Baim, D. C. Harrison, *Am. Heart J.* **102**, 819 (1981).
7. E. Müller-Schweinitzer, *J. Cardiovasc. Pharmacol.* **2**, 645 (1980).
8. M. Prinzmetal, P. Kennamer, R. Merliss, T. Wada, N. Bor, *Am. J. Med.* **27**, 375 (1959).
9. Segal *et al.* [S. A. Segal, D. L. Pearle, R. A. Gillis, *Eur. J. Pharmacol.* **76**, 447 (1981)] reported that they had produced coronary artery spasm in vagotomized cats by intravenous administration of picrotoxin. However, they observed no excessive vasoconstriction of the large epicardial coronary arteries and it is unclear why electrocardiographic ST-segment elevation of 0.30 mV occurred along with only a 25 percent increase in coronary vascular resistance yet the mean arterial pressure increased to 36 percent with picrotoxin administration.
10. The role of histamine in the cardiovascular system is complicated [K. J. Broadley, *Br. J. Pharmacol.* **54**, 511 (1975); R. Levi, G. Allan, J. H. Zavecz, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 1942 (1976)]. However, it has been accepted that the cardiac histamine system is analogous to the adrenergic system; that is, histamine H_1 receptors are analogous to α-adrenergic receptors and histamine H_2 receptors are analogous to β-adrenergic receptors [M. R. Bristow, R. Ginsburg, D. C. Harrison, *Am. J. Cardiol.* **49**, 249 (1982)].
11. P. D. Henry and M. Yokoyama, *J. Clin. Invest.* **66**, 306 (1980).
12. A brief report of this model was made at the International Teach-in, Regulation of Cardiac Function and Metabolism, 11 to 12 April 1983, Osaka, Japan. Supported in part by Scientific Research grants 56570338, 57440053, 57570353, and 58870058 and Developmental Scientific Research grant 587067 from the Ministry of the Education, Science and Culture Japan and Takeda Science Foundation, a grant from the Chiyoda Mutual Life Foundation, and a grant from the Taisha Ijo Foundation. We thank M. Mori for technical assistance and M. Ohara for critical reading of the manuscript.
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Deformed Whiskers in Mice Infected with Certain Exogenous Murine Leukemia Viruses

Abstract. *Mice infected at birth with replication competent Friend, Moloney, Cas-Br-M, C2S-M, and 1504-A murine leukemia viruses developed abnormalities of the vibrissae consisting of erratic curvature, shortening, and loss. A number of other virus strains, as well as endogenous AKR-type ecotropic virus and AKR-type, mink cell focus-inducing (MCF) viruses, did not produce these abnormalities. In mice with erythroid and myeloid leukemia, the perivibrissal sinus is the site of extramedullary hematopoiesis, but this did not appear to be the basis of the deformities. Genetic evidence indicated that newly arisen MCF-type recombinant viruses are involved in the pathogenesis of the abnormalities, at least with some of the virus systems studied.*

Although interest in mouse retrovirus infections has naturally focused on cells of the hematopoietic system, various retroviruses can infect a wide variety of cell types in vivo. Other than hematologic malignancies and a form of demyelinating brain disease (*1*), disease manifestations of infection with nondefective, replication competent murine retroviruses have not been observed.

In the course of examining mice that had been infected at birth with a replication competent Friend helper virus, free of the spleen focus-forming component, we noticed that their vibrissae were markedly abnormal. The whiskers were sparse, short, thin, and erratically curved (Fig. 1). The anomalies ranged from a few clearly altered hairs to virtually complete loss of vibrissae. The rest of the pelage has shown no anomalies.

The Friend helper virus on which most of these observations were made is the NB-tropic virus that was molecularly cloned by Oliff *et al.* (*2*). In newborn NFS/N mice this virus induces erythroleukemia within 5 to 10 weeks. The abnormalities of the vibrissae often precede the splenic enlargement, in some cases by as much as 3 or 4 weeks. The N-tropic Friend murine leukemia virus (MuLV), F-S, also induces the whisker abnormalities, but with a longer latent period and less marked effects.

We have examined mice infected with a number of exogenous and endogenous murine retroviruses for the whisker anomalies (Table 1). Four viruses consistently induced the changes: Friend and Moloney viruses, which are laboratory-passaged, highly pathogenic variants, and two horizontally transmitted viruses recovered from wild mice, Cas-Br-M and 1504-A. Another isolate from wild mice, C2S-M, also induced the changes in a small proportion of mice; this low response rate was probably due to the effect of a gene segregating in the study mice, as described below, rather than to inefficiency of the virus. In contrast, other highly pathogenic viruses, such as Gross Passage A, SL3, and a variety of thymomagenic recombinant MuLV's of the mink cell focus-forming (MCF) type

Table 1. Frequency of abnormal vibrissae in mice inoculated as newborns with various helper-independent MuLV's and in mice carrying life-long high titers of endogenous AKR-type MuLV. The inoculated mice were of genotypes that are permissive for replication of the inoculated virus; most of the animals were NFS/N or AKR, or hybrids derived from them. All viruses were grown in tissue culture and had been biologically cloned by two cycles of limiting dilution purification. Mice from cages in which there was any suspicion of barbering were excluded.

Virus	Major type of disease induced	Number of mice with abnormal vibrissae/number examined (%)				
		All mice by age				Leukemic mice only
		< 2 months	2 months	3 to 6 months	> 6 months	
		Inoculated mice				
Friend MuLV-NFS	Erythroleukemia	28/33 (85)	16/17 (94)			33/34 (97)
Friend MuLV-C57BL-derived strains with resistance to erythroleukemia	T lymphoma		4/5	22/22 (100)		1/1
Moloney MuLV	T lymphoma		6/29 (21)	31/58 (53)	7/11 (64)	13/37 (35)
FM-I2*	T lymphoma		4/5	27/37 (73)		12/16 (75)
Gross MuLV	T lymphoma		2/24 (8)	1/8	0/5	0/20 (0)
SL3 (3)	T lymphoma		1/30 (3)	0/8		0/21 (0)
Class I MCF viruses	T lymphoma		0/21 (0)	2/72 (3)	2/34 (6)	2/61 (3)
Cas-Br-M (4)	Neurologic			13/17 (76)	0/1	
C2S-M (5)	Myeloid leukemia			0/2	4/12 (33)	2/4
1504-A (4)	Lymphoma			4/4	14/16 (88)	1/1
		Uninoculated high-ecotropic carriers				
AKR and AKR F1's	T lymphoma			1/25 (4)	1/30 (3)	1/19 (5)
NFS congenic for various ecotropic V loci	T and B lymphomas			0/20 (0)	3/80 (4)	0/18 (0)

*FM-I2 is an in vitro recombinant between molecularly cloned Friend and Moloney viruses constructed by P. Chatis and N. Hopkins.

were essentially negative, as were the AKR-type endogenous viruses present in high titers. Moloney virus was much less effective in producing the vibrissal changes than Friend virus; the changes required several months to develop and were generally not as extensive as in mice infected with Friend virus. Mice that developed Friend erythroleukemia after being infected with Friend virus complex as weanlings did not show whisker changes.

The vibrissal anomalies in mice reported in Table 1 did not correlate with the occurrence of leukemia. In most of the categories, about half the animals examined were leukemic while the others had not yet developed disease; in each case the incidence of deformed vibrissae was comparable in the leukemic and preleukemic animals.

Further evidence that the strain of virus rather than type or occurrence of disease was the determining factor is shown by the results with Friend virus. The type of disease induced by the Friend helper virus depended on the genotype of the host; in some strains, including NFS/N, the virus induced erythroleukemia almost exclusively, whereas in C57BL and hybrids derived from it the virus induced lymphomas after a longer incubation period (*6*). In both systems the NB-tropic Friend helper virus regularly induced the whisker lesions (Table 1).

The mechanism by which the vibrissae were damaged is not known. The inoculated virus replicated to moderately high titers in the affected areas; the skin of the nose of affected mice showed titers in the range of $10^{5.5}$ to 10^6 plaque-forming units of virus per gram of tissue. Preliminary histopathologic studies have not indicated a consistent lesion. In a few animals there was some disorganization of the hair bulb, whereas in others the central root sheath appeared irregular. The most striking histopathologic observation was on the vibrissae of the mice with erythroleukemia induced by the Friend helper virus, but it may not have been related to the origin of the deformities. Normal vibrissae are enclosed in a blood-filled sinus, the lower two-thirds of which contains a loose network of stromal cells. In the erythroleukemic mice this network was infiltrated with large numbers of hematopoietic cells, primarily erythroblasts. Comparable infiltration with myeloblasts was seen in the perivibrissal sinus of two mice with myeloid leukemia induced by the C2S-M MuLV, but in one of the animals the vibrissae showed no deformity. Animals with whisker lesions induced by the Moloney or Cas-Br-M virus did not show cellular infiltration of the sinus. These observations, together with the finding that the whisker deformities could significantly precede the onset of erythroleukemia, indicate that this ectopic hematopoiesis is not the cause of the anomalies.

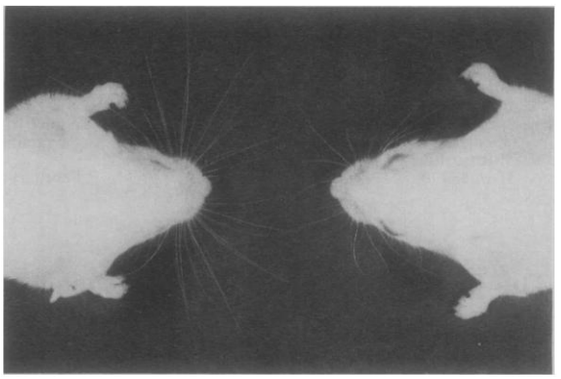

Fig. 1. Normal mouse (left) and mouse inoculated with Friend MuLV (right). The normal vibrissae are dense, thick, straight, or slightly curved, and give a "starburst" pattern. The whiskers of the infected mouse are asymmetrical, sparser, shorter, more curved, and in some cases bidirectionally curved.

An interesting observation was made that indicates that MCF-type recombinant viruses are involved in the pathogenesis of the vibrissal changes. During the replication of ecotropic or amphotropic MuLV's in mice, recombinant viruses arise that have acquired a new viral envelope (*env*) gene, and in some cases other viral genetic regions as well (*7*). These *env* gene recombinants are known as MCF viruses because they characteristically produce foci of cellular damage in a tissue culture line of mink lung cells. In the case of certain virus systems inducing thymic lymphomas (*8*) or erythroleukemias (*9*), the MCF recombinants are essential to the development of the disease, possibly by conferring a new tissue host range on the virus population. Workers at our laboratory have recently described a gene, *Rmcf* on mouse chromosome 5, the dominant (r) allele of which specifically reduces the sensitivity of the cells to infection by MCF viruses (*10*). A number of the mice we observed for whisker deformities were from crosses segregating for this gene [in coupling or repulsion with the closely linked morphological marker gene hammertoe (*Hm*)]. Overall, there was a highly significant association between inheritance of the resistance allele at *Rmcf* and decreased frequency of the anomalies (Table 2); also there was a distinct tendency for positive $Rmcf^{s/s}$ mice to have more extensive alterations of the whiskers than positive $Rmcf^{r/s}$ mice (data not shown). Thus, even though the cloned virus inocula were free of MCF viruses, MCF-type recombinants appeared to participate in the genesis of the lesions in at least some of the virus systems studied. Clearly, not all MCF viruses could affect the vibrissae, since the thymotropic MCF's, as shown in Table 1, were virtually inactive (*11*).

Table 2. Effect of a gene for resistance to MCF viruses on incidence of whisker deformities. The congenic mice were NFS mice heterozygous for $Rmcf^r$ and *Hm* in coupling; they were mated to NFS and the progeny were inoculated with virus and scored for hammertoe. Since *Rmcf* and *Hm* show only 2 percent recombination (*10*), the hammertoe phenotype is highly predictive of the $Rmcf^{r/s}$ genotype. In the NFS × (NFS.Hm × DBA/2)F_1 cross, NFS and NFS.Hm (a line of NFS congenic for the marker gene *Hm*) are $Rmcf^s$, whereas DBA/2 is $Rmcf^r$; thus the $Rmcf^r$ and *Hm* alleles are in repulsion, and the normal toed segregants would be $Rmcf^{r/s}$.

Virus	Number of mice with whisker lesions/number examined (%)	
	$Rmcf^{s/s}$	$Rmcf^{r/s}$
NFS.Rmcf^r Hm congenics		
Friend*	27/36 (75)	12/35 (34)
1504-A	15/15 (100)	13/20 (65)
Cas-Br-M	5/6	5/7
C2S-M	3/3	1/11
Moloney	16/35 (46)	10/30 (33)
FM-I2	21/23 (91)	10/19 (53)
NFS × (NFS.Hm × DBA/2)F_1		
Moloney	9/14 (64)	3/10 (30)
Total	96/132 (73)	54/132 (41)
	$\chi^2 = 26.0$, $P << 0.001$	

*These mice were scored at 45 to 60 days, near the end of the latent period of erythroblastosis; subsequently they all developed whisker deformities.

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References and Notes

1. P. G. Stansly, *Prog. Exp. Tumor Res.* **7**, 224 (1965); M. B. Gardner *et al.*, *J. Natl. Cancer Inst.* **51**, 1243 (1973).
2. A. I. Oliff, G. L. Hager, E. H. Chang, E. M. Scolnick, N. W. Chan, D. R. Lowy, *J. Virol.* **33**, 475 (1980).
3. R. C. Nowinski and E. F. Hays, *ibid.* **27**, 13 (1978); F. S. Pederson, R. L. Crowther, D. Y. Tenney, A. M. Reinhold, W. A. Haseltine, *Nature (London)* **292**, 167 (1981).
4. J. W. Hartley and W. P. Rowe, *J. Virol.* **19**, 19 (1976); S. Rasheed, M. B. Gardner, E. Chan, *ibid.*, p. 13.
5. W. P. Rowe, J. W. Hartley, T. N. Fredrickson, in preparation.

6. J. Silver and T. N. Fredrickson, in preparation.
7. P. J. Fischinger, S. Nomura, D. P. Bolognesi, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5150 (1975); J. W. Hartley, N. K. Wolford, L. J. Old, W. P. Rowe, *ibid.* **74**, 789 (1977).
8. W. P. Rowe, M. W. Cloyd, J. W. Hartley, *Cold Spring Harbor Symp. Quant. Biol.* **44**, 1265 (1980).
9. S. Ruscetti, L. Davis, J. Feild, A. Oliff, *J. Exp. Med.* **154**, 907 (1981).
10. J. W. Hartley, R. A. Yetter, H. C. Morse, *ibid.*, in press.
11. A portion of this work was done on Contract NO1-AI-22673 at Microbiological Associates, Bethesda, Md. We thank J. Silver and J. Hartley, of this laboratory, for supplying some of the mice for examination, and T. N. Fredrickson for examining the histological sections.

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The Immune Response Evokes Changes in Brain Noradrenergic Neurons

Abstract. *A decreased noradrenaline turnover in the hypothalami of rats was observed at the peak of the immune response to sheep red blood cells. The decrease in noradrenergic neuronal activity was mimicked by injection of soluble mediators released by immunological cells activated in vitro. Noradrenaline also tended to decrease in the brainstem but not in the residual brain. It is suggested that products released from activated immunological cells during the immune response may induce the previously described autonomic and endocrine mechanisms that contribute to immunoregulation.*

The immune response is a phasic phenomenon that includes well-programmed interactions among different cell types and molecules. Signals, originating from cellular and molecular elements of the immune system itself, constitute a level of autoregulation. There is also evidence of another more integrative level of regulation mediated by neuroendocrine signals (*1*). That there are information channels between immunological cells and the central nervous system (CNS) is indicated by the finding that changes in firing rates of neurons of the medial hypothalamus occur during the immune response (*2*). Since aminergic neurons projecting to the hypothalamus are important controllers of hypothalamic neuronal activity, we have considered the possibility that immunological signals could affect aminergic circuits within the CNS, which in turn could mediate the noradrenaline (NA) changes in lymphoid organs (*3*) and the hormonal changes (*1*) observed during the immune response. We report here that the immune response elicits a decrease in NA synthesis in the hypothalamus and that soluble products of activated immunological cells induce a decrease in NA content in the hypothalamus.

Since hypothalamic catecholamine (CA) concentrations are rather stable under normal conditions (*4*), we studied turnover rates (*K*) and rate constants of amine loss (*k*) in control rats and in rats immunized with sheep red blood cells (SRBC) using α-methyl-*p*-L-tyrosine (α-MTP) (*5*). This drug blocks the rate-limiting step of CA synthesis, acting as a competitive inhibitor of tyrosine hydroxylase. At the time of the peak immune response to SRBC, that is, 4 days after antigenic challenge, the number of plaque-forming cells (PFC) was measured in the spleen, as was also the CA turnover rate in the hypothalamus. All data taken together indicated a tendency for a decreased NA turnover rate in immunized animals. Because our animals showed considerable variability in the magnitude of their response to SRBC, we considered the possibility that those animals that responded strongly to the antigen might show a more pronounced decrease in NA synthesis in the hypothalamus.

When the NA turnover rates in the hypothalamus of each animal were plotted against the corresponding number of PFC per spleen, it was apparent that the animals with a more evident decrease in NA synthesis in the hypothalamus were those that responded strongly to the antigen. The data were therefore assessed in terms of high and low responder animals. Those rats whose splenic PFC values were higher than the mean number of PFC were classified as high responders (high responders > mean PFC) and the others as low responders (low responders < mean PFC). By this criterion, high responders had two- to threefold more PFC in the spleen than low responders; about half of the number of animals fell in each category. This classification revealed that the high responders markedly decreased their NA turnover rates in the hypothalamus (Table 1) whereas the low responders had turnover rates almost equal to those of the nonimmunized controls.

No changes were noted on day 1 after immunization (data not shown), suggesting that this phenomenon was neither caused by stress during manipulation nor by the antigen itself. This view is reinforced by the fact that low responders

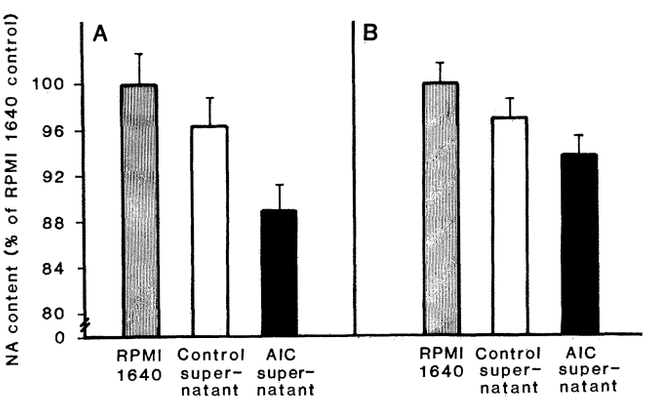

Fig. 1. Reduced NA content in (A) the hypothalamus and (B) the brainstem of rats after the injection of supernatants from activated immunological cells. One group of Holtzmann rats was injected intraperitoneally with 1 ml of the supernatant from activated immunological cells (*AIC*). These cells were obtained from the spleens of Lewis rats and were treated as described (*11*). Briefly, suspensions of 10^6 cells per milliliter in RPMI 1640 medium containing 10 percent heat-inactivated fetal calf serum were stimulated with Con A (5 μg/ml). After incubation of the mixture for 48 hours at 37°C, the supernatant was collected. Two other groups of rats were injected either with the same volume of RPMI 1640 medium or with control supernatant that was obtained as described above but Con A was added to the supernatant after collection. Two hours after the injection, the animals were killed and the hypothalamus, brainstem, and right hemisphere were removed as described in Table 1. Catecholamines were determined by high-performance liquid chromatography [column: 10 cm RP-18 Spheri 5 (Brownlee); mobile phase: 6.7 m*M* Na_2HPO_4; 13.3 m*M* citric acid; 2.5 m*M* octanesulfonic acid; 0.05 m*M* EDTA; 13 percent methanol, *p*H 3.3; flow rate: 1.3 ml/min] and electrochemical detection (detector potential against Ag/AgCl: 0.65 mV). Values are means ± standard error of the mean. Results are expressed as the percentages of those for rats injected with RPMI 1640 medium. The average NA values for this control ranged from 2913.78 ± 124.98 pg to 3506.70 ± 112.71 pg per milligram of tissue in the hypothalamus, and from 812.03 ± 13.94 pg to 960.73 ± 29.50 pg per milligram of tissue in the brainstem. In each experiment we used 13 to 16 animals per group. The NA content of the hypothalamus of rats injected with AIC supernatant differed significantly from controls injected with RPMI 1640 (Student's *t*-test, $P < 0.005$) or with control supernatant ($P < 0.05$). The NA content of the brainstem of rats injected with AIC supernatant differed significantly from the group injected with RPMI 1640 medium ($P < 0.02$).