humans given immunosuppressive treatment for renal or cardiac transplantation (5, 7-9). Laboratory mice have been used as a model for chronic CMV infection after experimental inoculation of virus (3, 4, 6). It is desirable to have an animal model of chronic CMV infection that does not require experimental virus inoculation. Laboratory mice are unsuitable in this regard, since they have a low prevalence (2 to 3%) of naturally occurring chronic CMV infection (10). However, chronic CMV infection with shedding of virus in saliva is apparently ubiquitous in wild mice (*Mus musculus*; 10) in both urban and rural environments, suggesting that feral mice might be an appropriate and readily available model for studying the effect of immunosuppression upon natural CMV infection. We report here that wild mice immunosuppressed with antithymocytic sera (ATS) show a high incidence of disseminated virulent CMV infection.

A total of 180 wild mice, equally divided by sex, freshly trapped from three widely separate areas in southern California, were treated with ATS. The mice weighed from 15 to 20 g, and were assumed to be of young adult age; they were individually housed in mason jars. Mouse ATS prepared in rabbits (from Microbiological Associates, Inc., Bethesda, Md.) was given subcutaneously, 0.3 ml twice weekly, until death. A total of 4,600 age-matched, untreated, aging wild mice from the same trapping areas served as controls (1). Sick or dead mice were necropsied, and microscope study was done when postmortem autolysis was not extremely severe. Five-micron sections, stained with hematoxylin and eosin, were prepared from formalin-fixed visceral and brain tissues.

A total of 100 of the 180 mice (55%) became sick and died within the first month. Of these 100 mice, 54 were examined with a microscope and 44 (81%) revealed the characteristic lesions of systemic, disseminated CMV infection. Of the remaining mice living for 2 to 6 months under ATS treatment, 46 were examined with a microscope and no evidence of CMV infection was found. The incidence of disseminated CMV infection was the same in mice from the three different trapping areas.

The diagnosis of disseminated CMV infection was based upon microscope examination. In each case the liver was involved, usually with extensive and confluent areas of coagulative necrosis. Characteristic intranuclear and intracytoplasmic eosinophilic inclusions within enlarged hepatocytes were always identified; only a scant acute inflammatory response accompanied this process. These histopathological findings are similar to those described in the liver of CMV-inoculated, cortisone-treated laboratory mice (3). Similar inclusions with less necrosis were often, but not invariably, noted in the acinar and ductal cells of the submaxillary gland and in enlarged mononuclear cells distending the splenic sinusoids. Inclusion-bearing cells were occasionally noted in the pulmonary alveolar septa, cardiac interstitium, and renal glomeruli. Inclusions were not seen in renal tubular cells or brain.

In an effort to confirm the histopathological evidence of disseminated CMV infection, isolation of CMV in vitro from a few remaining, stored 10% tissue extracts from 14 of the ATS-treated wild mice was attempted, and 10 of these mice showed the liver lesions of virulent

CMV infection. These extracts had been stored at −70 C for approximately one year. The extracts were thawed and assayed on Swiss embryo cells from the National Institutes of Health, Bethesda, Md. (NIH) for characteristic herpes cytopathic effect (11). Infected cells rounded up and clumped together, leaving clear plaques which eventually involved the entire monolayer. CMV was isolated in low titer ($10^{1.7}$ plaque-forming units per ml) from three of the ten ATS-treated mice showing the liver lesions, but not from four other mice, including one with lymphoma, that were given ATS but did not show liver pathology (Table 1). CMV was not isolated from similarly stored tissue extracts from 17 wild mice that bore other spontaneous tumors, but were not treated with ATS and showed no pathological evidence of disseminated CMV infection (Table 2).

However, because of the suspected inactivation of CMV thought to have probably resulted from such prolonged tissue storage, recovery of CMV was attempted from various freshly obtained tissue extracts and cultured cells from wild mice of the same trapping areas (Table 2). None of these mice had been treated with ATS,

TABLE 1. *Isolation of CMV in vitro from wild mice on ATS treatment*

Tissue	Preparation	Histopathological diagnosis	No. of mice positive for CMV[a]
Spleen	10% extract stored 1 year at −70 C	Disseminated virulent CMV infection	2/9
Submaxillary gland	10% extract stored 1 year at −70 C	Disseminated virulent CMV infection	2/5[b]
Liver	10% extract stored 1 year at −70 C	Disseminated virulent CMV infection	1/2[c]
Liver	10% extract stored 1 year at −70 C	Normal	
Liver	10% extract stored 1 year at −70 C	Lymphoma	0/1

[a] These extracts were thawed, and assayed on NIH Swiss embryo cells for characteristic herpes cytopathic effect. CMV was isolated from a total of 3 of 14 mice.

[b] These five mice were the same mice from which spleen extracts were assayed.

[c] One of these two mice was not included in the above group.

TABLE 2. *Isolation of CMV in vitro from healthy wild mice, wild mice with spontaneous tumors, and NIH Swiss controls*

Tissue	Preparation	Histopathological diagnosis[a]	No. of mice positive for CMV isolation per no. of mice tested[b]
Spleen	10% extract stored 1 year at −70 C	Lymphoma	0/10
Spleen/liver pool	10% extract stored 1 year at −70 C	Other tumors[c]	0/7
Spleen	Fresh 10% extract	Normal	5/20
Spleen	Fresh 10% extract	Lymphoma	5/10
Lymph node	Fresh 10% extract	Lymphoma	10/13
Saliva	Fresh mouth swab	Normal	9/20
Spleen	Primary culture	Normal	23/26
Spleen	Primary culture	Lymphoma	3/8
Lymph node	Primary culture	Lymphoma	9/14
Saliva	Fresh mouth swab	Normal NIH Swiss	0/15
Saliva	Fresh mouth swab	CMV-inoculated NIH Swiss[d]	12/12
Spleen	Primary culture	Normal NIH Swiss	0/10

[a] None of these mice were given ATS, and none showed histopathological evidence of disseminated virulent CMV infection.

[b] Extracts, supernatant fluids, and saliva were assayed on NIH Swiss embryo cells.

[c] Hepatomas, four; fibrosarcomas, two; breast carcinoma, one.

[d] These Swiss mice were inoculated intraperitoneally as newborns with a 10^{0} to 10^{-2} dilution of supernatant fluid from a primary culture of lymphoid cells from a spontaneous lymphoma of a wild mouse. The saliva was collected at 5 weeks of age, at which time the Swiss mice were clinically normal.

and none showed inclusions or other pathological evidence of disseminated CMV infection. CMV was isolated in high prevalence (15 out of 23 samples) from fresh extracts of lymphomatous spleen and lymph node, and in lower prevalence (5 out of 20 samples) from equally fresh extracts of normal spleen. The titer of CMV in these extracts was universally low ($10^{1.7}$ plaque-forming units per ml). CMV was directly isolated from the saliva of 9 of 20 normal wild mice, but from none of 15 normal NIH Swiss mice. CMV was also isolated in high prevalence and slightly higher titer ($10^{3.7}$ plaque-forming units per ml) from primary lymphoid cell culture fluids of normal (15 out of 16 samples) and lymphomatous spleens (3 out of 8 samples) and lymph nodes (9 out of 14 samples). Upon passage in vitro on NIH Swiss embryo cells, the titers increased to about 10^6 plaque-forming units per ml. CMV was not recovered from saliva (0 out of 15 samples) or primary spleen cultures (0 out of 10 samples) from normal NIH Swiss mice. However, CMV was recovered from the saliva of all (12 out of 12 samples) NIH Swiss mice inoculated as newborns with CMV of wild mouse tissue culture origin (Table 2).

Cells and supernatant fluids from three of the original wild mouse lymphomatous lymph node cultures and the three corresponding experimentally infected NIH Swiss cultures were all positive for mouse CMV by the complement fixation test which used antisera prepared in NIH Swiss mice. Mouse antibody production tests (10) for detection of 11 other murine viral antigens (lymphocytic choriomeningitis, murine adenovirus, polyoma, murine hepatitis virus, reovirus-3, K virus, Theiler's GD7 virus, Kilham rat virus, syncytial virus-5, Moloney sarcoma virus, and Sendai virus) were negative on these same six cultures. Electron microscope examination of four experimentally infected NIH Swiss cultures showed intranuclear and intracytoplasmic virus particles characteristic of CMV.

Disseminated, virulent CMV infection has never been histopathologically detected in the thousands of untreated control wild mice (from these same trapping areas) which were observed over their natural life span (1), although many mice did have histopathological evidence of spontaneous lymphoma or other tumors. No inclusions have ever been seen in the submaxillary glands or other organs of non-ATS-treated wild mice (1, 2).

Among the 80 mice surviving from 2 to 6 months on ATS treatment (46 were studied with a microscope), no unusual incidence of tumors was detected. However, glomerulonephritis was noted in 10 mice; this finding is very rare in untreated wild mice (1, 2).

Another group of young adult mice from one of the same trapping areas (no. 1) was given azathioprine (Imuran), 1 mg/10 g, twice weekly for 8 weeks. CMV activation was not observed by histopathological examination in 22 of these mice killed after 4 to 8 weeks.

These findings confirm the presence of chronic indolent subclinical CMV infection in high prevalence in healthy wild mice. They show that CMV is shed in saliva, and is also associated with leukocytes or lymphoid tissue in many apparently healthy wild mice and wild mice with spontaneous lymphomas. To what degree the virus genome is integrated nonproductively, as well as expressed as a low level productive infection in these or other tissues, has not been determined. These virus isolation results are similar to those obtained in CMV-inoculated, chronically infected laboratory mice (3). That CMV was not isolated in higher prevalence and titer from the stored extracts of ATS-treated mice, and mice with spontaneous tumors, is probably attributable to inactivation of virus from such prolonged storage. However, it is clearly evident that only ATS treatment has induced disseminated virulent CMV infection, since the ATS-treated mice alone became acutely ill and showed the inclusions and other histopathological features typical of disseminated CMV infection. These results add further evidence for the important role of cellular immunity in preventing activation of chronic or latent CMV (12). The wild mouse is thus an excellent animal model for studying the pathogenesis of naturally occurring, chronic CMV infection, the possible interaction of CMV with type C or other viruses, the early and late differential effects of immunosuppression on lymphocyte function (13), and the possible prevention of virulent CMV dissemination by various antiviral measures.

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