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Immune-Modulatory Drugs Alter *Candida albicans*-Induced Sleep Patterns in Rabbits

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TOTH, L. A. *Immune-modulatory drugs alter* Candida albicans*-induced sleep patterns in rabbits.* PHARMACOL BIOCHEM BEHAV **51**(4) 877–884, 1995.—To evaluate the influences of immune responsiveness on sleep alteration during infectious disease, sleep was monitored during *Candida albicans* infection in rabbits treated with immune-modulating drugs. Intravenous administration of *C. albicans* to normal rabbits initially increased and subsequently decreased both the amount of slow-wave sleep (SWS) and δ-wave amplitudes (DWA) during SWS. Cortisone treatment attenuated these effects. The immunosuppressive drug cyclosporine did not alter the initial enhancement of SWS, but did attenuate the *C. albicans*-induced reduction in SWS time and potentiate the reduction in DWA. In contrast, administration of incomplete Freund's adjuvant and prior immunization with killed *C. albicans*, which were expected to enhance immune responsiveness, did not markedly alter *C. albicans*-induced alterations in SWS. However, the immune stimulant levamisole potentiated the effects of *C. albicans* on SWS. These data indicate that pharmacologic treatments expected to alter immune responsiveness modulate microbially induced sleep, and are consistent with the hypothesis that facets of the immune response mediate sleep changes during infectious disease.

Sleep Infection *Candida albicans* Rabbit Cortisone Cyclosporine Levamisole Adjuvant Immunization

ENHANCED sleep, like fever, is a manifestation of the acute-phase response to microbial infection. In rabbits, acute bacterial, fungal, and viral infections induce an initial phase of increased sleep and a subsequent phase of reduced sleep (29,48,49). Several lines of evidence suggest that these effects may be mediated by facets of the immune response. For example, immunosuppressive doses of cortisone attenuate some of the sleep alterations induced by bacterial inoculation of rabbits (47), and sleep alterations do not occur in rabbits rendered tolerant to injections of influenza virus (29). To further evaluate the role of the immune response in microbially induced sleep alterations, the present study characterized sleep patterns in rabbits treated with immune-modulatory drugs before inoculation with the fungal organism *Candida albicans*. The data indicate that treatments designed to alter immune responsiveness also modulate microbially induced sleep patterns, and are consistent with the hypothesis that facets of the immune response mediate sleep changes during infectious disease.

METHOD

Animals and Experimental Design

Adult male New Zealand white rabbits (*Pasteurella multocida*-free; Myrtle's Rabbitry, Thompson Station, TN) weighing 4–5 kg were surgically implanted with electroencephalographic (EEG) electrodes and brain thermistors as previously described (48) and were allowed to recover for several weeks. Rabbits were housed individually in a temperature-controlled room (21° ± 2°C) on a 12L : 12D schedule. Before testing, rabbits were moved to experimental chambers maintained under the same conditions and were permitted an overnight period of adaptation. Sleep, brain temperature, and movement were then monitored for 4 consecutive days, with daily recording sessions beginning approximately 2 h after light onset. On day 1, baseline sleep was monitored. Beginning on day 2, rectal temperatures and blood samples were taken each morning, and experimental injections were administered immedi-

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ately after blood collection, as follows: cortisone, 20 mg/kg intramuscularly (IM) (Merck, Sharpe, and Dohme, West Point, PA) (n = 12); cyclosporine, 25 mg/kg subcutaneously (SC) (Sandoz, East Hanover, NJ) (n = 11); incomplete Freund's adjuvant (IFA), 0.25 ml/kg SC (Sigma Biochemical, St. Louis, MO; n = 8); or levamisole, 6 mg/kg SC (Pittman-Moore, Washington Crossing, PA) (n = 3). These treatments were repeated on days 3 and 4. On the same experimental days, control animals (n = 14) were also removed from the recording chambers for temperature measurement, blood collection, and IM injection of sterile pyrogen-free saline (0.9% NaCl). The effect of SC administration of saline was not evaluated independently because of previous work demonstrating that SC injections of saline do not alter sleep or hematologic parameters in rabbits (50). On day 3, all rabbits were inoculated intravenously with $6.1 \pm 0.8 \times 10^7$ colony-forming units (CFU) of *C. albicans*. Throughout the experiment, rabbits could move freely in their cages and had continuous access to food and water. Rabbits were euthanized with intravenous (IV) T-61 euthanasia solution (Hoechst-Roussel, Somerville, NJ) at the end of the experiment.

One group of rabbits (n = 8) was immunized with weekly IV injections of killed *C. albicans* for 8 weeks. Ten days after the final injection, sera were collected and measured for anti-*Candida* antibody titer by determining the highest serial dilution that would agglutinate formalin-fixed organisms. The median anti-*Candida* antibody titer in these rabbits was 1 : 64 (range 1 : 16 to 1 : 128). Sleep patterns in immunized rabbits were monitored for 24 h before and 48 h after inoculation with viable *C. albicans*, as described earlier.

Electrophysiologic Recording

EEG and brain temperature signals were measured via a flexible tether attached to a rotary commutator (Plastics One, Roanoke, VA) that permitted unrestricted movement by the rabbits. Animal movement was monitored via an acceleration transducer (Grass Instruments, Quincy, MA) suspended from the commutator. δ-wave (0.5–4.0 Hz) components of the EEG were quantified using band-pass filters (Buxco Electronics, Sharon, CT) and analog-to-digital conversion. Average EEG δ-wave amplitudes (DWA) were calculated for each 1-min interval of the recording period and stored in digital form on computer. The analog signals of EEG activity, the filtered rectified δ-wave component, animal movement, and brain temperature were also displayed on a polygraph (Grass Instruments).

Vigilance states were determined based on DWA, movement, and brain temperature data using computer-assisted scoring. Each animal's EEG tracing, filtered and rectified EEG δ-wave signal, and movement recording for the first 6 h of the baseline period were visually examined to determine a threshold DWA associated with slow-wave sleep (SWS). This value was used to determine the vigilance state of every animal during each 1-min interval throughout the recording period. An animal was considered to be in SWS whenever the average DWA during any 1-min interval exceeded the SWS threshold amplitude in the absence of movement. When not in SWS, the animal was either awake or in rapid-eye-movement sleep (REMS). REMS was identified by visually inspecting the polygraph record for a low-voltage EEG tracing associated with a rise in brain temperature and sporadic movements (occasional twitching). Sleep parameters were summarized across 2-h intervals. The percentage of time spent in SWS and REMS, the average DWA during SWS (which reflects the depth or intensity of SWS (4,5,37), and the average length of SWS bouts were calculated for each animal.

Preparation of Fungal Inocula

For preparation of the fungal inocula, *C. albicans* (American Type Culture Collection strain 310) was purchased as lyophilized cultures on Culti-loops (Scott Laboratories, Fiskeville, RI). Prewarmed Sabaroud agar plates were inoculated and incubated for 24 h at 37°C. Colonies were transferred to sterile pyrogen-free saline to achieve a concentration of approximately 2×10^9 CFU/ml, initially estimated using a Klett-Summerson photoelectric colorimeter and later verified by plating serial dilutions of the microbial suspension on Sabaroud agar plates. Killed organisms for rabbit immunization were prepared by suspension in thimerosal for several hours; they were washed twice with sterile saline, verified to be free of viable *C. albicans* by incubation on Sabaroud agar plates for 24 h, and stored in aliquots at −20°C until used.

Blood Analysis

Blood samples (1–3 ml) were collected from the central artery of the ear and immediately transferred into vacuum tubes containing EDTA. Total white blood cell (WBC) counts were measured with a model 2N cell counter (Coulter Electronics, Hialeah, FL). Differential WBC counts were made by classifying 100 WBCs on Wright-stained blood smears; final WBC counts were corrected for nucleated red blood cells (nRBC), if present. Fibrinogen concentrations were measured using a fibrometer (Becton-Dickinson, Towson, MD). Plasma triglyceride concentrations were measured with a Reflotron clinical chemistry analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN).

Postmortem blood cultures were performed using blood samples obtained by cardiac puncture immediately after euthanasia. Blood was incubated for 24–48 h at 37°C in brain-heart infusion broth. An aliquot was then transferred to Sabaroud agar for an additional 24–48 h incubation. Blood cultures were not performed on two rabbits that died spontaneously 24–48 h after *C. albicans* inoculation. One of these rabbits had received only *C. albicans*, and the other had received *C. albicans* in combination with levamisole.

Statistical Analysis

For the *Candida*-only group, the experimental design was a complete block without replication. Rabbit and time were the main effects. Several hypotheses were tested with t-tests, using the estimated pooled within-rabbit variance in the estimation of the standard error of the difference between two designated means (43). The first hypothesis was that during the baseline period there was no difference in sleep variables measured at a particular hour on day 1 compared to the same hour on day 2. Failure to reject this hypothesis was necessary to demonstrate that there was no effect of temperature measurement, blood collection, and IM injection of sterile pyrogen-free saline on the sleep variables during the baseline period. The second hypothesis was that inoculation with *C. albicans* had no effect on sleep variables measured at a particular hour on days 3–4 compared to the same hour on day 2. Rejection of this hypothesis indicated a significant effect of *C. albicans* on sleep variables.

The response over time of each treatment group was compared to the *Candida*-only group. The experimental design was the typical split-plot with rabbits nested within treatments

that were cross-classified with time. Several hypotheses were tested with *t*-tests, using the estimated pooled within-rabbits variance in the estimation of the standard error of the difference between the two designated means (43). The first hypothesis was that during baseline there was no difference between sleep variables measured at a particular hour on day 1 between the treatment and *Candida*-only groups. Failure to reject this hypothesis indicated that the groups were comparable at baseline. The second hypothesis was that there was no effect of the immunosuppressive or immune-stimulatory treatment on sleep variables measured at a particular hour on day 2 compared to the same hour on day 2 for the *Candida*-only group during the baseline assessment. Rejection of this hypothesis indicated a significant effect of the drug treatment on sleep. The third hypothesis was that there was no effect of the immunosuppressive or immune-stimulatory treatment in combination with *C. albicans* on sleep variables measured at a particular hour on day 3 compared to the same hour on day 3 for the *Candida*-only group, and at a particular hour of day 4 compared to the same hour on day 4 for the *Candida*-only group. Rejection of this hypothesis indicated a significant effect of the drug treatment in combination with *C. albicans* on sleep compared to *C. albicans* alone. The effects of drug and *C. albicans* were confounded by design with the explicit assumption that the effects of the particular drug were the same on days 3 and 4.

All data were analyzed with the generalized linear models procedure of the SAS statistical package (Cary, NC). To reduce the probability of type I errors, a significance level of $p \leq 0.03$ was used. At this level of significance, a sample size of eight to 12 rabbits was sufficient with a two-sided test to detect an effect size of 1.0–1.5 SD units at a power of 0.8, or an effect size of 1.5–2.0 SD units at a power of 0.5.

RESULTS

Sleep After C. albicans *Alone*

Inoculation with *C. albicans* alone ($n = 14$) induced biphasic alterations in sleep (Fig. 1). Relative to the baseline recording period (day 1), rabbits spent increased time in SWS during the initial 4–8 h after *C. albicans* inoculation. DWA during SWS and SWS bout lengths also increased during this period. During the 20–46 h interval after inoculation, time spent in SWS, DWA during SWS, and SWS bout lengths were decreased relative to corresponding baseline values. *Candida albicans* inoculation significantly reduced REMS for most of the postinoculation period. This effect was not significantly altered by any of the treatments discussed subsequently (data not shown).

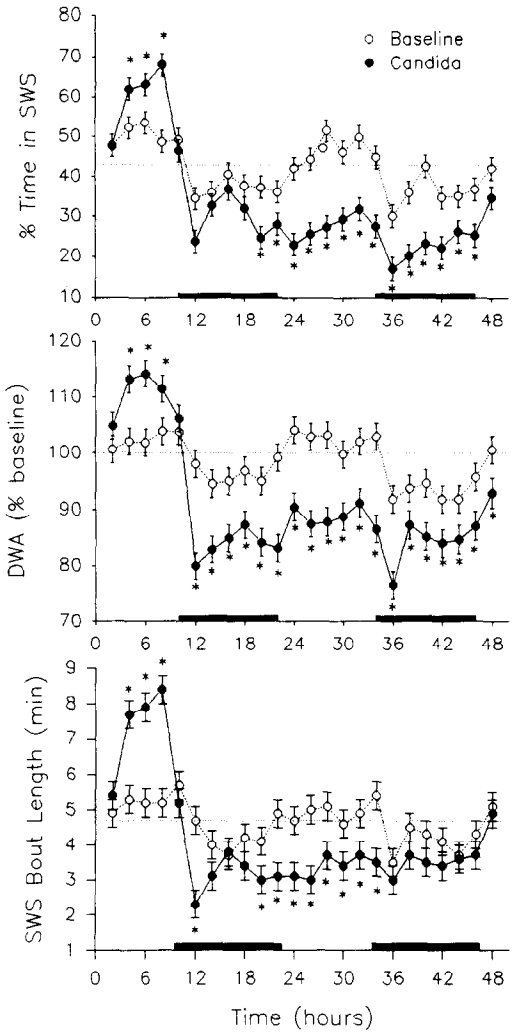

FIG. 1. Effects of *Candida albicans* inoculation on sleep in rabbits. Sleep was monitored for 48 h before (○) and 48 h after (●) inoculation of rabbits ($n = 14$) with *C. albicans*. Each data point represents the mean ± SEM of values obtained during the preceding 2 h. The dotted horizontal line indicates average values obtained on day 1 of recording. To facilitate visual comparison, baseline values (days 1–2) are superimposed over values obtained after inoculation (days 3–4). Baseline and postinoculation data also appear as shaded areas in Fig. 2 and 3. Bars on the abscissa designate the lights-off period. $*p <$ 0.03 relative to the comparable baseline interval.

Sleep During Immunosuppressive Treatments

Rabbits treated with immunosuppressive drugs (cortisone or cyclosporine) demonstrated significant alterations in *C. albicans*-induced sleep changes (Fig. 2). Cortisone alone (20 mg/kg, IM) increased DWA 6–10 h after injection (h 30–34, Fig. 2) and reduced DWA 22–24 h after injection (h 46–48, Fig. 2). Time spent in SWS was also reduced 16–18 h after cortisone injection (h 40–42, Fig. 2). During the 48-h period after *C. albicans* inoculation, cortisone-treated rabbits maintained a circadian rhythmicity of SWS time. Although they demonstrated modest increases and decreases in SWS time after *C. albicans* inoculation, the magnitude of these changes was markedly attenuated. Cortisone treatment also prevented the *C. albicans*-induced increase in DWA, but did not significantly alter the subsequent decrease in this parameter. *C. albicans*-induced increases and decreases in SWS bout length were attenuated in cortisone-treated rabbits.

Treatment of rabbits with the immunosuppressive drug cyclosporine (25 mg/kg, SC; $n = 11$) did not significantly alter normal sleep patterns during the 24 h after administration (h 24–48, Fig. 2), or markedly influence the increases in SWS time, DWA during SWS, or SWS bout length induced by *C. albicans* inoculation (Fig. 2). However, cyclosporine treatment reduced the *C. albicans*-induced suppression of SWS time (h 68–78, Fig. 2) and exacerbated the reduction in DWA (h 60–96, Fig. 2). SWS bout lengths were increased sporadically after *C. albicans* inoculation of cyclosporine-treated rabbits.

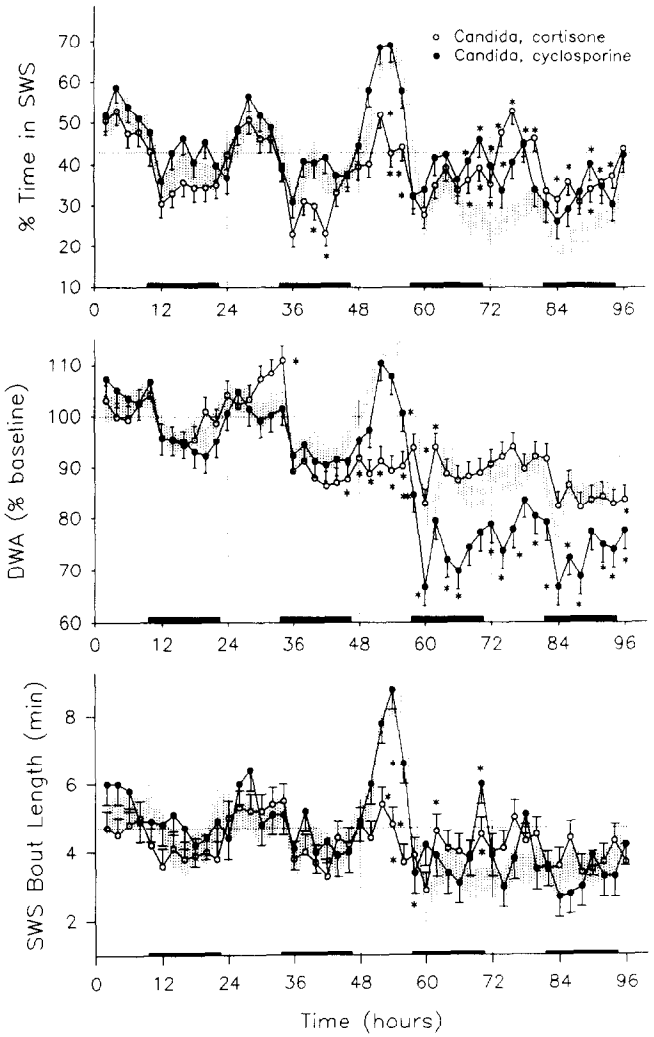

FIG. 2. Effects of immune-suppressive treatments on sleep in *Candida albicans*-inoculated rabbits. Sleep was recorded continuously for 96 h. Baseline sleep patterns were recorded during the initial 24 h. Rabbits were then injected IM with cortisone (n = 12; ○) or SC with cyclosporine (n = 11; ●); this treatment was repeated 24 and 48 h later. Treatments are indicated by vertical dotted lines. Rabbits were inoculated intravenously with *C. albicans* at 48 h. Each data point represents the mean ± SEM of values obtained during the preceding 2 h. Bars on the abscissa designate the lights-off period. To facilitate visual comparison, data from rabbits inoculated with *C. albicans* alone (see Fig. 1) are presented as shaded areas, with the dotted horizontal line indicating average values on day 1 of recording. *$p < 0.03$ relative to the comparable recording interval of rabbits receiving *C. albicans* alone.

Sleep During Immune-Stimulatory Treatments

Prior exposure to killed *C. albicans*, which induced serum titers of anti-*Candida* antibodies in rabbits, did not significantly influence baseline or *C. albicans*-induced sleep patterns (n = 8; data not shown). Treatment of rabbits with IFA (0.25 ml/kg, SC; n = 8) did not alter basal sleep patterns or *C. albicans*-induced sleep enhancement, but did attenuate the *C. albicans*-induced suppression of SWS time on the 2nd day postinoculation (h 84–96, Fig. 3). SWS bout lengths were not significantly altered by IFA.

Treatment of rabbits with the immune-stimulant levamisole (6 mg/kg, SC; n = 3) markedly reduced SWS time, DWA during SWS, and SWS bout length for 2–4 h after each injection. These sleep parameters subsequently demonstrated increases that persisted for at least 2 h. Levamisole treatment potentiated *C. albicans*-induced increases in SWS time and SWS bout length (h 52–60, Fig. 3) and exacerbated *C. albicans*-induced reductions in SWS time and in DWA during SWS (h 60–96, Fig. 3). Marked decreases in SWS bout length occurred 24–48 h after inoculation (h 74–96, Fig. 3). Rabbits treated with levamisole became moribund 24–48 h after *C. albicans* inoculation, and for humane reasons the number of rabbits in this experimental group was limited to three.

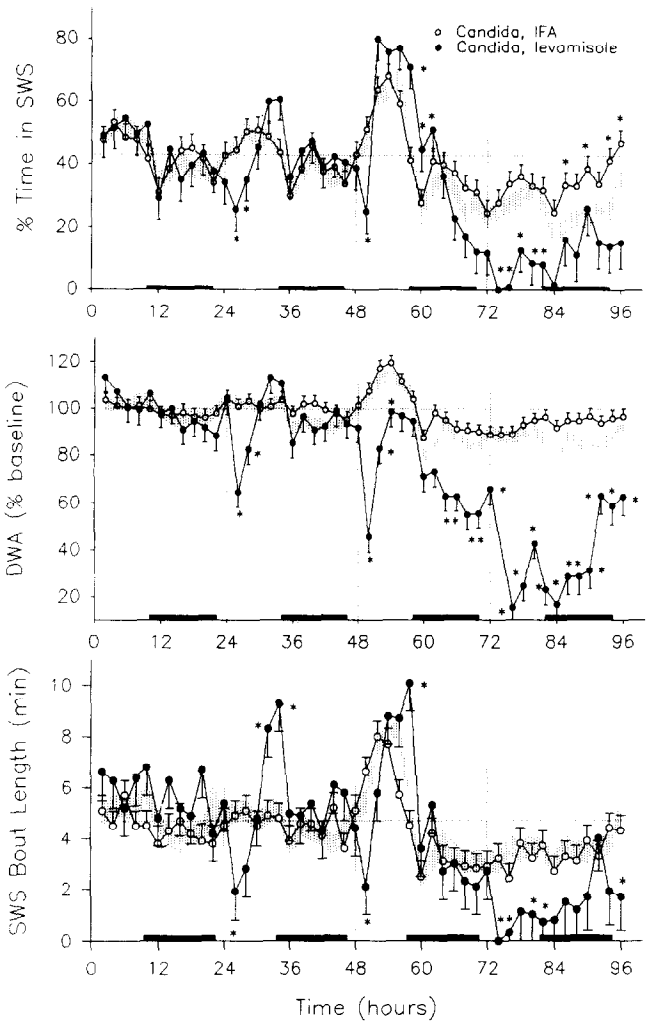

FIG. 3. Effects of immune-stimulatory treatments on sleep in *C. albicans*-inoculated rabbits. Sleep was recorded continuously for 96 h. Baseline sleep patterns were recorded during the initial 24 h. Rabbits were then injected SC with incomplete Freund's adjuvant (n = 8; ○) or levamisole (n = 3; ●); this treatment was repeated 24 and 48 h later. Treatments are indicated by the vertical dotted lines. Rabbits were inoculated intravenously with *C. albicans* at 48 h. Each data point represents the mean ± SEM of values during the preceding 2 h. Bars on the abscissa designate the lights-off period. To facilitate visual comparison, data from rabbits inoculated with *C. albicans* alone (Fig. 1) are presented as shaded areas, with the dotted horizontal line indicating average values on day 1 of recording. *$p < 0.03$ relative to the comparable recording interval of rabbits receiving *C. albicans* alone.

Febrile and Hematologic Effects

Table 1 shows the differences in the febrile and hematologic responses of the various treatment groups. Cortisone prevented *C. albicans*-induced fever and significantly reduced elevations in fibrinogen, triglycerides, and nRBCs. Cortisone also induced marked neutrophilia and lymphopenia before *C. albicans* inoculation, and exacerbated the neutrophilia that occurred subsequent to challenge. Cyclosporine attenuated *C. albicans*-induced lymphopenia, fibrinogenemia, and nRBC elevation. Immunized rabbits demonstrated enhanced neutrophilia and lymphopenia but reduced levels of triglycerides and nRBCs after *C. albicans* inoculation. IFA elicited neutrophilia and lymphopenia before fungal challenge and diminished *C. albicans*-induced increases in nRBCs, triglycerides, and fibrinogen. Levamisole induced mild lymphopenia when administered alone and exacerbated fever on the 1st day after fungal inoculation.

The frequency of positive postmortem blood cultures did not differ significantly across the various experimental groups (*C. albicans* only, eight of 13 (62%); cyclosporine, six of 11 (55%); IFA, five of eight (62%); cortisone, nine of 12 (75%); immunized, two of eight (25%); levamisole, two of two (100%), Fisher exact test). Notably, however, positive cultures were most prevalent in levamisole-treated rabbits, which were clinically the most severely affected, and least prevalent in immunized rabbits.

DISCUSSION

These findings indicate that treatment with immune-modulatory drugs can alter the somnogenic effects of *C. albicans* inoculation in rabbits, supporting the hypothesis that facets of the immune response elicit changes in sleep during infectious disease. The sleep alterations observed in microbially inoculated rabbits are characterized by an initial increase and a subsequent decrease in the amount and intensity of SWS (49). The initial sleep enhancement has been linked to production of somnogenic cytokines. For example, administration of the immune modulator interleukin 1 (IL-1) promotes sleep in rabbits and other species (33,45,46), and sleep enhancement induced in rabbits by administration of muramyl dipeptide, a synthetic analog of bacterial peptidoglycan, is attenuated by treatment with the IL-1 receptor antagonist (25). Similarly, serum antiviral activity, which presumably reflects serum concentrations of the somnogenic cytokine interferon, increases in temporal correlation with fever and sleep enhancement in influenza-inoculated rabbits (29). However, rabbits rendered tolerant to the virus by repeated exposure do not develop changes in sleep, temperature, or serum interferon

TABLE 1

TEMPERATURE AND HEMATOLOGIC PARAMETERS IN RABBITS INOCULATED WITH *CANDIDA ALBICANS* AFTER IMMUNE-MODULATING TREATMENTS

Group	Sample*	Temperature (° C)	Neutrophils (% WBC)	Lymphocytes (% WBC)	nRBC (per 100 WBC)	Triglycerides (mg/dl)	Fibrinogen (mg/dl)
Candida only	1	39.0 ± 0.1	28 ± 2	67 ± 2	0.1 ± 0.9	156 ± 63	305 ± 34
(n = 14)	2	38.9 ± 0.1	25 ± 2	70 ± 2	0.2 ± 0.9	134 ± 63	337 ± 33
	3	40.2 ± 0.1†	69 ± 2†	28 ± 3†	3.0 ± 1.0	373 ± 67†	634 ± 38†
	4	39.7 ± 0.1†	56 ± 3†	40 ± 3†	7.4 ± 1.0†	679 ± 67†	944 ± 36†
Cortisone	1	38.8 ± 0.1	25 ± 3	70 ± 3	0.7 ± 1.0	145 ± 69	300 ± 35
(n = 12)	2	38.9 ± 0.1	72 ± 3†‡	26 ± 3†‡	0.2 ± 1.0	99 ± 69	346 ± 40
	3	39.0 ± 0.1‡	85 ± 3†‡	14 ± 3†‡	0.8 ± 1.0	148 ± 69‡	443 ± 37†‡
	4	38.8 ± 0.1‡	75 ± 3†‡	24 ± 3†‡	2.2 ± 1.0‡	245 ± 69‡	658 ± 35†‡
Cyclosporine	1	39.0 ± 0.1	30 ± 3	66 ± 3	0.6 ± 1.0	166 ± 72	298 ± 42
(n = 11)	2	38.9 ± 0.1	32 ± 3	65 ± 3	0.0 ± 1.1	135 ± 77	283 ± 50
	3	39.8 ± 0.1†	62 ± 3†	39 ± 3†‡	0.7 ± 1.0	423 ± 72†	562 ± 40†
	4	39.6 ± 0.1†	52 ± 3†	45 ± 3†	2.8 ± 1.1‡	568 ± 77†	689 ± 40†‡
Immunized§	1	ND	ND	ND	ND	ND	ND
(n = 8)	2	38.7 ± 0.2	21 ± 3	76 ± 3	0.0 ± 1.2	138 ± 84	263 ± 47
	3	39.8 ± 0.2†	74 ± 3†	25 ± 3†	0.4 ± 1.2	273 ± 84	656 ± 43†
	4	39.6 ± 0.2†	67 ± 3†‡	28 ± 3†‡	4.0 ± 1.2†‡	254 ± 84‡	894 ± 43†
IFA¶	1	39.1 ± 0.2	28 ± 3	67 ± 3	0.1 ± 1.2	209 ± 84	282 ± 43
(n = 8)	2	39.1 ± 0.2	45 ± 3†‡	50 ± 3‡	0.1 ± 1.2	162 ± 84	378 ± 47
	3	40.1 ± 0.2†	68 ± 3†	30 ± 3†	2.4 ± 1.2	284 ± 84	629 ± 43†
	4	39.9 ± 0.2†	61 ± 3†	36 ± 3†	0.9 ± 1.2‡	218 ± 84‡	726 ± 47†‡
Levamisole	1	39.5 ± 0.4	27 ± 5	70 ± 5	0.0 ± 1.9	184 ± 137	185 ± 70
(n = 3)	2	39.6 ± 0.4	32 ± 5	54 ± 5†‡	0.0 ± 1.9	163 ± 137	225 ± 70
	3	41.2 ± 0.4†‡	69 ± 7†	26 ± 7†	7.5 ± 2.6†	470 ± 181	460 ± 93†
	4	39.6 ± 0.5	ND	ND	ND	ND	ND

*Blood samples and rectal temperatures were collected as follows: sample 1, immediately prior to drug administration on day 2 of the experiment; sample 2, immediately prior to *C. albicans* inoculation on day 3 of the experiment; sample 3, 24 h after *C. albicans* inoculation; sample 4, 48 h after *C. albicans* inoculation.

†$p < 0.03$ relative to the "sample 1" value for the same group.

‡$p < 0.03$ relative to the same sample number from the "*Candida*-only" group.

§ "Immunized" refers to rabbits with prior exposure to killed *C. albicans*.

¶IFA refers to incomplete Freund's adjuvant.

ND = not done.

concentrations after viral challenge (29). The mechanisms that mediate microbially induced sleep suppression are unknown, although the suppression appears to be actively generated (51). Some substances believed to negatively regulate the immune response [including α-melanocyte stimulating hormone, corticotrophin releasing factor, adrenocorticotrophin, and glucocorticoids (34,52)] also reduce sleep propensity (9,38,47), and may suppress sleep after microbial challenge. However, these relationships are highly speculative.

The manipulations used in these experiments were all intended to modify immune responsiveness in infected rabbits. Dosages and routes of administration of immune-modulatory agents were selected based on literature reports of efficacy in animal studies. Doses of cortisone similar to those used here suppress cellular immune responses and sleep responses in bacterially inoculated rabbits (35,47). Similarly, cyclosporine doses used were comparable to those reported to prevent rejection of allogeneic skin grafts in rabbits (22). Levamisole has not been evaluated extensively in rabbits, but in mice, guinea pigs, and rats, IP doses of 2.5–10 mg/kg are reported to augment immune responsiveness in several model systems (10,40, 42). Based on this information, an intermediate dosage (6 mg/kg, SC) was selected for use in the present study. The dosage of IFA was selected based on recommended volumes for rabbits immunization protocols (11). Finally, rabbits were immunized by exposure to killed organisms using methods similar to those previously reported to increase anti-*Candida* antibody titers (24,53).

With the exception of the immunized group, rabbits exposed to these immune-modulatory treatments demonstrated significant alterations in postinoculation sleep patterns as compared to the *Candida*-alone group. The experimental design did not evaluate *Candida*-independent drug effects on sleep during days 3–4 of the protocol, and the treatments alone could potentially produce sustained modulatory effects on sleep in uninfected rabbits. Nonetheless, the data clearly demonstrate that sleep patterns after infectious challenge are modified in animals that are treated concurrently with immune-modulatory drugs. These results thus support the involvement of immune processes in microbially induced sleep alterations. Although the observed effects cannot easily be incorporated into a comprehensive model of how the immune response influences sleep, possible mechanisms can be considered.

Several reports suggest that macrophage-mediated microbial processing and macrophage-derived somnogenic substances promote sleep enhancement during infectious disease (26,27), and the responses observed in cortisone- and levamisole-treated rabbits are consistent with macrophage involvement in microbially induced sleep alterations. Immunosuppressive doses of cortisone attenuate the biphasic changes in SWS time that occur after *C. albicans* inoculation. In contrast, the immune stimulant levamisole appears to exacerbate the sleep changes. Because only three levamisole-treated rabbits were evaluated, such a conclusion is tentative and cannot be claimed with certainty. Nonetheless, these apparently contrasting influences on sleep patterns may result from different effects of these agents on macrophage–monocyte function. Glucocorticoids suppress macrophage inflammatory response in at least three ways: a) by inhibiting the action of macrophage migration-inhibitory factor, thereby promoting the egress of macrophages from affected areas (3); b) by reducing antigen processing by macrophages (20,36); and c) by suppressing macrophage synthesis and release of the somnogenic cytokine IL-1 (31,44). Levamisole, however, promotes monocyte chemotaxis (39), facilitates phagocytosis and antigen clearance (1), and increases IL-1 production by monocytic cell lines and peritoneal macrophages (28).

The different immunologic properties of cortisone and levamisole are also apparent in their clinical effects during *C. albicans* infections. Cortisone reduced fever, as previously described in rabbits inoculated with bacteria (47), whereas fever was exacerbated by levamisole treatment. The effects of these substances on sleep enhancement and fever could reflect divergent influences on IL-1, a well-characterized endogenous pyrogen (30). Glucocorticoids, which suppress many of the physiologic effects of IL-1, also attenuate many of the symptomatic sequelae of microbial infections (2). In contrast, levamisole treatment of humans is associated with flulike side effects, including fever, muscle aches, and malaise (7,15), and similar effects occur in humans given IL-1 (13).

The two immunosuppressive agents cortisone and cyclosporine both reduced *C. albicans*-induced suppression of SWS time, and on the second day postinoculation, cyclosporine-treated rabbits, like the cortisone-treated group, showed circadian rhythms in SWS time. Cortisone and cyclosporine share some immunosuppressive actions, but it is uncertain which of these might reduce microbially induced sleep suppression. For example, cortisone and cyclosporine both inhibit some functions of antigen-presenting cells (17,18,23), which may be important in the generation of sleep-promoting substances during infections (26,27). In addition, both agents decrease interleukin 2 (IL-2) production (6,8,41,54), albeit via different mechanisms (16,21). Administration of IL-2 into the locus coeruleus of rats is reported to promote sleep (12), but somnogenic actions of IL-2 after peripheral administration have not been described. In addition to effects on IL-2, cyclosporine, like cortisone, may inhibit IL-1 release (8).

Despite some similarities, the somnogenic effects of cortisone and cyclosporine differed in other respects. For example, only cortisone blocked the *C. albicans*-induced increases in SWS time and in DWA during SWS that occurred during the first several hours after inoculation. In contrast, cyclosporine potentiated the *C. albicans*-induced reduction in DWA during sleep, whereas cortisone did not modify this parameter. Although the factors responsible for these divergent effects are unknown, they may reflect physiologic effects and mechanisms of action that differ between the two agents. For example, cyclosporine and dexamethasone produce different effects on human T-cell responses to various stimuli (16,19).

IFA administration and prior exposure to killed *C. albicans* were both intended to enhance immune responsiveness, but caused little or no change in the response of rabbits to *C. albicans* inoculation. These minimal effects could indicate that the treatments themselves were ineffective. Repeated prior exposure to killed organisms, for example, induced serum antibody titers against *C. albicans* in all immunized rabbits, and was expected to provide protective immunity. However, previous studies have indicated that anti-*Candida* antibodies are not always protective (24,53). Treatment of rabbits with the nonspecific adjuvant IFA produced only modest attenuation of *C. albicans*-induced suppression of SWS time and DWA during SWS. Although IFA is generally an adequate adjuvant for secondary immune responses, complete Freund's adjuvant (CFA) is commonly necessary for primary immunizations (11). Unfortunately, CFA contains somnogenic muramyl peptides in the form of mycobacterial peptidoglycan (14,32), and therefore was unsuitable for this study. Moreover, IFA is commonly administered IM as an emulsion containing the antigen, but in this study the antigen and adjuvant were ad-

ministered separately at different injection sites (IV and SC, respectively).

In summary, these data indicate that the somnogenic effects of microbial inoculation can be altered by pharmacologic treatments known to modulate immune function. These findings support the hypothesis that facets of the immune response may contribute to sleep alterations during infectious disease.

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