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BRIEF COMMUNICATION

Environmental Lighting Alters the Infection Process in an Animal Model of AIDS[1]

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McEACHRON, D. L., K. M. TUMAS, K. BLANK AND M. PRYSTOWSKY. *Environmental lighting alters the infection process in an animal model of AIDS.* PHARMACOL BIOCHEM BEHAV 51(4) 947-952, 1995.—In this study, we examined the effects of altered environmental lighting on the infection process of a murine leukemia virus, E-55(+), which induces a thymic lymphoma/leukemia in 100% of BALB.K mice inoculated as adults. One to two weeks after inoculation, high levels of proviral DNA are usually found. This is followed by an asymptomatic period of many weeks during which proviral DNA becomes essentially undetectable. Leukemia develops approximately 28 weeks postinoculation. In this experiment, one group of mice was exposed a consistent 10L : 14D cycle while a second was maintained in constant light (LL). A third group was exposed to a rotating cycle characterized by phase shifting a 10L : 14D cycle every three 24-h days (rLD). All cycles began 2 weeks prior to inoculation and were maintained thereafter. Animals were sacrificed at 1, 5, 10, and 15 weeks, and hematopoietic tissue was examined for proviral DNA content. At 1 week, LL- and rLD-exposed animals showed considerably less proviral DNA in bone marrow and spleen compared with controls. At 15 weeks, thymuses from controls were showing signs of infection whereas tissue from LL and rLD mice remained at background levels. We conclude that environmental lighting does alter the infective pattern displayed by this retrovirus, although whether this effect is mediated by changes in the target stem cells or through immunoenhancement has not yet been determined.

AIDS Leukemia Virus Circadian Light Mice Animal model Infection TH_1 Humoral TH_2 Cell mediated Immunity Resistance

NUMEROUS immune parameters show biological or circadian rhythms in both animals and humans (1,2,4,11,12,18,19). Clear circadian rhythms were uncovered in the number of peripheral and spleen lymphocytes in mice with peaks in the light (L) phase under both normal and reversed light : dark cycles (19). Studies in mice suggest that these rhythms have functional significance. Levi and colleagues (20) reported that the density of CD3 molecules on T lymphocytes is 200–300% greater in early L phase than in the evening or early dark (D) phase. Other reports demonstrated that T-cell proliferation in response to phytohemagglutin (PHA) was 200% greater when stimulated in early morning (0600–1000 h) compared to late evening (1800–2200 h) (18,34). One possible mediator of these effects is pineal melatonin, which has been implicated in the control of immune responses in mice (21–26) and rats (27,30). Using collagen-induced arthritis in DBA/1 mice, a model of autoimmunity, Hansson and colleagues found a more severe arthritis develops when animals are exposed to constant darkness, along with higher antibody titers and enlarged spleens (15). Measurement of serum melatonin displayed the predictable increase in DD compared with a 12L : 12D cycle and LL. In a later experiment, mice kept under LL but injected with

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melatonin also developed a more severe arthritis (16). In the latter experiment, the melatonin in animals exposed to LL but not injected was reduced and the circadian amplitude was damped. These data are consistent with the hypothesis that pineal melatonin plays an important role in regulating immune function, possibly through enhancement of the TH_2 type or humoral response. The purpose of this study was to determine if lighting schedules would alter the pattern of an infection of a murine retrovirus whose typical pattern resembles that of Human Immunodeficiency Virus (HIV).

The murine leukemia virus (MuLV) E-55(+) induces a thymic lymphoma/leukemia in 100% of BALB.K mice infected as adults after a latency period of 4–7 months (29). The pattern of viral integration as determined by detection of proviral DNA shows an initial surge in the number of infected cells (acute phase) 1–2 weeks after infection, followed by a latency period of 4–7 months during which viral DNA cannot be detected, even using sensitive polymerase chain reaction (PCR) methods (35). However, all animals develop the leukemia/lymphoma by approximately 28 weeks postinfection. This latter stage of the infection, where the E-55(+) virus appears to escape the immune system, is apparently due to a loss of antigenic epitopes as a result of genetic recombination involving the *env* gene (36). Thus, this process can be characterized as having three stages: 1) the *acute phase*, when the initial immune response reduces the detectable integrated provirus to background levels; 2) the *asymptomatic phase*, when the immune system maintains the virus at or near background levels; and 3) the *leukemic phase*, when the virus escapes the immune system to cause the development of leukemia/lymphoma. We investigated the effects of constant illumination and rotating light : dark cycles on the initial stages of infection with this retrovirus.

METHOD

Animals

Forty-seven adult BALB/c-H-2k (BALB.K) mice, 7–8 weeks old, were group housed in one of three light-tight cabinets. The animals were exposed to a light : dark cycle of 12L : 12D for 2 weeks and then each cabinet began its own individual environmental lighting regimen. Cabinet 1 provided a consistent 10L : 14D cycle (lights on 0600 EST). Cabinet 2 provided constant illumination (LL), and cabinet 3 furnished a rotating cycle. This latter cycle was characterized a 10L : 14D cycle that phase shifted every three 24-h days. The pattern consisted of the following repeating sequence: 6-h advance, 8-h advance, 8-h delay, 6-h delay. Lighting was supplied by 40-W incandescent bulbs. All animals were exposed to these cycles for 2 weeks before inoculation (intraperitoneal injection) with E-55(+) virus. All animals were maintained on these light cycles for the remainder of the experiment.

Virus and Cells

The E-55(+) murine leukemia virus was biologically cloned from virus obtained from an adult BALB.K mouse that had been injected with filtered (0.2 μ) cell-free culture supernatant from the leukemic cell line KgV (17). The leukemic cell line BALB.K-gvl (KgV) was obtained from the spleen of a leukemic BALB.K mouse that had been injected as a neonate with NB tropic MuLV (17). The cell line is being maintained in Dulbecco's minimal essential media (MEM) containing 10% fetal bovine serum (Hyclone Labs., UT), 0.015 mM glutamine, 2.4 μM asparagine, 1.3 nM folate, 5.5 μM arginine, 1.0 μM pyruvate, penicillin/streptomycin (100 U/ml and 50 μg/ml), and 0.25 μM β-mercaptoethanol. Cells are cultured at 37°C in 5% carbon dioxide.

Injection and Tissue Collection

Mice were injected intraperitoneally with 0.2 ml of filtered (0.2 μ) culture supernatant from Sc-1 cells infected with E-55(+) virus. The supernatant typically contains approximately 10,000 PFUs (plaque forming units)/ml of E-55(+) virus. Hematopoietc organs (bone marrow, thymus, and spleen) were collected from each mouse. The organs were minced and placed immediately into guanidine isothiocynate buffer and homogenized using an Omni International Model 1000 homogenizer (Omni International, CT). Homogenates were stored at −80°C until analyzed.

RNA/DNA Isolation

RNA and DNA were isolated from each organ using standard protocols (8) with the following modifications: RNA was extracted twice with *l*-butanol:chloroform (1 : 4) and DNA was treated overnight at 37°C with RNAase A and proteinase K.

Oligonuclelotides

The two oligonucleotide primers being used in the PCR protocol were synthesized by the DNA Synthesis Service of the Department of Chemistry at the University of Pennsylvania. The 3′ primer was:

5′GGCAGCATGGAGCTGCTGGAACTGCTG-3′

with the 5′ residue equal to nucleotide 1624 of the *env* gene and the 5′ primer was:

5′TTGGGACTACATCACAGTGGA-3′

with the 5′ residue equal to nucleotide 639 of the *env* gene. The primers span a region of 984 nucleotides containing the 3′ end of gp70 glycoprotein and the 5′ end of the p15E protein of the E-55(+) *env* gene.

PCR Assay

A sample of 0.5 μg of genomic DNA form each tissue was placed into a 100-μl reaction mixture containing 1 × PCR buffer [10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin], 200 μM of each dNTP (dATP, dCTP, dTTP, and dGTP), 0.25 μM 3′ oligonucleotide, 0.25 μM 5′ oligonucleotide, and 3 units of Taq polymerase (Perkin Elmer, CA). PCR amplifications was carried out in a Biocycler Oven (BIOS, CT) under the following amplification conditions: 92°C for 2 min, 55°C for 1 min, 72°C for 2 min for 30 cycles and then a 72°C 10-min elongation and a 22°C soak until the samples were removed from the instrument the next morning.

DNA Probes

DNA probe was created using amplified genomic DNA from the tumor cell line KgV. The 984-bp band was isolated from a 1% NuSeive (low melt) agarose (Sigma, MO) gel after removal of excess primer using Stratagene Prime Erase Quik Push columns (Stratagene, CA). This probe was designated E-55(+)984. The probe was labeled with [^{32}P]CTP (specific activity 3000 Ci/mmol, Amersham, IL) using a Stratagene Prime-It Random Primer Kit. Unincorporated radioactivity was separated out using a Boehringer Mannheim G50 Quick Spin column (Boehringer Mannheim, IN).

Analysis of PCR

Approximately 12% of each PCR reaction was separated by electrophoresis on a 1% agarose gel in Tris borate/EDTA buffer. Gels were then stained by ethidium bromide and photographed. After staining, gels were depurinated with 0.25 M HCl, denatured with 0.5 M NaOH, 1.0 M NaCl, and neutralized with 0.5 M Tris-HCl, 3 M NaCl, pH 7.25. Gels were blotted onto Hybond-N (Amersham) overnight using 20× SSC as the transfer buffer. The next day, the blots were UV cross-linked using a Stratagene UV Stratalinker 1800. Blots were prehybridized for 4 h at 42°C in prehybridization buffer containing 4× SSC, 5× Denhardts, 50 mM $NaPO_4$, pH 6.5, 1% SDS, 5% salmon sperm DNA, and 50% deionized formamide. Hybridization was done at 42°C overnight with 1× 10^7 counts of [^{32}P]E-55(+)984 in a hybridization buffer containing 4× SSC, 1× Denhardts, 20 mM $NaPO_4$, pH 6.5, 0.1% SDS, 1% salmon sperm DNA, 50% deionized formamide, and 10% dextran sulfate. The next day, blots were washed twice in 2× SSC plus 0.1% SDS at room temperature for 5 min and then washed twice in 0.1× SSC plus 0.1% SDS at 65°C for 30 min. Blots were exposed to x-ray film and analyzed using a PhosphorImager (Molecular Dynamics, CA) to quantify the presence of integrated virus. The relative level of *env* gene was determined using the following calculation: value from the PhosphorImager for tissue/value from the PhosphorImager for cell line KgV in the same assay.

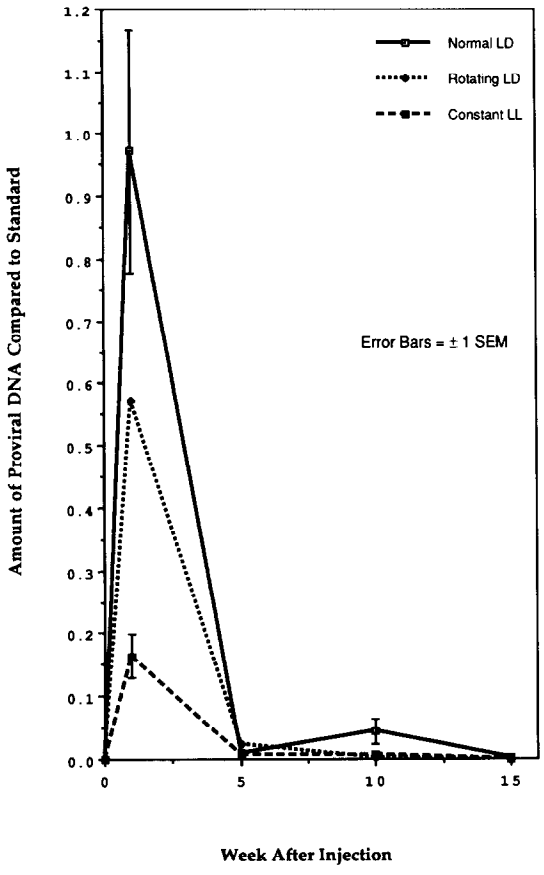

FIG. 1. Bone marrow results. The relative content of proviral DNA detected in the cells of bone marrow taken from E-55(+) infected mice under the three lighting conditions. Values are measured against those obtained from the KgV cell line. Error bars represent ± 1 SEM.

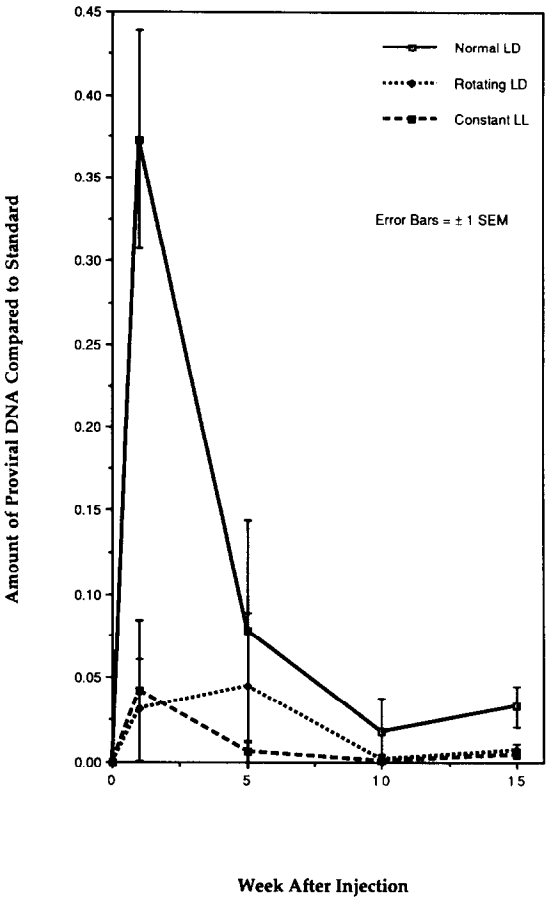

FIG. 2. Spleen cell results. The relative content of proviral DNA detected in the cells of spleens taken from E-55(+) infected mice under the three lighting conditions. Values are measured against those obtained from the KgV cell line. Error bars represent ± 1 SEM.

RESULTS

The results of this study are displayed in Figs. 1-3. Exposing mice to LL or rLD cycles caused a considerable reduction in the amounts of proviral DNA detectable in cells of bone marrow (Fig. 1) and spleen (Fig. 2). An analysis of variance (ANOVA) was run on bone marrow (Bm), spleen (Sp), and thymus (T) with the factors of light cycle and time of sacrifice as main effects (Statistica/MAC, StatSoft, Tulsa, OK). Time of sacrifice was a significant factor in all three tissues [Bm: $F(3, 26) = 162.5$, $p < 0.01$; Sp: $F(3, 27) = 27.8$, $p < 0.01$; T: $F(3, 25) = 3.77$, $p < 0.05$], substantiating that a temporal pattern exists in E-55(+) incorporation and infection in these mice. Light cycle was a significant factor in bone marrow, $F(2, 26) = 39.2$, $p < 0.01$, and spleen, $F(2, 27) = 34.4$, $p < 0.01$, with both LL and rLD cycles leading to significant reductions in detectable levels of proviral DNA. For example, the levels of proviral DNA detected in bone marrow were significantly less at week 1 in both LL-exposed ($p < 0.01$, Newman-Keuls, post hoc test) and rLD-exposed ($p < 0.01$, Newman-Keuls) mice compared with control animals exposed to the standard 10L : 14D cycle. The same results were found for spleens extracted 1 week postinoculation from LL-exposed ($p < 0.01$, Newman-Keuls) and rLD-exposed ($p < 0.01$, Newman-Keuls) mice compared with controls. Interestingly,

the amount of proviral DNA detected in bone marrow of LL-exposed animals was significantly less at 1 week than that found in marrow extracted from rLD-exposed mice ($p < 0.01$, Newman-Keuls), but no significant difference was uncovered with respect to the proviral DNA content in spleens obtained at 1 week from mice exposed to the two conditions. In addition, an increase in proviral DNA was detected in the thymus of mice maintained under 10L : 14D but not in animals exposed to either LL or rLD cycles (Fig. 3). To ascertain if this increase was artifactual, the data from control mice were compared to combined data from both LL and rLD mice. The difference was significant, $t(14) = 1.96$, $p < 0.05$ (one-tailed).

DISCUSSION

These results indicate that environmental lighting may have the ability to alter immune responsiveness to selected pathogens. The small sample size necessitates caution in interpretation of these results, but one model can be proposed that involves an effect of lighting on the balance between humoral and cell-mediated immunity.

Several researchers have proposed a model of immune regulation involving at least two subpopulations of helper T lymphocytes mediating different kinds of immune responses. TH_1 cells produce IL-2 and IFN-γ and promote a cell-mediated response whereas TH_2 cells generate IL-4, IL-5, IL-6, and IL-10, which foster the humoral immunity (28,31). These two immune states appear to be in a kind of competitive equilibrium, with IFN-γ inhibiting TH_2 activity whereas IL-4 and IL-10 suppress TH_1 responsiveness (13,14,31). Evidence has been gathered that indicates successful resistance to various pathogens and infectious agents is dependent upon the type of immune response that the host organism generates. Resistance to the protozoan parasite, *Leishmania major*, is poor in mice strains such as BALB/c, which respond with a predominantly humoral or TH_2-type reaction, whereas strains such as the CBA/J, with primarily cell-mediated or TH_1-type responses, are highly resistant (3). A similar phenomenon may occur in response to infection with the murine leukemia virus (MuLV), E-55(+). When comparing the infection process in BALB.K mice with that observed in the B10.BR strain (which express the same H-2 haplotype as BALB.K but differ in the genetic background), an interesting difference is revealed: B10.BR mice do not show the final leukemic phase, even when they have been maintained up to 13 months postinfection (unpublished observations). Insofar as BALB.K mice generate a predominantly TH_2 response to infection whereas B10.BR animals mount a predominantly TH_1 response (9,10), this would appear to be another example of the efficacy of cell-mediated

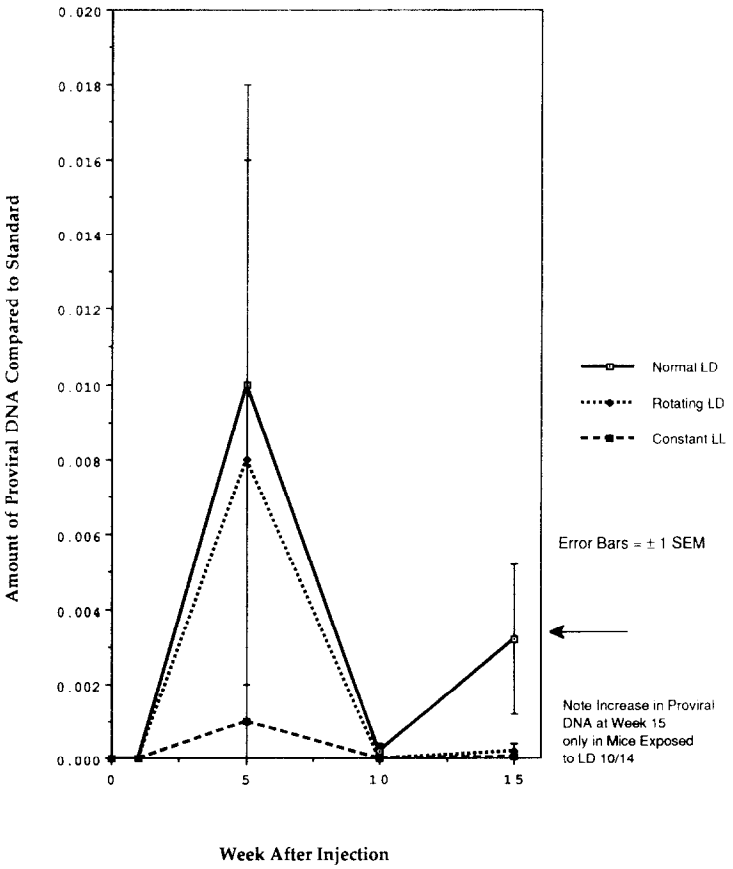

FIG. 3. Thymus cell results. The relative content of proviral DNA detected in the cells of thymus tissue taken from E-55(+) infected mice under the three lighting conditions. Values are measured against those obtained from the KgV cell line. Note increase in proviral DNA at week 15 only in mice exposed to 10L : 14D. Error bars represent ± 1 SEM.

immunity in disease resistance. However, the TH_1 response in not an immunological panacea. In response to helminth parasites, it is apparently the TH_2 response that confers successful resistance (37). Thus, when comparing the TH_1-favoring B10.BRs with the TH_2-dominant BALB.Ks, it is the BALB.Ks whose immune defense confers resistance to the parasites (9,10). An imbalance of TH_1- and TH_2-mediated immunity may also contribute to AIDS immune dysregulation (5–7,33). Defects in TH cell activities can be demonstrated in the absence of any significant alterations in CD4+ lymphocytes in asymptomatic HIV+ individuals (5,32), and TH cell function tests appear to be even more sensitive than PCR for detecting HIV infection (6). Furthermore, in tracking changes in HIV+ individuals over time, Shearer and Clerici (32) found that a high percentage of asymptomatic individuals (44%) showed immune responses indicative of a loss of TH_1 reactivity without a corresponding decrement in TH_2 immunity.

Melatonin has been primarily linked to increases in TH_2 or humoral immunity (22,23). An increase in these responses will suppress TH_1 immunity. In the case of E-55(+) infection, the more favorable response is the TH_1 type, and thus suppressing the TH_2 response might disinhibit cell-mediated immunity, leading to a more effective immune response. The hypothesis, then, is that light suppressed pineal melatonin, leading to a reduction in TH_2-mediated immunity that, in turn, disinhibited and enhanced the TH_1 response, leading to greater resistance to E-55(+). Clearly, more experimentation is needed to test this model and to determine if changes in environmental lighting schedules will increase the survival of infected BALB.K mice.

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