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Mouse Thymic Virus (MTLV; Murid Herpesvirus 3) Infection in Athymic Nude Mice: Evidence for a T Lymphocyte Requirement

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Mouse thymic virus (MTLV; murid herpesvirus 3) is a lymphotropic herpesvirus that cytolytically infects developing T lineage lymphocytes in the thymus of neonatal mice. MTLV establishes a persistent infection and can be recovered indefinitely from infected mice, but nothing is known about requirements for this persistent infection. In order to determine whether T lineage lymphocytes are required for infection, young adult athymic nude (nulnu) mice and euthymic littermates were infected with MTLV and tested for virus shedding. Although euthymic littermates regularly shed virus, in the nude mice only about 20% of isolation attempts up to 100 days postinfection were positive. Blind passage yielded an additional three isolations out of 14 samples (21%). In addition, unlike many other herpesviruses, the virus did not replicate in a number of epithelial and fibroblastic cell lines that were tested. These data confirm that the virus is preferentially T lymphotropic and suggest that infection may require T lineage lymphocytes.
the euthymic controls, all virus isolation attempts were positive through 100 days postinfection, with most of the inoculated pups developing macroscopic thymic necrosis (mean, 92%). In the athymic nude mice, by contrast, although virus was recovered at least once from each nude mouse (Table 1), only 5 of 27 primary isolation attempts from Day 6 through Day 98 were positive, giving an isolation rate of 18.5%. In positive isolations, fewer inoculated pups showed thymic necrosis (mean, 14.2%) than pups that received swab fluids from controls (Table 1).

For many viruses, virulence or tissue tropism can be altered by conditions of infection (7); introduction of MTLV into the nude mouse could therefore have altered virus infectivity. Blind passage is a standard method for restoring virulence and increasing assay sensitivity (1, 2). Although limitations in the number of litters available did not allow every negative sample to be tested, additional litters of normal newborn mice were inoculated with fresh homogenates (10–20%, w/v) of randomly selected negative thymuses and salivary glands, representing various test dates up to Day 48, from 14 assay litters that had received swab fluids from nude mice. Up to two blind passage cycles were performed. These passages yielded a total of three additional isolations (21%) (Table 2). In an additional biological passage, separate litters of normal neonatal mice were inoculated with swab fluids from each of the nude mice (Day 50 postinfection) and these newborns were allowed to reach adulthood. Homogenates of salivary glands and thymuses were prepared from these animals, and tested for MTLV by infectivity assay in fresh litters of newborn mice. One sample, from an animal that had received fluids from nude mouse 2, was positive on assay. This represented only the second isolation from this nude mouse, which was positive on primary isolation on Day 6 and had been consistently negative thereafter. Issues from uninfected mice (as negative controls), or other negative samples, passed at the same time as nude mouse samples were consistently negative.

Nude mice lack T lymphocyte function, but nonspecific immune functions, such as natural killer (NK) activity, are normal or even increased in the nude mouse (8). NK-deficient nude mice have recently been developed (9). In order to determine whether reduced virus shedding in the nude mice could have been due to active suppression by NK cells or similar mechanisms, eight NK-deficient BALB/c-C57 beige hybrid mice (Life Sciences, St. Petersburg, FL) were infected with MTLV and tested at various times after infection. There were six positive or possibly positive isolations out of a total of 46 attempts (13%) from 1 to 14 weeks postinfection. Virus was isolated consistently more than once from only one mouse, which was negative on Day 6, positive on Days 19, 26, and 41, positive with a lower level of virus shedding on Day 33, and negative on Day 48 and thereafter. One other nude mouse appeared to shed a low level of virus on Day 19 and was possibly positive on Day 34, but appeared negative on other isolation attempts including Days 6, 41, and 48 postinfection. The other six nude mice were negative on all isolation attempts (e.g., Days 6, 19, 26, 33–34, 41, 48 and thoracotomy through 14 weeks).

Thus, MTLV establishes a persistent infection in the nude mouse, but virus shedding may be reduced in comparison with euthymic mice. Aside from thymus-derived (T) lymphocytes, most other cell types appear to be normal in the nude mouse (5, 6). Therefore, if persistent MTLV infection were to involve mostly nonlymphoid cells, all isolations should have been positive

### TABLE 1

| Day postinfection | Group* | Percentage of inoculated pups showing thymic necrosis |
|------------------|--------|------------------------------------------------------|
| 6                | Nude (1) | 18.8                                                |
|                  | Control | 93.3                                                |
| 14–15            | Nude (0) | 0.0                                                 |
|                  | Control | 87.5                                                |
| 23               | Nude (1) | 5.6                                                 |
|                  | Control | 100.0                                               |
| 30               | Nude (1) | 13.6                                                |
|                  | Control | 100.0                                               |
| 34               | Nude (2) | 18.8                                                |
|                  | Control | 92.3                                                |
| 44               | Nude (0) | 0.0                                                 |
|                  | Control | 71.4                                                |
| 48               | Nude (0) | 0.0                                                 |
|                  | Control | 100.0                                               |
| 98               | Nude (0) | 0.0                                                 |
|                  | Control | 100.0                                               |

Note. Infected ICR Swiss athymic nude (nu/nu) and euthymic (+/nu) littermate controls (total number of mice, eight) were tested for virus shedding at the times shown. For virus assay, mouth swabs were taken using cotton swabs moistened with Dulbecco's modified Eagle's medium + 10% fetal bovine serum and 50 μg gentamycin per milliliter (DME-10), and tested for MTLV by infectivity assay in newborn BALB/c mice as described in the text. Additional infectivity assays in newborn ICR background Swiss mice gave the same results.

* Values in parentheses represent number of nude mice yielding positive isolations on that day. Infectivity assays from four nude mice through Day 30, three on Day 34, three on Days 44/48 together, and two on Day 98.
after initial infection. That this did not appear to occur suggests that T lineage lymphocytes might well be the major target cells for persistent infection. This has been demonstrated in Herpesvirus sylvilagus, a rabbit herpesvirus, which can be isolated from saliva and salivary glands of infected hosts, but the virus is associated with lymphocytes and not with epithelial cells (12). MTLV similarly persists in salivary gland (1, 2), but electron microscopy of salivary gland epithelial cells of infected animals revealed no abnormalities (1), and no viral antigens could be detected in salivary gland epithelial cells (2).

There are two likely explanations for the apparently low level of virus shedding in the nude mouse. Although nude mice lack mature functional T lymphocytes, early T lineage precursors are present in the nude mouse (6, 8, 10); under certain conditions, these cells in the nude mouse can also differentiate extrathymically into mature T lymphocytes (11). In addition, several reports indicate that a small number of apparently mature (bearing the T lymphocyte marker Thy 1) T lineage lymphocytes are present in nude mice throughout their lives (6, 10). A small population of this type could be persistently infected and responsible for the low level of virus shedding observed. This population reportedly increases with age or intercurrent infection (11). Infection with the coronavirus mouse hepatitis virus (MHV), a common murine pathogen endemic in many nude mouse colonies, has also been reported to induce extrathymic T cell differentiation in nude mice (11). Some of our preliminary results (data not shown) suggest that nude mice older at primary infection, or nude mice that have been infected with MHV, appear more likely to shed MTLV. Alternatively, there could be a secondary cell type (such as the macrophage) in which virus can persist at low levels even in the absence of T lymphocytes. Macrophage infection by another T lymphocytolytic virus, human immunodeficiency virus (HIV-1), has been documented (13). Our preliminary attempts to isolate MTLV from adherent peritoneal macrophages of persistently infected euthymic mice have been negative so far. However, low levels of infected cells might not be detected. Specific molecular probes, such as genomic DNA probes or monoclonal antibodies, are not presently available for this virus. We are in the process of producing specific high-titer antisera and other probes in order to test the presence of MTLV in various lymphoid and hematopoietic tissues and in nerve cells.

As regards other susceptible cells, original attempts to grow the virus in mouse embryo cells or mouse embryo kidney cells, among others, were unsuccessful (7). Our attempts to infect a variety of epithelial and fibroblastic cell cultures that support the replication of many other viruses have been uniformly negative. For infection, subconfluent monolayers of cells were incubated for 90 min with DME-10 containing 500–2000 ID50 MTLV and polybrene (30 µg/ml), then fed (DME supplemented with 5% fetal bovine serum), incubated, and tested for MTLV by infectivity assay at 5–7 days and at 2 weeks. Cells tested included AR42J rat glandular epithelium, BALB CL.7 mouse fibroblast, BALB 3T3 fibroblast, C1271 mouse mammary tumor, J774A.1 mouse macrophage, NCTC 1469 mouse liver, SC-1 feral mouse embryo, TCMK-1 mouse kidney, 32 I A rat hepatoma, A549 human lung carcinoma, 11Cp-2 human epidermoid carcinoma, MDCK canine kidney epithelium, Vero African green monkey kidney, W6/32 diploid human amniotic epithelium, 324K human kidney, and primary cultures of African green monkey kidney, cynomolgous monkey kidney, rabbit kidney, and rhesus monkey kidney. Replicate cultures, observed up to a month for cytolysis or focus formation, remained negative throughout. Additional cultures cocultivated with primary MTLV-infected thymocytes (5 × 10⁶/ml, harvested Day 5 postinfection) also remained uninfected. These results suggest that MTLV is unable to productively infect many cell lines that support the cytopathic replication of many viruses including other lymphotropic herpesviruses. Taken together, these lines of evidence appear to implicate the T lineage lymphocyte as the primary target of both acute and persistent MTLV infection.

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**TABLE 2**

**SUMMARY OF VIRUS ISOLATIONS FROM ICR SWISS ATHYMIC NUDE MICE**

| Mouse* | Primary | Passage | No. of samples | Isolation rate (%) |
|--------|---------|---------|----------------|-------------------|
| Nude 1 | 1       | 1       | 14             | 14.3              |
| Nude 2 | 1       | 1       | 10             | 20.0              |
| Nude 3 | 2       | 0       | 7              | 28.6              |
| Nude 4 | 1       | 1       | 10             | 20.0              |
| Controls | 16 | —       | 16             | 100.0             |

* Total number of mice, eight (four nulnu, four +lnu controls). This Table includes all positive isolations (primary or on passage) from the ICR Swiss nulnu mice. Infectious virus in mouth swabs or in passaged tissues (homogenates of pooled thymuses and salivary glands) was determined as described in text.
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