Brief Definitive Report

EXPRESSION OF ENDOGENOUS MURINE LEUKEMIA
VIRUSES DURING THE COURSE
OF A PROTRACTED IMMUNOLOGICAL DISORDER*

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Lymphoreticular tumors have been observed in mice with an experimentally
induced immunological disorder, the graft-versus-host reaction (GVHR) (1, 2). Young adult (BALB/cJ × A/J)F1 hybrid (CAF1) mice in which a protracted form
of GVHR is induced by the injection of immunocompetent BALB/cJ cells develop
tumors after some months. The incidence of tumors increases with age. Thus, at
12 mo the incidence is 27% and by 18 mo it is 55%, compared with a 5% incidence
among control CAF1 mice (2). Tumorigenesis in the GVHR appears to be due to
endogenous murine leukemia virus (MuLV) activated by the immune disturb-
ance, because cell-free extracts prepared from the reticular tissues of GVHR-
CAF1 mice: (a) induce lymphoreticular tumors in syngeneic recipients; (b)
contain MuLV complement-fixing antigens and both types of naturally occur-
ring mouse-tropic MuLV, namely, B-tropic and N-tropic MuLV (3). Experi-
ments to confirm that endogenous MuLV is indeed responsible for tumor induc-
tion in this experimental model have been hampered by the latency of the agent.
Cell-free extracts from GVHR-CAF1 mice cause tumors in only a minority of
recipients after at least 15 mo (3). However, serial in vivo passage of such
extracts has resulted in preparations that cause tumors in most recipients
within 12 mo (4). These preparations contain B-tropic MuLV, and when inocu-
lated into infant syngeneic mice, B-tropic MuLV is found in the reticular tissues
as early as 2 wk thereafter. The virus persists in the reticular tissues throughout
the latent period and is present in the tumors that subsequently develop.
However, when the same preparations are injected into young adult recipients,
there may be transient B-tropic MuLV replication, but the virus subsequently
disappears from the reticular tissues and no tumors develop (4).

To understand the mechanism by which the oncogenic potential of endoge-
 nous B-tropic MuLV is expressed in the GVHR model, we asked when B-tropic
MuLV first appears and how efficiently it replicates. Hirsch and his colleagues
reported the rapid activation of N-tropic MuLV in young CAF1 mice after the
injection of BALB/c spleen cells (5). However, their observations were limited to
the first 4 mo of the GVHR. We have looked for the presence of both B- and N-
tropic MuLV throughout the entire period between initiation of the GVHR and

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Fig. 1. Age-related appearance of B- and N-tropic MuLV among CAF/J male mice undergoing a graft-versus-host reaction (GVHR-CAF/J) and among normal control CAF/J male mice (NCAF/J). Percentage of mice with B-tropic (○ ○), with N-tropic (■ ■) MuLV. (∙ ∙ ∙) number of mice tested for MuLV.

development of tumors and have found that the GVHR accelerates the induction of both viruses in CAFj mice and preferentially enhances the replication of B-tropic MuLV.

Materials and Methods

Induction of GVHR. A GVHR was induced in 7-wk-old (BALB/cJ × A/J)F1 hybrid (CAF1) male mice by four weekly intraperitoneal injections of approximately 50 × 10⁶ cells prepared from the pooled spleens of 6- to 8-wk-old BALB/cJ male mice.

Virus Assay. Spleen cell suspensions (20 × 10⁶ cells/ml) were prepared in Eagle's minimal essential medium. Aliquots of 0.1 ml, or their 10-fold dilutions, were plated as infectious centers on both BALB/cN and NIH Swiss mouse embryo cells, and assayed for virus by the UV-XC procedure (6), modified as previously described (4).

Results

GVHR-CAF1, mice, and normal uninjected CAF1, and BALB/cJ male mice, were killed at ages ranging from 8 to 84 wk. We tested 209 individual spleen cell suspensions, 117 from GVHR-CAF1, mice, 59 from normal uninjected CAF1, mice, and 33 from normal uninjected BALB/cJ mice.

Expression of B- and N-tropic MuLV in GVHR-CAF1, mice and in normal control CAF1, mice is shown in Figs. 1 and 2. Both these viruses were detected as the mice aged. However, viral expression was considerably accelerated and enhanced in the GVHR group where both B- and N-tropic MuLV were detected earlier, in a greater proportion of mice, and in higher titer. In the GVHR-CAF1, mice, B- and N-tropic MuLV appeared 12 and 20 wk earlier, respectively, than in the control CAF1, mice (Fig. 1). Not only were the titers of both viruses higher in the GVHR mice than in the control group, but the titer of B-tropic MuLV was consistently about one log greater than that of N-tropic MuLV (Fig. 2). Another distinctive feature in the GVHR mice was the biphasic nature of viral expres-
sion. From 12 to 20 wk, and after a transient eclipse around 40–44 wk, both viruses rapidly surfaced in a majority of mice, quickly gaining substantial titers. Although N-tropic MuLV appeared first in both waves, the titer of B-tropic MuLV rose faster and higher. In the control CAF$_1$ group, neither virus was detected until 32 wk. Both then followed the same gradual, muted pattern of emergence, without remission, until 48 wk, when N-tropic MuLV began to wane.

Among the virus-positive mice, 58% in the GVHR group and 63% in the control CAF$_1$ group expressed both B- and N-tropic MuLV. Both types of MuLV were deemed to be present in an individual spleen cell suspension if the number of infectious centers on BALB/cN mouse embryo cells and that on NIH Swiss mouse embryo cells showed less than a 50-fold difference.

Expression of mouse-tropic MuLV in normal BALB/cJ male mice differed from the control CAF$_1$ group in that N-tropic MuLV was first detected rather early, at 20 wk (Fig. 3). The percentage of positive mice rose quite slowly, however, and did not exceed that in the control CAF$_1$ group. Emergence of B-tropic MuLV was similar in both groups of normal mice, as were the titers of both viruses, at least until 48 wk. Thereafter, the mean titer of B-tropic MuLV rose more steeply in the BALB/c group.

Four spleen cell suspensions, each prepared from a pool of 12 spleens removed from 6- to 8-wk-old BALB/cJ male mice, and used to induce the GVHR in CAF$_1$ male mice, were also tested for MuLV. All four pools contained neither B- nor N-tropic MuLV.

**Discussion**

The cells of mice contain the necessary genetic information to code for the constituents of MuLV (7). In mice such as the AKR strain, the appearance of infectious MuLV in substantial titer early in postnatal life is associated with a
high spontaneous incidence of lymphoma within a year (8). This high leukemia strain is of the Fv-1<sup>nn</sup> genotype, and its tumors are caused by endogenous N-tropic MuLV (9).

Evidence that endogenous B-tropic MuLV may also be oncogenic has come from study of BALB/c mice. Here, however, neoplasms are rare before 1 yr of age (10). Thereafter, the incidence of tumors increases with age, the commonest of the malignant neoplasms being lymphoreticular tumors which occur in 19% of BALB/c mice over 2-yr old (10). B-tropic MuLV has been etiologically incriminated in the development of these tumors (11). A study of MuLV expression during the life-span of BALB/c mice showed that although 90% of mice under 6 mo were virus negative, the incidence of demonstrable infectious mouse-tropic MuLV rose rapidly thereafter (12). Most of the viral isolates before 1 yr were N-tropic; isolations of B-tropic MuLV became more frequent in older mice, but only after 24 mo did the isolation rate exceed that of N-tropic MuLV (12).

Our study of MuLV expression in normal and GVHR-CAF<sub>1</sub> mice shows that both B- and N-tropic MuLV are normally detected after 6 mo of age, but that the GVHR accelerates induction of both viruses and preferentially enhances replication of B-tropic MuLV. Since CAF<sub>1</sub> mice are of the Fv-1<sup>bb</sup> genotype, B-tropic MuLV should replicate more efficiently than N-tropic MuLV (9). It is not clear, however, why such preferential replication is not seen in either normal CAF<sub>1</sub> mice or normal BALB/c mice (also of the Fv-1<sup>bb</sup> genotype) until relatively late in life. This might reflect greater inducibility of N-tropic MuLV, coupled with resistance to spread of infection, and relative absence of cells in which either mouse-tropic MuLV could replicate. Oncornaviruses need dividing cells for replication (13). The GVHR provides a large pool of dividing lymphocytes, an admixture of donor thymus-derived (T) cells and host bone-marrow-derived (B)
cells, recruited through allogeneic activation (14). The dividing B cells may preferentially replicate the induced viruses, since lipopolysaccharide-stimulated B cells do support MuLV growth (15).

It is not known why two mouse-tropic MuLV are expressed in CAF1 and BALB/c mice as they age, nor how each virus influences the other. However, the earlier appearance and enhanced replication of B-tropic MuLV in GVHR-CAF1 mice is probably causally related to induction of tumors, since the same tumors are reproduced by serially passaged GVHR extracts containing B-tropic MuLV, but no N-tropic MuLV (4). Xenotropic MuLV (16) is not measured in our assay, but we are currently studying its possible role in tumor induction.

In this model, the oncogenic potential of endogenous oncornavirus is revealed through an immunological disorder. Dividing cells available early in the GVHR may enable endogenous MuLV to passage through successive generations of cells, resulting not only in earlier and higher titers of infectious virus, but possibly also in the emergence of oncogenic variants.

Summary
Mice of the low leukemia (BALB/cJ x A/J)F1 hybrid (CAF1) strain express B- and N-tropic infectious murine leukemia virus (MuLV) after the age of 6 mo. Initiation of a protracted immunological disorder, the graft-versus-host reaction (GVHR), at 7 wk of age, accelerates the induction of both these mouse-tropic endogenous viruses, and preferentially enhances the replication of B-tropic MuLV. The earlier appearance of B-tropic MuLV in a greater proportion of mice and in higher titer is thought to be causally related to the eventual development of lymphoreticular tumors in the GVHR mice, since previous studies have shown that these same tumors can be reproduced by inoculating syngeneic recipients with serially passaged GVHR extracts containing B-tropic MuLV.

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