Lymphocytic cyclic AMP levels were elevated in a dose-dependent manner as the concentration of adenosine was increased up to 75 μM (Fig. 2). At all concentrations of adenosine tested, the elevation of cyclic AMP was markedly higher in the presence of the deaminase inhibitor. Purified (*13*) human peripheral blood lymphocytes also exhibited a dose-dependent increase in cyclic AMP in response to exogenous adenosine; a concentration of 3.8 μM adenosine caused a 2.0-fold increase in cyclic AMP after 30 minutes of incubation.

Several lines of evidence indicate that the ability of adenosine to inhibit cytolysis is unrelated to the inhibition of pyrimidine nucleotide synthesis described by Green and Chan (*3*). First, the inhibitory effect of adenosine is not prevented by the presence of a tenfold molar excess of uridine; uridine itself has no effect on cytolysis. Second, the inhibitory effect of adenosine is spontaneously reversible during prolonged incubation of cytolytic lymphocytes and adenosine. Third, high-pressure liquid chromatographic analysis (*14*) of the nucleotides of the lymphocyte population after 60 minutes of incubation with adenosine showed no effect on the pyrimidine nucleoside triphosphate pools.

The mechanism by which adenosine inhibits LMC appears to involve an elevation of lymphocytic cyclic AMP; the concentrations of adenosine which inhibit LMC (Table 1) are also effective in causing an increase in lymphocytic cyclic AMP (Fig. 2). The time-dependent decay of adenosine-enhanced cyclic AMP to control levels (Fig. 1) precedes the gradual recovery of cytolytic activity observed when the cells were incubated with adenosine (with or without EHNA) before the cytolytic assay (Table 2). This observation [in addition to the observation that cyclic AMP concentrations rapidly decrease after removal (washout) of adenosine and EHNA] is consistent with the hypothesis that the inhibitory effect of adenosine on LMC is mediated via raised cyclic AMP concentrations in the effector lymphocytes and that this inhibitory activity is reversed once the cyclic AMP concentration falls below some critical value.

It is apparent from this in vitro model that the absence of adenosine deaminase in lymphoid tissue would render these cells particularly sensitive to inhibition of an immune effector mechanism by adenosine. Adenosine formation and its subsequent reutilization (via adenosine kinase) or catabolism (via adenosine deaminase) appear to occur as a normal feature of cellular purine nucleotide metabolism (*15*). Deficiency of the enzyme adenosine deaminase may allow an abnormal expansion of the lymphoid pool of adenosine and thus lead to immunosuppressive effects such as that demonstrated in this report.

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Cell-Mediated Immunity to Antigens Associated with Endogenous Murine C-Type Leukemia Viruses

Abstract. *Splenic lymphocytes from (BALB/c × A/J) F_1 mice are cytotoxic for BALB/c fibroblasts infected with an endogenous C-type leukemia virus, but are not cytotoxic for uninfected BALB/c fibroblasts. These results indicate that mice do not exhibit cell-mediated immune tolerance to antigens associated with endogenous C-type viruses.*

Most, if not all, mouse strains carry within their cells the necessary genetic information to code for the production of endogenous C-type leukemia viruses (*1*). Expression of such virus genetic information varies greatly among strains, and may be related to the subsequent development of lymphomas. Little is known of the host's natural immune responses to endogenous C-type viruses. Recent studies have indicated that inbred mice develop humoral antibodies to several endogenous C-type viral antigens (*2*). We report here studies that indicate that mice may also develop cell-mediated cytotoxic responses against antigens associated with endogenous C-type viruses.

Male (BALB/c × A/J) F_1 mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Two BALB/c embryo fibroblast cell lines of common origin were established. One cell line was infected with an endogenous mouse-tropic C-type virus activated during a graft-versus-host reaction in a (BALB/c × A/J) F_1 mouse injected with BALB/c splenocytes (*3*). After 35 passages, 90 to 100 percent of these cells were producing infectious virus as determined by an infectious center assay previously described (*4*). The other BALB/c cell line was not infected, but was passed in parallel with the virus-infected line; this line remained virus-negative for both mouse-tropic and xenotropic C-type viruses, as determined by RNA-dependent DNA polymerase assays performed according to techniques described by Lieber *et al.* (*5*). These two fibroblast cell lines, histocompatible with the hybrid responder cells, served as target cells for all cytotoxicity assays.

Spleen suspensions were prepared from individual (BALB/c × A/J) F_1 mice of varying ages ranging from 3½ weeks to 9 months. Lymphoid cells were purified on Ficoll-Hypaque gradients (*4*), and adherent cells were removed by two 30-minute passages in plastic culture dishes at 37°C. The remaining nonadherent cells were 98 to 100 percent viable (trypan blue exclusion) and had the morphological appearance of small to medium-sized lymphocytes.

The microcytotoxicity assays were performed as previously described (*4*), and measured both cytotoxicity and inhibition of target cell replication. Fibroblast target cells were placed in Falcon Microtest II plates (approximately 150 cells per well). After 18 hours, splenic lymphocytes were added to each well. In most instances, lymphocyte–target cell ratios were 2 to 4×10^3 to 1. The plates were incubated for 48 hours, lymphocytes were removed, and the target cells were fixed, stained, and counted. Spleens were also tested for the presence of mouse-tropic C-type viruses by using the XC cytopathogenicity assay of Klement *et al.* (*6*), as described previously (*3*). The assay was performed on continuous feral mouse embryo cell cultures (SC-1 cells), obtained from Dr. W. P. Rowe. SC-1 cells support replication of both NIH-Swiss–tropic (N-tropic) and BALB/c tropic (B-tropic) viruses (*7*).

As shown in Table 1, splenic lymphocytes from (BALB/c × A/J) F_1 mice had variable effects on uninfected target cells; in some instances target cell numbers were increased, and in many others they were decreased. In contrast, the number of fibroblasts infected with endogenous type C viruses was uniformly reduced by lymphocytes from F_1 animals, regardless of age tested. The median reduction of infected target cells was 45 percent, while that of uninfected target cells was 21 percent. When percent reductions of target cells by the same lymphocyte preparations were compared within individual experiments, there was greater reduction of infected target cells than uninfected cells in 20 of 22 experiments ($P < .01$, chi-square). By summation of chi-squares derived from differences within individual experiments, the reactivity of the lymphocytes against the infected target cell population was significantly greater at the $P < .001$ level when compared with reactivity against uninfected target cells.

Splenocytes from animals older than 6 months were occasionally positive for mouse-tropic C-type viruses. However, spleens from younger mice were negative for detectable mouse-tropic virus. Previous studies have also indicated that (BALB/c × A/J) F_1 mice have a low incidence of both mouse-tropic and xenotropic virus positivity at various ages tested (*3, 8*).

The in vitro expression of endogenous murine C-type virus genetic information is controlled by intrinsic regulatory mechanisms that can be influenced by age, chemicals, radiation, and certain other stimuli (*1*). Regulation of virus expression in vivo is dependent not only on intrinsic cellular factors but also on the status of the host's immune responses. Previous studies have indicated that inbred mice can apparently produce various antibodies against several antigens associated with endogenous mouse-tropic or xenotropic viruses (*2*). These include RNA-dependent DNA polymerase, certain viral envelope antigens, and probably the group-specific antigens (gs-1 and

Table 1. Reactivity of splenic lymphocytes from (BALB/c × A/J) F_1 mice against BALB/c embryo target cells, either infected with endogenous C-type virus or uninfected. Each mean value (± standard error) was derived from at least ten replicate observations.

Age (months)	Mean number of target cells and reduction after reaction with lymphocytes						Selective reduction of infected cells (percentage points)
	Infected target cells			Uninfected target cells			
	Without lymphocytes	With lymphocytes	Reduction with lymphocytes (%)	Without lymphocytes	With lymphocytes	Reduction with lymphocytes (%)	
1	45 (± 4)	9 (± 1)	79	140 (± 4)	111 (± 11)	21	58
2	105 (± 6)	92 (± 7)	12	94 (± 4)	91 (± 6)	4	8
2	99 (± 7)	50 (± 5)	50	83 (± 5)	40 (± 5)	51	− 1
2	51 (± 2)	42 (± 4)	18	53 (± 2)	40 (± 2)	23	− 5
2	140 (± 7)	87 (± 5)	38	256 (± 11)	192 (± 9)	25	13
2	73 (± 5)	53 (± 7)	28	44 (± 5)	52 (± 6)	− 18	46
2	43 (± 4)	15 (± 2)	66	56 (± 3)	21 (± 1)	62	4
2	43 (± 4)	22 (± 2)	43	56 (± 3)	49 (± 4)	13	30
2	33 (± 4)	6 (± 1)	82	47 (± 4)	37 (± 3)	21	61
2	33 (± 4)	12 (± 2)	44	47 (± 4)	51 (± 5)	− 8	52
2	146 (± 9)	59 (± 3)	66	127 (± 8)	66 (± 5)	47	19
2	54 (± 7)	4 (± 1)	93	62 (± 5)	43 (± 4)	31	62
2	100 (± 8)	66 (± 6)	34	94 (± 6)	98 (± 8)	− 4	38
2	100 (± 8)	87 (± 10)	13	94 (± 6)	108 (± 4)	− 15	28
2	39 (± 3)	15 (± 1)	61	112 (± 5)	91 (± 3)	19	42
4	45 (± 4)	9 (± 2)	80	140 (± 4)	110 (± 6)	22	58
4	156 (± 13)	118 (± 9)	31	61 (± 3)	47 (± 3)	22	9
4	155 (± 9)	76 (± 7)	52	16 (± 1)	13 (± 2)	20	32
5	103 (± 7)	62 (± 3)	41	86 (± 4)	66 (± 4)	24	17
5	131 (± 5)	36 (± 3)	73	104 (± 5)	97 (± 5)	7	66
9	199 (± 7)	112 (± 8)	44	170 (± 19)	107 (± 21)	37	7
9	199 (± 7)	111 (± 8)	45	170 (± 19)	144 (± 26)	16	29
Mean	95 (± 11)	51 (± 8)	45	96 (± 12)	76 (± 9)	19	26*
Median			45			21	

* The selective reduction of infected target cells by F_1 lymphocytes was significant ($P < .001$) as determined by a chi-square summation test.

gs-3). Our studies show that mice also develop cell-mediated responses against antigens on histocompatible cells infected with endogenous mouse-tropic C-type viruses.

We have not been able, as yet, to rule out the possibility that these responses might be directed against antigens shared by both mouse-tropic and xenotropic viruses or against virus-induced cellular antigens. Nor have we determined what class of lymphocytes is responsible for these cell-mediated responses. In any event, it appears that at least some strains of mice may be exposed to antigens associated with C-type viruses at an early age, prior to detectability of infectious virus in these animals, and that they can respond with both humoral and cell-mediated immune responses against these antigens. Variations in the production of these immune responses may markedly influence the likelihood of C-type virus replication and subsequent oncogenesis. For example, cell-mediated virus elimination could be facilitated by cytotoxic antibodies or, conversely, inhibited by enhancing antibodies. Similarly, it has been suggested that production of certain antibodies to viral antigens might decrease the likelihood of neoplasia while increasing the chance of immune-complex glomerulonephritis (*2*). Depression of cell-mediated responses with antiserum to lymphocytes or cyclophosphamide in the presence of chronic antigenic stimulation, on the other hand, may increase the amount of endogenous C-type virus replication (*9*). Virus, once activated, may itself further depress host responses, either directly (*10*) or indirectly by induction of cell clones with autoaggressive reactivity (*4*). Thus, the interactions among endogenous C-type viruses, host immune responses, and neoplasia are complex. The unraveling of these complexities will require longitudinal studies in various mouse strains of specific humoral and cell-mediated responses to both viral and tumor antigens. Understanding these relationships may well provide a rational basis for eventual vaccination programs against endogenous oncogenic viruses.

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Vomeronasal Organ: Critical Role in Mediating Sexual Behavior of the Male Hamster

Abstract. *Sexual behavior in male hamsters is totally abolished by bilateral removal of the olfactory bulbs. This operation eliminates sensory input from both the olfactory and the vomeronasal systems. We previously demonstrated that peripheral destruction of the olfactory receptors caused anosmia but did not impair male hamster mating behavior. Here we demonstrate that peripheral deafferentation of the vomeronasal system produces severe sexual behavior deficits in approximately one-third of the treated animals. Combined deafferentation of both the vomeronasal and the olfactory systems eliminates copulation in 100 percent of the animals. This is the first experimental demonstration of a functional role for the vomeronasal organ in a mammalian species.*

Many aspects of reproductive behavior and physiology are influenced by the sense of smell (*1*). In the male rat, removal of the olfactory bulbs produces a deficit in mating behavior, whereas in male mice and hamsters, copulation is completely abolished (*2*). Olfactory bulb ablation interrupts sensory input from both the olfactory and vomeronasal receptors, and additionally destroys central components of these two systems. Attempts have been made to determine which of these variables is most relevant to the olfactory regulation of various behavioral processes (*3*).

We have previously reported that passing a 5 percent zinc sulfate solution through the nasal cavities of male hamsters reliably eliminates their ability to detect amyl acetate and female hamster vaginal odor, but does not impair their mating behavior (*4*). According to other studies, however, hamsters made peripherally anosmic fail to copulate (*5*). Consequently the importance of peripheral olfactory stimulation in this species remains unclear and the extent to which interference with the vomeronasal system might have contributed to these behavioral results has not been established. The experiments reported here differentiate between olfactory and vomeronasal organ effects on sexual behavior and for the first time provide experimental evidence for a functional role of the vomeronasal organ system in mammalian species.

The vomeronasal organs in most mammals are bilaterally paired tubes which lie on either side of the septum in the ventral portion of the nasal cavities (*6*). The tubes are only open anteriorly, near the nares. Axons from sensory receptors in the luminal epithelium of the organ project via the vomeronasal nerves to the accessory olfactory bulb (*7*), whereas the primary olfactory receptors innervate the main olfactory bulb. Main and accessory olfactory bulbs, in turn, maintain anatomically separate connections with nuclei of the amygdaloid complex and other forebrain areas (*8, 9*). Because the amygdaloid nuclei which receive input from the vomeronasal system in turn project to hypothalamic regions known to influence sexual behavior