ROLE OF PINEAL MELATONIN AND MELATONIN-INDUCED-IMMUNO-OPIOIDS IN MURINE LEUKEMOGENESIS

ARIO CONTI,1 NECHAMA HARAN-GHERA2 and GEORGES J. M. MAESTRONI1

1Laboratory for Experimental Pathology, Istituto Cantonale di Patologia, via in Selva 24, 6604 Locarno, Switzerland
Department of Chemical Immunology, The Weizmann Institute of Science, 76100 Rehovot, Israel

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The relationship between the pineal gland, melatonin and melatonin-induced-immuno-opioids with the response of C57BL/6 mice to A-RadLV induced T cell lymphomas was investigated. Mice were injected at day 0 with A-RadLV and from day 10 they were treated chronically with melatonin 4 mg/kg body weight, naltrexone 1 mg/kg or phosphate buffered saline, throughout the experiment. In another protocol, groups of mice were a) surgical pinealectomized at day-14, b) functional pinealectomized (24:24 hours light) from day -20 and c) sham pinealectomized. At day 0 each group was inoculated intrathymically with A-RadLV. The results show that melatonin accelerated (p < 0.005) leukemogenesis whereas the surgical pinealectomy and the functional pinealectomy delayed it (p < 0.005 and p < 0.01). Moreover, the action of melatonin was blocked by naltrexone (p < 0.005), indicating the involvement of melatonin-induced-immuno-opioids in the development of the lymphomas.

Key Words: Pineal Gland, Melatonin, Melatonin-Induced-Immuno-Opioids, Leukemia, Mice survival

INTRODUCTION

The interaction between the nervous, the endocrine and the immune systems1 is now well recognized. Neural and hormonal stimuli may modulate the immune responses,1 while products from immunocompetent cells such as lymphokines can affect neuroendocrine mechanisms.2,3 Besides this, immunocompetent cells can synthesize and release true hormones like ACTH, growth hormone, prolactin, and opioids.4,5

In our previous work we demonstrated that the pineal gland and its most studied product, melatonin, have immunomodulating and anti-stress properties.6,4 Melatonin is working directly on activated T helper cells (CD4+), stimulating the synthesis and/or release of melatonin-induced-immuno-opioid peptides (MIIO) which in turn exert the observed immunostimulating and anti-stress effects.9 The activities of melatonin and MIIO were naltrexone sensitive, indicating the involvement of opioid receptors.9,10 In fact, target cells of the circadian immunomodulating action of MIIO seem to involve thymic cells and peripheral immunocompetent cells bearing specific opioid receptors.9,10

On the other hand, the thymus and its microenvironment have been implicated in leukemogenesis in experimental murine models.11

Young, 5 to 8 weeks old C57BL/6 mice are highly sensitive to the leukemogenic action of A-RadLV when the virus is inoculated intrathymically.11,12 Injection of this leukemia virus produces an impairment of humoral and cellular response to SRBC,13 via a suppressive effect on T helper cells.14 Leukemic cells infiltrates are found in thymus, mesenteric and axillar lymphnodes, spleen, liver and kidney.11,16 The majority of the A-RadLV induced leukemias consist in the proliferation of T lymphocytes bearing high levels of surface H-2 antigens. Cellular targets for A-RadLV were shown to be corticosterone and radiation resistant CD4+ and/or CD8+ thymocytes.17 As reported above, we have demonstrated that melatonin has an immunoenhancing and anti-stress effect in antigen primed mice by acting on CD4+ T cells.7,9 On this basis, we hypothesized that melatonin could counteract the immunosuppressive effect of A-RadLV.

The aim of the present study was to define the role of the pineal gland, melatonin and MIIO in leukemogenesis using the experimental model represented by C57BL/6 mice inoculated intrathymically with A-RadLV.

To whom correspondence should be addressed
MATERIALS AND METHODS

Mice

C57Bl/6 female mice, aged 3 to 4 weeks, and CD1 outbred, female mice, aged 14 weeks old, were purchased from Charles River, Calco, Italy. Mice were maintained under a light-dark cycle of 12 hours (6 a.m. light on, 6 p.m. light off) at 22 ± 1 °C. Great care was taken to avoid environmental stress before and during the course of the experiments (noise, smells, cage crowding, and so on). Tap water and fodder were given ad libitum.

Virus

The virus isolate was prepared from thymic lymphomas induced in adult C57Bl/6 mice by A-RadLV as described previously. The same intrathymic route of virus administration (20 μl/injection) was used in all experiments. A-RadLV expressed reverse transcriptase activity of 131,248 cpm/20 μl as assayed according to the method of Goff et al. Mice were injected with A-RadLV 14 days after surgical pinealectomy and 20 days after functional pinealectomy at the age of 66 days.

Drugs and treatments

Melatonin was purchased from Biosynth, Inc, Staad, Switzerland and naltrexone from Sigma Co., St. Louis, MO, USA. Melatonin was dissolved in 100 % ethanol and then diluted in phosphate buffered saline to a final concentration of 1 % ethanol, while naltrexone was dissolved in phosphate buffered saline. Route, dose and schedule of administration were: 4 mg/kg body weight, 0.5 ml s.c. at 4.30 p.m. naltrexone, 1 mg/kg, 0.5 ml s.c. at 4 p.m. All drugs were injected for 5 days per week throughout the experiment.

Pinealectomy

Surgical pinealectomy was performed using a stereotactic apparatus (Rheuma Labortechnic, Hofheim, Germany) and a dental drill with the appropriate steel bur. Mice were anaesthetized by injecting intramuscular 100 μl of a 1:10 dilution of 50 % Rompun (2 % of 2-(2-6-xilidino)-5,6-dihydro-4H-1,3 thiazine hydrochloride) and 50 % Vetalar (ketamin hydrochloride) and 0.5 ml s.c. at 4.30 p.m. naltrexone, 1 mg/kg, 0.5 ml s.c. at 4 p.m. All drugs were injected for 5 days per week throughout the experiment.

Urine collection and 6-hydroxy-melatonin-sulfate RIA

We showed that C57Bl/6 mouse have a clearcut circadian rhythm of endogenous melatonin in serum. Nevertheless it is also possible to evaluate endogenous melatonin production by measuring its main metabolite 6-hydroxy-melatonin-sulfate secreted in the urine. Urine was collected using metabolic cages (Techniplast, Gazzada, Italy) over periods of 12 hour light on and 12 hour light off from surgical pinealectomized and sham pinealectomized mice and over 24 hour light (2 x 12 hour) from functional pinealectomized mice. Mice were 6 per group. During collection, the urine recipient was maintained at a temperature of around 0 °C. After collection, the urine was mixed with active peat charcoal (Norit A, Serva Fein Biochemica, Heidelberg, Germany), incubated for 15 minutes at 4 °C, centrifuged and kept frozen at −30 °C until assayed. The urinary concentration of 6-hydroxy-melatonin-sulfate was determined by a commercially available RIA kit (IBL-GMBH, Hamburg, Germany). The assay was run in tricine buffer pH 7.4. The tracer, constituted of 3H-hydroxy-melatonin-sulfate was added to standards and unknown samples of urine (diluted 1:10 in buffer) together with an appropriate dilution of rabbit anti-6-hydroxy-melatonin-sulfate antiserum and incubated for 18 hours at 4 °C. The cross-reactivity of the antiserum was < 1 % against melatonin, hydroxymelatonin, 5-methoxy-indol-3-acetic acid, 5-methoxy-trypophan, 5-methoxytryptophan, 5-hydroxy-tryptophan, 5-hydroxy-tryptamine, tryptamine, 5-hydroxy-D-trypophan-L, 5-hydroxyindol-3-acetic acid, and N-acetyl-5-hydroxytryptamine. At the end of the first incubation, sheep anti-rabbit IgG was added as solid phase and the mixture was further incubated for 1 hour at 4 °C. Then the tubes were centrifuged at 2,000 × g and the pellet resuspended in 1 ml of 0.1 % Triton-X 100 and transferred into the.