European Archives of Oto-Rhino-Laryngology (2019) 276:735–744
https://doi.org/10.1007/s00405-018-5219-6

RHINOLOGY

Differential expression of immune markers in the patients with obstructive sleep apnea/hypopnea syndrome

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Received: 8 September 2018 / Accepted: 23 November 2018 / Published online: 18 December 2018

Abstract

Purpose To evaluate phenotypic changes of various immune cells in the peripheral blood in the patients with sleep apnea/hypopnea syndrome (OSAHS).

Methods This is a case–control study. The peripheral venous blood was collected. A subset of T cells, B cells, natural killer cells, and dendritic cells was analysed using various markers and flow cytometry. Regression curve analysis was made to examine the correlation between the change of immune cells and aponea hypoxia index (AHI) and oxygen desaturation.

Results The percentage of $CD3^+/CD4^+$ T lymphocytes ($P<0.001$) and $CD19^+$ B cells ($P<0.001$) and the $CD4^+/CD8^+$ ratio ($P<0.001$) in the OSAHS patients were significantly increased compared with those in the control group without OSAHS, and $CD4^+/CD8^+$ ratio positively correlated with aponea hypoxia index ($r=0.37$, $P<0.001$) but negatively correlated with the lowest SaO_2 ($r=-0.2$, $P<0.001$), whereas a greater reduction in the percentage of $CD3^+/CD8^+$ T cells ($P<0.001$). Moreover, the ratios of $CD3^+/CD16^+/CD56^+$ natural killer (NK)-like T cells ($P<0.05$) and $CD3^-/CD16^+/CD56^+$ NKT cells ($P<0.001$) were significantly lower in the OSAHS group than those in the control group. However, no significant difference was observed in the percentage of $CD3^+$ total T cells, $CD8^+/CD28^+$ T cells, $CD8^+/CD28^-$ T cells, DC1, DC2, and DC1/DC2 dendritic cells between the OSAHS and control groups.

Conclusion Our study showed differential responses of various types of immune cells in the peripheral blood in patients with OSAHS and their correlation with severity of oxygen desaturation.

Keywords T cells · B cells · Natural killer cells · Dendritic cells · Flow cytometry · Obstructive sleep aponea

Introduction

Obstructive sleep apnea/hypopnea syndrome (OSAHS) is a clinical condition of a sleep-related breathing disorder involving intermittent partial obstruction of airflow (hypopnea) or complete obstruction of airflow (apnea) during sleep [1], resulting in hypoxemia and hypercapnia [2]. The prevalence is high with 14% of men and 5% of women suffering from OSAHS [3]. Overall, the rate of morbidity accounts for approximately 1–4% [4, 5].

The pathogenesis of OSAHS has been associated with multifactorial aetiology, such as narrowed airway, chronic nasal congestion, excess weight, and smoking, resulting in multisystem damages in the body [6, 7]. It has been shown that OSAHS is closely related to the incidence and progression of coronary heart disease [8, 9] and obesity [10]. OSAHS also significantly increases the risks of the central nervous system disorder such as stroke and metabolic diseases [7, 11]. Growth retardation, mental decline, and personality changes have been reported in children with OSAHS [4, 12–14]. OSAHS thus adversely affects physical, emotional, psychological well-being, and profoundly impacts patients' quality of life [15].

OSAHS associated with a series of inflammatory reaction has been extensively studied [16]. It has been shown that the immune system is significantly affected by poor sleep with interruption of normal circadian patterns [17, 18]. The activation of monocytes has been reported in OSA [19]; Moreover, several studies showed that OSA might be associated with activation and proliferation of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes counts in peripheral blood and tonsillar lymphoid tissues but with inconsistent results [5, 20–25]. Apart

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from T cells, a reduction in the number of peripheral B cells and natural killer T cells (NKT) but increased percentage of NK cells have recently been shown in OSAHS patients [26, 27]; however, dendritic cells in OSAHS patients is scarecely studied. Up to now, few studies have been focused on systematically evaluate the effects of OSAHS on the cellular immune system. Various phenotypic changes of different types of immune cells and their roles in response to OSAHS are less evaluated in OSAHS, and thus, we aimed to examine different phenotypes of immune cells of patients with OSAHS to fully understand the effects of OSAHS on immunity and to perform an effective treatment. The effects of OSAHS on the cellular immunity of adult OSAHS patients were assessed by collecting peripheral venous blood samples and detecting subsets of T cells, B cells, NK cells, and dendritic cells.

Materials and methods

Patient recruitment

Patients with OSAHS were hospitalised in the Department of Otorhinolaryngology Head and Neck Surgery in Beijing Shijitan Hospital, Capital Medical University. A total of 39 patients with OSAHS (female: $n=15$, male: $n=24$) and healthy volunteers (Total: $n=35$; male/female: 15/10, average age: 36.90 ± 9.80, Table 1) were recruited. Patients recruited had no history of infection, acute infection, immunological diseases, allergy, tumor, and chronic renal dysfunction. Subjects in the control group met these criteria of recruitment as well and had no night snoring and excessive daytime sleepiness. Their sleep was monitored on the day of hospitalisation and screened for OSA by Epworth sleepiness scale and underwent full polysomnography. The diagnosis of OSAHS was confirmed based on results of polysomnography. The severity of OSAHS was classified into mild, moderate, and severe according to the values of AHI and the lowest oxygen saturation ($LSaO_2$) based on the criteria of OSAHS developed by Society of Otorhinolaryngology of Chinese Medical Association (CMA) [28, 29].

Table 1 Demographic and polysomnographic characteristics of the subjects (mean ± SE)

	Control	Patients	*P* values
N (M/F)	35 (25/10)	39 (24/15)	NS
Average age (years)	36.90 ± 9.80	36.50 ± 1.76	NS
Average height (cm)	170.85 ± 1.36	167.87 ± 1.29	NS
Average weight (kg)	70.41 ± 2.12	81.52 ± 3.11	$P<0.01$
Average BMI (kg/m^2)	23.97 ± 0.47	28.63 ± 0.88	$P<0.001$

NS no significant change

Apnea is defined as an absence of airflow of 10 s or longer; Hypopnea as an airflow reduction (> 50%) lasting ≥ 10 s with a decrease in oxygen saturation over 4%. Obstructive apnea is defined as the absence of airflow in the presence of thoracic–abdominal motion. The AHI is calculated based on the average number of apnea plus hypopnea episodes per hour of recording time.

Blood collection and preparation and flow cytometry analysis

The following day of hospitalisation, morning fasting venous blood was examined for pre-operation and peripheral T cells and B cells. Detection of T cells and B cells from whole blood was made using standard operative procedures of flow cytometry (Beckman FC500 flow cytometer, USA). Whole blood was collected from each participant to evaluate the phenotypic characterisation of lymphocytes. Briefly, 4 mm of whole blood were collected in EDTA tubes. Peripheral blood mononuclear cells (PBMCs) were obtained by BD FACS Lysing Solution. Fifty μL blood samples were stained with 10 μL monoclonal antibodies (mAbs) anti-CD3, anti-CD4, anti-CD8, anti-CD56, anti-CD16, anti-CD56, anti-CD19, anti-CD25/CD127, anti-CD8/anti-CD28, anti-DC1, or anti-DC2 antibody. The samples were kept from light for 15 min and then were added with 400 μL BD lysis solution, followed by keeping from light for 8 min. Two mL of PBS was added and centrifuged at 1500r/min for 5 min, the supernatant was discarded, and the residual cells were kept in 400 μL PBS for flow cytometry analysis (Beckman FC500).

Statistical analysis

GraphPad PRISM 6 analysis software was used to collect, process, and analyse data. Values are presented as mean ± SE (standard error). Regression lines were calculated for the statistics of the correlation between various lymphocyte cell phenotypes and AHI, the lowest SaO_2 and SaO_2 by the least squares method, and the correlation coefficient r was calculated. Student's *t* test was used to determine statistical significance of differences ($P<0.05$).

Results

Demographic and sleep characteristics of subjects

A total of 35 (25 males and 10 females, median age: 36.90 ± 9.80 years) in the control group (AHI < 5) and 39 patients (24 males and 15 females, median age: 36.50 ± 1.76 years) in the OSAHS group

(AHI = 39.75 ± 4.99) and were analysed. The characteristics of the study population are shown in Table 1.

Changes of cytotoxic and regulatory T-cell subsets in OSAHS patients

T-cell subset analysis showed that no significant difference was observed in the percentage of $CD3^+$ total T cells between the OSAHS group and the control group (Fig. 1a, 64.47 ± 3.20% vs. 64.48 ± 3.10%, $P > 0.05$). The percentage of $CD3^+/CD8^+$ T-cytotoxic cells (Tc) in the patients with OSAHS was markedly decreased when compared to that in the control group (Fig. 1b, 23.33 ± 0.69% vs. 28.8 ± 0.69%, $P < 0.001$). However, no significant difference was observed in both $CD8^+/CD28^-$ T cells (Fig. 1c, 13.97 ± 0.62% vs. 15.69 ± 0.57, $P > 0.05$) and $CD8^+/CD28^+$ T cells (Fig. 1d, 15. 00 ± 0.72 vs. 13.87 ± 0.37, $P > 0.05$).

T-cell subset analysis further showed that the percentage of $CD3^+/CD4^+$ (T-helper cells) in the patients with OSAHS was markedly greater than that in the control group (Fig. 2a, 43.85 ± 1.12% vs. 38.94 ± 0.58%; $P < 0.001$); however, no significant changes were observed in $CD4^+CD25^+/CD127^+$ regulatory T cells (Fig. 2b, 2.98 ± 0.18% vs. 2.50 ± 0.18%, ns). Moreover, the OSAHS group had a significantly higher ratio of $CD4^+/CD8^+$ than that the control group (Fig. 2c, 1.96 ± 0.55% v. s. 1.38 ± 0.04%, $P < 0.001$).

The OSAHS group had a significantly higher percentage of $CD19^+$ total B lymphocytes than that of the control group (Fig. 3a, 15.21 ± 1.52% vs. 13 ± 1.92%; $P < 0.001$). Moreover, in the OSAHS group, the ratio of both $CD3^-/CD16^+CD56^+$ total NK cells (Fig. 3b, 11.6 ± 0.56% vs. 13.99 ± 0.47%, $P < 0.001$) and $CD3^+/CD16^+CD56^+$ NK-like T cells (Fig. 3c, 3.50 ± 0.19% vs. 3.5 ± 0.74%; $P < 0.05$) was significantly lower than those in the control group.

No changes in peripheral dendritic cells in OSAHS patients

There is no significant difference between the two groups in the percentage of DC1, DC2, and DC1/DC2 (Fig. 4a–c, $P > 0.05$), and no correlation was observed between DC1, DC2, or DC1/DC2 with AHI, lowest SaO_2, and mean SaO_2, indicating that OSAHS has no significant effect on peripheral dendritic cells and no correlation between the severity of OSAHS and dendritic cells.

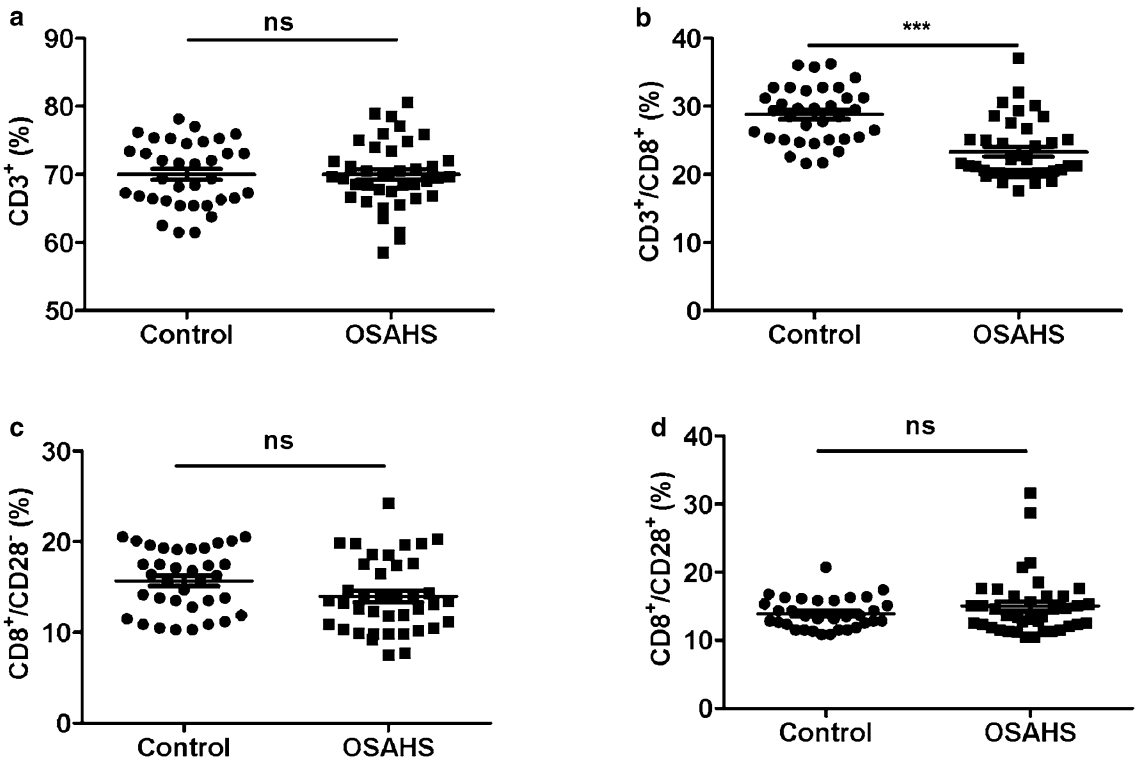

Fig. 1 Cell subset analysis results showing the percentage of $CD3^+$ (**a**), $CD3^+/CD8^+$ (**b**), $CD8^+/CD28^-$ (**c**), and $CD8^+/CD28^+$ (**d**) T lymphocytes in the patients with and without OSAHS. Circles indicate the control, whereas squares indicate the patients with OSAHS. The data are presented as median (minimum, maximum). ns: no significance, $^*P < 0.05$ vs. the control group; $^{**}P < 0.01$ vs. the control group, $^{***}P < 0.001$ vs. the control group

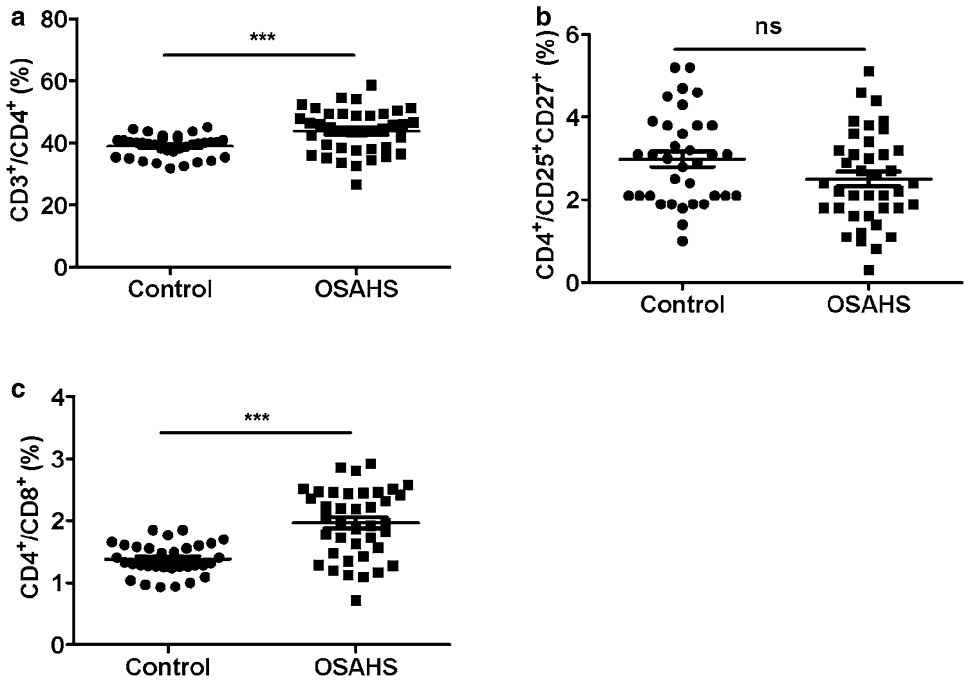

Fig. 2 Cell subset analysis results showing the percentage of $CD3^+/CD4^+$ T-helper cells (**a**), $CD4^+/CD25^+/CD127^+$ T regulatory cells (**b**), and $CD4^+/CD8^+$ (**c**) in the patients with and without OSA. Circles indicate the control, whereas squares indicate the patients with OSAHA; ns: no significance

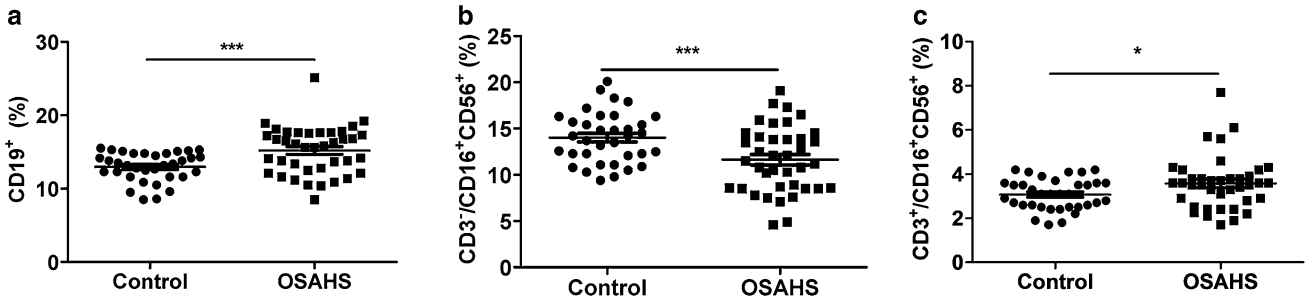

Fig. 3 Cell subset analysis results showing the percentage of $CD19^+$ B cells (**a**) $CD3^-/CD16^+CD56^+$ (**b**), and $CD3^+/CD16^+CD56^+$ NK cells (**c**) in the patients with and without OSAHS. Circles indicate the control, whereas squares indicate the patients with OSAHS. The data are presented as median (minimum, maximum). $^*P<0.05$ vs. the control group, $^{***}P<0.001$ vs. the control group

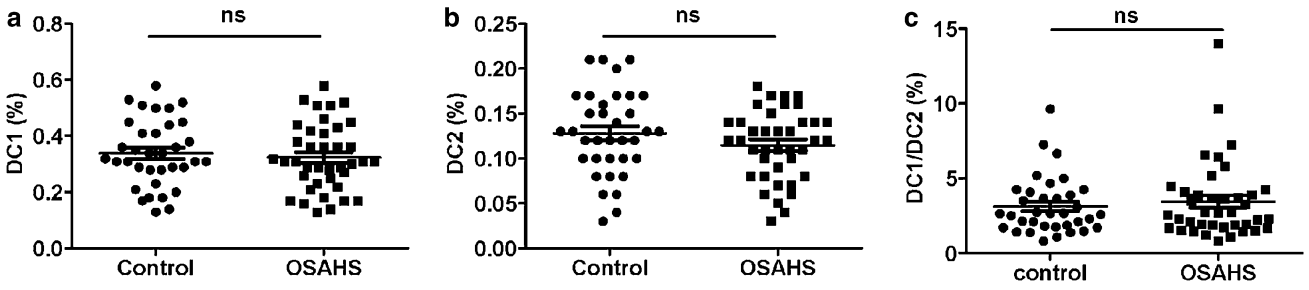

Fig. 4 Cell subset analysis results showing the percentage of DC1 (**a**), DC2 (**b**), and the ratio of DC1/DC2 (**c**) dendritic cells in the patients with and without OSA. Circles indicate the control, whereas squares indicate the patients with OSAHA. The data are presented as median (minimum, maximum); ns: no significance vs. the control group

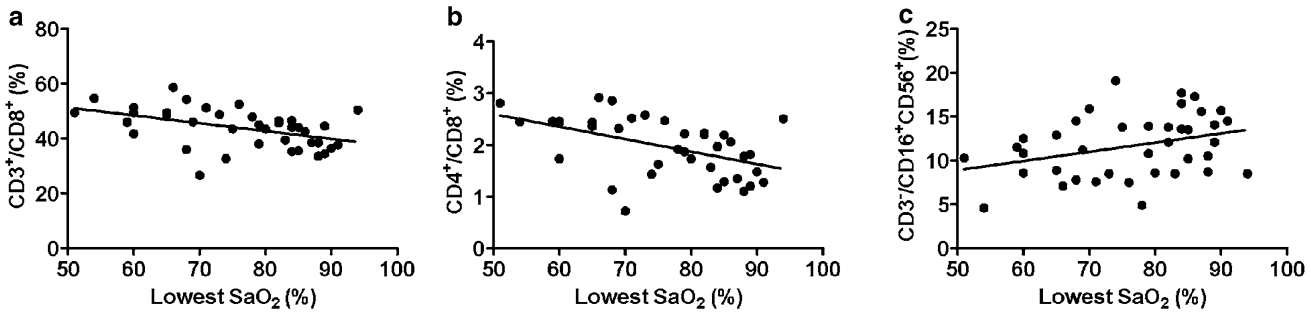

Fig. 5 Scatterplots and linear regression analysis between the lowest $SaCO_2$ and the percentage of $CD3^+/CD8^+$ (**a**), $CD4^+/CD8^+$ (**b**), and $CD3^-/CD16^+/CD56^+$ (**c**). **a** Coefficient of correlation: $r = -0.2$, $P < 0.01$. **b** Coefficient of correlation: $r = -0.2$, $P < 0.001$. **c** Coefficient of correlation $r = 0.1$, $P < 0.05$

Correlation of the expression of immune markers with AHI, the lowest SaO_2, and mean SaO_2

Regression curve analysis showed that the lowest SaO_2 and $CD3^+/CD8^+$ Tc (Fig. 5a, $r = -0.2$, $p < 0.001$) and $CD4^+/CD8^+$ (Fig. 5b, $r = -0.2$, $P < 0.001$) has negative correlation, by contrast, and there was a significant positive correlation between the lowest SaO_2 and $CD3^-/CD16^+/CD56^+$ total NK cells (Fig. 5c, $r = 0.1$, $P < 0.05$) (Fig. 6).

Regression curve analysis also showed mean SaO_2 negatively correlated with $CD4^+/CD8^+$ (Fig. 6a, $r = -0.2$, $P < 0.01$) and $CD3^+/CD4^+$ T-helper cells (Fig. 6b, $r = -10.2$, $P < 0.01$). Whereas both $CD3^-/CD16^+/CD56^+$ total NK cells (Fig. 6c, $r = 0.18$, $P < 0.01$) and $CD4^+/CD25^+/CD127^+$ Tregs (Fig. 6d, $r = 0.1$, $P < 0.05$) positively correlated with mean SaO_2.

AHI was found to positively correlate with $CD4^+/CD8^+$ (Fig. 7a, $r = 0.37$, $P < 0.001$); There was significant negative correlation between AHI with $CD3^-/CD16^+/56^+$ total NK cells (Fig. 7b, $r = -0.1$; $p < 0.05$) and $CD4^+/CD25^+CD17^+$ regulatory T cells (Fig. 7c, $r = -0.18$, $P < 0.01$).

Discussion

The present study showed that $MSaO_2$ and $LSaO_2$ were significantly decreased in the OSAHS group, and there are significant higher percentage of $CD3^+/CD4^+$ T cells and $CD19^+$ B cells and the greater ratio of $CD4^+$ and $CD8^+$

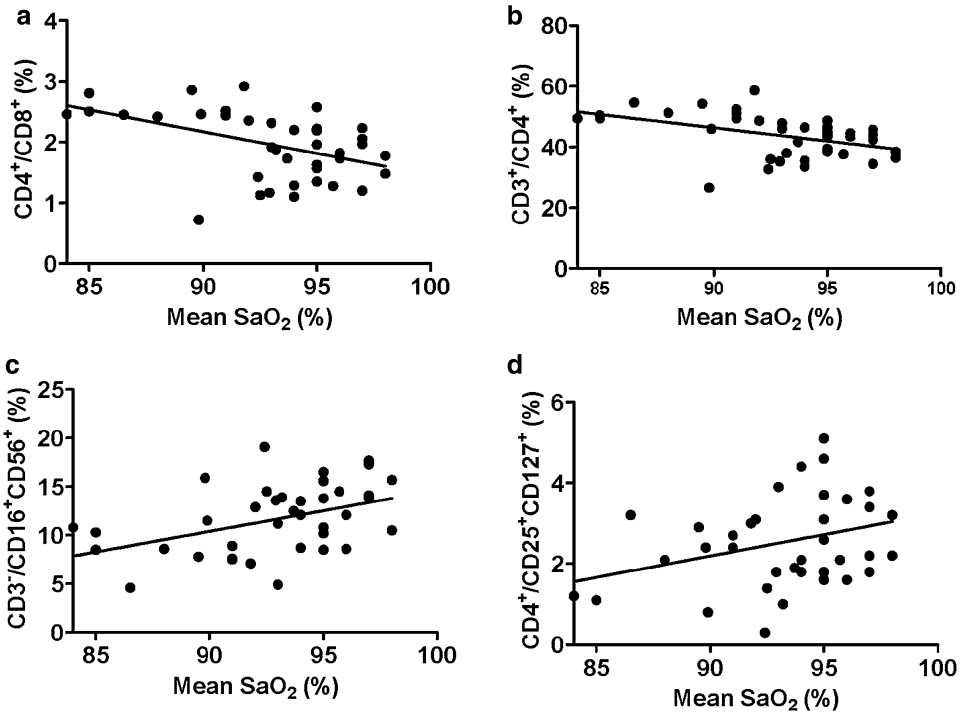

Fig. 6 Scatterplots and linear regression analysis showing correlation between mean $SaCO_2$ and the percentage of $CD4^+/CD8^+$ (**a**), $CD3^+/CD4^+$ (**b**), $CD3^-/CD16^+/CD56^+$ (**c**), and $CD4^+/CD25^+/CD127^+$ (**d**). **a** Coefficient of correlation: $r = -0.2$, $P < 0.01$. **b** Coefficient of correlation: $r = -0.2$, $P < 0.001$. **c** Coefficient of correlation: $r = 0.18$, $P < 0.01$. **d** Coefficient of correlation: $r = 0.1$, $P < 0.05$

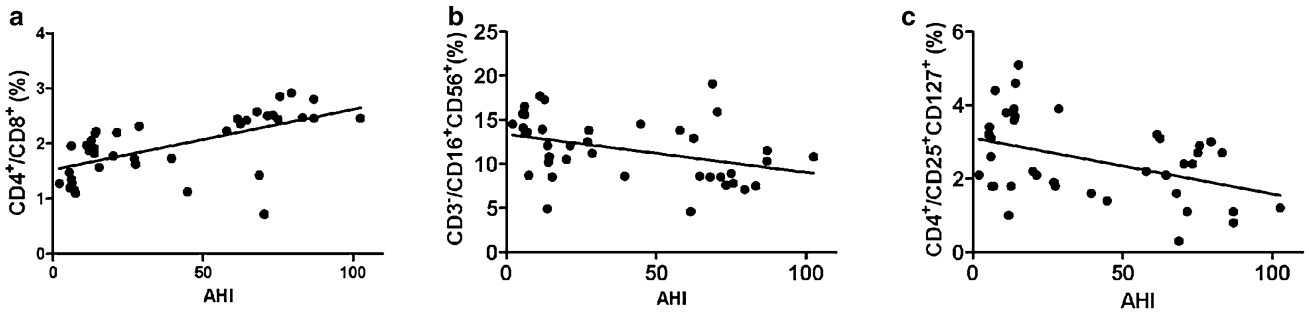

Fig. 7 Scatterplots and linear regression analysis showing correlation between AHI and the percentage of $CD4^+/CD8^+$ (**a**), $CD3^-/CD16^+/CD5^+$ (**b**), and $CD4^+/CD25^+/CD127^+$ (**c**). **a** Coefficient of correlation: $r = 0.3$; $P < 0.05$. **b** Coefficient of correlation: $r = -0.1$; $P < 0.05$. **c** Coefficient of correlation: $r = -0.18$, $P < 0.01$

but a greater reduction in the percentage of $CD3^+/CD8^+$ T cells in the OSAHS patients when compared with those in the control group. Moreover, regression curve analysis showed that $CD4^+/CD8^+$ ratio positively correlated with AHI, but negatively correlated with the lowest SaO_2. By contrast, the ratios of $CD3^+/CD16^+/CD56^+$ NKT cells and $CD3^-/CD16^+/CD56^+$ NKT cells were significantly lower in the OSAHS group than those in the control group. However, no significant difference was observed in the percentage of $CD3^+$ total T cells, $CD8^+/CD28^+$ T cells, $CD8^+/CD28^-$ T cells, DC1, DC2, and DC1/DC2 dendritic cells between the OSAHS and control groups. A significant positive correlation was found between the lowest SaO_2 and $CD3^-/CD16^+/CD56^+$ total NK cells. Mean SaO_2 positively correlated with both $CD3^-/CD16^+/CD56^+$ total NK cells and $CD4^+/CD25^+/CD127^+$ Tregs, but negatively correlated with $CD4^+/CD8^+$ and $CD3^+/CD4^+$ T cells. Moreover, AHI negatively correlated with $CD3^-/CD16^+/56^+$ total NK cells and $CD4^+/CD25^+CD17^+$ (regulatory T cells). These results are important to further understanding the response of individual types of immune cells to the OSAHS, which could provide the guidance for future clinical study and treatment.

OSA has intermittent hypoxia/reoxygenation cycles and sleep fragmentation, inducing systemic inflammation which has been extensively studied [30–32]. In contrast, few studies have been conducted to systematically examine phenotypic changes of different types of immune cells in OSAHS. It is known that lymphocytes in the peripheral blood develop and differentiate into subsets with various could regulate immune functions and actively involves functions. The proportions of T cells and B cells are well maintained in the normal human body, and they interact mutually to keep immune functions normal. Activation of T cells release of inflammatory mediators [5, 33, 34], and CD3 is used as a marker for all T lymphocytes, which includes T-cytotoxic cells ($CD3^+CD8^+$ cells) and T-helper cells ($CD3^+CD4^+$ cells). An increased number of $CD8^+$ T lymphocytes in the peripheral blood have been reported in both the children and adult patients with moderate or severe OSAHS than those of the control group [23, 24]. Functionally, cytotoxicity against endothelial cells for CD8+ T cells with TNF-alpha dependence has also been reported in OSA patients [5, 20]. Moreover, the activation of $CD8^+$ T cells has been reported to be changed with time from an initial increased activation to decreased activation after prolonged exposure to hypoxia in a rat model of hypoxia [35]. In contrasts, a recent study reported that a gross reduction in the perforin positive $CD3^+$ γδ-T cells in the peripheral blood was associated with OSA [25]; similarly, our results showed that $CD3^+/CD8^+$ cytotoxic T cells were significantly decreased in OSAHS patients. Moreover, we showed that $CD3^+/CD8^+$ negatively correlated with the lowest SaO_2, suggesting that the more oxygen desaturation induces lesser depression of the cytotoxic T cells. The discrepancy in these results may be related to the different paradigm of OSAHS and duration of OSA used in these studies. The alternation of cytotoxic T cells is possibly associated with the severity of OSA and could be tissue- or disease specific. For example, increased cytotoxic $CD8^+$ T cells have been shown to be closely associated with atherogensis [21, 23], whereas the reduced cytotoxic T cells have been implicated in depression of cytotoxicity within epithelial tissues [36], prevention of colonic inflammation [37], and tumor-induced immunosuppression [38].

CD28 as a costimulatory molecule stimulates the secondary signal for the activation of T cells, $CD8^+CD28^-$ and $CD8^+CD28^+$, are two subsets of $CD8^+$ T cells according to whether or not CD28 is expressed [39]. It has been shown that shortening telomere length in $CD8^+$ $CD28^-$ T cells is prone to occur among people with poor global sleep quality and high stress [40] and advancing age [40, 41]. Currently, no studies have been performed to compare changes of the two subsets in response to OSAHS. Although our studies for the first time examined whether any changes of the two subsets in the patients with OSAHS occurred, the way in which the balance of the two subsets affects the development of OSAHS remains to be evaluated.

Several $CD4^+$ T-cell subsets with different functions have been identified, including T helper 1 (Th1) and Th2 based on cytokine production, regulatory T cells, T follicular helper cells, T-helper type 17 (Th17) cells, and T-helper type 22 (Th 2) cells. The previous studies have shown that there was a significant decrease in $CD4^+$ T lymphocytes in adult patients and children with OSAHS [24, 26]. Whereas our results showed that increased $CD3^+$/$CD4^+$ T-helper cells in OSAHS patients and negatively correlated with oxygen desaturation, which is consistent with a recent study showing an increase in CD4 + T-cell frequency in OSA and the increase is associated with cell proliferation [42]. Our results also showed that there was no change in Treg cell expressing $CD4^{+/}CD25^+/CD127^+$, which was different from a previous study, showing that reduced Treg population expressing $CD4^+CD25^+$ in OSA [33]. Treg cells are a subpopulation of cells that maintain immune homeostasis and self-tolerance by inhibitory regulation on other immune cells [43]. The possibility of no change in Treg cells but with increased $CD3^+/CD4^+$ T-helper cells is that $CD4^+$ T cells is heterogeneous in phenotype and function, other $CD4^+$T-cell subsets such as a significant increase in peripheral Th17 cells could contribute to the increased $CD3^+/CD4^+$ T-helper cells in response to OSA [44], and it has been shown that Th17 cells are associated with inhibition of Treg differentiation [45].

Our results showed increased T-helper-($CD3^+CD4^+$) and decreased T-cytotoxic-($CD3^+CD8^+$) cells; thus, the ratio of CD4/CD8 was increased and negatively correlated with the severity of oxygen desaturation. In general, an increase in $CD4^+$/ $CD8^+$ ratio indicates an enhanced cell immune function [46]; nevertheless, there was no change in the percentage of $CD3^+$ total T cells, which may reflect haemostasis of immune status in the body via modulating both helper and cytotoxic T cells under OSAHS.

CD16 and CD56 are identified as classical markers of NK cells, which involve the direct killing of cells [47, 48]. Our results showed that both total NK cells and NKT cells were markedly decreased in OSAHS patients when compared with the control group, and closely associated with severity of oxygen desaturation as demonstrated by the fact of a positive correlation between total NK cells and the lowest SaO_2 and mean SaO_2. Our findings were consistent with the previous studies, showing that the patients with OSA have significantly reduced levels of circulating NKT cells and impaired NKT-cell function [27]. OSA-related sympathetic activation and catecholamine release have been shown to regulate immune activation [48], and catecholamine has been shown to inhibit NK activity [50]. The decreased NK cell count has linked to atherosclerotic plaque instability and the risk of acute cardiovascular events [51], and it also implicates low immunity and an increased risk of cancer due to OSA-associated hypoxia impairing NK cell function, resulting in increased apoptosis, reduced cytotoxicity, and impaired interferon gamma secretion [27].

B cells are antigen-presenting cells that can present antigens to T cells to generate effective immune responses and involve adaptive immunity by producing high-affinity antibodies [52]. Our findings showing that an increased number of $CD19^+$ B cells in the OSAHS patients, which was in contrast to a recent study showing that a decreased number of B cells in the peripheral blood of OSA patients overlapped with chronic obstructive pulmonary disease (COPD) and correlated with metabolic disorder and obesity [26]. The discrepancy might be due to the patients we recruited were excluded OSAHS from comorbidities. It is speculated that overlapping syndrome OSA/COPD results in much severer hypoxic condition than OSA alone. The severity of hypoxia in OSAHS with various oxygen gradients could produce different effects on controlling B-cell fate and function via regulating the level and duration of hypoxia-inducible factor activation [53].

Dendritic cells (DC) are a class of bone-marrow-derived cells arising from lympho-myeloid haematopoiesis that form an essential interface between innate and adaptive immunity [54]. Myeloid dendritic cells (DC1) and plasmacytoid DCs (DC2) are two main subpopulations of human DCs. Although human DC1 and DC2 have a high intrinsic capacity to interact and present antigens to activate CD8+ T cells and to promote T-helper type 1 and NK responses, DC1 cells primarily induce Th1 differentiation but DC2 cells mainly promote a Th2 response apart from activation of Th1 and CD8+ T cells [54, 55]. To our knowledge, for the first time, our study attempted to examine the effect of OSAHS on peripheral dendritic cells; nevertheless, our results showed that there were no significant changes in the percentage of DC expressing markers DC1 and DC2 and in the ratio of DC1/DC2; moreover, no correlation was found between the severity of OSAHS and DC, indicating that dendritic cells were not obviously affected by OSAHS; nevertheless, hypoxia has been reported to trigger phenotypic and functional changes in DC [56].

It is worthwhile to notice that DC markers we used in this study may be insufficient to truly identify DC, which could account for non-remarkable change; other markers such as CLEC9A, CASM1, and BTLA have been proposed to be used for confirmation and increase the accuracy of identification for DC subset [57, 58]. Moreover, the traditional classification of DCs based on phenotypic and functional properties has led to difficulties in cell identification, a recent ontogenetic definition which is independent of functional or phenotypic properties has been proposed, which could provide a way to evaluate the role of DC in the immune response in an unbiased manner [59].

It is worthwhile to consider the limitations of our study. Sample size is relatively small. Moreover, a confounding factor such as BMI should be considered for its correlation with the changes of immune cells. In our study, the median BMI was 23.97 ± 0.47 kg/m^2 in the control group and 28.63 ± 0.88 kg/m^2 in the OSAHS group ($P < 0.001$, Table 1); there is a statistically significant difference between the two groups. The previous studies have shown that nearly, 60% of moderate to severe cases of patients with obstructive sleep apnoea can be caused by a BMI more than 25 kg/m^2 [60]; Moreover, obesity is believed to predispose to the patients with obstructive sleep apnoea because of mass loading in the upper airway [61]. Furthermore, the risk in obesity is also associated with gender. Men has a higher risk of OSA than woman [62]. In addition, thus, further study should be conducted to examine the correlation among the level of cholesterol in the blood, BMI, or more specific measure such as neck or waist circumference with different types of immune cells in OSAHS in patients with different genders. Furthermore, the integration of exercise program for weight reduction in the patients with OSAHS [63], and the detection of the changes in the immune cells would provide therapeutic value for achieving better clinical outcome.

Conclusion

In summary, our studies have systematically shown phenotypic changes of various types of immune cells in the peripheral blood in patients with OSAHS and their correlation with severity of oxygen desaturation. The interaction between various types of immune cells may be associated with complications of OSAHS. Further research needs to be conducted to examine how OSAHS regulates immune responses, which could facilitate our understanding of how each subset of immune cells contribute to the pathogenesis of OSAHS and will be helpful to make therapeutic innovation targeting specific immune cells, ultimately, reducing OSAHS-induced complications, and improving clinical outcome and patients' quality of life.

Compliance with ethical standards

Conflict of interest Hong Xie has no conflict of interest, Jinshu Yin has no conflict of interest, Yunbo Bai has no conflict of interest, Hong Peng has no conflict of interest, Xiaohong Zhou has no conflict of interest, and Juan Bai has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All participants were informed about the study and signed an informed consent form. This study was approved by the Ethical Committee of Beijing Shijitan Hospital.

References

1. Mbata G, Chukwuka J (2012) Obstructive sleep apnea hypopnea syndrome. Ann Med Health Sci Res 2(1):74
2. Gale SD, Hopkins RO (2004) Effects of hypoxia on the brain: neuroimaging and neuropsychological findings following carbon monoxide poisoning and obstructive sleep apnea. J Int Neuropsychol Soc 10(1):60–71
3. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM (2013) Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol 177(9):1006–1014. https://doi.org/10.1093/aje/kws342
4. Mingari MC, Ponte M, Bertone S, Schiavetti F, Vitale C, Bellomo R, Moretta A, Moretta L (1998) HLA class I-specfiic inhibitory receptors in human T lymphocytes: interleukin 15-induced expression of CD94/NKG2A in superantigen-or alloantigen-activated CD8 + T cells. Proc Natl Acad Sci U S A 95(3):1172–1177
5. Dyugovskaya L, Lavie P, Lavie L (2005) Lymphocyte activation as a possible measure of atherosclerotic risk in patients with sleep apnea. Ann N Y Acad Sci 1051:340–350
6. Valham F, Mooe T, Rabben T, Stenlund H, Wiklund U, Franklin KA (2008) Increased risk of stroke in patients with coronary artery disease and sleep apnea: a 10-year follow-up. Circulation 118(9):955–960. https://doi.org/10.1161/CIRCULATIONAHA.108.783290
7. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP (2010) Pathophysiology of sleep apnea. Physiol Rev 90(1):47–112. https://doi.org/10.1152/physrev.00043.2008
8. Peker Y, Carlson J, Hedner J (2006) Increased incidence of coronary artery. Eur Respir J 28(3):596–602
9. Drager LF, McEvoy RD, Barbe F, Lorenzi-Filho G, Redline S, INCOSACT Initiative (International Collaboration of Sleep Apnea Cardiovascular Trialists) (2017) Sleep apnea and cardiovascular disease: lessons from recent trials and need for team science. Circulation 136(19):1840–1850
10. Strohl KP, Redline S (1996) Recognition of obstructive sleep apnea. Am J Respir Crit Care Med 154(2 Pt 1):279–289
11. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V (2005) Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 353(19):2034–2041
12. Wieckowski EU, Visus C, Szajnik M, Szczepanski MJ, Storkus WJ, Whiteside TL (2009) Tumor-derived microvesicles promote regulatory T cell expansion and induce apoptosis in tumor-reactive activated CD8 + T lymphocytes. J Immunol 183(6):3720–3730
13. Azagra-Calero E, Espinar-Escalona E, Barrera-Mora JM, Llamas-Carreras JM, Solano-Reina E (2012) Obstructive sleep apnea syndrome (OSAS): review of the literature. Med Oral Patol Oral Cir Bucal 17(6):e925–e929
14. Zhang Z, Wang C (2017) Immune status of children with obstructive sleep apnea/hypopnea syndrome. Pak J Med Sci 33(1):195–199
15. Coman AC, Borzan C, Vesa CS, Todea DA (2016) Obstructive sleep apnea syndrome and the quality of life. Clujul Med 89(3):390–395. https://doi.org/10.15386/cjmed-593
16. Fleming WE, Ferouz-Colborn A, Samoszuk MK, Azad A, Lu J, Riley JS, Cruz AB, Podolak S, Clark DJ, Bray KR, Southwick PC (2016) Blood biomarkers of endocrine, immune, inflammatory, and metabolic systems in obstructive sleep apnea. Clin

Biochem 49(12):854–861. https://doi.org/10.1016/j.clinbiochem.2016.05.005
17. Geiger SS, Fagundes CT, Siegel RM (2015) Chrono-immunology: progress and challenges in understanding links between the circadian and immune systems. Immunology 146(3):349–358. https://doi.org/10.1111/imm.12525
18. Murphy K (2017) Janeway's immunobiology (9 th. edn.). Garland Science Taylor & Francis Group, LLC, New York
19. Gozal D, Farré R, Nieto FJ (2015) Putative links between sleep apnea and cancer: from hypotheses to evolving evidence. Chest 148(5):1140–1147. https://doi.org/10.1378/chest.15-0634
20. Dyugovskaya L, Lavie P, Lavie L (2003) Phenotypic and functional characterization of blood gammadelta T cells in sleep apnea. Am J Respir Crit Care Med 168(2):242–249
21. Dyugovskaya L, Lavie P, Hirsh M, Lavie L (2005) Activated CD8 + T-lymphocytes in obstructive sleep apnoea. Eur Respir J 25(5):820–828
22. Kim J, Bhattacharjee R, Dayyat E, Snow AB, Kheirandish-Gozal L, Goldman JL, Li RC, Serpero LD, Clair HB, Gozal D (2009) Increased Cellular Proliferation And Inflammatory Cytokines In Tonsils Derived From Children With Obstructive Sleep Apnea. Pediatr Res 66(4):423–428. https://doi.org/10.1203/PDR.0b013e3181b453e3
23. Qin YH, Cai Z, Qiu YR (2010) T cell subsets and NK cell level in patients with obstructive sleep apnea/hypopnea syndrome. Guangdong Med J 31(5):615–616
24. Zhang Z, Wang C (2017) Immune status of children with obstructive sleep apnea/hypopnea syndrome. Pak J Med Sci 33(1):195–199. https://doi.org/10.12669/pjms.331.11959
25. Staats R, Rodrigues R, Barros A, Bacelar-Nicolau L, Aguiar M, Fernandes D, Moreira S, Simões A, Silva-Santos B, Rodrigues JV, Barbara C, de Almeida AB, Moita LF (2018) Decrease of perforin positive $CD3_{+}\gamma\delta$-T cells in patients with obstructive sleep disordered breathing. Sleep Breath 22(1):211–221. https://doi.org/10.1007/s11325-017-1602-6
26. Domagała-Kulawik J, Osińska I, Piechuta A, Bielicki P, Skirecki T (2015) T, B, and NKT Cells in systemic inflammation in obstructive sleep apnoea. Mediators Inflamm 2015:161579. https://doi.org/10.1155/2015/161579
27. Gaoatswe G, Kent BD, Corrigan MA, Nolan G, Hogan AE, McNicholas WT, O'Shea D (2015) Invariant natural killer T cell deficiency and functional impairment in sleep apnea: links to cancer comorbidity. Sleep 38(10):1629–1634. https://doi.org/10.5665/sleep.5062
28. Editing committee of Chinese Journal of Otorhinolaryngology Head and Neck Surgery (2002) Diagnostic criteria for OSAHS and criteria for outcome evaluation and indication of Uvulopalatopharyngoplasty. Chin J Otorhinolaryngol Head Neck Surg 37:403–404
29. Ye JY, Li WY (2009) Diagnosis and guideline for surgical treatment of OSAHS. Chin J Otorhinolaryngol Head Neck Surg 44:91–94
30. Ryan S, Taylor CT, McNicholas WT (2006) Predictors of elevated nuclear factor-kappaB-dependent genes in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 174(7):824–830
31. Unnikrishnan D, Jun J, Polotsky V (2015) Inflammation in sleep apnea: an update. Rev Endocr Metab Disord 16(1):25–34. https://doi.org/10.1007/s11154-014-9304-x
32. Vicente E, Marin JM, Carrizo SJ, Osuna CS, González R, Marin-Oto M, Forner M, Vicente P, Cubero P, Gil AV, Soler X (2016) Upper airway and systemic inflammation in obstructive sleep apnoea. Eur Respir J 48(4):1108–1117. https://doi.org/10.1183/13993003.00234-2016
33. Bollinger T, Bollinger A, Skrum L, Dimitrov S, Lange T, Solbach W (2009) Sleep-dependent activity of T cells andregulatory T cells. Clin Exp Immunol 155(2):231–238. https://doi.org/10.1111/j.1365-2249.2008.03822.x
34. Freire AX, Kadaria D, Avecillas JF, Murillo LC, Yataco JC (2010) Obstructive sleep apnea and immunity: relationship of lymphocyte count and apnea hypopnea index. South Med J 103(8):771–774. https://doi.org/10.1097/SMJ.0b013e3181e6dabf
35. McNamee EN, Korns Johnson D, Homann D, Clambey ET (2013) Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function. Immunol Res 55(1–3):58–70. https://doi.org/10.1007/s12026-012-8349-8
36. Vroom TM1, Scholte G, Ossendorp F, Borst J (1991) Tissue distribution of human gamma delta T cells: no evidence for general epithelial tropism. J Clin Pathol 44(12):1012–1017
37. Punit S, Dubé PE, Liu CY, Girish N, Washington MK, Polk DB (2015) Tumor necrosis factor receptor 2 restricts the pathogenicity of CD8(+) T cells in mice with colitis. Gastroenterology 149(4):993–1005.e2. https://doi.org/10.1053/j.gastro.2015.06.004
38. Akbarpour M, Khalyfa A, Qiao Z, Gileles-Hillel A, Almendros I, Farré R, Gozal D (2017) Altered $CD8^{+}$ T-Cell lymphocyte function and TC1 Cell stemness contribute to enhanced malignant tumor properties in murine models of sleep apnea. Sleep 40(2). https://doi.org/10.1093/sleep/zsw040
39. Borthwick NJ, Lowdell M, Salmon M, Akbar AN (2000) Loss of CD28 expression on $CD8^{+}$ T cells is induced by IL-2 receptor γ chain signalling cytokines and type I IFN, and increases susceptibility to activation-induced apoptosis. Int Immunol 12(7):1005–1013
40. Prather AA, Gurfein B, Moran P, Daubenmier J, Acree M, Bacchetti P, Sinclair E, Lin J, Blackburn E, Hecht FM, Epel ES (2015) Tired telomeres: Poor global sleep quality, perceived stress, and telomere length in immune cell subsets in obese men and women. Brain Behav Immun 47:155–162. https://doi.org/10.1016/j.bbi.2014.12.011
41. Effros RB, Dagarag M, Spaulding C, Man J (2005) The role of CD8+ T-cell replicative senescence in human aging. Immunol Rev 205:147–157
42. Said EA, Al-Abri MA, Al-Saidi I, Al-Balushi MS, Al-Busaidi JZ, Al-Reesi I, Koh CY, Hasson SS, Idris MA, Al-Jabri AA, Habbal O (2017) Altered blood cytokines, CD4 T cells, NK and neutrophils in patients with obstructive sleep apnea. Immunol Lett 190:272–278. https://doi.org/10.1016/j.imlet.2017.08.009
43. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133(5):775–787. https://doi.org/10.1016/j.cell.2008.05.009
44. Ye J, Liu H, Zhang G, Li P, Wang Z, Huang S, Yang Q, Li Y (2012) The Treg/Th17 imbalance in patients with obstructive sleep apnoea syndrome. Mediators Inflamm 2012:815308. https://doi.org/10.1155/2012/815308
45. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S (1998) Immunologic self-tolerance maintained by CD25 + CD4 + naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 10(12):1969–1980
46. Thounaojam MC, Dudimah DF, Pellom ST Jr, Uzhachenko RV, Carbone DP, Dikov MM, Shanker A (2016) Bortezomib enhances expression of effector molecules in antitumor CD8+ T lymphocytes by modulating Notch-NF-kB-miR-155 crosstalk. Oncotarget 6(32):32439–32455. https://doi.org/10.18632/oncotarget.5857
47. Loza MJ, Perussia B (2004) Differential regulation of NK cell proliferation by type I and type II IFN. Int Immunol 16(1):23–32
48. Freud AG, Mundy-Bosse BL, Yu J, Caligiuri MA (2017) The broad spectrum of human natural killer cell diversity. Immunity 2017;47(5):820–833. https://doi.org/10.1016/j.immuni.2017.10.008
49. Bisogni V, Pengo MF, Maiolino G, Rossi GP (2016) The sympathetic nervous system and catecholamines metabolism in

obstructive sleep apnoea. J Thorac Dis 8(2):243–254. https://doi.org/10.3978/j.issn.2072-1439.2015.11.14
50. Ben-Eliyahu S, Shakhar G, Page GG, Stefanski V, Shakhar K (2000) Suppression of NK cell activity and of resistance to metastasis by stress: a role for adrenal catecholamines and β-adrenoceptors. Neuroimmunomodulation 8(3):154–164
51. Backteman K, Ernerudh J, Jonasson L (2014) Natural killer (NK) cell deficit in coronary artery disease: no aber'[rations in phenotype but sustained reduction of NK cells is associated with low-grade inflammation. Clin Exp Immunol 175(1):104–112. https://doi.org/10.1111/cei.12210
52. LeBien TW, Tedder TF (2008) B lymphocytes: how they develop and function. Blood 112(5):1570–1580
53. BBurrows N, Maxwell PH (2017) Hypoxia and B cells. Exp Cell Res 356(2):197–203
54. Collin M, Bigley V (2018) Human dendritic cell subsets: an update. Immunology 154(1):3–20. https://doi.org/10.1111/imm
55. Bachem A, Güttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, Salama A, Movassaghi K, Opitz C, Mages HW, Henn V, Kloetzel PM, Gurka S, Kroczek RA (2010) Superior antigen cross-presentation and XCR1 expression define human CD11c + CD141 + cells as homologues of mouse CD8+ dendritic cells. J Exp Med 207(6):1273–1281. https://doi.org/10.1084/jem.20100348
56. Winning S, Fandrey J (2016) Dendritic cells under hypoxia: how oxygen shortage affects the linkage between innate and adaptive immunity. J Immunol Res 2016:5134329. https://doi.org/10.1155/2016/5134329
57. Ahrens S, Zelenay S, Sancho D, Hanč P, Kjær S, Feest C, Fletcher G, Durkin C, Postigo A, Skehel M, Batista F, Thompson B, Way M, Reis e Sousa C, Schulz O (2012) F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. Immunity 36(4):635–645. https://doi.org/10.1016/j.immuni.2012.03.008
58. Zhang JG, Czabotar PE, Policheni AN, Caminschi I, Wan SS, Kitsoulis S, Tullett KM, Robin AY, Brammananth R, van Delft MF, Lu J, O'Reilly LA, Josefsson EC, Kile BT, Chin WJ, Mintern JD, Olshina MA, Wong W, Baum J, Wright MD, Huang DC, Mohandas N, Coppel RL, Colman PM, Nicola NA, Shortman K, Lahoud MH (2012) The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. Immunity 36:646–657
59. Schraml BU, Reise Sousa C (2015) Defining dendritic cells. Curr Opin Immunol 32:13–20. https://doi.org/10.1016/j.coi.2014.11.001
60. Young T, Peppard PE, Taheri S (1985) Excess weight and sleep-disordered breathing. 99, 1592–1599
61. Shelton KE, Woodson H, Gay S, Suratt PM (1993) Pharyngeal fat in obstructive sleep apnea. Am Rev Respir Dis 148:462–466
62. Morselli LL, Temple KA, Leproult R, Ehrmann DA, Van Cauter E, Mokhlesi B (2018) Determinants of slow-wave activity in overweight and obese adults: roles of sex, obstructive sleep apnea and testosterone levels. Front Endocrinol (Lausanne) 9:377. https://doi.org/10.3389/fendo.2018.00377. eCollection 2018
63. Hudgel DW, Patel SR, Ahasic AM, Bartlett SJ, Bessesen DH, Coaker MA, Fiander PM, Grunstein RR, Gurubhagavatula I, Kapur VK, Lettieri CJ, Naughton MT, Owens RL, Pepin JD, Tuomilehto H, Wilson KC (2018) American Thoracic Society Assembly on Sleep and Respiratory Neurobiology. The role of weight management in the treatment of adult obstructive sleep apnea. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med 198(6):e70–e87. https://doi.org/10.1164/rccm.201807-1326ST