predict that female HVc neurons will respond to a broader array of conspecific song stimuli than is observed in male HVc, in which neurons respond specifically to the bird's own song.

A challenge remaining is to determine how song perception develops in female birds that do not normally sing. It has been proposed (*17*) that neurons in male HVc acquire their pronounced song selectivity through a mechanism similar to that presented in the motor theory of human speech perception (*18*). This theory posits that, to perceive a song syllable, a bird must first convert the sound it hears into the motor commands required to produce that sound. Male HVc neurons develop song selectivity during the sensorimotor phase of song learning (6). Song production and song perception are thus viewed as being functionally linked in males. Female birds that do not normally sing presumably do not develop the entire set of motor commands required in male HVc for the production of full adult song. However, females learn to perceive even subtle differences among the songs of conspecific males (*8, 19*). It thus seems unlikely that such perceptual learning in female birds can be explained fully by a motor theory of song perception, at least as proposed for males (*17*). Rather, the sexes may differ in the mechanisms by which neurons in song nuclei develop their selectivity to conspecific courtship signals.

REFERENCES AND NOTES

1. J-P. Ewert, R. R. Capranica, D. J. Ingle, Eds., *Advances in Vertebrate Neuroethology* (Plenum, New York, 1983).
2. F. Nottebohm, in *Handbook of Physiology*, F. Plum, Ed. (Williams & Wilkins, Baltimore, 1987), Section 1, pp. 85–108.
3. F. Nottebohm and A. Arnold, *Science* **194**, 211 (1976); M. Baker *et al.*, *Brain Res.* **295**, 85 (1984).
4. F. Nottebohm, in *Biological Perspectives on Language*, D. Caplan, A. Lecours, A. Smith, Eds. (MIT Press, Cambridge, 1984), pp. 65–95; E. Brenowitz and A. Arnold, *Soc. Neurosci. Abstr.* **12**, 314 (1986).
5. D. Kelley and F. Nottebohm, *J. Comp. Neurol.* **183**, 455 (1979); L. Katz and M. Gurney, *Brain Res.* **221**, 192 (1981).
6. D. Margoliash, *J. Neurosci.* **6**, 1643 (1986); S. Volman and M. Konishi, *Soc. Neurosci. Abstr.* **13**, 870 (1987).
7. Captive female canaries may produce a song-like vocalization if isolated for several months. This vocalization is much simpler and less stable in structure, and is produced less often, than normal male song [A. Pesch and H-R. Guttinger, *J. Ornithol.* **126**, 108 (1985); Nottebohm (*2*); personal observation]. There are no reports of song in wild female canaries.
8. All birds were kept in a short-day photoperiod (light:dark, 8:16 hours) for at least 2 months before the start of the experiment. They were then gradually shifted to a long-day photoperiod (light:dark, 14:10 hours) to bring them into breeding condition. When a female developed a vascularized brood patch, she received two subdermal implants of estradiol (inner diameter, 0.76 mm; outer diameter, 1.65 mm; length = 12 mm) to induce copulation solicitation behavior in response to conspecific song.
9. The conspecific stimulus was a 5-min sequence of male roller canary song containing 19 syllable types and consisting of bouts of 4.8 ± 2.3 phrases (x ± SD), lasting 5.4 ± 3.0 s, and separated by silent intervals of 1.8 ± 0.9 s. [See F. Nottebohm and M. E. Nottebohm, *Z. Tierpsychol.* **46**, 298 (1978)]. In preliminary studies, female canaries only responded to songs produced by males of the same strain. The heterospecific stimulus was a white-crowned sparrow song recorded from the *Z.l. nuttalli* dialect in northern California. [See L. Baptista, *Univ. Calif. Publ. Zool. 105* (1975)]. White-crowned sparrow song was used because it contains frequency-modulated syllables as does canary song but has a very different syntactical structure. The single sparrow song lasted 1.55 s, repeated at intervals of 2.45 s for 5 min. Songs of both species were played at a maximum root-mean-square amplitude of 90 dB sound pressure level at 50 cm. The two songs were presented in random order each day, at 3-hour intervals to minimize facilitation or habituation effects.
10. A. King and M. West, *Science* **195**, 1002 (1977); M. Baker *et al.*, *ibid.* **214**, 819 (1981); W. Searcy and P. Marler, *Z. Tierpyschol.* **66**, 128 (1984); W. Searcy and E. Brenowitz, *Nature* **332**, 152 (1988).
11. Lesions were made by passing 100 μamps of anodal direct current for 2 min through insulated tungsten wire. Sites of lesions were confirmed histologically.
12. For sham lesions, birds were anesthetized, and an electrode was lowered to the stereotactic coordinates of HVc, but no current was delivered to the electrode.
13. For statistical analysis I used the median number of each bird's displays obtained from her full set of pre-lesion and post-lesion playback sessions. There were 1 to 4 pre-lesion and 1 to 13 post-lesion playback sessions per bird.
14. Ranges for the median number of solicitation displays are as follows: (i) effectively lesioned birds, pre-lesion canary song, 5 to 20 displays; post-lesion canary song, 4 to 16.5 displays; pre-lesion sparrow song, 0 to 1 display; post-lesion sparrow song, 3 to 11 displays; (ii) sham-lesioned birds, pre-lesion canary song, 5 to 12 displays; post-lesion canary song, 5 displays; pre-lesion sparrow song, 0 display; post-lesion sparrow song, 0 to 1 display.
15. It is unlikely that lesions acted by disrupting fibers of passage between song nuclei other than HVc. Axons projecting from lateral MAN (magnocellular nucleus of the anterior neostriatum) to RA travel lateral and ventral to HVc [F. Nottebohm, D. Kelley, J. Paton, *J. Comp. Neurol.* **207**, 344 (1982)]. Only lesions of medial caudal HVc induced responses to sparrow song. Projections between other song nuclei do not pass adjacent to HVc.
16. To determine whether effective lesions of HVc eliminated all song discrimination, I played the songs of different bird species to one female canary 7 months post-lesion. She responded strongly (≥5 solicitation displays during 5 min of playback) to songs of the northern mockingbird (*Mimus polyglottos*), the bay wren (*Thryothorus nigricapillus*), and the buff-breasted wren (*T. leucotis*). These songs have different syntactical structures, but are similar to each other and to canary song in that each has syllables with pronounced frequency modulation. The same female did not respond to rufous-and-white wren (*T. rufalbus*) song, which contains little frequency modulation. These observations suggest that lesions of medial caudal HVc eliminated the species-specificity of responses and resulted in a more generalized pattern of signal discrimination. The altered pattern of response may be related functionally to reports that HVc neurons respond more selectively to species-specific song stimuli than do neurons in the afferent auditory nucleus, field L (*6*).
17. H. Williams and F. Nottebohm, *Science* **229**, 279 (1985).
18. A. M. Liberman, *Am. Psychol.* **37**, 148 (1982).
19. D. Miller, *Anim. Behav.* **27**, 376 (1979); *Nature* **280**, 389 (1979); see M. Baker *et al.* (*10*).

20. Thanks to M. Beecher, S. Bottjer, K. Canady, G. Klump, P. Loesche, K. Nordeen, G. Rose, E. Rubel, D. Sengelaub, S. Volman, H. Zakon, and two anonymous referees for comments. S. Bottjer and A. Arnold offered advice during preliminary studies. B. Nalls provided technical assistance. Supported by NIH DC 00487, NSF BNS 86-02469, and a Sloan fellowship.

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The Effect of Anti-Neoplastic Drugs on Murine Acquired Immunodeficiency Syndrome

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The murine acquired immunodeficiency syndrome (MAIDS) is associated with proliferation of target cells that have been infected by a defective retrovirus. To control the growth of this primary neoplasia, virus-inoculated mice were treated with anti-neoplastic drugs. Paradoxically, cyclophosphamide, which is also immunosuppressive, was very effective in preventing the appearance and progression of the disease, in restoring a normal T cell function, and in depleting the number of infected target cells. This result suggests that the proliferating infected target cells were responsible for the immunodeficiency.

THE DUPLAN STRAIN (*1*) OF MURINE leukemia virus (MuLV) induces a severe immunodeficiency syndrome in mice (*2–6*). The disease shows many similarities with human AIDS (*7, 8*), including abnormal T and B lymphocyte functions, polyclonal B-cell proliferation, lymphadenopathy, splenomegaly, hypergammaglobulinemia, enhanced susceptibility to infections, and late appearance of B cell lymphomas (*2–9*). The etiologic agent of this murine AIDS (MAIDS) has been identified as a defective retrovirus (*10, 11*).

The mechanism by which the immunodeficiency arises in these mice remains unknown, but viral replication does not seem to be required for the appearance of the

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disease. Target cells proliferated and emerged as oligoclonal populations in lymph nodes of diseased mice that had been inoculated with helper-free stocks of defective Duplan retrovirus (*12*). Therefore, it appears that the defective virus is oncogenic in infected animals, and induces a primary neoplasia in association with an acquired immunodeficiency syndrome (*12*).

If this model of pathogenesis is correct, treatment with the anti-neoplastic drugs cyclophosphamide (Procytox^r), 5-aza-2'deoxycytidine (5-aza-dC), and arabinosylcytosine (Ara-C) (Cytosar^r) should be beneficial. The median lethal dose (LD_{50}) of cyclophosphamide and 5-aza-dC, were respectively, 530 mg/kg and 25 mg/kg in C57BL/6 mice (Charles River, Canada). Sublethal doses of cyclophosphamide (300 mg/kg) and of 5-aza-dC (15 mg/kg) induced a slight (<20%) reduction of body weight, but Ara-C (2000 mg/kg) was not toxic in this strain of mouse.

In group A, 30- to 40-day-old C57BL/6 mice were inoculated once intraperitoneally with a helper-free stock of defective Duplan (Du5H) retrovirus, as described (*12*). Treatment with anti-neoplastic drugs was begun 6 days after inoculation, at which time virus entry and integration has occurred but no signs of disease are detectable. Mice that received the drugs alone remained healthy throughout the experiment (Table 1, groups A-2, A-3, and A-4). All mice that received the virus alone developed MAIDS, as measured by the appearance of splenomegaly and lymphadenopathy (Table 1, group A-5), but the disease was more pronounced when the virus was given in two separate inoculations (compare groups A-5 and B-4). All virus-inoculated mice that received cyclophosphamide were free of macroscopic lesions when the mice were killed. Ara-C and 5-aza-dC had no effect on the incidence of the disease, although the lymph nodes of mice treated with 5-aza-dC were not as enlarged (20% of the enlargement seen in untreated inoculated mice).

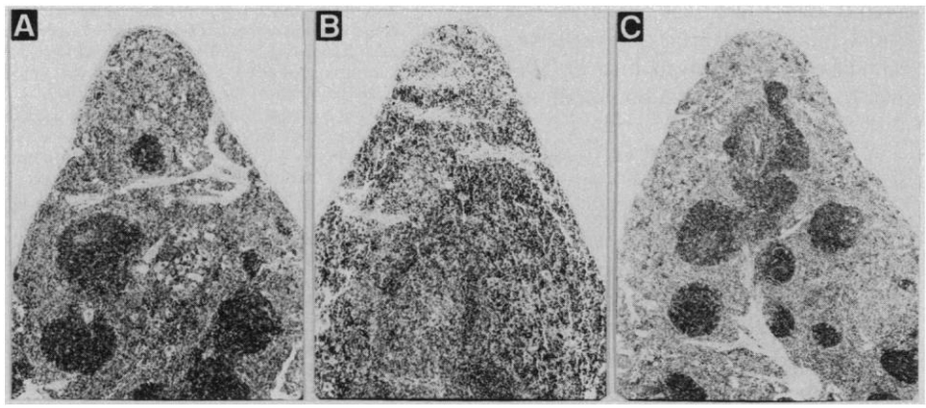

Fig. 1. Representative histological sections of spleens from normal, diseased, and cyclophosphamide-treated mice. (**A**) Spleen from control mouse treated with cyclophosphamide alone. (**B**) Spleen from a diseased mouse inoculated with the helper-free defective Duplan (Du5H) virus. (**C**) Spleen from a virus-inoculated mouse treated with cyclophosphamide (group B-5). Hematoxilin-eosin stain (× 95).

In group B, drug treatment was started 48 days after viral inoculation. Two infected mice that were killed at this time had enlarged spleens ($\bar{X}$ of 274 mg) and lymph nodes ($\bar{X}$ of 238 mg), and their splenocytes were not stimulated by concanavalin A (Con A) (*13*), a good indication of the progression of the disease.

Drug treatment was stopped 2.5 to 4 weeks before the mice were killed (starting at day 96 after virus inoculation). Treatment

Table 1. Effect of drug therapy on the appearance of MAIDS.

Group	Day of virus inoculation*	Drug administered† (beginning no. days after virus inoculation)	No. diseased mice† / No. mice inoculated	Weight ($\bar{X}$ ± SEM) Total (g)	Spleen (mg)	Lymph node (mg)§	Response to Con A ($\bar{X}$ ± SEM cpm)‖	IgM ($\bar{X}$ ± SEM mg/liter)¶
A-1	None	None	0/6	24.6 ± 0.8	93.2 ± 6.1	<10	127,619 ± 7,139	N.D.
A-2	None	Cyclo. (6)	0/5	20.4 ± 0.5	89.2 ± 6.8	<10	110,027 ± 7,047	N.D.
A-3	None	5-aza-dC (6)	0/4	24.0 ± 1.1	108.2 ± 4.7	<10	71,546 ± 9,074	N.D.
A-4	None	Ara C (6)	0/5	23.2 ± 0.5	83.2 ± 5.6	<10	92,751 ± 10,400	N.D.
A-5	0	None	11/11	24.1 ± 0.5	185.9 ± 20.9	655.5 ± 169.5	18,146 ± 6,388	N.D.
A-6	0	Cyclo. (6)	0/9	20.4 ± 0.9	93.4 ± 10.5	<10	113,037 ± 12,620	N.D.
A-7	0	5-aza-dC (6)	8/8	24.0 ± 0.4	180.6 ± 16.5	132.0 ± 21.1	21,557 ± 9,242	N.D.
A-8	0	Ara C (6)	11/11	23.7 ± 0.4	234.2 ± 48.5	598.4 ± 257.0	25,057 ± 11,446	N.D.
B-1	None	None	0/5	24.2 ± 0.3	109.6 ± 6.7	<10	147,106 ± 34,794	294.6 ± 9.4
B-2	None	Cyclo. (48)	0/4	22.1 ± 1.5	70.2 ± 4.9	<10	133,190 ± 12,845	277.4 ± 75.6
B-3	None	5-aza-dC (48)	0/2	24.1 ± 0.2	92.5 ± 9.5	<10	102,007 ± 33,843	N.D.
B-4	0 + 14	None	11/11	25.3 ± 0.6	317.7 ± 102.4	1226.3 ± 318.7	3,703 ± 1,675	544.8 ± 47.2
B-5	0 + 14	Cyclo. (48)	0/9	22.3 ± 0.6	94.9 ± 4.7	<10	100,589 ± 11,862	216.8 ± 17.0
B-6	0 + 14	5-aza-dC (48)	2/10	24.1 ± 0.4	111.7 ± 10.7	25.7 ± 14.0	52,375 ± 10,678	N.D.

*ψ2 packaging cell line transfected by defective Du5H DNA (*12*) was used to produce stocks of helper-free viruses. Mice of groups A-1 through A-8 received one intraperitoneal injection of virus (1.5 ml) or saline at day 0. Mice of groups B-1 through B-6 received two consecutive injections at days 0 and 14, respectively. †All drug-treated mice received 0.8 ml of optimal drug concentration diluted in sterile 0.9% NaCl. Treatment regimens were: Cyclophosphamide 300 mg/kg in one injection every 14 days for four consecutive treatments, starting at day 6 (groups A-2 and A-6) or for three consecutive treatments starting at day 48 (groups B-2 and B-5) after virus inoculation. 5-aza-dC, 15 mg/kg, divided into three injections every 6 hr at intervals of 14 days for respectively six (groups A-3 and A-7) and three (groups B-3 and B-6) consecutive treatments. Cytosine arabinoside, 2000 mg/kg divided in three injections every 6 hr at intervals of 14 days for six consecutive treatments (groups A-4 and A-8). ‡Based on macroscopically detectable signs of splenomegaly and lymphadenopathy at autopsy. §Total weight of the nodes in each mouse. Lymph nodes larger than 10 mg were considered significantly enlarged compared to those of untreated controls. The paramandibular, axillary, inguinal, mediastinal, and mesenteric nodes were weighed. ‖Con A assays were performed on spleen cells, as previously described (*10*). Each well received 200,000 cells and 0 to 20 μg/ml Con A. For each dilution of Con A, triplicates were done. Plates (Nunc) were incubated for 3 days prior addition of [^{3}H]-thymidine (1 μCi per well). The cells were collected 16 hr later and processed for radioactivity detection (*10*), and mean values determined. The counts obtained in the presence of Con A were substracted from the background (counts obtained without Con A). Maximal activities (usually obtained at 10 μg/ml of Con A) of each group are reported. The differences between the groups of virus-inoculated non-treated and drug-treated mice (groups A-5 and A-6, $P = 0.0001$), (groups B-4 and B-5, $P = 0.0001$), (groups B-4 and B-6, $P = 0.0053$) are statistically significant as revealed by a two way analysis of variance with multiple comparisons. No statistically significant differences were found between groups A-5 and A-7 ($P > 0.5$) and groups A-5 and A-8 ($P > 0.5$). ¶Serum IgM levels were determined with the use of mouse IgM radial-immunodiffusion test plates (ICN), as recommended by the manufacturer. N.D., not determined.

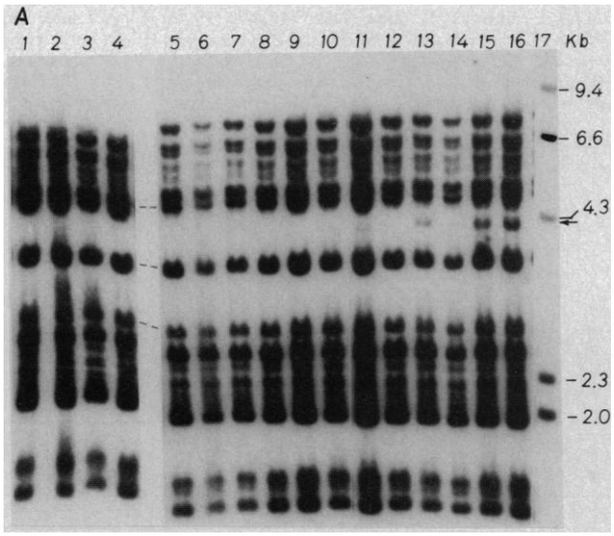

Fig. 2A. Southern (DNA) blot analysis of DNA from spleens of diseased and cyclophosphamide-treated mice. DNA from individual virus-inoculated cyclophosphamide-treated mice (group B-5) (lanes 1 to 4 and 6 to 10), from a normal cyclophosphamide-treated C57BL/6 mouse (lane 5), or from individual, virus-inoculated, untreated mice (group B-4) (lanes 11 to 16). Cellular DNA (20 μg per lane) was digested with Pst I, fragments were separated by electrophoresis in 1% agarose gels, transferred to nitrocellulose membranes, and hybridized with a ^{32}P-labeled D34 probe as described (*12*). ^{32}P-labeled Hind III–digested λ DNA was used as marker (lane 17). Arrow indicates the defective viral genome.

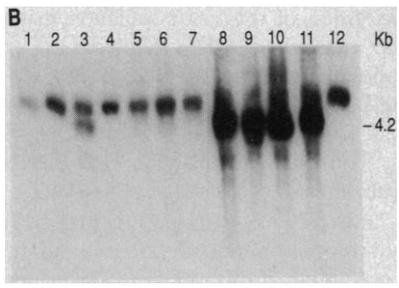

Fig. 2B. Northern (RNA) blot analysis of RNA from spleens of diseased and cyclophosphamide-treated mice. Total RNA from control normal (lane 1) or cyclophosphamide-treated (lane 2) C57BL/6 mouse, individual virus-inoculated cyclophosphamide-treated mice (group B-5) (lanes 3 to 7), or individual virus-inoculated untreated diseased mice (group B-4) (lanes 8 to 12). RNA (20 μg per lane) was extracted from spleens as described (*25*) and separated by electrophoresis in 1% formaldehyde-agarose gels (*26*). After blotting on nitrocellulose membranes, the samples were hybridized with a ^{32}P-labeled D30 probe as before (*12*).

of virus-inoculated mice with cyclophosphamide was very effective; no mice showed signs of disease (Table 1, group B-5). Eight of the ten virus-inoculated mice that had been treated with 5-aza-dC were also free of macroscopic disease, and the lymph node and spleen masses of all 5-aza-dC–treated mice were markedly reduced (group B-6).

As has previously been shown (*2, 3, 10*), we observed a severe decline of the Con A response in all mice of both groups of untreated, virus-inoculated mice (Table 1). In groups A and B, most virus-inoculated mice treated with cyclophosphamide (groups A-6 and B-5) and with no macroscopic evidence of disease had a normal Con A response, which indicates that the treatment had indeed restored this T cell function. All the mice treated with 5-aza-dC or Ara-C in group A were macroscopically diseased and most of them had a poor Con A response, which was statistically comparable to the control untreated virus-inoculated mice ($P > 0.5$) (Table 1). In group B, several mice treated with 5-aza-dC had no macroscopic disease; these also had a Con A response significantly better ($P = 0.005$) than the untreated virus-inoculated mice (Table 1). Together, these results indicated that some anti-neoplastic agents are effective in restoring one important immune function in these diseased mice.

Mice with MAIDS have elevated levels of serum IgM (*2, 3, 10*). Cyclophosphamide lowered the levels of serum IgM in these mice (Table 1) and hence restored another parameter of immunity.

As previously reported (*14–16*), the splenic architecture of all (5 of 5 tested) diseased mice was profoundly disorganized (Fig. 1). In contrast, the splenic architecture of most (eight out of nine) cyclophosphamide-treated virus-inoculated mice (group B-5) was almost indistinguishable from that of control mice treated with cyclophosphamide (Fig. 1).

We next quantitated the defective proviral DNA in spleens of control and drug-treated virus-inoculated mice. We chose the restriction endonuclease Pst I, which cleaves the defective viral DNA internally within the long terminal repeats (LTRs) and generates a 4.2 kb fragment (*12*). DNA from spleens of most (8 out of 11) of the control virus-inoculated mice contained the 4.2 kb proviral DNA (Fig. 2A). In spleen DNA from virus-inoculated mice that had been treated with cyclophosphamide (group B-5), the 4.2 kb proviral DNA was not detected, except very faintly in one mouse. This represents a significant ($P = 0.009$) difference as measured by the Fisher's exact test for the equality of two proportions. By comparative end-point dilution between these groups, we estimated that the disappearance of proviral DNA from most treated mice represented a 20-fold reduction in proviral DNA in the infected spleens (*17*). This represents a 200- to 400-fold reduction of defective viral DNA, as calculated from the total weight of their respective lymphoid organs. The amount of the 4.2 kb viral RNA from spleens of most (8 of 9) virus-inoculated mice that had been treated with cyclophosphamide was much lower than in most (9 of 11) control virus-inoculated mice (Fig. 2B), again a significant difference ($P = 0.003$). By end-point dilution, we estimated that viral RNA had decreased by 160- to 320-fold after drug treatment in most mice (*17*).

As no replicating MuLVs, other than xenotropic MuLV, were detectable in mice inoculated with helper-free stocks of defective Duplan virus (*12*), these results strongly suggest that the infected target cells have been depleted by the treatment. To confirm the absence of replicating helper MuLV in diseased tissues, the Northern (RNA) blots were rehybridized (*12*) with a probe representing BALB/c endogenous, ecotropic, MuLV; with a probe specific for env of ecotropic MuLV; and with a probe specific for the U3 LTR of Moloney MuLV (*18*). In control lymph nodes of mice inoculated with defective virus and rescued with the ecotropic G_6T_2 MuLV, the representative and the ecotropic MuLV–specific probes were able to detect the helper 8.0-kb full-length and env-specific RNA, as expected. None of the three probes detected similar helper RNA species in spleens of mice inoculated with helper-free Duplan virus, which confirms that these virus stocks and this disease are helper-free.

The drugs used have been demonstrated to be effective antileukemic agents in mice (*19, 20*). Cyclophosphamide is also effective against some immune diseases (*21*). It appears paradoxical that this anti-neoplastic drug, which is normally immunosuppressive (*22, 23*), is so efficient in treating an immunodeficiency syndrome.

The anti-neoplastic regimen probably succeeds by killing proliferating cells. These cell populations would probably expand again if the mice were left untreated for a longer period of time. We previously postulated that the important initiating event in MAIDS is the expansion of the infected target cells after infection with the helper-free stock of defective virus (*12*). Our present data are consistent with the hypothesis that these proliferating target cells are responsible for the appearance of the disease.

Expansion of this cell population may initiate a cascade of events that leads to immunodeficiency. Other uninfected cells, including B cells, T cells, and other hematopoietic cells, also proliferate in this disease (*2, 3, 5, 9*). The cyclophosphamide treatment was probably also cytostatic for these cells.

This model of pathogenesis may be relevant to other immunodeficiency syndromes. For example, if this mouse model of immunodeficiency shares some aspect of its pathogenesis with human AIDS, and especially if a critical target cell population is proliferating in human AIDS, this type of therapy may be considered for prevention and treatment of human AIDS. A detailed comparison of the pathogenesis of both diseases will be necessary to test the validity of this approach in humans. The recent isolation of a simian immunodeficiency virus variant that induces a severe gut lymphoproliferative disorder with loss of T and B lymphocytes (*24*) suggests that a link may exist, as in MAIDS, between lentivirus-induced immunodeficiency and cell proliferation.

REFERENCES AND NOTES

1. R. Latarget and J. F. Duplan, *Int. J. Radiat. Biol.* **5**, 339 (1962).
2. E. Legrand, R. Daculsi, J. F. Duplan, *Leuk. Res.* **5**, 223 (1981).
3. D. E. Mosier, R. A. Yetter, H. C. Morse, III, *J. Exp. Med.* **161**, 766 (1985).
4. D. E. Mosier, *Immunol. Invest.* **15**, 233 (1966).
5. S. P. Klinken, T. N. Fredrickson, J. W. Hartley, R. A. Yetter, H. C. Morse, III, *J. Immunol.* **140**, 1123 (1988).
6. R. M. L. Buller, R. A. Yetter, T. N. Fredrickson, H. C. Morse, III, *J. Virol.* **61**, 383 (1987).
7. F. Wong-Staal and R. C. Gallo, *Nature* **317**, 395 (1985).
8. A. S. Fauci, *Science* **239**, 617 (1988).
9. P. K. Pattengale *et al.*, *Am. J. Pathol.* **107**, 362 (1982).
10. D. C. Aziz, Z. Hanna, P. Jolicoeur, *Nature* **338**, 505 (1989).
11. S. K. Chattophadhyay, H. C. Morse, III, M. Makino, S. K. Ruscetti, J. W. Hartley, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3862 (1989).
12. M. Huang, C. Simard, P. Jolicoeur, *Science* **246**, 1614 (1989).
13. Stimulation of splenocytes by Con A was performed as described in Table 1. The Con A response of both diseased mice was 1.5% and 6.7% of that of noninfected controls.
14. J. F. Duplan, P. Monnot, P. M. Mistry, *Bull. Cancer (Paris)* **57**, 23 (1970).
15. P. B. Mistry and J. F. Duplan, *Bull. Cancer (Paris)* **60**, 287 (1973).
16. J. W. Hartley, T. N. Fredrickson, R. A. Yetter, M. Makino, H. C. Morse, III, *J. Virol.* **63**, 1223 (1989).
17. The spleen DNA (Fig. 2A, lane 13) and RNA (Fig. 2B, lane 8) samples were diluted up to 1 in 40 and compared by hybridization with those of group B-5.
18. C. Simard and P. Jolicoeur, unpublished data.
19. E. Frei, F. M. Schabel, A. Goldin, *Cancer Res.* **34**, 184 (1974).
20. R. L. Momparler, *Pharmacol. & Ther. Part B Gen. & Syst. Pharmacol.* **30**, 287 (1985).
21. A. S. Fauci, *J. Allergy Clin. Immunol.* 66, 5 (1990).
22. J. J. Marchaloris, *The Lymphocyte, Structure and Function* (M. Dekker, New York, ed. 2, 1988).
23. J. L. Turk and L. W. Poulter, *Clin. Exp. Immunol.* **10**, 285 (1972).
24. D. S. Dewhurst, J. E. Embretson, D. C. Anderson, J. I. Mullins, P. N. Fultz, *Nature* **345**, 636 (1990).
25. R. Chomezynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987).
26. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).

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Evidence for Biased Gene Conversion in Concerted Evolution of Ribosomal DNA

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Concerted evolution is the production and maintenance of homogeneity within repeated families of DNA. Two mechanisms—unequal crossing over and biased gene conversion—have been the principal explanations of concerted evolution. Concerted evolution of ribosomal DNA (rDNA) arrays is thought to be largely the result of unequal crossing over. However, concerted evolution of rDNA in parthenogenetic lizards of hybrid origin is strongly biased toward one of two parental sequences, which is consistent with biased gene conversion as the operative mechanism. The apparent gene conversions are independent of initial genome dosage and result in homogenization of rDNA arrays across all nucleolar organizer regions.

ALTHOUGH CONCERTED EVOLUTION of DNA sequences (the homogenization of repeated units within individuals and populations) has been documented for several gene families (*1*), the proposed mechanisms, which include unequal crossing over (*2*) and biased gene conversion (*3*), remain controversial. The debate over the relative importance of these mechanisms primarily has involved theoretical, rather than empirical, studies (*2–4*; but see *5, 6*). Unisexual hybrids provide a unique opportunity to study interchromosomal mechanisms of concerted evolution because they contain entire arrays of repeated gene families that initially are fixed within chromosomes but differ between chromosomes (*6*). Moreover, unisexual hybrid lineages provide an opportunity to distinguish between unequal crossing over and biased gene conversion because the predicted patterns of variation produced by these two mechanisms differ in these "permanent hybrid" lines.

We have investigated the tandemly repeated ribosomal RNA genes and their associated spacer regions (rDNA) within a group of unisexual parthenogenetic lizards and their sexual relatives, which together comprise the *Heteronotia binoei* complex (*7*). We conducted this study to determine whether concerted evolution occurs in parthenogenetic lineages, and, if so, to determine which of the proposed mechanisms of concerted evolution is consistent with the observed patterns of variation.

The *H. binoei* complex consists of several cryptic sexual species and a large number of parthenogenetic lines of hybrid origin (*7, 8*). The two sexual species known to be involved in the origination of the parthenogens have been designated CA6 and SM6, pending their formal description (*8*). These two species apparently hybridized in the past, and backcrosses to each of the sexual species produced many independent, triploid, parthenogenetic lineages (*8*). Each of these lineages contains one haploid set of chromosomes from one of their sexual ancestors and two from the other (that is, 1CA6/2SM6 or 2CA6/1SM6). Unlike most other unisexual vertebrates, there is clear evidence from allozyme studies for multiple origins of these parthenogenetic lineages (*8*).

Parthenogenetic species that arise through hybridization are usually fixed heterozygotes at all loci that are diagnostic in the parental species (*9*). In *Heteronotia*, each of the parental haploid genomes contains one nucleolar organizer region (NOR), which contains the rDNA cluster, so each triploid lineage should contain three NORs inherited from the two parental species at the time of lineage formation. Some lineages

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