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Sleep after Vaccination Boosts Immunological Memory

Tanja Lange,*† Stoyan Dimitrov,*† Thomas Bollinger,‡ Susanne Diekelmann,* and Jan Born*†

Sleep regulates immune functions. We asked whether sleep can influence immunological memory formation. Twenty-seven healthy men were vaccinated against hepatitis A three times, at weeks 0, 8, and 16 with conditions of sleep versus wakefulness in the following night. Sleep was recorded polysomnographically, and hormone levels were assessed throughout the night. Vaccination-induced Th cell and Ab responses were repeatedly monitored for 1 y. Compared with the wake condition, sleep after vaccination doubled the frequency of Ag-specific Th cells and increased the fraction of Th1 cytokine-producing cells in this population. Moreover, sleep markedly increased Ag-specific IgG1. The effects were followed up for 1 y and were associated with high sleep slow-wave activity during the postvaccination night as well as with accompanying levels of immunoregulatory hormones (i.e., increased growth hormone and prolactin but decreased cortisol release). Our findings provide novel evidence that sleep promotes human Th1 immune responses, implicating a critical role for slow-wave sleep in this process. The proinflammatory milieu induced during this sleep stage apparently acts as adjuvant that facilitates the transfer of antigenic information from APCs to Ag-specific Th cells. Like the nervous system, the immune system takes advantage of the offline conditions during sleep to foster adaptive immune responses resulting in improved immunological memory. The Journal of Immunology, 2011, 187: 283–290.

The immune system and the CNS share the ability to respond to external stimuli and to form memory (1). Vaccination induces a multistep immune response eventually leading to differentiation of Ag-specific memory T and B cells as effectors of immune protection (2, 3). After vaccination, APCs take up the Ag and migrate to lymphatic tissues to present part of the Ag to the cell surface to Th cells, thereby forming the so-called immunological synapse and initiating the transfer of the antigenic information into long-term storage in Ag-specific T and B cells (1, 3). Formation of the immunological synapse represents an early stage in the immune response that determines quantity and quality of immunological memory, and it is at this stage adjuvants act to enhance the immunogenicity of the vaccine (4).

Sleep generally regulates immune functions in a supportive manner (5–7). Sleep impacts APC and Th cell traffic and facilitates the release of endogenous adjuvants like growth hormone (GH), prolactin, and APC-derived IL-12, whereas it lowers the release of immunosuppressive hormones like cortisol (7–9). In this study, we used an experimental vaccination approach against hepatitis A virus (HAV) to examine whether these effects of sleep can boost immunological memory formation. To our knowledge, we show for the first time that sleep indeed enhances the vaccine-driven induction of immunological memory in the expansion, contraction, and maintenance phase as reflected by increased formation of Ag-specific Th cells and IgG1.

Materials and Methods

Subjects

Participants were 27 healthy, nonsmoking young men with a mean (±SEM) age of 26.1 ± 0.7 y (body weight: 81.4 ± 1.9 kg; body mass index: 24.5 ± 0.5). They presented with a normal nocturnal sleep pattern and did not take any medication at the time of the experiments. None had a medical history of any chronic disease or mental disorder. Acute illness was excluded by physical examination and routine laboratory investigation, including chemistry panel, C-reactive protein concentration <6 mg/l, and a WBC count <9000/µl. Naive immune status against HAV and hepatitis B virus was explored by medical history and vaccination card and confirmed by HAV–Ab levels <5 IU/ml and hepatitis B virus surface Ag (HBs)–Ab levels of 0.5 IU/ml, respectively. In addition, HBs and hepatitis B core–Ab were negative in all subjects.

The men were synchronized by daily activities and nocturnal rest. They had a regular sleep–wake rhythm for at least 6 wk before the experiments. All subjects were adjusted to the experimental setting by spending at least one adaptation night in the laboratory that took place before the experiment proper. The presence of signs of sleep disturbances including apnea and nocturnal myoclonus was excluded by interview and by recordings during this adaptation night. For private reasons, three subjects dropped off at the second vaccination and two further subjects at the third vaccination. Twelve subjects were available at a follow-up examination 1 y later. The study was approved by the Ethics Committee of the University of Lübeck. All men gave written informed consent prior to participating in accordance with the Declaration of Helsinki.

Experimental design and procedure at days of vaccination

The participants were randomly assigned to a condition of sleep and a condition of continuous wakefulness (wake). Both groups were comparable for age, body weight, and body mass index (p > 0.5). Each participant in both groups was injected with a vaccine into the deltoid muscle of the nondominant arm at 8 Am three times (i.e., at weeks 0, 8, and 16), with 1 ml of a hepatitis A vaccine that was combined with a hepatitis B vaccine (Twinrix, 720 IU HAV, 20 µg HBs; GlaxoSmithKline Biologicals, Rixensart, Belgium) (see Fig. 1B for study protocol). All primary vaccinations were performed in February and March, and the third shot was done in June or July 2007, with no difference in timing between the sleep and wake groups (p > 0.6). After immunizations, subjects were instructed to refrain
from physical or psychological stress and not to nap during the daytime. Adherence to these instructions was confirmed by questionnaires. The participants returned to the laboratory at 9 pm for preparing blood sampling and, in the regular sleep condition, for standard polysomnographic recordings.

Sleep in the sleep group was allowed between 11 pm (lights off) and 6:30 am (lights on) in the morning. In the wake condition, subjects stayed awake in bed during this period in a half-supine position, watching TV, reading, listening to music or even talking to the experimenter. Subjects consoled their normal room light (~300 lx). Subjects of the wake condition were not allowed to sleep until 8 pm the following day. For hormonal analyses, blood was sampled repeatedly during the experimental nights via an i.v. forearm catheter, which was connected to a long, thin tube and enabled blood collection from an adjacent room without the subject’s awareness or disturbing sleep. To prevent clotting, 5 to 10 ml of saline solution was infused throughout the experimental period in both conditions.

All subjects rated their sleepiness (Stanford Sleepiness Scale) and mood [using the short form of the German version of the Multidimensional Mood Questionnaire (10)] in the morning of the first night after the vaccinations. As expected, participants of the wake group scored higher in subjective ratings of sleepiness and lower in mood than the sleep group (p < 0.05 for the three vaccinations).

Sleep, slow-wave activity, and hormones
Sleep was recorded by standard polysomnography including the electroencephalogram (EEG) (from C3 and C4, referenced to an electrode attached to the nose), electrooculography, and submental electromyography. EEG signals were filtered between 0.15 and 35 Hz and sampled at 200 Hz. Subsequent 30-s epochs of polysomnographic recordings were scored offline by two experienced technicians according to standard criteria as sleep, wake stages 1–4, and rapid eye movement (REM) sleep (11). Sleep stages 3 and 4 were combined to slow-wave sleep (SWS).

Determination of slow-wave activity (SWA) was based on power spectral analyses. For these analyses, first, all epochs with artifacts and arousals were rejected semi-automatically (movement artifacts in the electromyography exceeding ±50 μV followed by visual inspection). Fast Fourier transformation, applied to successive 10.24-s epochs (2048 data points, Han ning window), was performed on all artifact-free epochs scored as non-REM sleep stages 2–4. SWA was defined by the mean power density within 0.68–1.17 Hz, covering the slow oscillation frequency band. Data were averaged per subject across the entire night and across both recording sites (C3, C4). Because of technical problems in two subjects, SWA could not be analyzed.

GH, prolactin, and cortisol were measured in serum using a commercial immunoassay kit (DPC-Biermann, Bad Nauheim, Germany). Epinephrine and norepinephrine were measured in plasma by standard HPLC (ChromSystems, Munich, Germany). Serum/plasma samples were kept frozen at −70°C until assay. Because hormonal levels were remarkably stable for each subject across the three nights, for further analyses data were collapsed across these nights.

Follow up procedures
During the 7 d after each inoculation subjects were monitored with respect to activities, stress, core body temperature and potential side effects. Blood samples to measure Ag-specific responses were drawn at 8 am on 13 occasions: immediately before and 1, 2, and 4 wk after each inoculation and again 1 y after the first inoculation (i.e., at weeks 0, 1, 2, 4, 8, 9, 10, 12, 16, 17, 18, 20, and 52). At these time points, subjects were also asked with respect to the occurrence of stressful events, infections, side effects and sleep habits during the prior week(s).

Measurement of the Ag-specific Th cell response
PBMCs were immediately isolated from 30 ml heparinized blood by Ficoll-Paque gradient centrifugation in Leucosope tubes (Greiner Bio- One, Frickenhausen, Germany) according to the manufacturer’s instruction. PBMCs were washed twice, and then left in tissue culture medium RPMI 1640 supplemented with 2 mM l-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (all from Sigma-Aldrich, Seelze, Germany). Five million cells in 0.5 ml medium were placed in 50 ml polypropylene tubes (Sarstedt, Nümbrecht, Germany) and stimulated at 37°C and 5% CO2 for 6 h with a pool of HAV surface peptides, a pool of HBs peptides, or co-stimulation and brefeldin A alone. Costimulation consisted of anti-CD28 and anti-CD49d (BD Biosciences, San Jose, CA), both at 1 μg/ml, and anti-CD20 (BD Biosciences, San Jose, CA), both at 1 μg/ml, and anti-CD49d (BD Biosciences, San Jose, CA), both at 1 μg/ml, and anti-CD20 (BD Biosciences, San Jose, CA), both at 1 μg/ml, and anti-CD49d (BD Biosciences, San Jose, CA), both at 1 μg/ml. The HBs antigen pool consisted of 54 peptides of 15 aa overlapping by 11 made from HBs (adw serotype, final concentration for each peptide 3 μg/ml, PEPScreen; ProImmune).

After a stimulation period of 6 h cells were surface-stained for CD3 and CD4 for 15 min at room temperature. After washing, fixation, and permeabilization according to the manufacturer’s instructions (Cytofix/Cytoperm Kit; BD Biosciences), cells were stained intracellularly for cytokines and CD154/CD40L (which detects both intracellular and surface Ag) (12, 13). The following combination of mAb reagents were used in the main experiment: CD3 PerCP, CD4 PE, CD154/CD40L allophycocyanin, IL-4 PE-cyanin 7 (Cy7), IFN-γ allophycocyanin-Cy7, IL-2 FITC, and TNF-α PE-Cy7. The Abs were all bought from or prepared as custom conjugates (IL-4 PE-Cy7: clone 5H1 from BD Biosciences; IFN-γ, clone B27) by BD Biosciences. A minimum of 200,000 CD3+CD4+ events were collected for each sample on a FACSCanto (BD Biosciences). Data were analyzed using FACS Diva software. The FACS plots are displayed as 5% probability with outliers.

For each sample, the percentages of total CD40L+ cells or CD40L+ cells coexpressing different cytokines in relation to total CD3+CD4+ cells were according to the main experiment with anti-CD28/anti-CD49d alone subtracted from the percentages for the Ag-specific stimulation.

Measurement of the Ag-specific Ab response
HAV- and HBs-specific Ab of the subtypes IgG1, IgG2, IgG3, and IgG4 were determined by ELISA. Microtiter plates were either precoated with HAV (anti-HAV enzyme immunoassay; Mediadnost, Reutlingen, Germany) or coated with HBs (incubation of 96 well microtiter plates (Nunc, Wiesbaden, Germany) with 0.04 μg HBs (Aldevron, Nürnberg, Germany) for 9 h at 4°C). After washing and blocking, serum was added for four dilutions (1:5, 1:25, 1:125, and 1:625) for 2 h and thereafter mouse anti-human IgG subtype biotin-conjugated Ab (Sagard-Aldrich, Steinheim, Germany) for 1 h. Then, avidin–HRP conjugate (Sagard-Aldrich) was added for 30 min, followed by incubation with the enzyme substrate tetramethylbenzidine (Invitrogen, Karlsruhe, Germany) for 5 min. The reactions were stopped with 100 μl 1 M H2SO4, 100 μl 3% H2O2, and 0.4 μl H2O. OD was measured at a 450-nm wavelength with a microtiter plate reader (MRXII; Dynex, Denkendorf, Germany). For each IgG subtype, OD was then determined as weighted average across all dilutions. In addition to IgG subtype analyses, total HBs-Abs were assessed at week 20 by quantitative microparticle enzyme immunoassay (AxSYM AUSAB; Abbott, Wiesbaden, Germany) to assess the responder status of each subject with respect to the recommendations of the permanent commission for vaccinations (14).

Statistical analyses
Data are presented as means ± SEM. Normal distribution was confirmed by the Kolmogorov–Smirnov test for all variables of interest, except for the fraction of cytokine positive cells within the population of Ag-specific Th cells. These values were therefore log transformed prior to statistical analyses. Analyses of differences between the effects of sleep versus wake relied on ANOVA with a group factor sleep/wake and a repeated measures factor time covering the different times of measurement after vaccination (immune response: weeks 0–20; hormones: 11PM–7 AM; side effects: weeks 0–20; scores at injection site and fatigue, and these time interactions were revealed 3). The following combination of mAb reagents were used in the main experiment: CD3 PerCP, CD4 PE, CD154/CD40L allophycocyanin, IL-4 PE-cyanin 7 (Cy7), IFN-γ allophycocyanin-Cy7, IL-2 FITC, and TNF-α PE-Cy7. The Abs were all bought from or prepared as custom conjugates (IL-4 PE-Cy7: clone 5H1 from BD Biosciences; IFN-γ, clone B27) by BD Biosciences. A minimum of 200,000 CD3+CD4+ events were collected for each sample on a FACSCanto (BD Biosciences). Data were analyzed using FACS Diva software. The FACS plots are displayed as 5% probability with outliers.

For each sample, the percentages of total CD40L+ cells or CD40L+ cells coexpressing different cytokines in relation to total CD3+CD4+ cells were calculated.

Results
Clinical course after vaccinations
All vaccinations were well tolerated. Subjects reported only minor side effects like pain at the injection site and fatigue, and these reports did not differ for the three vaccinations. As expected,
reported fatigue was higher in the wake than sleep condition in the morning after the first postvaccination night \((p = 0.02)\). Questionnaires did not reveal any difference between the sleep and wake groups with regard to self-reported activities or stressful events during the respective follow-up periods after the vaccination.

**Sleep**

Sleep variables confirmed that subjects slept normally under laboratory conditions with SWS predominant during the first night-half and REM sleep dominating the second night-half. Table I summarizes sleep variables of all three nights with no significant differences from session to session.

**Sleep enhances the HAV-specific Th cell response to vaccination**

Vaccination elicited a robust increase in CD40L+ HAV-specific Th cells, which was already evident 2 wk after each inoculation and reached a maximum between 2 and 4 wk after completion of the three-shots schedule (collapsed across sleep and wake conditions: 0.124 ± 0.014 versus 0.012 ± 0.001% measured at baseline, \(n = 22\); \(p < 0.001\)). Although the Ag-specific Th cell response to HAV vaccination has not been characterized previously, the magnitude of the response and its time course were comparable in size with those following HBs vaccination (15; see also results below), and it clearly shows the phases of expansion (weeks 1 and 2, 8–10, and 16–18) and contraction/maintenance (weeks 2–8, 10–16, and 18–52) (see also Supplemental Fig. 4). Importantly, when the participants had slept on the night after inoculations, the increase in HAV-specific Th cells was clearly enhanced when compared with the condition of continuous wakefulness. Overall (i.e., across weeks 0–52), we found a >2-fold higher percentage of Ag-specific Th cells during the sleep than during the wake condition (0.123 ± 0.022 versus 0.060 ± 0.009%, \(n = 12\); \(p = 0.001\)) (Figs. 1A, 2A). This boosting effect of sleep became significant already 8 wk after the first inoculation (0.031 ± 0.005 versus 0.019 ± 0.001% in the wake group, \(n = 27\); \(p = 0.04\)) and was most prominent after the second and third inoculations with a maximum at weeks 18–20 (0.161 ± 0.024 versus 0.093 ± 0.007%, \(n = 22\); \(p = 0.001\)) (Fig. 1A). The enhancement in HAV-specific Th cells was remarkably persistent. We still observed doubling of HAV-specific Th cells in the sleep group in the maintenance phase (i.e., 1 y after initial vaccination (0.099 ± 0.022 versus 0.044 ± 0.008%, \(n = 12\); \(p = 0.004\)) (Fig. 1A).

**Sleep boosts the Th1 immune response developing after vaccination**

In addition to the increase in Ag-specific Th cells after vaccination, we monitored the functional aspect of cytokine expression in these cells. We found that vaccination induced a general predominance of proinflammatory and Th1 cytokine activity that primarily supports cellular adaptive immune responses (IL-2 > IFN-γ ≈ TNF-α > IL-4) (Supplemental Fig. 1). Specifically, vaccination induced a marked but short-lived increase in the fraction of IFN-γ+ cells within the CD40L+ Th cell subset, reaching a maximum 2 wk after the first Ag inoculation (collapsed across sleep and wake conditions: 38.0 ± 3.5 versus 19.2 ± 2.7% at baseline, \(n = 27\); \(p < 0.001\)), whereas the fraction of IL-2+ Th cells gradually rose after each vaccination reaching an ~2-fold increased level at weeks 18–20 (71.2 ± 1.6 versus 43.0 ± 5.7% at baseline, \(n = 22\); \(p < 0.001\)). In addition, we observed significant increases in the fraction of TNF-α+ and IL-4+ Th cells, although these were less pronounced (Supplemental Fig. 1).

Interestingly, in subjects who slept the night after the first inoculation, the transient vaccination-induced increase in the fraction of IFN-γ+ cells within the CD40L+ Th cell subset at weeks 0–8 was significantly more pronounced than in those subjects who had stayed awake on this night (32.2 ± 2.6 versus 22.9 ± 3.1%, \(n = 27\); \(p = 0.03\)) (Supplemental Fig. 1). The boosting effect of sleep on vaccine-induced increases in HAV-specific Th cells was indeed most prominent in the subset of IFN-γ+CD40L+ Th cells (Fig. 2B, 2C).

**Sleep enhances the HAV-specific IgG1 response**

Promoting both cellular and humoral defense mechanisms, the Th cell response to vaccination represents a most immediate indicator of effective antigenic memory formation (2, 3). Nevertheless, in addition to the Th cell response, we determined the Ag-specific B cell response by measuring Ag-specific IgG titers, which are commonly used for quantification of the Ag-specific immune response in the clinical setting. As expected (16), we found opsonizing HAV-specific IgG1 and IgG3, which markedly increased at weeks 12 and 20 \((p < 0.01)\) (Fig. 3A, 3B). In contrast, HAV-specific IgG2 and IgG4 remained unchanged. When subjects slept during the nights after inoculations, analogous to the Th cell response, IgG1 levels were significantly higher than in those subjects who had stayed awake at weeks 8–16 \((p < 0.05)\). Indeed, enhancing effects of sleep on Ab titers followed those on Th cells, as indicated by significant correlations between IgG1 levels at

**Table I. Sleep parameters**

<table>
<thead>
<tr>
<th></th>
<th>First Night (min)</th>
<th>Second Night (min)</th>
<th>Third Night (min)</th>
<th>(t) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time</td>
<td>440.2 ± 3.6</td>
<td>447.4 ± 5.0</td>
<td>420.2 ± 18.0</td>
<td>0.16</td>
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<tr>
<td>Wake</td>
<td>52.7 ± 12.1</td>
<td>20.4 ± 6.1</td>
<td>20.9 ± 6.5</td>
<td>0.28</td>
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<tr>
<td>Stage 1</td>
<td>32.1 ± 2.8</td>
<td>31.7 ± 3.1</td>
<td>23.8 ± 2.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Stage 2</td>
<td>221.1 ± 14.4</td>
<td>242.2 ± 9.1</td>
<td>228.7 ± 13.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Stage 3</td>
<td>43.6 ± 4.5</td>
<td>47.2 ± 4.8</td>
<td>48.9 ± 4.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Stage 4</td>
<td>41.6 ± 5.5</td>
<td>16.1 ± 5.9</td>
<td>10.7 ± 6.2</td>
<td>0.74*</td>
</tr>
<tr>
<td>SWS</td>
<td>58.2 ± 6.8</td>
<td>63.3 ± 6.6</td>
<td>59.6 ± 8.7</td>
<td>0.60(*)</td>
</tr>
<tr>
<td>REM</td>
<td>73.1 ± 3.7</td>
<td>84.5 ± 5.8</td>
<td>83.0 ± 9.6</td>
<td>0.25</td>
</tr>
<tr>
<td>Sleep onset latency</td>
<td>35.1 ± 4.0</td>
<td>25.8 ± 2.9</td>
<td>21.7 ± 5.1</td>
<td>0.01</td>
</tr>
<tr>
<td>SWA (μV²)</td>
<td>101.1 ± 13.7</td>
<td>102.5 ± 11.4</td>
<td>109.5 ± 20.6</td>
<td>0.72*</td>
</tr>
</tbody>
</table>

Mean ± SEM total sleep time and time (in min) spent awake (wake), in non-REM sleep stages S1–S4, SWS equivalent to S3 plus S4) and in REM sleep during the nights after inoculations in the sleep condition. Sleep onset latency (in min) is indicated with reference to 11 pm (lights off). Bottom line indicates SWA (power density in the 0.68-1.17 Hz frequency band during S2 and SWS, in μV²). Right column indicates correlation coefficients between respective sleep parameter (collapsed across the participant’s three nights) and HAV-specific Th cells at weeks 18–20 \((n = 9)\). Note most consistent relationship with SWS stage 4 and SWA.

*Significant coefficients are bold.

\(*p < 0.05, (**p = 0.09.\)
FIGURE 1. Sleep enhances the HAV-specific Th cell response to vaccination. A. Emergence of CD40L+ HAV-specific Th cells (percentage of total Th cells) after HAV vaccination (three shots at weeks 0, 8, and 16 – vertical syringes) in two groups of subjects who either slept (●) or stayed awake (○) in the night following inoculations. y-axis is log transformed. Means ± SEM are indicated. n = 12–27 for both groups. **p < 0.01, *p < 0.05, (*) p < 0.1 for comparisons between sleep and wake conditions. B, Study design. Two groups of subjects were tested who either slept (sleep group, n = 13) or stayed awake (wake group, n = 14) on the night after inoculations with HAV vaccine, taking place three times (i.e., at weeks 0, 8, and 16). Immediately before vaccinations, 1, 2, and 4 wk after each vaccination, and again 1 y after the first inoculation, blood was sampled to assess the Ab response. The schema below the study design provides more details about the procedure on each of the 3 d of inoculation. Inoculation took place at 8 AM. Subjects of the sleep group slept between 11 PM and 6:30 AM. Subjects of the wake group stayed awake until 8 AM on the following day. Dots indicate time points of blood sampling. Sleep was monitored by polysomnography.

A

B

Means SEM are indicated. n = 13 or 14 (**p < 0.01, *p < 0.05, (*) p = 0.08) in the night stage 4 and the percentage of HAV-specific Th cells at weeks 18–20 (r = 0.72; p = 0.02) as well as 1 y later (r = 0.90; p = 0.02) (Table I). Likewise, increased EEG SWA during non-REM sleep was significantly associated with increased HAV-specific Th cell percentages at weeks 18–20 (r = 0.72; p = 0.03) and 1 y later (r = 0.94; p = 0.005) (Fig. 4A). Other measures, including REM sleep-related parameters, were not predictive (p > 0.20) (Table I). These data demonstrate a strikingly strong association between adaptive immune responses and SWS.

The endocrine milieu during SWS predicts the HAV-specific Th cell response to vaccination

Brain–immune interactions during sleep are conveyed mainly via endocrine pathways, apart from direct neuronal influences on lymphatic tissue (1, 18). Immunostimulating hormones like GH and prolactin as well as immunosuppressive hormones such as cortisol and catecholamines are strongly regulated by sleep (7, 8, 19). We sampled blood during the nights after vaccination (every 1.5 h) to assess a potential impact of these hormones on the HAV-specific Th cell response (Supplemental Fig. 2). As expected, sleep induced a profound increase in concentrations of GH and prolactin when compared with wakefulness (p < 0.001). The increase in GH occurred during periods of SWS dominating the early night. In contrast, sleep was associated with decreased cortisol nadir levels during the early SWS-rich night-half (p < 0.02). This decrease was compensated by enhanced morning cortisol levels after sleep (p = 0.008). Sleep also diminished catecholamine levels, which was somewhat more robust for norepinephrine (p < 0.001) than for epinephrine (p = 0.006). To be noted, although relatively increased compared with sleep, plasma concentrations of epinephrine and norepinephrine during nocturnal wakefulness (12.8 ± 1.1 and 263.7 ± 18.7 pg/ml, respectively) were still distinctly below the levels observed during daytime waking (measured before vaccinations, 32.6 ± 3.3 and 392.9 ± 33.2 pg/ml; n = 14; p < 0.001 for both comparisons) and were far from reaching any level commonly associated with acute stress (20). This in conjunction with the finding that nocturnal average concentrations of the primary stress hormone cortisol were closely comparable between the sleep and wake conditions (p = 0.81) clearly excludes that responses to vaccination in the wake condition were substantially affected by acute stress.

To dissociate the relative contributions of these hormones to the HAV vaccination response, we performed regression analyses on average concentrations of the hormones between 0:30 and 2 AM (i.e., the time interval with predominant SWS and most prominent hormonal changes for all five hormones) (Supplemental Fig. 2). The percentage of HAV-specific Th cells was used as dependent
variable. We found that GH, prolactin, and cortisol significantly contributed to the fully developed HA V-specific Th cell response as measured at weeks 18–20, as well as to the response 1 y later, explaining 48% \((p = 0.002)\) and 56% \((p = 0.02)\) of the variance, respectively. Adding epinephrine or norepinephrine concentration to the analyses did not increase explained variance, which agrees with previous findings showing that the effects of catecholamines on the vaccination response are inconsistent \((18)\). The combined action of GH, prolactin, and cortisol on the formation of the HA V-specific immune response is best described by an “adjuvant factor” summarizing the average hormone concentrations (0:30–2 AM) during postinoculation nights by the formula \(GH^{3} \text{prolactin}/\text{cortisol}\). This factor showed correlations as high as \(r = 0.71\) \((p < 0.001)\) and \(r = 0.80\) \((p = 0.002)\) with the percentage of HA V-specific Th cells at weeks 18–20 and week 52, respectively (Fig. 4B). These analyses suggest that GH, prolactin, and cortisol synergistically contribute to the sleep-dependent enhancement of the vaccination response.

**HBs-specific Th cell and Ab response**

The use of a combined hepatitis A/B vaccine allowed us to directly compare the response to HAV with that to HBs. Data regarding the response to HBs essentially confirmed findings after HAV vaccination, and therefore, the figures are presented as supplemental material (Supplemental Fig. 3). Vaccination induced an increase in CD40L+ HBs-specific Th cells, which started 2 wk after the first, and already 1 wk after second and third inoculation, respectively, and reached a maximum 2 wk after completion of the three-shot schedule (collapsed across sleep and wake conditions: \(0.100 \pm 0.017\) versus \(0.004 \pm 0.001\%\) measured at baseline, \(n = 22; p < 0.001\)), with this pattern well agreeing with a previous report \((15)\). Compared with wakefulness, sleep on the nights after HBs vaccination significantly enhanced both the CD40L+ HBs-specific Th cell response and the Ab response, although overall, these effects appeared to be less consistent than for the HAV vaccination. The enhancing effect of sleep on the frequency of HBs-specific Th cells became significant after the second inoculation \((p = 0.05, \text{for weeks 8–16})\) and continued during weeks 16–20 after the third inoculation \((p = 0.05)\) (Supplemental Fig. 3A). At the follow-up 1 y later, HBs-specific Th cell percentages in the sleep group were still on average distinctly higher than in the wake group, but this difference failed to reach significance \((0.057 \pm 0.018\) versus \(0.037 \pm 0.010\%, \quad n = 12; \quad p = 0.18)\).

In line with previous reports \((21)\), the Ab response to HBs vaccination was restricted to the IgG1 subtype reaching peak concentrations toward the end of the 20-wk vaccination period. When the participants slept on the nights following vaccination, this peak was distinctly enhanced \((14.25 \pm 2.34\) versus \(7.21 \pm 1.72, \quad n = 22; \quad p = 0.04)\) (Supplemental Fig. 3B). This sleep-induced increase in HBs-specific IgG1 was remarkably persistent and was also revealed at the follow-up examination 1 y later \((p = 0.02)\) (Supplemental Fig. 3B). Assessment of HBs-specific total IgG at the end of the vaccination period (week 20) revealed that three subjects failed to reach the clinical criterion Ab level of 100 mIU/ml IgG (indicating sufficient immunity) and had to undergo additional inoculation. All three subjects were in the wake group.

**FIGURE 2.** Sleep boosts the Th1 immune response developing after vaccination. Average frequencies of CD40L+ HAV-specific Th cells (percentage of total Th cells) \(A\) and average frequencies of these cells (CD40L+ HAV-specific Th cells) \(B\) that were specifically positive for the cytokines of interest (i.e., from left to right: IFN-\(\gamma\), IL-2, TNF-\(\alpha\), and IL-4). Means \pm SEM values are shown for the sleep (○) and wake conditions (●) during respective time intervals after inoculations: that is, weeks 0–8, weeks 8–16, weeks 16–20, and for the whole 1-y observation period (weeks 0–52); \(n = 12–27\) for both groups. ***\(p < 0.001\), **\(p < 0.01\), *\(p < 0.05\) for comparisons between sleep and wake condition. C, Representative dot plots of CD40L+ Th cells after HAV-specific in vitro stimulation at week 18 in one subject from the sleep and wake group, respectively. FSC, forward scatter; SSC, side scatter.
Discussion
The immunoregulatory functions of sleep are not well understood. By comparing the immune response after HAV vaccinations in healthy men who either slept or stayed awake in the night following inoculations, we provide, to our knowledge, first-time evidence that sleep acts like an adjuvant to enhance the Ag-specific Th cell and Ab response. Sleep SWA and the associated hormonal milieu in the postvaccination night were strong predictors of the subsequent Th cell response.

Mounting an adaptive immune response that eventually results in Ag-specific memory is a slow multistep process taking a minimum of several days. Our findings of an enhanced Ag-specific Th cell response after sleep occurring within 24 h postvaccination indicates an effect during the early stages of the immune response, most likely on APC–Th cell interactions taking place during this time window in lymphatic tissues (3, 4). Our analyses of sleep and endocrine activity suggest a scenario in which SWS plays a key role (Supplemental Fig. 4). SWS, a brain state hallmarked by EEG SWA, stimulates release of immunostimulating hormones like GH and prolactin and inhibits immunosuppressive cortisol (7, 8, 19). These hormones are not only uniquely regulated by sleep but also affect the interaction between APC and Th cell and the response to vaccination, which is increased by GH and prolactin but reduced by cortisol (22–24). Interestingly, such hormonal effects appear to be most pronounced within the first 24 h after inoculation (i.e., the time window of interest in the current study) (23, 24).

The sleep-associated hormonal changes prompt a shift of the type 1/type 2 cytokine balance toward enhanced activity of proinflammatory and Th1 cytokines (TNF-α, IL-12, IL-2, and IFN-γ) favoring cellular aspects of adaptive immune responses over activity of anti-inflammatory or Th2 cytokines (like IL-10, not measured in this study, and IL-4) (7, 8). Sleep as well as prolactin strongly enhance APC-derived IL-12 that, along with Ag stimulation, is an important signal switching Th cell differentiation toward increased Th1 cytokine production (9, 17, 25, 26). In line, sleep during the nights following the three inoculations improved the development of adaptive Th1 immune responses at two functional levels. First, once early differentiation of IFN-γ-producing HA V-specific Th cells (i.e., within 2 wk) is increased, sleep promotes IFN-γ-dependent effector functions. Second, through increased formation of IL-2 expressing HA V-specific Th cells, sleep enhances T cell survival, which enforces the induction of Ag-specific memory (2, 15).
Our findings support a concept in which SWS establishes a unique endocrine milieu that exerts adjuvant activity by stimulating the production of proinflammatory cytokines, eventually enhancing the development of stable adaptive immunity both at the T and B cell sites. Interestingly, proinflammatory cytokines and muramyl dipeptide (like many other vaccine adjuvants) have SWS-promoting properties speaking for the presence of a feed-forward loop where proinflammatory cytokines consolidate central nervous SWS during an ongoing immune response (27, 28). Animal data confirm such an assumption, because the amount of SWS predicts the survival rate after pathogen challenge in rabbits (29). Although REM sleep deprivation is known to impact immune parameters, some of which are also involved in regulating adaptive immune responses (30), correlation analyses of the current study argue against REM sleep playing a major role for immunological memory formation. Further studies are needed to determine the specific functions of REM sleep in this process, which, given that REM sleep follows SWS, could complement effects initiated during the prior periods of SWS (as it was hypothesized for memory formation in the neurobehavioral domain) (31).

The observed sleep-induced increases in HAV- and HBs-specific IgG1 explain observations from two previous studies in humans in which under conditions of sleep restriction or deprivation total IgG was slightly reduced shortly after vaccination (influenza and HAV) (32, 33). Likewise, 7–8 h of sleep deprivation in rodents immediately following antigenic challenge suppressed the secondary Ab response, an effect that was completely prevented by administration of proinflammatory adjuvants like IL-1 and muramyl dipeptide (34). However, these human and animal studies focused on the clonal expansion phase early after vaccination and not on the contraction/maintenance phase reflecting memory formation (3). In addition, other animal studies could not confirm these results and if antigenic challenge took place after short-term sleep loss even an opposing (i.e., immunoenhancing) effect on early defense mechanisms became evident (34–36). Overall, the available data point out that experimental timing of Ag challenge, sleep deprivation, and respective immunological outcome measures is essential to uncover the adjuvant like actions of sleep on immunological memory formation.

We were able to show an enhancing effect of sleep also for the adaptive immune response to HBs. It is difficult to explain that this enhancement was overall less robust than after HAV vaccination but could be related to differences in Ag uptake, processing, and presentation by APCs (37). Interestingly, in comparisons of morning and evening inoculations, Ab titers measured 4 wk later were revealed to be higher after HAV vaccination in the morning, whereas for HBs vaccination, this effect was reversed, with higher Ab titers after vaccination in the evening (38–40). It is likely that sleep is most effective in supporting antigenic memory formation during a critical time window when the immunological synapse between APC and Th cell is forming. If as a result of the specific protein structure APC need a longer time for taking up and processing HAV than HBs, then sleep occurring ∼15 h after a morning vaccination might optimally cover this critical window for HAV but to a lesser extent for HBs.

Our data corroborate the basic concept that sleep serves the formation of long-term memory, which so far has been elaborated primarily for neurobehavioral memory. In providing evidence that the offline period of sleep with distinctly reduced environmental Ag challenge is used to promote the formation of Ag-specific memories, our study extends this concept to the immune system. Our analyses suggest a key role for SWS in the transfer of antigenic information from APC into Ag-specific Th cell responses reflecting memory. Curiously, this observation finds likewise a parallel in the neurobehavioral domain where SWS and underlying SWA have been identified as a brain state critically supporting the transfer of information about episodes experienced during prior wakefulness from temporary to long-term stores (31, 41). Beyond adding to an integrative concept of the memory function of sleep, our observation has a number of immunological and clinical implications. Indicating a supporting effect of sleep on adaptive immunity, our findings can explain immunodeficiency and poor responsiveness to vaccination in the course of aging known to be accompanied by a particular decline in SWS, GH, prolactin, and APC-derived IL-12 (7, 42–45). Considering the stability of sleep characteristics, as observed in this study across the three postvaccination nights, they highlight the contribution of habitually good sleep to immunocompetence and longevity in successful aging (44, 46). In the past decades, modern lifestyle led to a decrease in average sleep duration also in young and middle-aged subjects. Cross-sectional and longitudinal epidemiological studies indicate that this sleep curtailment relates to negative health outcomes that may involve immune pathways (46). Interestingly, habitual short sleep duration is associated with an increased susceptibility to experimental rhinovirus infection and predicts poor Ab titers and poor clinical protection after three doses of hepatitis B vaccination in 125 middle-aged men and women (47, 48). Hence, the suppressing effects of acute experimental sleep loss on immunological memory formation observed in the current study seem to apply also to natural variations in sleep, underscoring the clinical relevance of our findings. Prolonged experimental sleep restriction likewise consistently revealed a negative impact on immune functions (7, 49). However, as habitual short sleep and experimental sleep restriction may not always lead to reduced SWS or SWA (50, 51), such immunosuppressive effects may stem rather from more pronounced endocrine changes like overall increases in cortisol levels (8).

Most important, our findings prompt to consider sleep as well as the sleep-induced temporary increase in proinflammatory cytokine and hormone activity as possible tools for optimizing vaccination strategies. Especially the synergistic action of multiple proinflammatory factors like GH and prolactin hopefully stimulates new designs of vaccine adjuvants (52). In this context, additional hormones that are known to support the immune response after vaccination and were not investigated in this study, like melatonin [which presumably was slightly lower in our waking subjects (26)], could have contributed to the immunosupportive effect of SWS (53). Whatever the hormonal mediators, our data indicate that improving SWS may provide a therapeutic tool for enhancing efficacy of vaccinations.

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Disclosures
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References


