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Murine Leukemia: A Virus-Induced Autoimmune Disease?

Abstract. *Thymocytes from mice carrying Moloney murine leukemia virus since birth are cytotoxic for normal syngeneic fibroblasts; they are much less cytotoxic for the same cells infected with this virus. The cytotoxic thymocytes appear to increase in number with age of the carrier mice and are present both during preleukemic and leukemic periods. These results suggest that lymphomas in carrier mice result from a sequence of events initiated by intrathymic destruction of normal cells by virus-infected cells, and culminating in the unrestricted proliferation of autoaggressive clones in the thymic cortex.*

The concept that clones of lymphoid cells with antiself activity may be involved in the pathogenesis of both autoimmune and lymphoproliferative disorders is not new (*1*). It has been proposed that viruses might trigger the proliferation of these clones of lymphocytes, particularly when normal immune surveillance mechanisms are disturbed (*1, 2*). These hypotheses still lack necessary experimental support, although there is evidence that autoimmune reactivity, viruses, and oncogenesis may be related (*3*). We now report that, during the course of murine leukemia virus (Moloney Strain, MuLV-M) leukemogenesis, thymus cells of congenitally infected mice, both in preneoplastic and neoplastic periods kill normal syngeneic fibroblasts in an in vitro assay but spare identically derived but MuLV-M infected fibroblasts.

We used male mice from a colony of C3/HeJ mice (Jackson Laboratories) carrying MuLV-M since birth (carriers). Normal C3H/HeJ mice served as controls. C3H/HeJ embryo cell lines of common origin infected with MuLV-M or not infected were used as target cells for thymocyte cytotoxicity assays. By an infectious center assay, 90 to 100 percent of the infected target cells were producing infectious MuLV. Uninfected target cells were negative for virus. The establishment of the carrier colony, the target cell lines, and the infectious center assay have been described (*4*).

Thymocyte suspensions were prepared from the pooled thymus glands of three to four carrier or normal mice (*4*). Both weanling (4 weeks old) and adult (12 weeks old) mice were used, depending on the experimental design. After purification of the cells on Ficoll-Hypaque gradients (*5*), adherent cells were removed by incubating the cells recovered from the gradient interface in plastic culture dishes for 30 minutes at 37°C. The procedure was repeated once, and the remaining nonadherent cells were then used. Thymocyte viability was 98 to 100 percent (trypan blue exclusion). These cells had the morphological appearance of small- to medium-sized lymphocytes. Thymocyte extracts were prepared by sonic disruption of cell suspensions (Raytheon Sonifier; 3 amp for 2 minutes).

The thymocyte cytotoxicity assay (*4*) measures both cytotoxicity and inhibition of cell replication. The cells were placed in Falcon Microtest II plates (approximately 150 target cells per well). After 18 hours, thymocytes (5×10^5) were added to each well. The plates were incubated for 48 hours, the thymocytes were removed, and the target cells were fixed, stained, and counted. When uninfected fibroblasts are placed in Falcon Microtest II plates 48 ± 1.4 percent of the cells survive for 18 hours whereas 39 ± 1.4 percent of infected fibroblasts survive for 18 hours after their addition to the wells. These differences are significant ($P < .01$ by paired *t*-tests) and are reflected in Tables 1 and 2. However, the differences in the number of cells that survive do not interfere with interpretation of the table, since interpretation of reductions in number of target cells by carrier thymocytes should always be compared with reduction of that same target cell by normal thymocytes (group comparisons in Tables 1 and 2 should be made horizontally).

Thymocytes from adult carrier mice were combined in vitro with syngeneic normal or MuLV-M infected target cells. These thymocytes were cytotoxic for normal target cells, but less so or not at all for infected target cells ($P < .01$ by paired *t*-test) (Table 1). The

Table 1. Reactivity of thymocytes from MuLV-M carrier and normal C3H/HeJ mice against infected and noninfected target cells. Each mean value (± standard error) was derived from at least ten replicate observations. Both cell lines are routinely passed in parallel and are used at the same passage level for a given experiment; NT, not tested.

Target cells	Mean number of target cells remaining and reduction (%) after reaction with thymocytes							
	Adult normal	Reduction*	Adult carrier	Reduction	Weanling normal	Reduction	Weanling carrier	Reduction
Infected	63 ± 3.3	0	58 ± 3.6	8	75 ± 6.3	0	74 ± 3.3	0
Noninfected	108 ± 8.6	0	26 ± 2.5	76†	112 ± 8.3	0	70 ± 4.5	38†
Infected	18 ± 1.9	0	21 ± 1.4	0	17 ± 1.2	6	18 ± 9.0	0
Noninfected	38 ± 14.6	0	8 ± 0.2	79†	38 ± 1.1	0	28 ± 1.0	26†
Infected	55 ± 4.8	0	62 ± 6.3	0	NT		NT	
Noninfected	62 ± 5.8	0	39 ± 3.2	37†	NT		NT	
Infected	45 ± 2.7	0	40 ± 3.3	11	NT		45 ± 3.6	0
Noninfected	157 ± 13.1	0	35 ± 2.8	78†	NT		50 ± 3.6	68†

* Percent reduction of infected or noninfected target cells is relative to the reduction by normal thymocytes on infected or noninfected target cells. † Significant differences ($P < .01$ by paired *t*-tests) compared with the effect of adult normal or weanling normal thymocytes on noninfected target cells.

Table 2. Reactivity of thymocytes from normal and thymoma-bearing MuLV-M carrier C3H/HeJ mice against infected and noninfected target cells. Mean values and derivation of thymocytes are as described in Table 1; NT, not tested.

Target cells	Mean number of target cells remaining and reduction (%) after reaction with thymocytes from:					
	Adult normal	Reduction*	Thymoma bearing	Reduction	Adult carrier	Reduction
Infected	NT		NT		NT	
Noninfected	124 ± 5.4	0	26.2 ± 1.8	79†	26.4 ± 2.1	79†
Infected	49.9 ± 4.8	0	43.0 ± 5.2	14	NT	
Noninfected	81.6 ± 9.7	0	12.0 ± 1.5	86†	NT	

* Percent reduction is relative to the reduction by normal thymocytes on infected or noninfected target cells. † Significant differences ($P < .01$ by paired t-tests) compared with the effect of normal adult thymocytes on noninfected target cells.

carrier animals appeared healthy, and there was no gross evidence of lymphoma. However, their thymus glands were smaller than those of normal 12-week-old mice (17.9 ± 1.2 mg for carriers; compared with 33.6 ± 1.1 mg for normal mice; $P < .01$ by paired t-test). One lobe was often smaller than the other. This is evidence of the premature thymus involution accompanying MuLV-M induced leukemia (*6*). The sizes of the thymus glands from weanling age carrier mice were not appreciably different from those of normal weanling age mice. Thymocytes from 4-week-old carriers were also cytotoxic for uninfected target cells but were not cytotoxic for MuLV-M infected target cells (Table 1).

Previous experiments have shown that, unlike thymocytes, peripheral lymphocytes from 8- to 10-week-old carrier mice were not reactive against noninfected target cells; such peripheral lymphocytes from seven out of nine of these mice were cytotoxic for infected fibroblasts, suggesting the presence of cell-mediated immunity against MuLV-M (*4*).

The carrier thymocytes were cytotoxic against noninfected target cells even after the thymus had become overtly lymphomatous (Table 2) at 15 to 25 weeks of age. In two experiments, such thymocytes reduced the number of noninfected target cells by 79 to 86 percent. Thus, the number of cytotoxic thymocytes in carriers appears to increase with age and progression of lymphoma.

Dose-response studies indicated that thymocytes from carrier mice were cytotoxic for uninfected target cells at ratios of 100 : 1 or greater, whereas these thymocytes were not at all reactive against infected target cells at this ratio. At thymocyte-to-target-cell ratios of 1000 : 1 or greater, there was some cytotoxicity against both target cell populations although there was always a significantly greater degree of cytotoxicity against the noninfected fibroblasts (*7*).

To determine whether viable thymocytes were necessary for cytotoxic reactivity by carrier thymocytes, normal and carrier thymocytes were prepared from 12-week-old mice and equal amounts of sonicated (zero percent viable by trypan blue exclusion) or whole thymocytes (98 to 100 percent viable) were added to infected or noninfected target cells. Whole carrier thymocytes caused 75 percent greater reduction of noninfected target cells than did whole normal thymocytes. Normal and carrier thymocyte sonicates caused about the same percent reduction of both infected or noninfected target cells (34 to 45 percent).

Our results indicate that during their entire life after weaning MuLV-M carrier mice have thymocytes that are vigorously cytotoxic for fibroblasts from a normal syngeneic cell line; these thymocytes are much less or not at all cytotoxic for the same cells infected with MuLV-M. Among the aggressor carrier thymocytes, cells are present which are infected with and shedding MuLV-M virus particles (*7*). The thymus is believed to be a primary target organ for murine viruses known to cause lymphomas (*6*, *8*). Metcalf found that mice infected with MuLV-M underwent a premature asynchronous thymus involution which was preceded by medullary expansion, cortical "thinning" and the abnormal appearance of germinal centers in the thymus (*6*). Involution was accompanied by marked cellular destruction and was followed by the focal appearance of tumor cells in the thymus cortex. During the course of these events, such mice develop hyporesponsiveness to certain specific antigens, such as sheep erythrocytes (*9*) and to nonspecific mitogens, such as phytohemagglutinin (*7*). Metcalf interpreted the appearance of germinal centers and cellular destruction in the thymus of MuLV-M infected mice as probably representing a localized immune response by normal immunocompetent cells against virus-infected thymus cells. Our data suggest that the converse reaction may also occur. It appears that a subpopulation of thymocytes can be altered by MuLV-M infection so that these cells recognize normal noninfected cells as foreign. We do not know whether the pattern of reactivity in vitro we observed is representative of autoimmunity in vivo. Destruction of normal thymocytes or their precursors by autoaggressive cells could lead to depression of T cell dependent immunological functions such as tumor rejection. Thus, autoaggressive cells infected by the shedding MuLV-M would increasingly avoid recognition as the numbers of normal cells decreased. Replication and eventual spread of these autoaggressive cells could account for metastatic lymphoproliferative disease.

Although as yet we have no conclusive evidence that the aggressor carrier thymocytes are selectively those infected with MuLV-M, preliminary studies suggest that this may be the case. Nonlytic infection of cells by certain viruses can result in functional alterations of such cells (*10*). Bretscher (*2*) has proposed that certain viruses replicating in or expressing new antigens on the surface of thymus dependent T cells could indirectly lead to a nonspecific induction and clonal growth of preexisting but repressed antiself cells. He further suggested that the thymus would be the primary site for such an event. Our results are compatible with this interpretation.

Studies (*11*) have shown that normal fetal and neonatal murine thymocytes can react against normal isogenic cells in vitro, but that this property disappears as the mice mature. However, autoimmune responses may develop in aged mice of certain strains (*12*). A similar pattern has been described for overt signs of endogenous MuLV infection; fetal and neonatal murine cells frequently possess MuLV-associated antigens or infectious virus particles that disappear shortly before or after birth, then reappear with aging (*13*). Perhaps the expression of MuLV is necessary for autoreactivity and the continued presence of MuLV in the carrier animal merely results in the maintenance and accentuation of this normal fetal-neonatal pattern.

The accumulating evidence for an association between virus infections, auto-

immune phenomena, and lymphoproliferative disorders in several models, including New Zealand Black mice (*3, 14*) and chronic graft versus host disease (*3*), suggests that the same relationships may exist in man. In all of these situations, the common thread may be a virus-induced autoaggressive reaction that may or may not progress to neoplasia depending on the interrelationships between clonal proliferation of autoimmune cells and antiviral and antitumor immunity.

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Chemical Facilitation of Thermal Conduction in Physiological Systems

Abstract. *Experimental evidence supports the concept that solutes capable of reversible chemical reaction can passively facilitate thermal conduction within their solutions. Myoglobin and bicarbonate are suggested as energy carriers in muscle, having the combined capacity for conveying all waste metabolic heat produced under normal physiological conditions. The concept is extended to convective heat transfer in vivo; this implicates hemoglobin.*

There are a number of physiological systems in which the transmission of a gas in solution can be passively facilitated by the diffusion of a carrier for which it has a reversible chemical affinity, for example, oxygen in combination with myoglobin (*1*). A similar regenerative process is proposed here for the conduction of heat.

Let us consider a closed system in which a particular substance can exist in two chemical states, A and B, which have different enthalpies, H, and are readily interconverted according to the rapid reversible reaction:

$$\underset{(1-x)}{\mathrm{A}} \rightleftarrows \underset{(x)}{\mathrm{B}} + \text{heat}\ (-\Delta H) \quad (1)$$

where the position of equilibrium, and hence the degree of conversion, x, are determined at any point by the local temperature, T, according to the Van't Hoff isochore.

If the system is macroscopically static, and opposite sides are maintained at different temperatures, T_1 and T_2 (Fig. 1), then the substance must act as a passive energy carrier if it is capable of translational movement within the medium. Molecules moving up the temperature gradient will tend to assume form A, with absorption of heat,

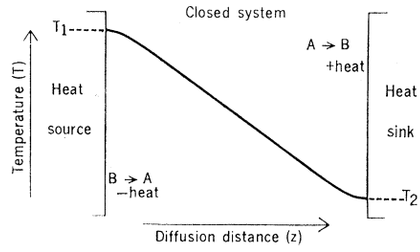

Fig. 1. A closed system with opposite sides maintained at different temperatures, T_1 and T_2.

while those whose random thermal motions take them to cooler regions will tend to revert to B with release of heat. Thus the transfer of energy from the region of the source, T_1, to that of the sink, T_2, can be effected by the diffusion of carrier molecules which can also exchange heat transmitted by simple thermal conduction. An analysis of the system in Fig. 1 has been made (*2*) and gives expressions exactly analogous to those for facilitated diffusion (*3*). For the ideal case of local chemical equilibration, these reduce to a simple form giving the overall steady-state heat flux ($\dot{Q}$) as:

$$\dot{Q}\,\bar{z} = K(T_1 - T_2) + DC(\Delta H)(x_2 - x_1) \quad (2)$$

where $\bar{z}$ is the effective mean path length; K is the thermal conductivity without carrier; D is the mean diffusion coefficient of the carrier whose molar concentration is C; and x_1 and x_2 are the degrees of conversion at temperatures T_1 and T_2 respectively. The first term on the right side in this equation represents simple conduction, while the second represents chemical facilitation.

As a simple test of this thermal carrier hypothesis, alternating current was used to generate heat at a fixed rate in a thermistor whose temperature was recorded by a d-c circuit. The thermistor did not reach as high a temperature immersed in hemolyzed human blood at $P_{O_2} = 53$ mm-Hg and $P_{CO_2} = 38$ mm-Hg as it did when the hemoglobin was "poisoned" by the substitution of 1 percent CO for N_2 in the equilibration gas at the same oxygen and carbon dioxide tensions. In another series of experiments, the temperature was slightly higher for normal saline than for isotonic bicarbonate solution at $P_{CO_2} = 38$ mm-Hg.

Since convection is dependent upon conduction, whichever of these processes was primarily responsible for the thermistor cooling, it is difficult to invoke any interpretation of these findings other than an increase in the thermal conductivity. At least, this holds when one solute is present under conditions conducive to its reversible chemical transformation over the prevailing temperature gradients, namely, Hb at $P_{O_2} = 53$ mm-Hg and HCO_3^- at $P_{CO_2} = 38$ mm-Hg according to the reactions:

$$\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2} + 18\ \text{kcals} \quad (3)$$

$$\mathrm{H^+} + \mathrm{HCO_3^-} \rightleftarrows \mathrm{H_2CO_3} + 2\ \text{kcals} \quad (4)$$

The above findings are consistent with the unusually high heat transfer rates in the gaseous phase that have been