Endogenous glucocorticoid receptor signaling drives rhythmic changes in human T-cell subset numbers and the expression of the chemokine receptor CXCR4

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ABSTRACT In humans, numbers of circulating naive T cells strongly decline in the morning, which was suggested to be mediated by cortisol, inducing a CXCR4 up-regulation with a subsequent extravasation of the cells. As a systematic evaluation of this assumption is lacking, we investigated in two human placebo-controlled studies the effects of the glucocorticoid receptor (GR) antagonist mifepristone (200 mg orally at 23:00) and of suppressing endogenous cortisol with metyrapone (1 g orally at 04:00) on temporal changes in CXCR4 expression and numbers of different T-cell subsets using flow cytometry. Mifepristone attenuated, and metyrapone completely blocked, the morning increase in CXCR4 expression on naive T cells. In parallel, both substances also hindered the decline in naive T-cell numbers with this effect, however, being less apparent after mifepristone. We identified, and confirmed in additional in vitro studies, a partial agonistic GR effect of mifepristone at night (i.e., between 02:00 and 03:30) that could explain the lower antagonistic efficacy of the substance on CXCR4 expression and naive T-cell counts. CXCR4 expression emerged to be a most sensitive marker of GR signaling. Our studies jointly show that endogenous cortisol, specifically via GR activation, causes the morning increase in CXCR4 expression and the subsequent extravasation of naive T cells, thus revealing an important immunological function of the morning cortisol rise. Besedovsky, L., Born, J., Lange, T. Endogenous glucocorticoid receptor signaling drives rhythmic changes in human T-cell subset numbers and the expression of the chemokine receptor CXCR4. FASEB J. 28, 67–75 (2014). www.fasebj.org

Key Words: circadian rhythm • cortisol • CXCL12

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; GH, growth hormone; GR, glucocorticoid receptor; MFI, median fluorescence intensity; MR, mineralocorticoid receptor; PBS, phosphate buffered saline; REM, rapid eye movement; SWS, slow-wave sleep

T cells continuously migrate between blood, lymphoid organs, and tissue in search of their specific antigen. This traffic is under tight control of the circadian system (1–3). The resulting rhythm is hallmarked, in humans, by a pronounced decline of blood T-cell numbers that occurs in the early morning hours, after peak counts were reached during nocturnal sleep (4). We have recently shown in humans that this decrease can be mimicked by exogenous cortisol administration and mainly reflects changes in T cells expressing lymph node homing receptors, i.e., naive and central memory T cells (5). Numbers of these subsets decline by as much as 40% during the early morning when cortisol levels rise (5). Already ~30 yr ago, Fauci et al. (6, 7) demonstrated that glucocorticoids selectively affect T cells with lymph node-homing capacity and redirect these cells from the blood to the bone marrow. As the bone marrow produces high levels of the chemokine CXCL12, up-regulation of the respective receptor CXCR4 on T cells by glucocorticoids represents a likely but yet unproven mechanism of this redistribution (5, 8, 9).

Cortisol acts via two receptor types, i.e., high-affinity mineralocorticoid receptors (MRs), which are already occupied to a great extent during nadir cortisol levels, and low-affinity glucocorticoid receptors (GRs), which only become activated during high levels of cortisol present during stress and during the circadian rise of cortisol in the morning hours (10, 11). Against this backdrop, it is believed that the morning decline in blood T-cell numbers is mediated via GRs (5). Yet, this

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view lacks experimental proof. Indirect evidence comes from in vitro studies showing that the glucocorticoid induced up-regulation of CXCR4 on T cells is blocked by the GR antagonist mifepristone (RU486; refs. 9, 12, 13). Here, we scrutinized the notion that the morning decline in blood T-cell numbers is mediated via GR-induced up-regulation of CXCR4 in humans. For this purpose, we measured in two placebo-controlled human studies the time course of peripheral cell numbers and CXCR4 expression for 8 T-cell subsets with different stages of differentiation and lymph node homing capacities (naive, central memory, effector memory, and effector CD4\(^+\) and CD8\(^+\) T cells), after oral intake of mifepristone or metyrapone, a cortisol-synthesis inhibitor.

**MATERIALS AND METHODS**

**Subjects**

Sixteen healthy men participated in study 1 (mean ± SEM age: 23.31 ± 0.91 yr) and another 18 healthy men (mean ± SEM age: 22.39 ± 0.63 yr) in study 2. All subjects had a regular sleep/wake pattern, did not take any medications at the time of the experiment, and were nonsmokers. Acute and chronic illness was excluded by medical history, physical examination, and routine laboratory investigation. The men were synchronized by daily activities and nocturnal rest. All subjects spent one adaptation night in the laboratory in order to become accustomed to the experimental setting. The study was approved by the Ethics Committee of the University of Lübeck, and all participants gave written informed consent.

**Experimental design**

Both studies were performed according to a double-blind, randomized crossover design. Each participant spent two experimental nights in the sleep laboratory (placebo vs. active treatment). The two nights were separated by ≥2 wk to ensure clearance of the drug. The order of conditions was balanced across subjects. On experimental nights, subjects arrived at the laboratory at 21:00 for preparing blood sampling and polysomnographic recordings. Sleep was allowed between 23:00 (lights off) and 07:00. In study 1, subjects orally received either 200 mg of mifepristone (Exelgy Laboratories, Paris, France; peak plasma concentration after 90 min, plasma half-life 12–72 h) or placebo right before lights out. In study 2, subjects orally received either metyrapone (1 g, Alliance Pharmaceuticals, Chippenham, UK; peak plasma concentration after 1 h) or placebo at 04:00. To keep conditions comparable across studies, potential side effects of mifepristone or metyrapone, respectively, were evaluated in the morning by questionnaire. Standard polysomnographic recordings were obtained to ensure normal nocturnal sleep. Blood pressure was assessed before subjects went to sleep and every 30 min between 07:00 and 10:00. During this time, subjects performed psychological tests, results of which are reported elsewhere (14).

**T-cell subpopulations**

Absolute counts of CD3\(^+\) total T lymphocytes, CD4\(^+\) T-helper lymphocytes, and CD8\(^+\) cytotoxic T lymphocytes, as well as their naive (CD45RA\(^-\)CD62L\(^+\)), central memory (CD45RA\(^+\)CD62L\(^+\)), effector memory (CD45RA\(^-\)CD62L\(^-\)), and (terminally differentiated) effector (CD45RA\(^-\)CD62L\(^-\)) subsets were determined by a "lyse no-wash" flow cytometry procedure. Briefly, 50 µl of an undiluted blood sample was immunostained with anti-CD3/Horizon V500, anti-CD8/PerCP, anti-CD4/Horizon V450, anti-CD62L/FITC, anti-CD45RA/PE, and anti-CD184 (CXCR4)/APC, in Trucount tubes (all from BD Biosciences, San Jose, CA, USA). After 15 min of incubation at room temperature, 0.9 ml of fluorescence-activated cell sorting (FACS) lysing solution (BD Biosciences) was added, followed by incubation for 15 min. Finally, samples were mixed gently, and ≥10,000 CD3\(^+\) cells were acquired on a BD LSRII flow cytometer using DIVA software (BD Biosciences). The absolute number of cells per microliter blood was calculated using the following formula: cells/microliter = (acquired cell events in the respective gate) × (sample volume, µl). To study changes in relative quantity of CD184 (CXCR4) on the cell subsets, the median fluorescence intensity (MFI) of the labeled anti-CD184-antibody was analyzed.

For practical reasons, the antibody panel in study 2 was different from study 1 and consisted of the following fluorochrome conjugates: anti-CD3/Horizon V500, anti-CD62L/Horizon V450, anti-CD45RA/Alexa Fluor 700, anti-CD184 (CXCR4)/PE (all from BD Biosciences), anti-CD8/Qdot 605 (Invitrogen, Carlsbad, CA, USA), and anti-CD4/PerCP (Biorad, Dublin, CA, USA).

**Hormone assays, CXCL12 assay, and sleep analyses**

Samples for measuring hormone and CXCL12 concentrations were kept frozen at −80°C until assay. Serum cortisol, plasma ACTH, and plasma aldosterone were measured using commercial assays (cortisol, ACTH: Immulite, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA; aldosterone: IBL International, Hamburg, Germany). Epinephrine and norepinephrine were measured in plasma by standard high-performance liquid chromatography. CXCL12 (SDF-1\(x\)), the endogenous ligand of CXCR4, was measured in plasma using a commercial assay (R&D Systems, Minneapolis, MN, USA). Sensitivity was as follows: cortisol, 0.2 µg/dl; ACTH, 9 pg/ml; aldosterone, 10 pg/ml; epinephrine, 15
pg/ml; norepinephrine, 15 pg/ml; CXCL12, 18 pg/ml. Intra-assay and interassay coefficients of variation for all assays were <13.4%.

Sleep stages were determined offline from polysomnographic recordings following standard criteria (15). For each night, sleep onset (with reference to lights off at 23:00), total sleep time, and the time as well as percentage of total sleep time spent in the different sleep stages (wake, stages 1, 2, SWS, and REM sleep) were determined.

**In vitro** experiments

Whole blood from 13 healthy subjects was sampled during early sleep (at 03:30) and again in the morning at 08:00 to cover periods of lowest and highest endogenous cortisol levels comparable to the in vivo experiment. To test the influence of mifepristone on the expression of CXCR4, blood samples were incubated with phosphate-buffered saline (PBS) at 37°C in the absence or presence of 5 μM mifepristone (representing expected peak blood concentration after oral intake of 200 mg mifepristone) for 2 h. Samples were then labeled with anti-CD3/Horizon V500, anti-CD4/Horizon V450, anti-CD62L/FITC, anti-CD45RA/Alexa Fluor 700, anti-CD184 (CXCR4)/APC (all from BD Biosciences), and anti-CD8/Qdot 605 (Invitrogen), and subsequently processed via a lyse no-wash flow cytometry procedure, as described for the in vivo experiments.

A subset of 8 subjects was orally given metyrapone (1 g) at 04:00 on an additional experimental night before blood was sampled at 08:00 for subsequent in vitro studies. This was done in order to scrutinize whether effects of mifepristone on CXCR4 expression depended on endogenous cortisol levels.

**Statistical analyses**

Data are presented as means ± SEM. Analyses generally relied on analysis of variance (ANOVA), examining repeated-measures factors for the administered substance (mifepristone/metyrapone vs. placebo), time (reflecting the different time points during the observation period), and early/late (23:00–03:30 vs. 05:00–09:30). For analyses of CXCR4 MFI, differences between conditions in baseline measures (i.e., at 23:00 and 02:00 in study 1 and study 2, respectively) were used as covariates to correct for day-to-day variations in flow cytometer performance. Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Values of F and effect sizes for all relevant ANOVA are summarized in Supplemental Table S1. Paired t tests were applied to analyze post hoc differences at single time points once ANOVA indicated significant effects, to assess in vitro effects of mifepristone in comparison to the control, and to analyze differences in sleep parameters and blood pressure. A value of P < 0.05 was considered significant.

**RESULTS**

**Study 1: mifepristone vs. placebo**

*Mifepristone induces systemic changes in hormone levels but leaves CXCL12 concentration unaffected*

Blood levels of hormones displayed the typical temporal patterns across the night-time and morning period of monitoring (between 23:00 and 10:00). Compared with placebo, mifepristone, by blocking feedback inhibition, persistently increased ACTH levels from 07:30 on (see Fig. 1 for pairwise statistical comparisons at the different time points). Mifepristone induced a first slight and transient reduction in cortisol levels (around 03:30) followed by a strong persistent increase in cortisol, as well as in aldosterone levels, starting at 08:00 (Fig. 1). Prolactin concentrations were higher after mifepristone between 02:00 and 04:15 and around 10:00 (Supplemental Fig. S1; P<0.023, for substance × time interactions for ACTH, cortisol, aldosterone, and prolactin levels). Mifepristone did not alter levels of GH, norepinephrine, and CXCL12 (Supplemental Fig. S1). Epinephrine levels were below detection threshold most of the time (data not shown).

*Fig. 1. ACTH, cortisol, and aldosterone concentrations in blood after mifepristone administration. Means ± sem are indicated; n = 16. *P < 0.05, **P < 0.01, for pairwise comparisons between the effects of mifepristone (solid circles) and placebo (open circles) at single time points.*

Mifepristone exerts opposing effects on T-cell parameters in the night and morning hours

In the placebo condition, absolute counts of naive, central memory, and effector memory CD4⁺ T cells, as well as of naive and central memory CD8⁺ T cells, showed the typical variation over time (P<0.005, refer to Supplemental Table S1 for detailed information on respective ANOVA results), generally peaking in the early night and strongly decreasing in the morning. As expected (5), terminally differentiated effector CD8⁺ T cells showed an opposite pattern, with peak numbers in the morning (P=0.031; Fig. 2A, C). Mifepristone unexpectedly reduced numbers of naive CD4⁺ T cells at 03:30 (P=0.016; for respective ANOVA interaction; P=0.011) and of naive CD8⁺ T cells also at 03:30 (P=0.016; for respective ANOVA interaction: P=0.064; Fig. 2A, C). We suspected that
this reducing effect of mifepristone masked a subsequent antagonistic increasing effect of the substance developing in the morning. Therefore, as an estimate of the antagonistic effect that is independent of the changes occurring earlier in the night, we calculated the slope of the decrease in cell counts in the morning hours between 06:30 and 09:30. During this time, cortisol concentrations reach levels (≥10 μg/dl) that preclude any agonistic actions of mifepristone to persist (see Discussion), and thus allow for unraveling antagonistic effects of the drug. Indeed, mifepristone was found to significantly attenuate this morning downward slope of naive CD4+ and CD8+ T cells (P<0.049; Fig. 3A).

Expression of CXCR4 in the placebo session showed temporal changes on all T-cell subsets, with lowest levels in the early night and an increase in the morning (P≤0.029; Fig. 2B, D). Mifepristone affected CXCR4 expression on most T-cell subsets (P≤0.028). The modulating effect was overall less robust in effector CD4+ T cells (P=0.086) and only missing in central memory CD8+ T cells. It comprised a significant increase in CXCR4 expression around 02:00 (P≤0.038), whereas expression in the morning (at 09:30) was distinctly reduced in naive and central memory CD4+ T cells and naive, effector memory, and effector CD8+ T cells (P≤0.045; Fig. 2B, D). In naive, central memory, and effector memory CD4+ T cells and also in naive and effector memory CD8+ T cells, the reducing (antagonistic) action of mifepristone on CXCR4 expression was also evident for the slope of morning increases in CXCR4 expression (P≤0.036; Fig. 3B, D).

In vitro experiments reveal cortisol-level dependency of mifepristone effects

Changes in blood cell counts and CXCR4 expression after administration of mifepristone in vivo could be secondary to systemic effects on hormone release that might also affect T-cell migration. To control for such confounding factors, we evaluated the direct effect of the substance on CXCR4 expression in vitro (using blood from different donors). Mifepristone added to blood sampled during the early night, with low endogenous cortisol concentration, increased expression of CXCR4 on all CD4+ and CD8+ T-cell subsets to a similar extent, compared with a PBS control (Fig. 4). In contrast, mifepristone added to blood sampled in the morning, with high endogenous cortisol concentration, generally reduced CXCR4 expression. To scrutinize whether the opposing effects of mifepristone were...
owing to the different concentrations of endogenous cortisol, we pretreated a subgroup of subjects \( (n=8) \) on an additional night with metyrapone (1 g, at 04:00) to block cortisol synthesis. Incubation of morning blood with mifepristone after metyrapone pretreatment indeed increased CXCR4 expression \( (P \leq 0.032, \text{ for all comparisons; Fig. 4}) \).

**Study 2: metyrapone vs. placebo**

Metyrapone reduces cortisol levels and increases ACTH and aldosterone concentration in blood

Metyrapone treatment effectively reduced cortisol levels in all subjects, with this suppression starting at 05:00 and persisting throughout the recording period \( (P<0.001; \text{ Fig. 5}) \). Cortisol levels during this period were mostly <5 \( \mu \text{g/dl} \) and thus well comparable to levels typically occurring around the natural circadian nadir. Because of lacking feedback inhibition, ACTH levels were persistently increased after metyrapone in-
take, with this increase starting at 06:00 (P<0.001; Fig. 5). Aldosterone concentrations were transiently (at 07:30) reduced after metyrapone, with this effect followed by increased aldosterone concentrations toward the end of the recording period (P<0.001; Fig. 5). Metyrapone is known to inhibit aldosterone synthesis by blocking activity of the steroid 11β-hydroxylase (16), and this effect indeed can be accompanied by a compensatory increase (17). Levels of GH, prolactin, nor-epinephrine, and CXCL12 remained unchanged after metyrapone (Supplemental Fig. S2). Epinephrine levels were below detection threshold most of the time (data not shown).

Morning increase in CXCR4 expression and decrease in naive T-cell numbers are completely blocked following metyrapone administration

Metyrapone suppressed the morning decline in numbers of naive, central memory, and effector memory CD4+ and CD8+ T cells, although to different degrees in the subpopulations. The effect was most pronounced for the two naive subpopulations (P<0.001), but only approached significance for effector memory CD8+ T cells in the overall ANOVA (P=0.054; see Fig. 6A, C for statistical comparisons at the different time points). Also, metyrapone abolished the morning increase in CXCR4 expression on all subpopulations (P<0.002; Fig. 6B, D) except for effector CD4+ T cells. Like the effect on absolute cell counts, the effect of metyrapone on CXCR4 expression was also most pronounced for naive T-cell subsets.

Sleep, blood pressure, and side effects after mifepristone and metyrapone intake

Nocturnal sleep was comparable between active treatment and placebo conditions in both studies, except that mifepristone significantly reduced time spent in REM sleep (P=0.025) and tended to increase time awake (P=0.091). Despite subjects being aroused around 04:00 for drug administration, sleep architecture overall was normal under laboratory conditions. Neither mifepristone nor metyrapone changed blood pressure, and they also did not produce any side effects. Subjects were not able to correctly indicate whether they received an active agent or placebo.
DISCUSSION

Many immune parameters show variations across the 24-h cycle, which is assumed to optimize different functions of immunity associated with the active and rest phase of this cycle, respectively (3, 18, 19). The strong morning decline in circulating numbers of naive T cells is one of the hallmarks of this circadian regulation (5, 20). Although it has been suspected for some time that this decline is mediated by a cortisol-induced up-regulation of CXCR4 expression with subsequent extravasation of the T cells to the bone marrow (5, 6, 9), this study provides direct experimental support for this notion. We demonstrate that blocking the action of endogenous cortisol on GRs can completely prevent the morning decline in T-cell blood counts, as well as the morning increase in CXCR4 expression on these cells, with the size of the effect depending on the specific T-cell subset. In vivo, these effects were most clear after administration of metyrapone that produced cortisol levels similar to those during the circadian nadir of endogenous cortisol release. At this time, MRs but not GRs are activated (10, 11). The effects of metyrapone on both cell counts and CXCR4 expression were strongest in naive CD4⁺ and CD8⁺ T cells, which also showed the most pronounced circadian rhythm and the highest CXCR4 expression among these subsets (5, 21). The complete suppression of the circadian rhythm in these naive T-cell parameters following metyrapone administration shows that cortisol is the main regulator of naive T-cell counts under physiological conditions, indeed proving causality for the consistent finding of a negative correlation between rhythms of cortisol levels and blood T-cell counts (5, 22, 23).

Effects of the GR antagonist mifepristone to prevent the morning increase in CXCR4 expression and decline in T-cell numbers were less strong, which, at a first glance, might surprise. Yet, this is explained by the specific consequences of mifepristone administered in vivo. First, systemic administration of mifepristone strongly increases cortisol levels, owing to the removal of feedback inhibition of pituitary-adrenal activity accompanying GR blockade (24). Enhanced cortisol release at least partially also compensates for the blockade of T cellular GR by mifepristone, a view similarly expressed by others (25, 26). Second, we showed that with low cortisol concentration, mifepristone behaves as a partial GR agonist. This agonistic effect manifested itself in a transient increase in CXCR4 expression at 02:00, followed by a decline in numbers of both naive T-cell subsets and of cortisol levels around 03:30. As a consequence, cell numbers and CXCR4 expression already differed between the placebo and mifepristone conditions in the early night, with this difference possibly masking subsequent antagonistic effects of mifepristone expected to occur in the morning. Indeed, T-cell numbers were comparable for both conditions in the morning. However, as a measure that is unbiased by any agonistic effect of mifepristone at night, we calculated the slope of the change in cell numbers and CXCR4 expression between 06:30 and 09:30, when endogenous cortisol is naturally enhanced. This slope measure in fact revealed a diminished decrease in naive CD4⁺ and CD8⁺ T cells after mifepristone compared to placebo, thus indicating that the drug attenuated the decline in cell numbers to a significant extent. The fact that this antagonistic effect of mifepristone on cell counts was evident only for the slope of the morning decrease in T-cell numbers but not for the absolute cell numbers fits with several previous studies that failed to find antagonistic effects of mifepristone on counts of various leukocyte subsets (25–27).

Considering the partial agonistic effect of mifepristone at low cortisol concentrations, its antagonistic effects on peripheral processes might be best revealed in conditions of naturally enhanced cortisol levels, e.g., when administered at the morning peak of cortisol (28). To block the morning GR activation, we needed to give mifepristone before the circadian cortisol peak. However, administration of the drug later at night, when cortisol levels are enhanced, may have avoided agonistic effects and thus produced more prominent antagonistic effects.

Our in vitro experiments corroborated the view of a partial agonistic GR effect of mifepristone, depending on the level of cortisol. As in the in vivo study, mifepristone reduced CXCR4 expression in blood sampled in the morning when cortisol is high, but increased CXCR4 expression in blood sampled during the night when cortisol is low. Mifepristone added in vitro to blood sampled in the morning from individuals pretreated with metyrapone to suppress cortisol synthesis again increased CXCR4 expression. Thus, agonistic and antagonistic effects of mifepristone are clearly related to differences in endogenous cortisol levels. At high cortisol levels, the substance acts as an antagonist, competing with cortisol for GR but displaying much lower intrinsic activity at this receptor. However, at low cortisol levels, this intrinsic activity is high enough to produce agonistic effects (29). This concept explains that we could detect agonistic and antagonistic effects on one and the same parameter, which goes beyond previous studies demonstrating such opposing effects of mifepristone only in different types of tissues (30, 31). Changes in GR expression on T cells could also add to the agonistic vs. antagonistic effects of mifepristone (31). However, in preliminary experiments of our laboratory, metyrapone did not affect GR levels in T cells (unpublished results), which rules out that differences in GR expression substantially contributed to the opposing effects of mifepristone depending on whether subjects were pretreated with metyrapone.

Although known for some time (9, 32), partial agonistic properties of mifepristone are not well characterized in humans. To the best of our knowledge, in vivo, so far they have only been shown in one study in patients with adrenal insufficiency after administration of rather high doses of the substance (33). Here, we demonstrate agonistic actions of mifepristone in healthy men in two different systems (T cells and pituitary-adrenal system) using a dose of only 200 mg. Moreover, our data corroborate, and extend to in vivo conditions, the view that GR signaling enhances CXCR4 expression, which was based so far solely on in vitro experiments with mifepristone (9, 12, 13).

Contributions of aldosterone and MR activation to the observed effects on T-cell counts and CXCR4 expression can be ruled out. In previous studies, MR blockade by spironolactone did not affect CXCR4
expression on T-cell subsets (34). Irrespective of CXCR4 signaling, MR activation at a time of low GR signaling can reduce blood T-cell numbers (34, 35). However, as aldosterone increased in the morning hours after both metyrapone and mifepristone, this should have further decreased rather than increased T-cell numbers, i.e., counteracted the effects of mifepristone and metyrapone during this time. Of note, MR blockade at a time of high GR signaling in the morning did not affect the circadian decrease in naive T cells (34), which fits well with the present findings that this morning decline is specifically mediated via GR.

Reductions in peripheral lymphocyte numbers are one of the most prominent changes after corticosteroid treatment (36). In the present studies, CXCR4 expression was revealed as a marker of GR signaling even more sensitive than blood cell counts. Our data suggest CXCR4 expression could be considered for measuring mifepristone efficacy, for example, in the treatment of Cushing’s syndrome. Mifepristone has been proposed as a therapy for this disease, although with the drawback that there is currently a lack of available drug-monitoring data (37–39).

The exact mechanism underlying the cortisol-induced up-regulation of CXCR4 on T cells is not known. It seems to involve cytosolic receptors, as membrane-bound GRs have not been found on T cells (40), and activation of the CAMP-protein kinase A pathway (13, 41, 42). Overall, GR expression in T-cell subsets does not correlate with the subset’s sensitivity to the lymphodepletive effects of glucocorticoids (5, 7). However, GR-mediated effects on CXCR4 signaling are reported to depend on the T-cell state, with glucocorticoids enhancing chemotaxis in resting T cells, but not in activated T cells (43). This finding is consistent with the present observation that most pronounced circadian and treatment-induced changes in cell counts occurred in the naive T-cell subsets.

Although in humans the destination of T cells leaving the blood cannot be readily followed, in animals, after injection of glucocorticoids, these cells accumulated in the bone marrow (6, 44). T-cell entry to the bone marrow depends on CXCR4-CXCL12 interactions and is most effective in naive and central memory T cells (45). Interestingly, like naive T cells, hematopoietic stem cells are released into the circulation during sleep and become redistributed to the bone marrow during the activity period following increases in their CXCR4 expression and in bone marrow levels of CXCL12 (3, 46, 47). CXCR4 also mediates the homing of T cells to lymph nodes (48). However, redistribution to this compartment is unlikely, given the consistent evidence that glucocorticoids impair lymphocyte migration to lymph nodes (36, 44, 49). The biological function of a T-cell redistribution to the bone marrow in the morning is presently not known. It has been suggested that in the bone marrow, T cells contribute to the regulation of hematopoiesis, receive survival signals, or serve the generation of primary immune responses to blood-borne antigens (45, 50–53). As such, the circadian morning increase in endogenous cortisol might, indeed, serve an important function for T-cell immunity.

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