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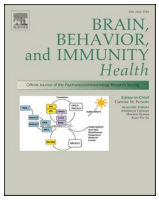

The temporal expression pattern of classical MHC class I in sleep-restricted mice: Generalizations and broader implications

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ABSTRACT

The intricate relationship between sleep and leukocyte trafficking has garnered intense attention, particularly their homing dynamics to secondary lymphoid organs under normal and restricted sleep (SR). Considering the scarcity of information regarding circadian rhythms in major histocompatibility class I (MHC-I) expression in SR, we designed a study that assessed the temporal expression of MHC-I in murine lymph nodes and spleen and the subsequent effects of sleep recovery. Male C57BL/6, housed in 12:12 light/dark cycle, were grouped into control (C) and SR. SR was carried for one week before lymphoid tissues were sampled at selected time points and assessed for leukocyte number and MHC-I expression.

SR resulted in 21% decrease in granulocyte and 24% increase in agranulocyte numbers.

In C, MHC-I expression pattern in lymph nodes was bimodal and relatively higher than splenocytes during the animal's active phase (110.2 ± 1.8 vs 81.9 ± 3.8, respectively; $p = 0.002$). Splenocytes; however, showed a bimodal pattern upon SR, with higher protein levels during the rest than the activity period (154.6 + 36.2 vs 99.5 + 15.9, respectively; $p = 0.002$), suggesting preparedness for a potential infection. Furthermore, SR caused a significant drop in MHC-I expression at the onset of rest with 57% and 30% reduction in lymph nodes and splenocytes, respectively. However, the overall protein expression collectively taken from both lymphoid tissues remained stable, emphasizing its indispensable role in immunological homeostasis. This stability coincided with the restoration of protein levels to baseline after a short sleep recovery period, resembling a reset for MHC-I antigen presentation following a week of SR.

Understanding the interplay between MHC-I expression and contextual factors could enhance treatment protocols, refining the efficacy and time precision of glucocorticoid-based therapies in immune modulation.

1. Introduction

Sleep is part of a daily biological rhythm necessary for the individual to maintain homeostasis. It serves as a crucial regulator of the immune system, overseeing physiological processes essential for its balance. Both the quality and quantity of sleep can affect immune function by modifying the activity of the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system (Asif et al., 2017; Majde et al., 2005; Manzar et al., 2012; Moldofsky, 1995; Rico-Rosillo et al., 2018). Primary and secondary lymphoid organs are innervated by sympathetic, peptidergic, and sensory nerve fibers (Felten et al., 1985). Circulatory and tissue-resident leukocytes can sense neurotransmitters and neuropeptides released by nerve endings (Ordovas-Montanes et al., 2015). In turn, leukocytes respond by releasing cytokines, hormones, and neuropeptides that can act in a paracrine manner on the brain, inducing a state of sleep and fatigue in case of infection.

Furthermore, the circadian rhythms of hormones, including cortisol, exhibit nighttime drops in their plasma levels, promoting various immune system activities (Antonini et al., 2000; Yamaguchi et al., 1974, 1978). The circadian rhythm and sleep collaborate to support both the innate and adaptive immune response. Some studies have revealed the significant impact of sleep restriction (SR) on immune cell function, cytokine levels and inflammatory markers, leading to decreased activity in natural killer cells (NKCs), T lymphocytes, and monocytes (Irwin et al., 1996; Born et al., 1997; Lange et al., 2010). Despite low cellular activity, total peripheral leukocyte counts, mainly neutrophils, showed increased levels in SR-induced conditions (Boudjeltia et al., 2008; Wilder-Smith et al., 2013). Furthermore, monocytes and the subpopulations

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of lymphocytes exhibited peak times during the night (Ackermann et al., 2012) that might lose the rhythmicity and migratory norm of leukocyte circulation dynamics upon sleep loss.

Monitoring the trafficking dynamics of leukocytes can be via the blood circulation or lymphoid tissue isolates. Redistribution of white blood cell rhythmicity upon sleep disturbance can vary between various leukocyte subsets. However, examining the overall activity of isolated leukocytes might be more significant than examining their subset counts. This can be achieved via genome microarray analysis, cytokine level testing, or as presented in this study through the quantitative assessment of surface expression levels of Major Histocompatibility Complex (MHC) class I molecules. Using whole genome microarray, the expression levels of MHC I-related signaling pathways in leukocytes seemed to be down-regulated upon sleep restriction (Aho et al., 2013).

MHC class I molecules are pivotal components of the immune system. Their cell surface expression and presentation of intracellular antigens regulate the activation of NKCs and $CD8^+$ T lymphocytes (Chaplin, 2010; Whitton, 1998). For instance, their downregulation or absence at the cell surface of cancer or infected cells activates NK cell killing. Elevated or aberrant MHC class I daily expression patterns in the context of circadian misalignment and sleep restriction could indicate heightened immune activity, possibly in response to infections or cellular stress exacerbated by the disrupted circadian dynamics of T cell rhythm (Besedovsky et al., 2019). Conversely, a reduction in MHC class I expression might signify compromised immune function or evasion strategies employed by viral infected or tumor cells (Cohen et al., 1994; Hewitt, 2003; Zhou, 2009; Sari et al., 2023; Garcia-Lora et al., 2003). Therefore, considering the scarcity of information about the role of sleep on MHC-I expression patterns, quantitatively assessing MHC class I temporal expression under conditions of circadian misalignment and altered sleep patterns offers a nuanced perspective on the immunological landscape. This approach not only provides insights into potential underlying pathological conditions, but also underscores the intricate interplay between circadian regulation of sleep and immune responses (Besedovsky et al., 2012; Dickstein et al., 1999; Ganz, 2012). Such information is vital for advancing our understanding of the complex physiological relationships between the suprachiasmatic nucleus (master clock), sleep, and immune function (Gillette, 2004), with implications for both research and clinical applications in the fields of immunology and sleep medicine.

While a number of studies have demonstrated the fundamental role of nocturnal sleep and circadian rhythms on the time-of-day dependent variation in leukocyte counts and cell dynamics, including their homing into secondary lymphoid organs (Yang et al., 2010; Lasselin et al., 2015; Lange et al., 2022), very little is known about the possible interaction between inadequate sleep and the circadian timing system in influencing potential daily cycles in MHC cell dynamics in the lymph nodes and spleen. Noteworthy, these secondary lymphoid organs have gained research attention in view of their central role in antigen presentation and priming NKCs, dendritic cells, thereby orchestrating between innate and immune responses. In one study, we described the role of sleep restriction in promoting a state of inflammation possibly mediated by MHC class II-dependent mechanisms (Ghanem et al., 2019). Therefore, knowing that no information is available about circadian regulation of MHC class I in sleep restricted states, we designed the present study with the following aims: 1) to assess the temporal expression profile of MHC class I in the axillary lymph nodes and spleen under normal and acute sleep restriction in mice and 2) to examine whether 3–4 days of recovery following restriction might have potential effects on MHC class I timely expression. This was to test the hypothesis that leukocyte homing to secondary lymphoid organs might be associated with a heightened antigen presentation activity manifest as a disruption in the amplitude (presumably increase) and/or rhythmic pattern of MHC class I molecules.

2. Materials and methods

2.1. Animals and housing conditions

Male C57BL/6 mice (purchased from the American University of Beirut animal facility), 10–17 weeks old and weighing on average 25 g, were used. Animals were placed in an air-conditioned room at 22 °C and 50% humidity under a 12:12 light/dark cycle [lights on at local time 0700 h, defined as *Zeitgeber time* (ZT) 0] and were allowed food and water ad libitum.

Mice used in the study were maintained and treated in accordance with the guidelines dictated by the "Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2011). The study was approved internally by the University Institutional Review Board (IRB) at NDU.

2.2. Reagents

Fluorescence-activated cell sorting (FACS) buffer [0.02% sodium azide, 0.2% Bovine serum albumin (BSA), 10% fetal calf serum (FCS), 1X phosphate-buffered saline (PBS)] was used as wash buffer. To fix the cells, 4% paraformaldehyde (PFA) was used. Permeation buffer [1% BSA, 10% FBS, and 0.3 M Glycine in 0.1% phosphate-buffered saline Tween 20 (PBST)] was used to allow for intracellular staining. Propidium Iodide (Geiger et al., 2015) [(10 μg/ml) diluted in PBS from 1 mg/ml stock solution] was used to test for cell viability or integrity (dead cells stain with PI).

2.3. Experimental procedure

All mice were acclimatized to the housing conditions for at least one week prior to experimentation. At the beginning of the experimental period, they were randomly divided into 3 groups: control mice (C) (n = 18) kept in standard polypropylene cages throughout the duration of the experiment; sleep restricted mice (SR) (n = 18), subjected to the multiple platform method on a daily basis for one week; and recovery group (R) (n = 9), allowed sleep recovery following the 7-day SR period by placing them in standard propylene cages for 58, 71, and 91 h.

2.4. Sleep restriction (SR)

Sleep restriction was carried by placing mice in a tiled water cage (18 x 30 × 21 cm) for 18 h (beginning at ZT9) for 7 days. This method was a modified version of the Multiple Platform that allows the animal to move around by leaping from one platform to another or to hold onto the cage lid. The SR animals in this study could be considered as particularly REM sleep-deprived during the time spent in the multiple platform cage since this method suppresses REM sleep and, possibly, causes some NREM sleep loss (Zager et al., 2007). Throughout the SR procedure, mice were allowed to rest for 6 h (from ZT3 toZT9) a day. One week after the experimental period (7 days of SR), lymph nodes and spleens were collected to analyze the expression of MHC I molecules from isolated cells. C and SR animals were sacrificed at each of the following time points: ZT0, ZT5, ZT10, ZT13, ZT18, and ZT23 as depicted in Fig. 1. As for the R group, animals were sacrificed and the lymph nodes and spleens were collected 58, 71, and 91 h following the end of the SR period (i.e., at ZT10 and ZT23 on the second day of recovery and at ZT18 on the third day of recovery).

2.5. Isolation and processing of lymph nodes and spleens

Spleen and auxiliary lymph nodes were removed from all mice and placed in Eppendorf tubes with 500 μl DMEM at 4 °C until further processing. The excised organs were smashed into small pieces in 2 ml PBS. Tissue fragments were further homogenized using a 70 μm cell strainer attached to a 50 ml conical tube (remaining cells were washed

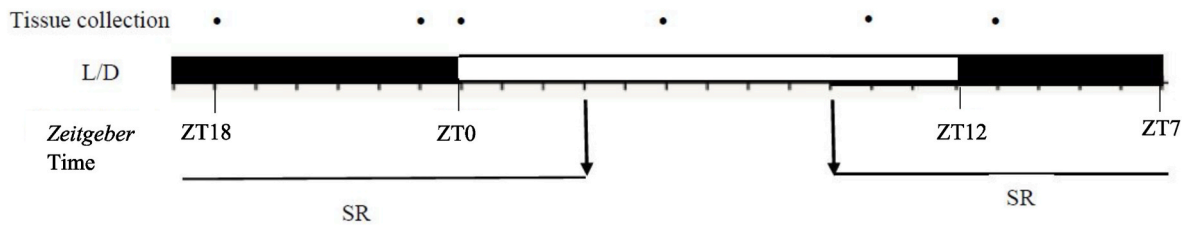

Fig. 1. Schematic representation of the time points for lymphoid tissue collection in mice maintained on a 12:12 light/dark cycle or under sleep restriction (SR). Dots represent different time points of tissue collection; black bars, dark period (ZT12); open bars, light period (ZT0); arrows point at the beginning and end of the SR time period.

out of the strainer with 5 ml PBS solution) and the rest of the vial was filled with 10 ml PBS. The cell suspension was centrifuged at 300×*g* for 5 min at room temperature (RT). The pellet was retrieved and reconstituted with 9 ml NH_4Cl to eliminate red blood cell contamination, then incubated for 10 min at RT, and centrifuged again at 300×*g* for 5 min. The pellet was re-suspended with 0.5 ml of freezing medium (40 ml RPMI, 10% DMSO, and 10% FCS). Eppendorf tubes were stored in an isopropanol chamber overnight in the freezer at −80 °C and then transferred to a cryobox at −80 °C (modified from Zager et al., 2007).

2.6. Processing isolated cells for flow cytometry

A protocol modified from Abcam was used. 1 ml FACS buffer was added to each Eppendorf tube containing either homogenized lymph node or spleen. Cells were centrifuged twice at 300×*g* for 5 min at 4 °C and re-suspended in FACS buffer. Samples were transferred into a 96 well plate (100 µl/well) and centrifuged at 300×*g* for 5 min at 4 °C. Cells were fixed with 100 µl 4% PFA for 10 min on ice and then washed with *PBS*. Cells for propidium iodide (P)I staining were re-suspended in 100 µl FACS buffer, while other wells were incubated with 1% BSA/10% FBS/0.3M glycine in 0.1% PBS-Tween for 1 h on ice to permeabilize cells and block non-specific binding. Cells were then washed with 100 µl FACS buffer for 3 min followed by plate centrifugation at 300×*g* for 5 min. Cells were incubated in anti-MHC I antibody[34-1-2S] (Phycoerythrin), Abcam ab95571, directed against the H-2Db and H-2Kb allotypes (1:40 dilution, for 1 h at room temperature (RT) in the dark.; C cells were left untreated in 5 µl FACS buffer. Cells were then centrifuged twice with 0.2 ml FACS buffer at 300×*g* for 5 min at 4 °C, suspended in 1.2 ml FACS buffer, and transferred to a FACS cuvette for data acquisition using flow cytometry.

For PI staining, 10 µl PI was added to each Eppendorf tube containing 100 µl FACS buffer for 1 min in the dark at room temperature and suspended in 1.1 ml FACS buffer for FACS reading using Cyflow Cube 8 (CY–S-3068, PARTEC).

2.7. Flow Cytometry analysis

Cyview software version 1.3 was used for FACS analysis. Flow cytometry reading was restricted to 100,000 cells/sample, with specific gain and threshold settings. Results were analyzed and mean fluorescence intensity (MFI) calculated using Flowjo software. For anti-MHC I antibody, FL2 laser was used (excitation: 488 nm, emission: 575 nm).

2.8. Statistical analysis

Student t-test was used to compare MFI between C and SR group, and between SR and R groups. One-way ANOVA was used to compare different time points extracted from C and SR groups. IBM SPSS statistics software (version 19) was used to perform statistical analysis and determine the p-values. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Characterization of cell isolates

Splenocyte isolates were plotted by flow cytometric analysis based on their forward (FSC) and side (SSC) scatter. Three distinct pools of cells were detected. The pool with the smallest size or FSC was labeled lymphocytes, followed by monocytes, and the largest pool as granulocytes (Fig. 2A). Moreover, staining cells with PI, a DNA intercalating agent that is permeant to dead cells, revealed a ~99% of cells as alive. This demonstrates the capability of isolating cells without any early apoptotic or necrotic signs (Fig. 2B).

3.2. Effect of sleep restriction on leukocyte count in secondary lymphoid tissues

Sleep restriction in mice for one week resulted in an average decrease of 27.5% in granulocyte count in SR group when compared to control values (16.7 % ± 1.99 vs 44.2 % ± 1.9, respectively; $p=.004$), as depicted by the forward (FSC) and side scatter (SSC) signals of representative dot plots in Fig. 3.

When considering agranulocytes, the number of lymphocytes and monocytes increased in SR compared to control (C) mice by around 26% (SR: 79.7 % ± 3.19 vs C: 53.6% ± 1.13; $p = .009$). Despite the changes in leukocyte subset counts upon SR, the relative MFI signal of total MHC class I surface expression levels examined from lymph node leukocyte isolates at ZT5 showed insignificant slight increase (Fig. 4).

3.3. Diurnal expression of MHC-I molecules in lymph node and spleen cell isolates of control and sleep-restricted mice

3.3.1. a. MHC-I expression in control mice

The mean MHC-I MFI signal in the lymph node and spleen tissues of control mice across the selected time points was 135.7 ± 42.7 and 147.2 ± 100.6, respectively. As shown in Fig. 5, the diurnal pattern of protein expression varied between the lymph node and spleen cells, being cyclical and exhibiting a bimodal pattern (with an average inter-peak interval of 9 h) in the lymph nodes. In lymph nodes, MHC-I levels were high at the beginning of the rest period (peaked at ZT0 - light onset), decreased to relatively low levels during the first part of the rest period, increased to relatively high levels (peaked at ZT18) in the second part of the rest period (few hours before activity onset), decreased in the first part of the activity period, and increased again in the second part of the activity period (Fig. 5A). This fluctuation in cellular protein expression was not observed in spleen tissue, especially between ZT5 and ZT13 (Fig. 5B). During this period, MHC-I level was less robust in the spleen than lymph nodes, being significantly lower in the spleen at ZT13 (82.7 ± 3.5 vs 99.0 ± 4.2, respectively; $p = 0.005$). In the lymph nodes, there was a significant difference in MFI across the different time points (one-way ANOVA: $F(5,9) = 8.481$, $p = 0.003$). Furthermore, comparing expression levels during the rest and activity periods, a significant difference in protein expression between the lymphoid tissues was observed during the animals' active phase (Table 1).

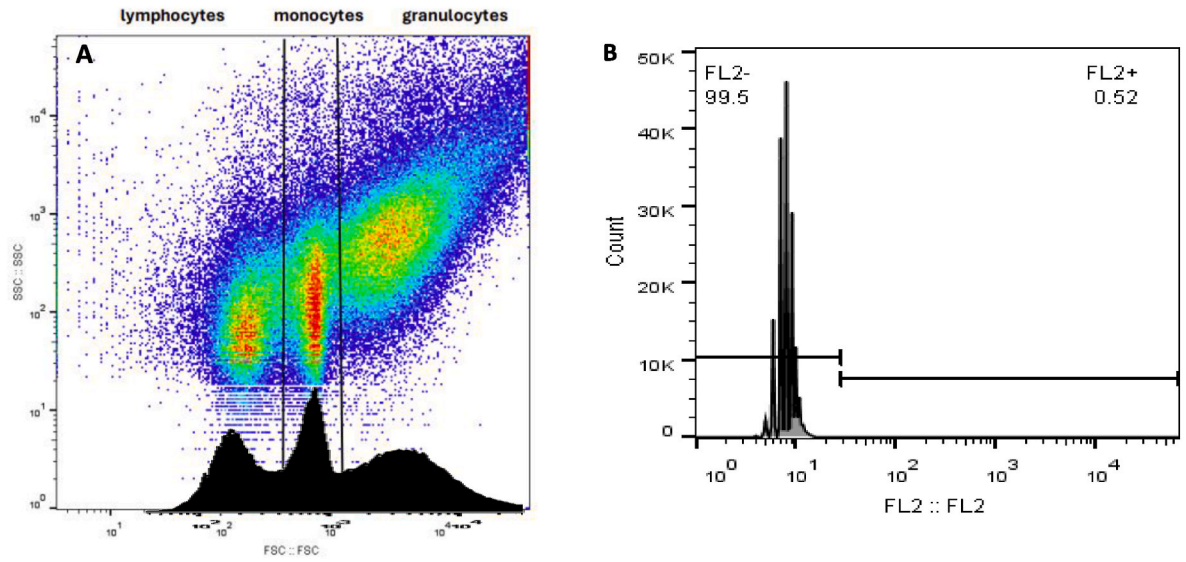

Fig. 2. Identifying different subsets of leukocytes and their viability from isolated mouse splenocytes. A) Three pools of leukocytes, represented by 3 distinct peaks, were distinguished based on cell size or FSC signal at ZT5. In terms of increasing size, lymphocytes have the smallest FSC signal, followed by monocytes and granulocytes. B) Cell viability was monitored by tracking PI signal by flow cytometry. Almost all cells showed negative PI fluorescence as depicted by low FL2 signal. This demonstrates a minute death of cells upon isolation and homogenization.

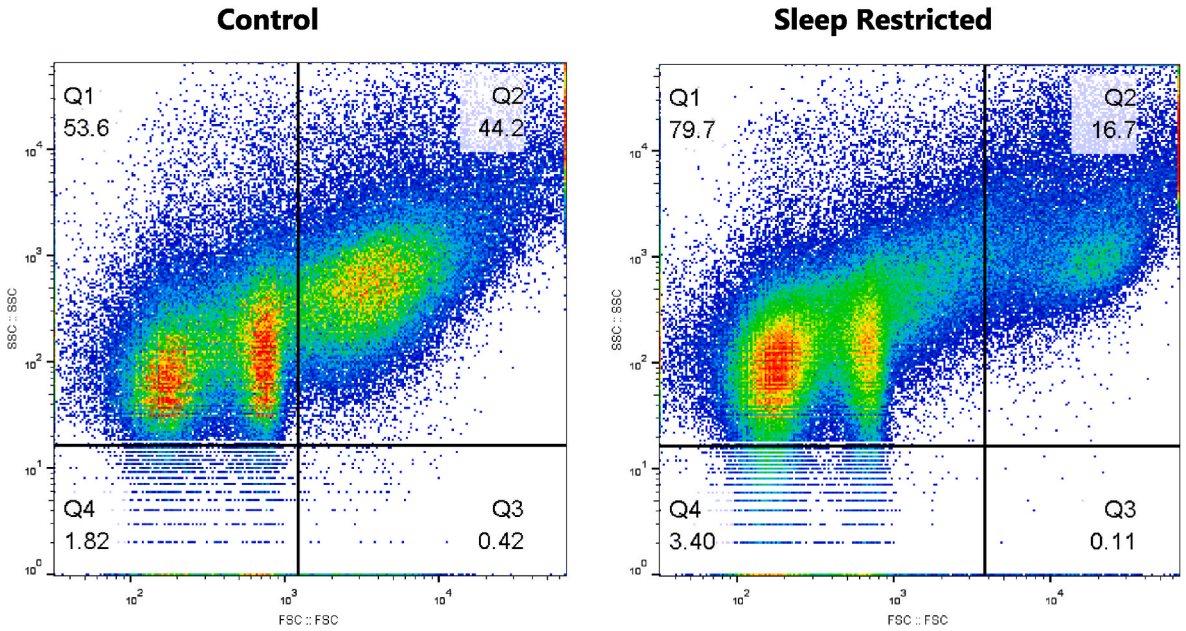

Fig. 3. A representative scatter plot showing granulocyte number in isolated lymphoid tissue of control and sleep restricted mice. The total cell count, as well as the relative proportions of agranulocytes, were determined based on SSC and FSC signals at ZT5. Compared to control, SR animals had lower granulocyte number (16.7 % ± 1.99 vs 44.2 % ± 1.9, respectively; *p=.004);* higher lymphocyte and monocyte counts (79.7% ± 3.19 vs 53.6% ± 1.13, respectively; *p= .009*). FSC, forward scatter; SR, sleep restriction; SSC, side scatter.

3.3.2. MHC-I expression in sleep restricted mice

Under sleep restricted conditions, the mean MHC-I MFI signal in the lymph node and spleen tissues was 113.7 ± 53.4 and 106.9 ± 26.2, respectively. Fig. 5 shows the diurnal pattern of protein expression in both lymphoid tissues of SR animals. Unlike the observation in control mice, both lymph node and spleen cells showed a synchronous temporal fluctuation in MHC-I levels when sleep is restricted to 6 h daily. In both tissues, protein expression decreased at the end of the rest phase of the animal (ZT3 – ZT9), yet fluctuated throughout the activity period and attained relatively high levels towards the beginning of the rest period (ZT3). There was a significant difference in MFI in both lymphoid tissue across the measured time points (one-way ANOVA: lymph nodes, $F(5,7) = 6.084$, $p = 0.0017$; spleen, $F(5,9) = 5.363$, $p = 0.015$). Furthermore, comparing protein expression levels during the rest and activity periods, the spleen showed a significantly higher MFI signal during the rest than activity period (Table 2).

3.3.3. Effects of sleep restriction on the diurnal expression pattern and levels of MHC I in the lymph nodes and spleen

Compared to C conditions, SR resulted in a change in the timing of the rise in MHC-I expression, as well as in the overall pattern of expression across the selected time points, especially in spleen tissue

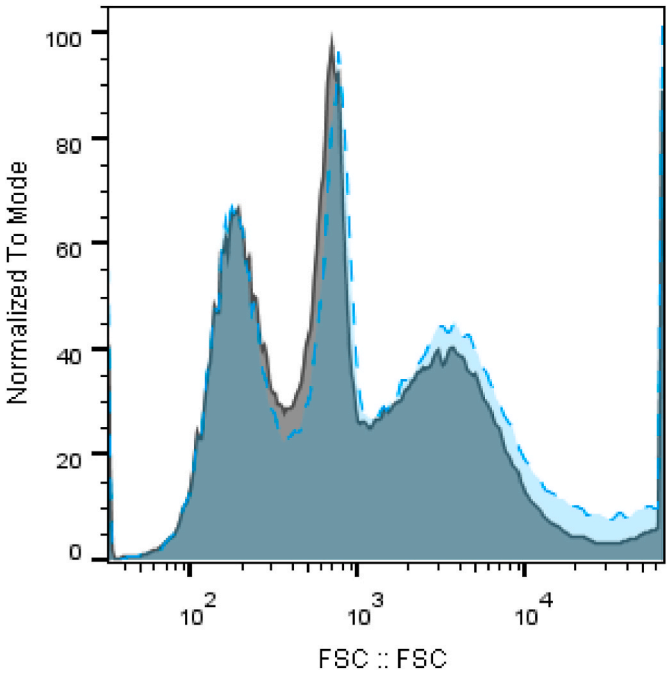

Fig. 4. A representative MHC expression profile in lymph node isolates collected at ZT5 from C57BL/6 mice. The relative MFI counts of MHC I immunoreactive cells were plotted in C (solid line) and SR (dashed line) animals. The number of immunolabelled cells in SR groups showed a slight increase in MHC class I expression. C, control; SR, sleep restriction.

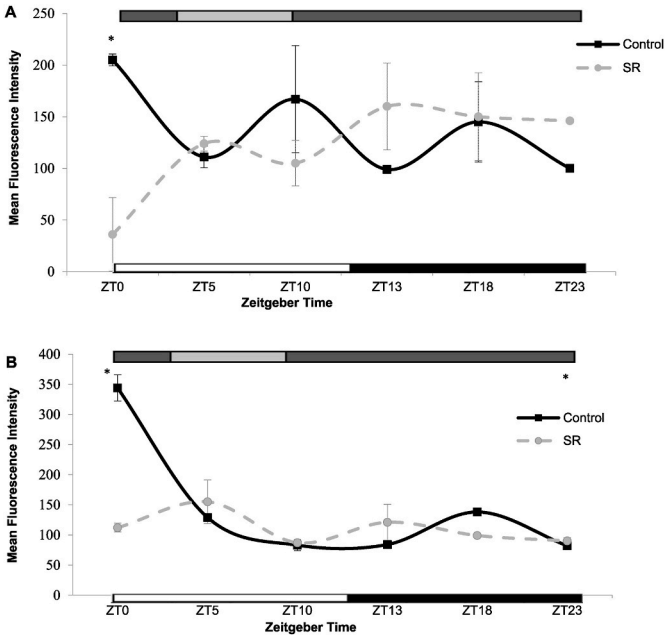

Fig. 5. Graphical representation of the diurnal MFI MHC-I signals in the lymph node (A) and spleen (B) of control and sleep restricted mice. A) Unlike splenocytes, lymph node cells displayed a bimodal pattern of protein expression, with relatively high levels at ZT0, ZT10, and ZT18, under C conditions. This pattern was altered following SR, where MHC-I levels increased at ZT5 and reached maximal levels at ZT13. B) In splenocytes, MHC-I levels were relatively low throughout most of the selected time points (except at ZT0 and ZT18), whereas SR caused a different pattern of protein expression during the day, with 2 peaks at ZT5 and ZT13. MFI, mean fluorescence intensity; C, control group; SR, sleep restricted group; bottom open bar, light period (lights on at 0700 h or ZT0); bottom black bar, dark period; top open bar, rest period of SR (onset at ZT3); top gray bar, activity period of SR (18 h duration). * p-value <0.05 indicates significant difference between C and SR groups. Values represent mean ± standard deviation.

(Fig. 5). MHC-I expression in both lymph node and spleen cells occurred earlier (a phase advance of about 5 h) in SR than C. Furthermore, the presumptive cyclical expression of the protein in SR tissues was discordant and seemingly out-of-phase to that in C. Significant differences in MFI were notable in the spleen at ZT18 (C: 138.1 ± 3.0 vs SR: 99.0 ± 2.7, p = 0.001) and ZT0 (C: 343.8 ± 21.7 vs SR: 112.0 ± 7.3, p = 0.001), as well as in the lymph nodes at ZT0 (C: 204.6 ± 5.8 vs SR: 35.7 ± 10.0, p = 0.000).

3.4. *Effect of sleep recovery on MHC I expression*

Mice were subjected to a recovery period extending between 3 and 4 nights following sleep restriction. Throughout this period, samples were collected at 58, 71, and 91 h corresponding to time points ZT10, ZT23, and ZT18, respectively. There was no significant difference in MHC-I expression at any of the sleep recovery durations as compared with C and SR groups, neither in lymph node cells nor in splenocytes (Fig. 6).

4. Discussion

The intricate interplay between sleep and the trafficking of leukocytes has been a subject of intense investigation, with a focus on understanding the homing dynamics of immune cell subsets to secondary lymphoid organs during normal and sleep-restricted conditions (Lange et al., 2022; Geiger et al., 2015). Although the expression level of adhesion molecules, ICAM-1 and LFA-1, on the surface of leukocytes has been explored to understand the diurnal homing activity to lymphoid organs (Milicevic et al., 2004), the signals of MHC class I expression during SR has received limited attention. In our study, we observed a noteworthy shift in leukocyte population counts in secondary lymphoid organs, namely spleen and axillary lymph nodes, upon sleep restriction with a decrease in granulocytes and an increase in lymphocytes and monocytes (Fig. 3). We addressed whether such changes in leukocyte counts affect the temporal signals of MHC class I molecules upon SR.

We first investigated MHC class I expression in secondary lymphoid organs at a specific time (1200 h or ZT5, almost mid-rest period). Contrary to our expectations, MHC class I expression levels were not altered despite sleep restriction. Further exploration of temporal cell dynamics

Table 1
Comparison of MHC-I expression in the lymph nodes and spleen of control mice during the animals' rest and activity periods. Independent sample *t*-test comparisons between groups (across: rest vs activity; down: lymph nodes vs spleen). * p-value <0.05 denotes significant difference.

Tissue	Rest (MFI Signal mean ± SD)	Activity (MFI Signal mean ± SD)	p
Lymph nodes	144.6 ± 43.5	100.2 ± 1.8*	0.109
Spleen	162.3 ± 106.5	81.9 ± 3.8*	0.223
p	0.597	0.002	

Table 2
Comparison of MHC-I expression in the lymph nodes and spleen of sleep restricted mice during the animals' rest and activity periods. Independent sample *t*-test comparisons between groups (across: rest vs activity; down: lymph nodes vs spleen). * p-value <0.05 denotes significant difference.

Tissue	Rest (MFI Signal mean ± SD)	Activity (MFI Signal mean ± SD)	p
Lymph nodes	124.0 ± 7.07	111.8 ± 58.2	0.781
Spleen	154.6 ± 36.2*	99.5 ± 15.9*	0.002
p	0.362	0.472	

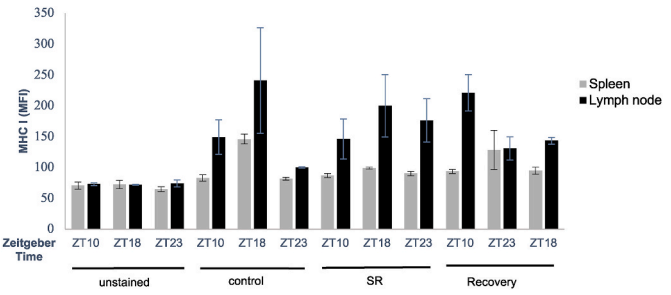

Fig. 6. Graphical comparison of MHC-I expression in the lymph nodes and spleen of control and experimental group before and following recovery sleep. There was no significant difference in protein expression among all groups at the selected time points in both lymphoid tissues. Values are expressed as mean ± standard deviation.

across various time points revealed a bimodal pattern, with peaks in lymph nodes mirroring the normal trend, while new peaks emerged in the spleen during both rest and active periods when sleep was restricted. Early shifts in these peaks prompted questions about their significance and potential implications for immune responses. The circadian regulation of immune function was demonstrated by Druzd et al. (2017) in mice (Druzd et al., 2017); they showed a strong circadian oscillation in lymph node lymphocyte cellularity that involved interaction between the central oscillator (i.e. the biological clock in the hypothalamus) and the lymphocyte intrinsic clock (i.e. cell or peripheral oscillators). Briefly, they described a time-of-day-dependent variation in the number of lymphocyte subpopulations and dendritic cells, always concertedly peaking in the second hour of the night phase (dark phase or ZT13 in 12:12 L/D; CT 13 in DD corresponding to the animal's subjective activity period). Furthermore, genetic disruption of lymphocyte-specific proteins which characterize their cellularity, namely the chemokine receptor (CCR7) and the sphingosine-1-phosphate-receptor (S1P1), abolished their adaptive immune function. To relate to our findings, it is possible that the timing of the MHC-I peak we observed in the lymph nodes around the beginning of the dark phase and those reported for lymphocytes and dendritic cells few hours later may reflect a physiologically important phase-relationship in the circadian cycles of the components of the adaptive immune pathways and which is presumably regulated by the circadian timing system. The importance of temporal synchronization between antigen presentation by antigen presenting cells (APCs) and circadian $CD8^+$ T cell responses has been demonstrated by immunization studies where optimal adaptive immune responses are achieved when vaccines are administered at times of day corresponding to peak $CD8^+$ T cell activity (Nobis et al., 2019; Fortier et al., 2011).

Surprisingly, examining the overall expression of MHC class I levels between the rest and active periods did not show significant difference in the lymph nodes, suggesting that the tested 21-day SR duration might not be sufficient to disrupt the rhythmic circulation dynamics in these nodes and that antigen presentation via MHC class I molecules is not affected. However, a stark difference emerged in the spleen of sleep-deprived mice, with increased levels in the rest period following SR. This phenomenon may be attributed to an increased migration of leukocytes from the lymph nodes to the spleen for enhanced antigen presentation, hinting at an alert or simulated infection state. This state can be harmful as increased levels of MHC class I presentation at the surface of splenocytes might activate naive cytotoxic $CD8^+$ T cells without a real cause of viral infection. We then assessed whether a chrono disruption of MHC class I expression by splenocytes was achieved. Interestingly, exposing the mice to a short recovery period did revert MHC class I levels to basal levels. This is aligned with studies showing the restoration of NKCs and lymphocyte count in the blood after short sleep recovery period (Irwin et al., 1996; Born et al., 1997). But how far our body can accommodate the switch between sleep restriction and recovery (Simpson et al., 2016)? We speculate that chronic SR exposure cannot be restored and would augment MHC class I expression leading to chronic and systemic low-grade inflammation with increased rate for infectious pathologies, and impaired allotolerance manifested by autoimmune-related disorders and risks (Garbarino et al., 2021). Further studies are required to examine the cellular and molecular impact of altered MHC class I levels on T-lymphocyte differentiation upon SR and potential cross-presentation of self-peptides by MHC class II molecules. It is also intriguing to test the trafficking of MHC class I in complex with peptides upon SR, given that slight perturbation in its intracellular quality control can affect its peptide loading and functionality at the cell surface (Anjanappa et al., 2020; Ghanem et al., 2015; Howe et al., 2009; Jantz-Naeem et al., 2021; Springer, 2015).

Our investigation revealed a noteworthy observation in the augmented MHC class I expression during the early rest period, around ZT0 (0700 h). Strikingly, upon SR, there was a significant and abrupt drop-in MHC class I levels. The drastic reduction in MHC class I expression at the beginning of the rest period of both lymphoid organs prompts consideration of stress hormone involvement, presumably cortisol (corticosterone) or other endocrine and paracrine mediators, such as blood epinephrine, cytokines, and factors originating from both the central nervous system (CNS) and leukocytes (Dickstein et al., 1999; Dimitrov et al., 2009; Krueger et al., 2011). Elevated blood corticosterone levels, indicative of a chronic stress state, have been shown to impact immune function in a cell type- and context-specific manner (Quatrini et al., 2021). Based on *in vitro* and *in vivo* studies in mice, corticosterone controls the diurnal oscillations in T lymphocyte distribution and responses via inducing Il-7R and CXR4 expression, in addition to impairing the anti-tumor effect of immune checkpoint blockade via modulating (namely upregulating) the expression or function of PD-1 (immune checkpoint receptor) in cytotoxic T lymphocytes in tumor-infiltrating $CD8^+$ T lymphocytes from tumor-bearing mice. Knowing that the stress-associated glucocorticoids are under circadian control (Zavala et al., 2020) and that they regulate the rhythmic homing and activity of leukocytes in lymphoid tissues (Shimba et al., 2018), our findings may implicate the potential role of corticosterone in regulating MHC-I expression in the lymph nodes and spleen.

The sustained equilibrium in the expression levels of MHC class I along with reversible impacts in the spleen, elucidates the indispensable function of these molecules in maintaining immunological homeostasis. Finally, understanding the relationships between MHC class I expression and various contextual factors—such as stress, age, and health status—could lead to the refinement of treatment protocols, ultimately enhancing the efficacy and time-precision of glucocorticoid-based therapies in the context of immune modulation.

5. Conclusion

By developing strategies to harmonize MHC class I expression with the circadian disruptions prevalent in a wide array of people with lifestyle-associated sleep problems (such as shift work) (Akerstedt, 1995), healthcare providers can potentially mitigate the adverse health effects experienced by this group. Thus, the exploration of interventions or therapies capable of modulating diurnal MHC class I expression presents an exciting avenue for disease prevention and management. Should research identify specific approaches to manipulate MHC class I expression, this knowledge could translate into novel therapeutic interventions. These interventions could potentially target diseases at the molecular level, offering innovative strategies for the treatment and prevention of a wide range of health conditions.

CRediT authorship contribution statement

Colette S. Kabrita: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samar Al Bitar:** Writing – original draft, Visualization, Investigation. **Esther Ghanem:** Writing – review &

editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

Declarations of interest: none.

Data availability

Data will be made available on request.

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