Aus der Klinik für Psychiatrie und Psychotherapie, Campus Benjamin Franklin der Medizinischen Fakultät Charité – Universitätsmedizin Berlin,

DISSERTATION

Immunological alterations in severely obese patients with comorbid depression

Immunologische Veränderungen in einer Kohorte hochgradig adipöser Patienten mit komorbider Depression

zur Erlangung des akademischen Grades
Doctor rerum medicinalium (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Victoria Stiglbauer

aus Wien, Österreich

Datum der Promotion: 30.11.2023

Inhaltsverzeichnis

List of tables

List of figures

Supplementary Figures

List of abbreviations

AF 488	Alexa Fluor 488
APC	Allophycocyanin
APC Cy7	Allophycocyanin-cyanine 7 tandem
BV421	Brilliant violet 421
BV510	Brilliant violet 510
CRP	C-reactive protein
DC	Dendritic cells
FACS	Fluorescent-activated cell sorting
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
GAD-7	Generalized Anxiety Disorder-7
HC	Healthy Control
IL-6	Interleukin-6
MDD	Major Depressive Disorder
NK	Natural killer cell
NKc	Cytotoxic natural killer cell
NKreg	Regulatory natural killer cell
Ob	Obesity
Ob+D	Obesity + Depression
PBMCs	Peripheral mononuclear cells
PBS	Phosphate-buffered saline

PE	Phycoerythrin
PE-Cy7	Phycoerythrin-cyanine 7 tandem
PerCP-Cy5.5	Peridinin chlorophyll protein-cyanine 5.5 tandem
PHQ-9	Patient Health Questionnaire-9
SSRIs	Selective serotonin reuptake inhibitors
TCM	Central memory T cell
TEM	Effector memory T cell
TNF-α	Tumor necrosis factor α
T_{reg}	Regulatory T cells

1.1. Abstract

Prevalence of Major Depressive Disorder (MDD) and obesity are ever-increasing and a major public health concern. The two conditions are strongly linked on an epidemiological level, with both disorders favouring development of the other by approximately 50 % and increasing overall morbidity in the general population. This comorbidity could be in part driven by converging biological pathways, including inflammatory processes. As chronic low-grade inflammation is present in both MDD and obesity, the study of potential immunogenic shifts in patients with obesity and comorbid depressive symptoms may shed light on putative shared mechanisms.

Multicolour flow cytometry of peripheral blood mononuclear cells (PBMC) was used for characterization of immune phenotypes. Immune cell population frequencies of patients with severe obesity and comorbid depression (n = 10) were compared to obese (n = 17) and lean controls (n = 20) without psychiatric symptoms and correlated with clinical characteristics.

The Patient Health Questionnaire (PHQ-9) was used for assessment of depressive symptoms and depression defined by a cut-off ≥ 10.

Patients with obesity and comorbid depressive symptoms showed reduced amounts of cytotoxic natural killer cells, $CD8^+$ effector memory T cells and dendritic cells in comparison to normal-weight controls. Frequencies of regulatory T cells were increased in patients with obesity and comorbid depression, compared to obese patients without depression. The amount of circulating $CD4^+$ central memory T cells was elevated in obese patients with comorbid depression compared to lean controls. Furthermore, frequencies of cytotoxic natural killer cells, dendritic cells and $CD4^+$ central memory T cells significantly correlated with PHQ scores in the total sample. A higher waist-hip-ratio (WHR) was associated with lower frequencies of dendritic cells.

Together, these results are suggestive of a possible depression-specific immune dysregulation in patients with obesity.

1.2. Abstrakt

Depression und Adipositas sind hochprävalent in der Allgemeinbevölkerung und stellen eine starke Belastung für die Betroffen, Angehörigen und das allgemeine Gesundheitssystem dar. Epidemiologisch wurde eine stabile, bidirektionale Assoziation von Depression und Adipositas nachgewiesen, beide Erkrankungen erhöhen das Risiko für das Auftreten der jeweils anderen um circa 50 %. Dieser Zusammenhang könnte eventuell durch konvergierende pathophysiologische Vorgänge erklärt werden, da beide Krankheitsbilder mit chronischen, systemischen Entzündungsvorgängen assoziiert sind. Besonders Patienten mit atypischer Depressionssymptomatik, welche gesteigerten Appetit, Gewichtszunahme, Fatigue und Hypersomnie umfasst, zeigen signifikante Assoziationen mit erhöhten Entzündungsparametern im Blut. Die Immunphänotypisierung von Patienten mit hochgradiger Adipositas und komorbider Depression bietet eine Möglichkeit, diese Mechanismen genauer zu erforschen. Ziel dieser Arbeit war die Untersuchung der relativen Verteilung verschiedener Immunzellpopulationen im Blut adipöser Patienten mit depressiver Symptomatik (n = 10) im Vergleich zu einer adipösen (n = 17) und einer normalgewichtigen Kohorte (n = 20). Depression wurde durch eine Punktzahl von ≥ 10 des Patient Health Questionnaire (PHQ-9) Fragebogens definiert. Mittels Durchflusszytometrie wurden die zellulären Komponenten des angeborenen und des adaptiven Immunsystems analysiert, auf Gruppenunterschiede getestet und die Ergebnisse mit klinischen Parametern korreliert. Im Blut adipöser Patienten mit komorbider Depression fanden sich signifikant reduzierte Zahlen zytotoxischer natürlichen Killerzellen und dendritischer Zellen, sowie eine Zunahme von $CD4^+$ zentralen T-Gedächtniszellen und regulatorischen T Zellen im Vergleich zur gesunden Kontrollgruppe. Die Menge zytotoxischer natürlicher Killerzellen und $CD4^+$ zentraler T-Gedächtniszellen korrelierte signifikant mit dem Schweregrad der Depression, aber nicht mit Taille-Hüft-Verhältnissen. Die Reduktion dendritischer Zellen zeigte einen Zusammenhang mit Depression und Taille-Hüft-Verhältnissen. Diese Ergebnisse suggerieren potenziell depressionsspezifische Auswirkungen auf das Immunsystem bei Patienten mit hochgradiger Adipositas.

1.3. Introduction

Major Depressive Disorder

Major Depressive Disorder (MDD) is the most common psychiatric disorder which affects approximately 6 % of the general population each year[1]. The life-time prevalence of MDD lies around 20 %[1] and women are affected twice as often as men[1].

Depressed mood and anhedonia constitute the two core symptoms of MDD[2] and are regularly accompanied by changes in appetite and weight, sleep disturbances, psychomotor symptoms, fatigue and suicidal ideation[3]. The vast heterogeneity of clinical presentations has led to the establishment of several subtypes of MDD according to different symptom clusters[1], which might be partly driven by discrete biological mechanisms. Six subtypes according to different symptom characteristics are currently specified in the Diagnostic and Statistical Manual of Mental Disorder (DSM-V), including depression with melancholic, atypical and catatonic features, chronic, postpartum and seasonal depression[4]. Atypical depression affects between 15 – 30 % of patients with MDD[5] and is characterized by hyperphagia, weight gain, hypersomnia, fatigue and increased sensitivity to interpersonal rejection. Furthermore, atypical depression shows a higher prevalence in young female MDD patients and often leads to a chronic course of disease[5].

MDD not only greatly impairs quality of life and functioning, it is also often accompanied by a plethora of medical comorbidities that might contribute to the elevated risk of overall mortality in MDD patients, in addition to suicide[6]. MDD is associated with several cardiovascular diseases, including hypertension, coronary heart disease and stroke, several forms of cancer and metabolic dysregulations such as insulin resistance, diabetes, obesity and metabolic syndrome[6–9].

Obesity

The age-standardized prevalence of obesity ranges from 14.9 % in women to 10.8 % in men[10]. In Germany, 23 % of adult men and 24 % of adult women are obese[11]. There is a high co-prevalence of obesity and type 2 diabetes, cardiovascular disorders, cancers[12,13] and psychiatric comorbidities[10], in addition to reduced quality of life[13]. Obesity is defined by a Body mass index (BMI) $\geq$ 30 kg/m^2 or a waist circumference $\geq$ 80 in women and $\geq$ 90 in men[10]. Waist and hip circumference measurements can be used for calculating the waist-hip ratio (WHR), which is considered useful for risk assessment of metabolic and cardiovascular comorbidities[15]. Especially the presence of visceral fat (as opposed to subcutaneous fat) is supposedly a key factor for the development of severe comorbid diseases via the production of pro-inflammatory adipokines[10]. Moreover, obesity is associated with impaired immune function[16]. Even though the exact mechanisms are unknown, alterations in circulating T cell subsets, B cells and natural killer (NK) cells have been reported[17]. Furthermore, obese individuals show greater susceptibility to infections, sepsis and reduced antibody production in response to vaccines such as hepatitis B[16].

Epidemiological overlap between MDD and obesity

Both MDD and obesity are major public health concerns and associated with high socioeconomic costs[18]. MDD is among the leading causes of years lived with disability and therefore a major contributor to chronic disease burden worldwide[19]. Obesity is also associated with an increased risk of limitations with activities of daily living[20] and, due to an ever-increasing prevalence, increases disability in both high- and low-income countries[21].

A strong reciprocal epidemiological link exists between obesity and MDD, with MDD patients having a 58 % higher risk of becoming obese and obese people at a 55 % increased risk of developing depression later in life[14,18]. Interestingly, obesity and depression showed an even stronger association than overweight and depression[18]. A recently published study investigating the relationship of neuropsychiatric symptoms (including depression) and body mass index found the highest rates of depression in individuals with severe obesity compared to overweight and lean controls[22]. In the same study, the authors also reported higher measures of systemic inflammation (CRP and IL-6 levels) in subjects with severe obesity which also correlated with depressive symptoms, anxiety and fatigue[22].

While it might seem intuitive that obesity, which is often associated with social discrimination, numerous medical comorbidities, lower socioeconomic status and a tendency towards poor lifestyle behaviours[23], should facilitate the development of depressive symptoms, there is also evidence that lean MDD patients present with subclinical metabolic alterations[24].

Putative shared biological mechanisms – towards immunometabolic depression

Part of the association between MDD and obesity might be driven by shared pathophysiological pathways, including chronic low-grade inflammation and dysregulations of the innate and adaptive immune system[25–27].

A recent, large-scale metabolomics study in MDD patients revealed significant dyslipidemia, characterized by added presence of very low-density lipoprotein (VLDL) and triglycerides, next to reduced concentrations of high-density lipoprotein (HDL)[24]. These findings were independent of sex, age, or body mass index (BMI), suggesting

that these changes may present early manifestations of metabolic dysfunction in MDD which might facilitate the development of obesity over time.

In a mouse model of obesity (induced by a high-fat diet), it was shown that anxiety-like behaviour is a direct consequence of obesity-driven accumulation of pro-inflammatory senescent glial cells in proximity to the lateral ventricles. Senolytic treatment using the compounds Dasatinib and Quercetin led to the clearance of these cells and alleviated the anxiety-like behavioural phenotype[28].

Since common genetic risk factors for obesity and depression have been identified, heightened inflammation has been hypothesized to constitute a shared mechanism. Especially in patients exhibiting a symptom profile that resembles atypical depression (e.g., reporting an increase in appetite and weight gain) had a higher likelihood to carry genetic variants associated with obesity-related traits. This subgroup of patients showed an increased polygenic risk score for higher BMI and elevated blood levels of Leptin (a proteo-hormone that plays a role in body-weight regulation) and the inflammation marker C-reactive protein (CRP)[29]. A recent gene expression study performed on a subset of untreated MDD patients that reported hyperphagia and/or weight gain during an active episode suggests a contribution of inflammatory genes: Compared to patients with hypophagia, patients reporting increased appetite showed an elevated expression of genes associated with cytokine signalling, apoptosis and the NLRP3 inflammasome (a multimeric protein complex that regulates caspase-1 activity)[30]. Especially genes related to the NLRP3 inflammasome were highly expressed in MDD patients with hyperphagia[30]. An upregulation of NLRP3 genes has been described in adipocytes obtained from patients with obesity, suggesting a role for caspase-1 in adipogenesis and obesity-associated inflammatory processes[31].

Higher levels of pro-inflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α) and Interleukin 6 (IL-6) as well as increased CRP have been detected in both patients with MDD and obesity[32,33]. Moreover, a recently published study investigated the association of overweight and comorbid depression with CRP levels and showed that while both the presence of depression and overweight alone already increased the risk of elevated CRP levels compared to healthy controls, individuals affected by both conditions had the highest CRP measures of all groups[34].

Taken together, these observations led to the idea, that the comorbidity between obesity and MDD might be in part explained by shared inflammatory dysregulations leading to (chronic) inflammation. These ideas were summarized under the term "immuno-metabolic depression", which might particularly apply to patients with an atypical depression symptom profile[27]. This model offers an explanation on how changes in sleeping patterns, energy metabolism, appetite and weight, and the development of depressed mood could be influenced or even induced by a chronic pro-inflammatory state similar to "sickness-behaviour" observed in animals[27]. A further observation that supports the notion that inflammation might be a causal factor for the development of depression is the strong association of Interferon treatment (e.g. for the treatment of hepatitis C or cancers) and the development of depressive symptoms that has long been reported in clinical practice[35,36,37]. Inflammatory processes also exert a direct effect on metabolic regulation, favoring the development of leptin and insulin resistance, which has been frequently observed in both patients with MDD and people with obesity[27]. Recently, a review article on dietary regulation of immune function[38] highlighted how different macronutrients and their systemic metabolism affect cell metabolism of immune cells which in turn influences their functioning. Metabolism is tightly linked to immune function and

influences homeostasis overall. Immune-metabolic dysregulations in the form of chronic low-grade inflammation have been shown to negatively impact mitochondrial function, which has been linked to depression and persistent fatigue[39].

It is recognized that adipose tissue is highly immunologically active, producing adipocytokines, including the aforementioned hormone leptin as well as adiponectin, that both influence the development of insulin resistance and directly affect the innate and adaptive immune system[38,40]. Leptin possesses pro-inflammatory properties due to its structural similarity to inflammatory cytokines and favours the production of IL-6, IL-12 and TNF-α in monocytes and macrophages[40,41]. Leptin receptors are highly expressed on the surface of various immune cell (sub)populations and their activation affects T lymphocyte differentiation and function, next to maturation of dendritic cells[42]. Obesity is associated with hyperleptinemia[10,42] and leptin dysregulation has also been reported in MDD patients with atypical depression, for both current and remitted patients[43]. Since central inflammation may negatively impact leptin receptor function and lead to leptin resistance, it could constitute an underlying shared pathophysiological mechanism between MDD and obesity[43].

Cells of both the innate and adaptive immune system are strongly influenced by a chronic inflammatory state, not only concerning their function, but also their proliferative capacities and abundance in blood and peripheral tissues[44]. Relative abundances of immune cell subpopulations can be easily assessed by flow cytometry. Immunophenotyping by flow cytometry is a powerful tool to study different components of the human immune system[45]. While it is commonly used in preclinical and clinical research, immunophenotyping is also put to use in several clinical settings, including assessment of disease progression in HIV-infected patients and diagnosis and subsequent monitoring of leukemia and lymphoma[45]. Although

immunophenotyping does not assess the function of cell types, it is very useful for exploring the distribution of several immune subsets in clinical cohorts. Since the immune system is gaining attention in the context of psychiatric diseases, immunophenotyping of MDD patients could provide further insights into the putative involvement of different immune cell subsets in disease-specific inflammatory processes.

Several immunophenotyping studies of samples obtained from MDD patients have reported changes in the abundance of monocytes, natural killer cells and T lymphocytes, some of these changes were significantly associated with depression severity or sleep disturbance[46–49]. Moreover, flow cytometric analyses of blood samples obtained from patients with obesity and metabolic syndrome revealed changes in numbers of natural killer cells, granulocytes and memory T cells that correlated with BMI and visceral adipose tissue mass[17].

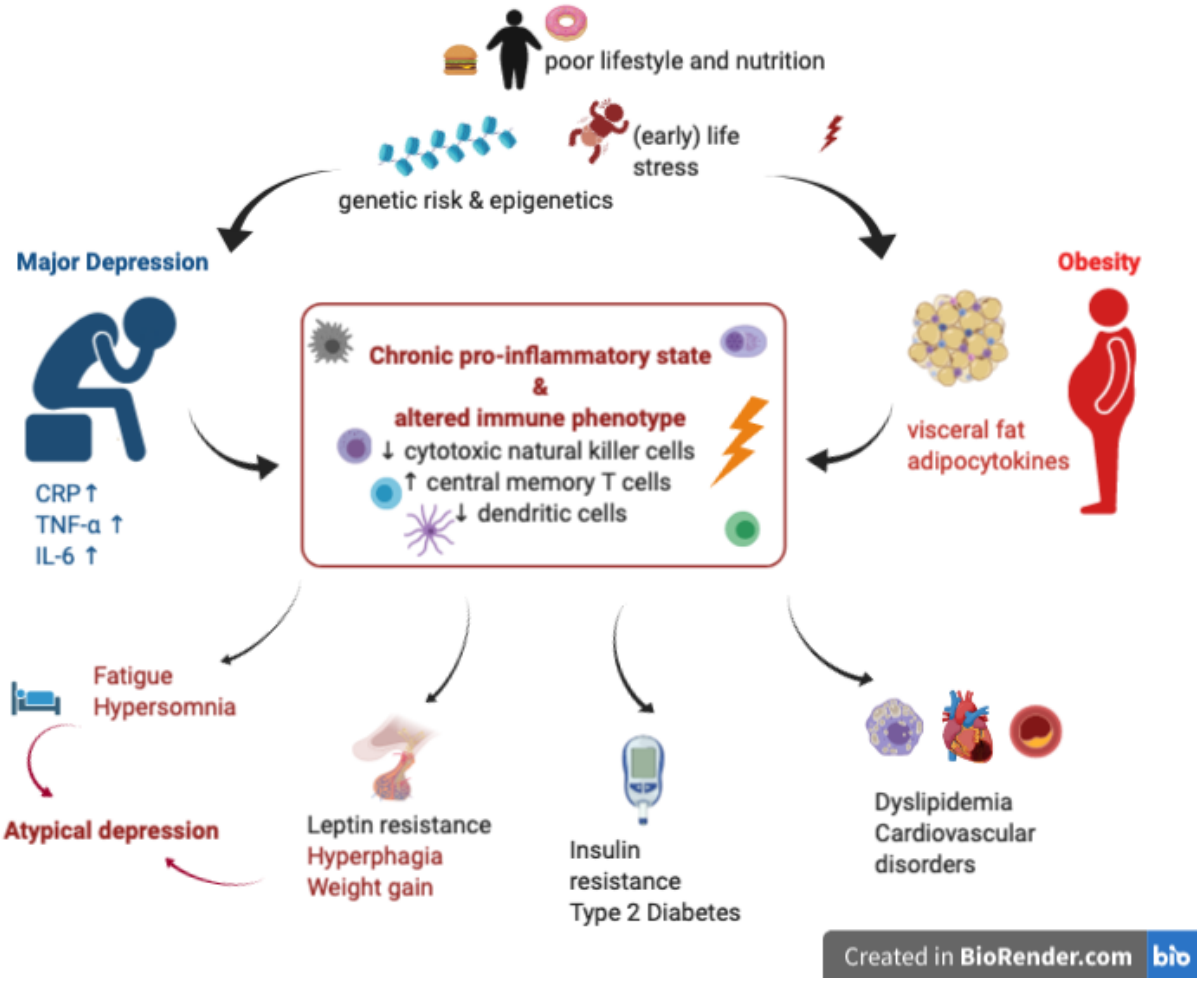

Figure 1 | Immunometabolic depression. Illustration of shared environmental and biological factors between major depression and obesity. Created using BioRender.com.(own representation: Victoria Stiglbauer)

Aim of the dissertation

Current findings from epidemiological, genetic, and immunological studies support the idea that low-grade inflammatory processes might be a contributing factor linking depression and obesity. Patients affected by both disorders therefore provide a valuable cohort for immunophenotyping studies. The aim of this dissertation was to perform an exploratory analysis of the distribution of innate and adaptive immune cell subpopulations in whole blood samples obtained from patients with obesity and comorbid depression in comparison to obese and lean controls and correlate the findings with clinical characteristics considered relevant for both depression and obesity.

1.4. Methods

Participants

A total of 47 participants were included in this study belonging to three different groups: lean healthy controls (n = 20), severely obese participants (n = 17) and severely obese patients with comorbid depressive symptoms (n = 15). The local ethics committee (Charité Universitätsmedizin Berlin, Campus Mitte, EA1/063/16) approved the study, including the extraction of peripheral mononuclear blood cells (PBMCs) from whole blood and their subsequent experimental analysis. Clinical parameters including blood pressure, waist circumference, height, weight, etc., were measured by trained staff. Depressive symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9)[50–52] and depression was defined by a score ≥ 10. Anxiety was assessed using the Generalized Anxiety Disorder-7 scale[53,54].

Healthy controls were recruited via online advertisements and bulletins and had no record of any significant medical illness (including insulin resistance or diabetes, hypothyroidism, or cardiovascular diseases) or psychiatric condition. Participants with severe obesity were recruited during their participation in a 6-month outpatient treatment program at the Department of Psychosomatic medicine (Medizinische Klinik mit Schwerpunkt Psychosomatik, Charité - Universitätsmedizin Berlin, Campus Benjamin Franklin), which included psychosocial and nutritional interventions, in addition to physical exercise programs. A description of demographic and clinical characteristics of the cohort can be found in Table 1.

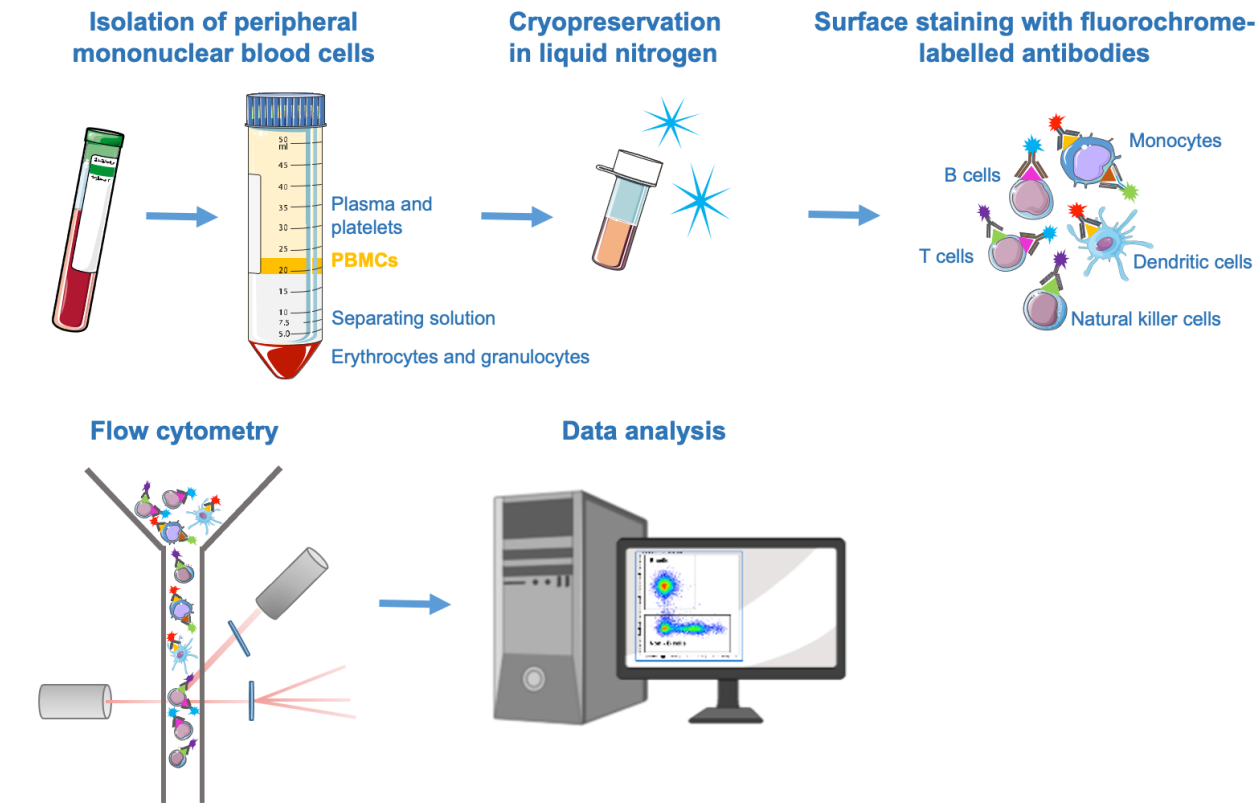

Figure 2 | Graphical representation of the experimental procedures, images courtesy of Servier SMART Medial Art and biorender.com, created using Powerpoint. (own representation: Victoria Stiglbauer)

Flow Cytometry

Four antibody panels were established to analyze different cell populations of the innate and adaptive immune system via multicolor flow cytometry. All antibody panels analyzing T cell subpopulations included PD-1 and KLRG1 as markers for cellular exhaustions and senescence. A table depicting all antibody panels and clones can be found in the supplements of the publication[55] (page 63).

In brief, the first panel aimed to identify regulatory T cells (Treg), including naïve and memory Treg. The following combination of fluorochrome-conjugated monoclonal antibodies was used (dilution indicated in brackets): CD25 BV421 (Clone M-A251,1:50; Biolegend, UK), CD3 BV510 (Clone UCHT1, 1:25; Biolegend, UK), KLRG1 AF488 (Clone 13F12F2, 1:25; Thermofisher Scientific, USA), PD-1 PE (Clone EH12.2H7, 1:25; Biolegend, UK), CD4 PerCP-Cy5.5 (Clone RPA-T4, 1:50;

Biolegend, UK), CD45RA PE-Cy7 (Clone HI100, 1:200; Biolegend, UK) and CD127 APC (Clone A019D5, 1:25; Biolegend, UK). The second panel served to distinguish naïve and several memory T cell populations of both the $CD4^+$ and $CD8^+$ compartment. Antibodies included in this panel were: CD3 BV421 (Clone UCHT1, 1:200; Biolegend, UK), CD8 BV510 (Clone RPA-T8,1:25; Biolegend, UK), KLRG1 AF488 (1:25), PD-1 PE (1:25), CD4 PerCP-Cy5.5 (1:50), CD45RA PE-Cy7 (1:200), CCR7 APC (Clone G043H7, 1:20; Biolegend, UK). For the identification of T cell subsets including helper T cells, the following antibodies were used: CXCR3 BV421 (Clone G025H7, 1:50; Biolegend, UK), CD3 BV510 (1:200), KLRG1 AF488 (1:25), PD-1 PE (1:25), CD4 PerCP-Cy5.5 (1:50), CCR6 PE-Cy7 (Clone G034E, 1:50; Biolegend, UK) and CCR4 APC (Clone L291H4, 1:25; Biolegend, UK). The fourth panel served to identify non-T cell populations and included: CD14 BV421 (Clone HCD14, 1:100; Biolegend, UK), CD3 BV510 (1:200), HLA-DR FITC (Clone LN3, 1:100; Biolegend, UK), CD56 PE (Clone HCD56, 1:50; Biolegend, UK), CD4 PerCP-Cy5.5 (1:50), CD20 PE-Cy7 (Clone 2H7, 1:200; Biolegend, UK) and CD16 APC (Clone 3G8, 1:200; Biolegend, UK). In addition, the Zombie NIR live/dead stain (1:1000; Biolegend, UK) diluted in PBS was used for all panels.

All antibody premixes were prepared in FACS buffer (PBS (Sigma Aldrich, USA) + 0.5 % bovine serum albumin (Miltenyi Biotec, Germany) + 2 mM EDTA, pH = 8, (Promega, Germany)). For the naïve and memory T cell panel, the CCR7 antibody was added directly to the live/dead marker in PBS.

For each donor, two aliquots of 10^7 cryopreserved PBMCs were thawed. Samples were transferred from liquid nitrogen to an ice box and then to a water bath at 37 °C for 1-2 minutes until only a small ice clump was visible in the vial. Under sterile conditions, the cell suspension was transferred dropwise into a 12 ml falcon tube filled with 10 ml RPMI 1640 + Glutamax medium (Gibco, Thermofisher Scientific,

USA) + 10 % FCS (Merck, Germany) that had been pre-warmed to 37 °C. The falcon tubes were then centrifuged at room temperature at 250 x g for 6 minutes (with brake) and the supernatant was removed. Cells were resuspended in 500 µl medium and the suspension was filtered into 10 ml falcon tubes using cell strainer filters at a size of 70 µm (Sarstedt, Germany) to expel cellular debris and clumps. Filters were washed three times with 500 µl PBS (4 °C). Cell counting was performed using a Neubauer chamber.

Following centrifugation at 350 x g at RT for 5 min and after removal of the supernatant, PBMCs were incubated with 50 µl of the live/dead marker solution for 15 min in the dark at room temperature. 50 µl of antibody premixes were then added to the respective tubes for each panel and incubated for an additional 15 min at RT protected from light. Following incubation, 1 ml of FACS buffer was added to each tube and samples were centrifuged at 350 x g for 5 min at 4 °C. Cells were resuspended in 150 µl FACS buffer and FACS tubes were kept on ice until measurement.

Measurements were performed on FACSCanto II flow cytometers (BD, Germany) using BD FACS Diva Software (BD, Germany). Due to technical problems with the first flow cytometer during the experiments, data was acquired by switching to another machine of the same type. Data was analyzed separately for both devices and then pooled for statistical analysis. Instrument setup and compensation were performed on both devices according to the same protocol.

Analysis and Statistics

Flow cytometric data was analyzed using FlowJo software version 10.1 (Trestar Inc., USA). PBMC subpopulations were identified using manual gating based on the recommendations by the Human Immunology Project[56].

A detailed description of the manual gating strategy can be found in the supplementary figures S1-S2 on pages 64-66. Fig. 3 provides a general overview of all immune cell (sub)populations that could be identified according to the antibody panels applied. In brief, lymphocytes were identified according to their forward scatter area (FSC-A) and side scatter area (SSC-A) characteristics. Then, doublets and dead cells were excluded. For the identification of regulatory T cells, the $CD3^+/CD4^+$ population was selected and further gated for CD127 negativity and CD25 positivity. Naïve regulatory T cells were identified based on CD45RA positivity. Furthermore, total regulatory T cells were clustered according to their expression of PD-1 and KLRG1.

To distinguish different naïve and memory T cell populations, both $CD8^+$ and $CD4^+$ T cells populations were analyzed for CD45RA and CCR7 expression. Naïve T cells ($CCR7^+/CD45RA^+$), central memory ($CCR7^+/CD45RA^-$), effector memory ($CCR7^-/CD45RA^-$) and effector memory T cells re-expressing CD45RA ($CCR7^-/CD45RA^+$) were additionally examined for their expression of PD-1 and KLRG1.

For the identification of T helper cell subsets, the $CD3^+/CD4^+$ lymphocyte population was analysed for CCR6, CXCR3 and CCR4 (co-) expression (adapted from[57]). In doing so, the following subsets could be classified: Th0 cells ($CXCR3^-$, $CCR6^-$, $CCR4^+$), Th1 cells ($CXCR3^+$, $CCR6^-$), Th1/Th17 ($CXCR3^+$, $CCR6^+$), Th2 ($CXCR3^-$, $CCR6^-$, $CCR4^-$). All subsets were also analyzed for PD-1 and KLRG1 expression.

Gating strategy for identification of T helper cell subsets is depicted in Figure S3 on page 49.

Starting with live lymphocytes, B cells were identified via the expression of CD20 after exclusion of T cells ($CD3^+$). Natural killer cells were defined as $CD3^-/CD20^-/CD14^-/CD56^+$ and further differentiated according to their expression of CD16, with cytotoxic NK cells defined as $CD16^{++}/CD56^+$ and regulatory NK cells defined as $CD16^{-/+}/CD56^{++}$. Monocytes were first selected according to their FSC-A and SSC-A characteristics, and after exclusion of doublets, dead cells, $CD3^+$, $CD20^+$ and $CD56^+$ cells, examined for their (co-) expression of CD14 and HLA-DR. Monocyte subsets were further classified due to their differential expression of CD14 and CD16, with classical monocytes defined as $CD14^+/CD16^-$, intermediate monocytes as $CD14^+/CD16^+$ and non-classical monocytes as $CD14^+/CD16^{++}$. Dendritic cells were identified from an overall "cell" gate, including monocyte and lymphocyte gates, and defined as $CD3^-/CD20^-CD14^-/CD56^-/HLA\text{-}DR^+$.

Relative frequencies of cell populations of interest were exported for subsequent statistical analysis, which was carried out using GraphPad Prism Version 7 software (GraphPad Software Inc., USA). Due to varying sample quality, it was decided to only include cell population frequencies of samples with a minimum of 100 events present in the respective parent gate. Therefore, the numbers of samples analyzed vary depending on the cell type (eg. there were more samples of sufficient quality to analyze overall $CD4^+$ T cells compared to $CD4^+$ Central memory T cells). First, PBMC subpopulation frequencies were subjected to an omnibus Kruskal-Wallis test in order to determine whether there are statistically significant differences between the study groups. If a p-value < 0.05 was detected, adjusted pairwise post-hoc comparisons (Dunn's multiple comparison test) were performed. Spearman's correlation analysis served to investigate the association of immune population frequencies with clinical

variables, including PHQ-9 and GAD-7 scores, WHR, systolic and diastolic blood pressure measurements. In addition, subitem scores of the PHQ-9 were correlated with the relative frequencies of cytotoxic NK cells, CD4+ central memory T cells and dendritic cells (Spearman's rank correlation) and a Holms-correction was performed to control for multiple testing. Figures were created using GraphPad Prism Version 7 and Heatmapper.ca.

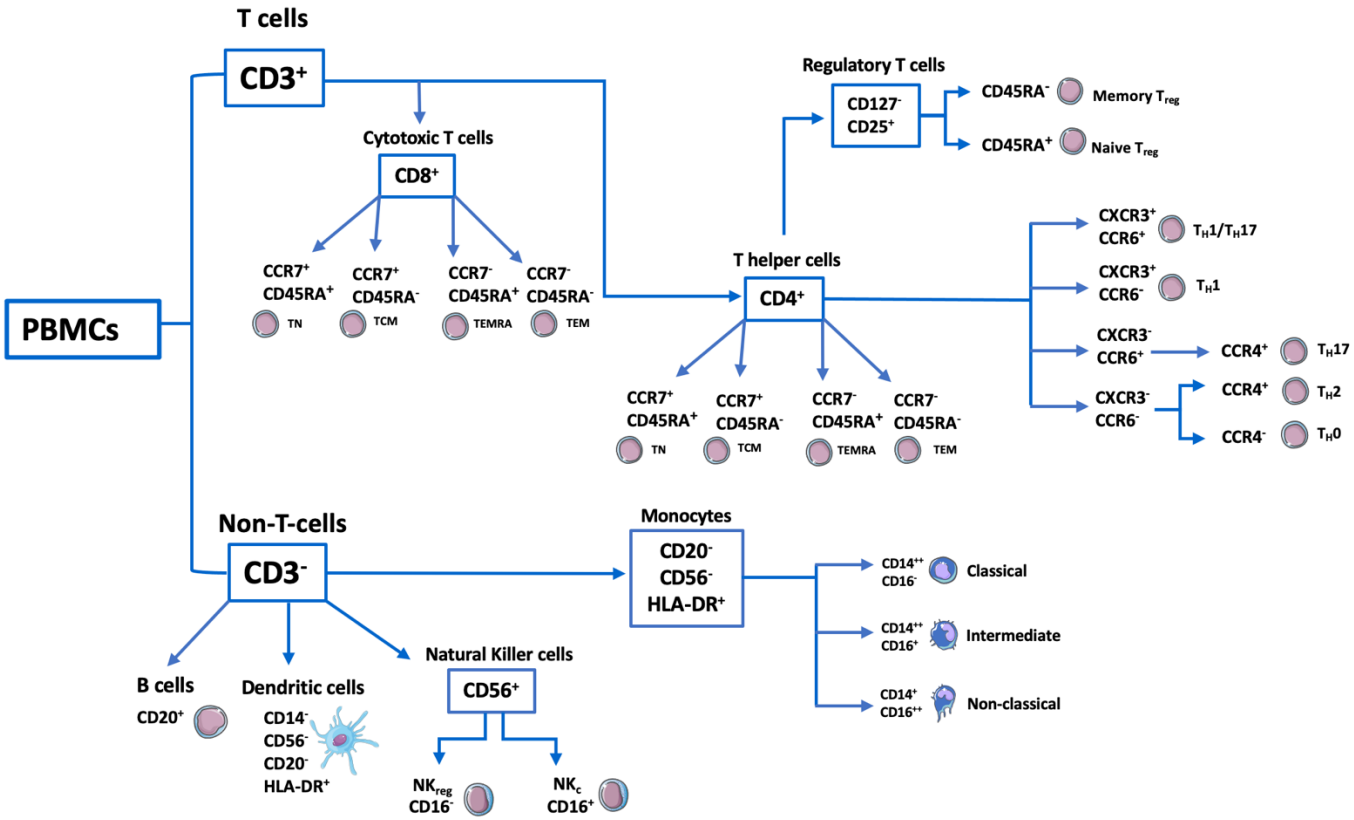

Figure 3 | Overview of leukocyte and lymphocyte populations identified and analyzed in this study. Graphic representation of cell populations courtesy of Servier SMART Medial Art, created using Powerpoint (own representation: Victoria Stiglbauer). TN = naïve T cells, TCM = central memory T cells, TEMRA = effector memory T cells re-expressing CD45RA, TEM = effector memory T cells, Treg = regulatory T cells, Th = T helper cell, NK = natural killer cells

1.5. Results

Sample characteristics

Lean healthy controls had a mean age of 38.8 ± 11.9 (SD), a BMI of 22.9 ± 1.6, WHR of 0.8 ± 0.1 and a PHQ-9 score of 3.1 ± 1.5.

Severely obese patients had a mean age of 47.7 ± 12.7, a BMI of 46.1 ± 5.3, a WHR of 0.9 ± 0.1 and a mean PHQ-9 score of 6.4 ± 2.5.

Patients with severe obesity and comorbid depressive symptoms had a mean age of 40.9 ± 15.2 (SD), a BMI of 44.6 ± 5.3, WHR of 0.9 ± 0.1 and a PHQ-9 score of 14.7 ± 3.3. They also scored significantly higher on the GAD-7 scale (8.4 ± 2.7) compared to obese patients without depressive symptoms (4.1 ± 3) and lean healthy controls (2.7 ± 1.9). The percentage of females was around 70 % in all three groups. The above information is summarized in Table 1 below. For a detailed summary of further demographic and clinical characteristics see Table 1 of the publication[55] on page 56.

Table 1: Demographic and clinical characteristics of study participants

	HC (n = 20)	***Ob (n = 20)***	***Ob + D (n = 15)***
Age [years]	36.6 ± 10.9	47.7 ± 11.5	40.9 ± 13.8
Height [cm]	171.8 ± 8.8	165.6 ± 13.2	169.8 ± 11.2
Weight [kg]	67.7 ± 8.3	128.3 ± 23	130.1 ± 26.1
BMI [kg/m^2]	22.9 ± 1.6	46.1 ± 5.3	44.6 ± 5.3
Waist circumference [cm]	76.9 ± 6	128.5 ± 13.6	126.6 ± 15.7
Waist to hip ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Systolic blood pressure [mmHg]	113.4 ± 12.7	130.8 ± 15.3	130.9 ± 8
Diastolic blood pressure [mmHg]	75.1 ± 10.6	83.7 ± 12	83.27 ± 10.6
% Females	75	70	73.3
PHQ-9	3.1 ± 1.5	6.4 ± 2.5	14.7 ± 3.3
GAD-7 sum	2.7 ± 1.9	4.1 ± 3.	8.4 ± 2.7

Table 1 | Demographic and clinical characteristics of healthy controls and patients included in this study, mean shown with SEM. BMI = Body Mass Index, PHQ-9 = Patient Health Questionnaire-9, GAD-7 = Generalized Anxiety Disorder-7 scale. (modified from *Stiglbauer et al., 2021)*[55]

Regulatory T cells

The relative amount of regulatory T cells (Treg) among $CD4^+$ T cells was significantly increased (KW $p = 0.023$) in patients with obesity and comorbid depression (Ob+D) when compared to obese controls (Ob). However, the frequency of Treg neither correlated with PHQ-9 scores or WHR measures. Relative frequencies of naive and memory Treg subpopulations remained unchanged across all groups. An increased expression of PD-1 (an inhibitory marker) on Tregs of Ob compared to healthy controls (HC) was observed (KW $p = 0.03$). See Fig. S3 on page 68.

Naïve and memory T cell subsets

In patients with Ob+D, the relative number of $CD4^+$ central memory T cells (TCM) among total $CD4^+$ T cells was significantly increased compared to HC (KW $p = 0.02$). Moreover, frequencies of $CD4^+$ TCM were significantly correlated to depression severity ($p = 0.0087$, r = 0.3711, Spearman's rank correlation), but not to WHR measurements.

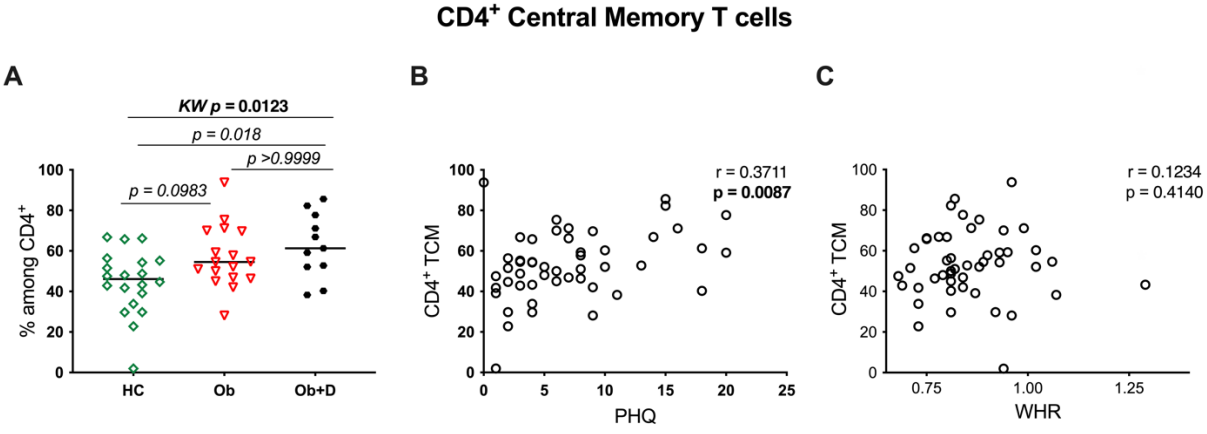

Figure 4 | $CD4^+$ Central memory T cells in patients with obesity and comorbid depression. Relative frequencies of $CD4^+$ TCM among total $CD4^+$ T cells, median shown **(A)**. Spearman's rank correlation of $CD4^+$ TCM relative frequencies PHQ-9 scores **(B)** and with WHR measures **(C)** in the

total sample. TCM = Central memory T cells; HC = Healthy controls; Ob = Patients with obesity; Ob+D = Patients with obesity and comorbid depression; PHQ-9 = Patient Health Questionnaire 9; WHR = waist to hip ratio. (modified from *Stiglbauer et al., 2021)*[55]

No relevant changes in population frequencies of $CD4^+$ naïve (TN), effector memory (TEM) or effector memory T cells re-expressing CD45RA (TEMRA) could be observed. No significant differences in the expression of KLRG1 or PD-1 were found, see Fig. S4 on page 69.

The frequency of $CD8^+$ TEM was significantly decreased in Ob compared to HC (KW $p = 0.03$). The amount of $CD8^+$ TEM in obese patients with comorbid depressive symptoms compared to HC was also reduced but not statistically significant. Relative frequencies of naïve $CD8^+$ TN, $CD8^+$ TCM or $CD8^+$ TEMRA remained unchanged among groups. Expression of PD-1 on the surface of $CD8^+$ TCM was significantly reduced in Ob+D compared to HC (KW $p = 0.006$). The amount of $CD8^+$ TCM double positive for PD-1 and KLRG1 expression was significantly lower in obese individuals compared to HC (KW $p = 0.03$). See Fig. S5 on page 69.

Helper T cells

No relevant alterations concerning the relative frequencies of any of the analyzed helper T cell subsets, including Th0, Th1, Th1/Th17, Th2 and Th17 cells, could be detected among the three groups. Since these results were not presented in the publication[55], see Figure 5 on page 26.

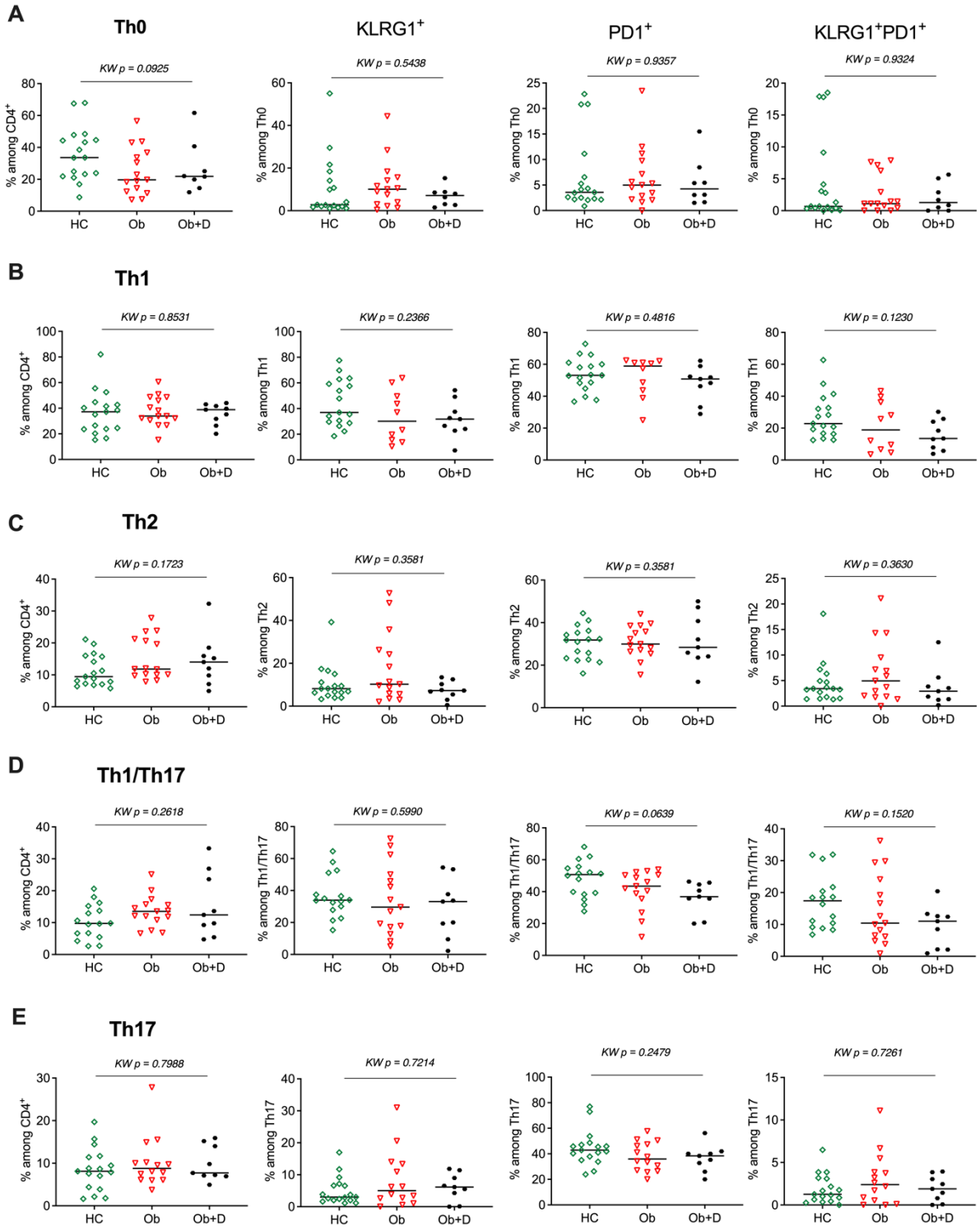

Figure 5 | Immune phenotype of helper T cell subsets in patients with obesity and comorbid depression. Relative frequencies of Th0 **(A)**, Th1 **(B)**, Th2 **(C)**, Th1/Th17 **(D)** and Th17 **(E)** cells including their respective expression of KLRG1 and PD-1, median shown. Gating strategy for the identification of T cell subsets is shown in **Figure S3** on page 49 of this manuscript. n (HC)= 17; n (Ob)= 15; n (Ob+D) = 9; HC = Healthy controls; Ob = Patients with obesity; Ob+D = Patients with

obesity and comorbid depression; KLRG1 = Killer cell lectin-like receptor subfamily G member 1; PD-1 = programmed cell death protein 1. (own representation: Victoria Stiglbauer)

Natural killer cells

The total amount of natural killer (NK) cells did not significantly differ among groups. However, cytotoxic natural killer cells (NKc) were significantly reduced in Ob+D compared to HC (KW *p = 0.006*), which was further supported by the post-hoc test. The frequency of regulatory NK cells (NKreg) showed no group differences, albeit a trend towards an increase of NKreg in patients with Ob+D could be observed. The frequency of NKc was significantly correlated with depression severity (r = -0.3862, *p = 0.01,* Spearman's rank correlation), but no association with WHR could be confirmed.

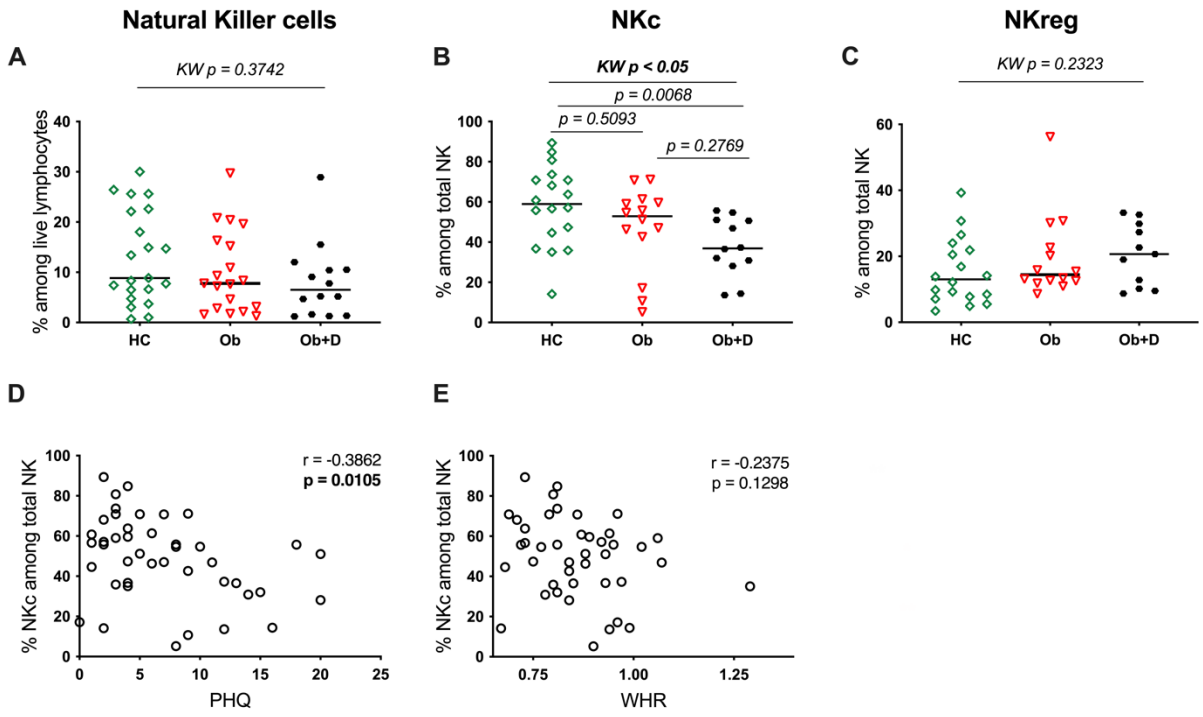

Figure 6 | NK cell subpopulations in in patients with obesity and comorbid depression. Relative frequencies of NK cells among live lymphocytes **(A)** and NKc **(B)** and NKreg **(C)** subsets among total NK cells, median shown. Spearman's rank correlation of NKc frequencies with PHQ-9 scores **(D)** and WHR measures **(E)**. Gating strategy for natural killer cells is depicted in **Figure S4B**. NK = natural killer cells; NKc = cytotoxic natural killer cells; NKreg = regulatory natural killer cells; HC

= Healthy controls; Ob = Patients with obesity; Ob+D = Patients with obesity and comorbid depression; PHQ-9 = Patient Health Questionnaire 9; WHR = waist to hip ratio. (modified from *Stiglbauer et al., 2021)*[55]

Dendritic cells

Dendritic cells showed a significant reduction in both Ob and Ob+D compared to HC (KW *p = 0.001*). Frequencies of dendritic cells significantly correlated with depression severity (r = -0.4167, *p = 0.0016*, Spearman's rank correlation) in the total cohort and WHR measurements (r = -0.4022, *p = 0.0026*, Spearman's rank correlation).

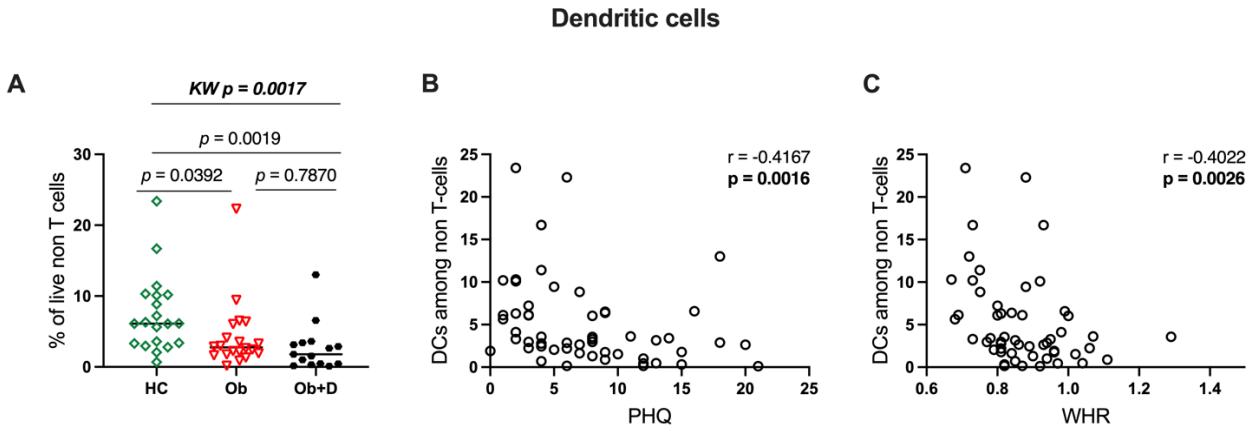

Figure 7 | Dendritic cells in in patients with obesity and comorbid depression. Relative frequencies of DCs (defined as HLA-DR$^+$ cells gated on live non-CD3/CD20/CD14/CD56 cells) among live non T cells, median shown **(A)**. Spearman's rank correlation of DC frequencies with PHQ-9 scores **(B)** and WHR measures **(C)**. Gating strategy for dendritic cells is displayed in **Figure S2** of the publication, see page 66. DCs = dendritic cells; HC = Healthy controls; Ob = Patients with obesity; Ob+D = Patients with obesity and comorbid depression; PHQ-9, Patient Health Questionnaire 9; WHR = waist to hip ratio. (modified from *Stiglbauer et al., 2021)*[55]

B cells and Monocytes

The relative frequencies of CD20$^+$ B cells and monocyte subsets (including classical CD14$^+$CD16$^-$, intermediate CD14$^+$CD16$^+$ and non-classical CD14$^+$CD16^{++} monocytes)

did not significantly differ among groups. See Fig. S6 on page 70 of the supplements of the publication[55].

Correlation analyses with PHQ subitems

During the revision process we further explored the correlation of our findings with depressive symptoms. Therefore, PHQ subitem scores were correlated with the relative abundances of $CD4^+$ TCM, NKc and DCs.

Correlation analyses of the relative frequencies of $CD4^+$ TCM (Spearman's rank correlation) revealed significant associations with the following PHQ item scores: PHQ item 1 (Loss of interest/pleasure, $p = 0.0415$, r = 0.2986), PHQ item 3 (difficulty sleeping, $p = 0.0473$, r = 0.2909), PHQ item 5 (changes in appetite, $p = 0.0168$, r = 0.3471) and PHQ item 7 (difficulty concentrating, $p = 0.0108$, r = 0.3687). After adjusting for multiple testing (Holm's correction), none of the p-values remained < 0.05.

Several PHQ subitems showed a significant correlation with NKc frequencies. These included PHQ item 2 (sadness/depression, $p = 0.0065$, r = -0.409), PHQ item 6 (negative self-perception, $p = 0.0164$, r = -0.3639) and PHQ item 8 (psychomotor symptoms, $p = 0.006$, r = -0.4126). After correcting for multiple testing (Holm's correction), none of these p-values reached the threshold for significance.

Regarding the frequency of DCs, a significant inverse correlation with both PHQ-9 score (r = -0.4167, $p = 0.0016$, Spearman's test) and WHR (r = -0.4022, $p = 0.0026$) was found. PHQ subitem scores were correlated with relative frequencies of DCs during explorative post hoc analyses. PHQ item 4 (low energy level, $p = 0.0066$, r = -0.3622), PHQ item 5 (changes in appetite $p = 0.0091$, r = -0.3485), PHQ item 6

(negative self-perception, *p = 0.019*, r = -0.3154), PHQ item 7 (difficulty concentrating, *p = 0.031*, r = -0.2912) and PHQ item 8 (psychomotor symptoms, *p = 0.0093*, r = -0.3478) were significantly correlated to the abundance of DCs, although no significant p-values were obtained following adjustment for multiple testing (Holm's correction).

A heatmap summarizing these exploratory correlation analyses is presented in Fig. 8.

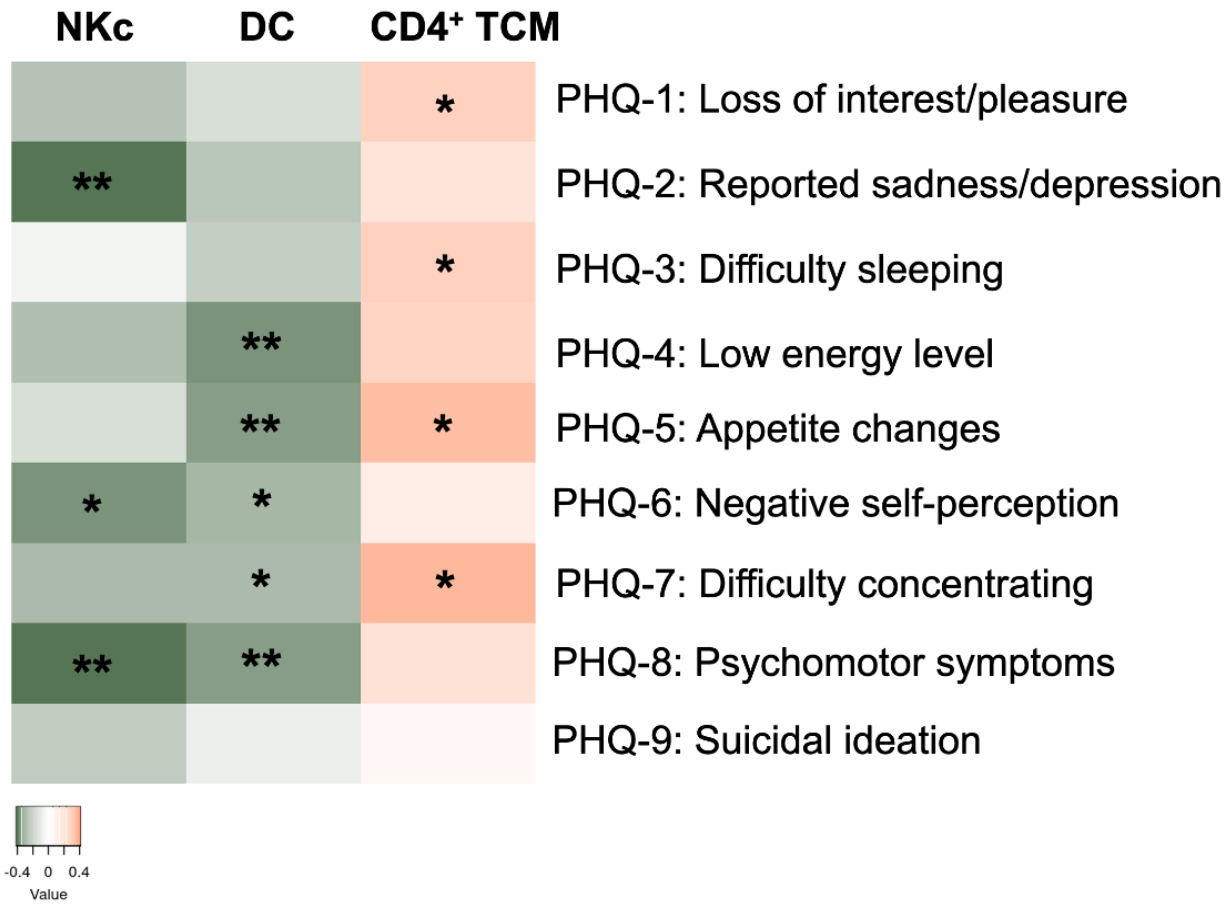

Figure 8 | Heatmap illustrating the correlation of relative abundances of cytotoxic natural killer cells, dendritic cells and CD4+ central memory T cells with PHQ subitem scores. * p-value < 0.05 (unadjusted), ** p-value < 0.001 (unadjusted). Created using Heatmapper.ca. (modified from *Stiglbauer et al., 2021)*[55]

1.6. Discussion and outlook

Using multicolour flow cytometry for detailed immunophenotyping this study identified changes in frequencies of several immune cell subsets in patients with obesity and comorbid depressive symptoms in comparison to non-depressed obese and lean controls. Concerning the innate immune system, frequencies of cytotoxic natural killer cells and dendritic cells were significantly decreased in patients with obesity and comorbid depression compared to obese and lean controls. In addition, higher frequencies of circulating $CD4^+$ central memory T cells were detected in patients with obesity and comorbid depression in comparison to lean healthy controls. Both changes in NKc and $CD4^+$ TCM frequencies were significantly correlated with depression severity as assessed by PHQ-9 scores, but not waist to hip ratio measures. These results therefore reveal possible depression-specific alterations in immune cell subsets in severely obese individuals. Reductions of dendritic cells were both significantly correlated with PHQ-9 scores and WHR, suggestive of additive effects of depression and obesity on this cell population.

Although correlation analyses of cell abundances with PHQ subitem scores were of a purely explorative nature, our results hint at symptom domain-specific changes in the abundances of $CD4^+$ TCM, NKc and DCs. In the case of NKc, their reduced frequencies were significantly correlated with sadness/depression, negative self-perception, and psychomotor symptoms. This combination of symptoms could be cautiously interpreted to resemble the melancholic subtype of MDD. Conversely, changes in the abundance of $CD4^+$ TCM were associated with changes in appetite/food intake and sleep (among other subitems). Alterations in appetite/food intake and sleeping patterns are considered key features of the atypical depression subtype, which is also associated with the concept of “immuno-metabolic depression”.

Similarly, DC frequencies were significantly correlated with low energy levels (which might be interpreted as fatigue) and alterations in appetite/food intake and thus similar to the results obtained for $CD4^+$ TCM. Therefore, it could be hypothesized that while changes in NKc might be primarily associated with melancholic depression, alterations in $CD4^+$ TCM and DC frequencies could be more closely linked to "immuno-metabolic depression", which tends to present itself with a symptom profile resembling the atypical subtype of depression[6]

MDD has been robustly associated with changes in the abundance[58] and impaired function of natural killer cells[58–60]. A recent immunophenotyping study of MDD patients reported that numbers of total NK cells were associated with depression severity in a subset of patients with inflamed depression, albeit with increased numbers of NK cells[46].

PBMC gene expression analysis of a large cohort including healthy controls, patients with current and remitted MDD from The Netherlands Study of Depression and Anxiety showed a downregulation of genes involved in natural killer cell mediated cytotoxicity (KEGG pathway). This association was stronger in patients currently experiencing a depressive episode compared to patients in remission[61]. Differential gene expression analysis of patients with MDD and hyperphagia revealed an even stronger association of enriched inflammatory pathways and MDD[30]. There is evidence that reduced NK cell cytotoxicity might be a consequence of depression and chronic stress[58,60]. Taken together, these findings are in accordance with the results of the experiments performed during this study, since reductions of cytotoxic NK cells are apparent in patients with comorbid depression and correlated with depression severity.

Reductions of NK cells have also been reported in severely obese patients compared to lean controls and are associated with insulin resistance in children with obesity[62]. In addition, changes in the expression of surface activation markers and decreased function of NK cells have been connected to obesity[62,63]. Interestingly, the detrimental effect of obesity on both number and function of NK cells can be attenuated by weight loss[62]. While there was a trend towards reduction of NKc apparent in the obese cohort of the current study, patients with obesity and comorbid depression showed significant reductions compared to healthy controls. Since these changes in abundance inversely correlated with PHQ-9 scores but not WHR measurements, our results are indicative of a depression-specific alterations in NKc abundance.

The contribution of dendritic cells to pathophysiological processes in psychiatric disorders has not been well studied so far. One reason for this might be the relatively low abundance of dendritic cells in the peripheral blood. One study reported a decrease of circulating dendritic cells in patients with Alzheimer's Disease which significantly correlated with depression severity[64]. Dendritic cells obtained from patients diagnosed with bipolar disorder exhibit functional impairments, which can be reversed by the effects of Lithium *in vitro*[65]. Obesity is associated with decreased numbers and impaired function of dendritic cells[66]. *In vitro* stimulation of DCs obtained from obese individuals resulted in significantly less upregulation of activation markers compared to lean controls[66]. Since both obesity and MDD are associated with greater susceptibility to (chronic) infections, impaired function of professional antigen presenting cells such as DCs might play a causal role[66]. In this study, dendritic cells were defined as $CD3^-/CD20^-CD14^-/CD56^-/HLA\text{-}DR^+$ according to recommendations by the Human Immunology Project[56] and found to be decreased in patients with obesity with and without comorbid depression. However, this

identification strategy lacks specific markers for dendritic cells, for example CD11c and CD123, which could additionally serve to further classify DCs into plasmacytoid and myeloid subsets[56,67]. Despite these obvious limitations, our preliminary findings might serve as a basis for future research on the role of dendritic cells in potentially shared pathobiological mechanisms of MDD and obesity.

Significantly increased frequencies of $CD4^+$ central memory T cells in obese patients with comorbid depressive symptoms compared to lean controls showed a significant correlation with depression severity but not waist to hip ratio. The underlying mechanisms and specific relevance of an altered abundance of memory T cells has not yet been extensively studied in MDD[48], but many studies suggest an involvement of the overall T cell compartment in the pathogenesis of MDD, since several phenotypic alterations of T cells have been reported in MDD patients[48,49,60,68–70]. Elevated amounts of circulating $CD4^+$ central memory T cells are usually associated with greater antigen exposure in the past and/or chronic infections[71]. Since patients with obesity and healthy controls showed no significant differences concerning their age, these changes could be partly driven by “immunological ageing”, meaning more frequent antigen exposure in depressed patients with obesity. A meta-analysis uncovered significant associations of infections with herpes simplex-1 virus, varicella zoster virus, Borna disease virus, Epstein-Bar virus and Chlamydophila trachomatis with MDD[72]. However, the studies included in this meta-analysis did not screen for antigen-specific T cells and relied on serological tests and the detection of viral particles, so the role of $CD4^+$ central memory T cells in MDD remains a matter of speculation.

$CD8^+$ effector memory T cells were significantly less abundant in obese patients compared to healthy controls. Studies on mouse models of obesity have found that

the infiltration of $CD8^+$ effector T cells contributes to adipose tissue inflammation[73]. One possible explanation for the reduction of circulating $CD8^+$ effector memory T cells observed in obese patients could be their recruitment to adipose tissue. However, since our results did not correlate to WHR, this finding needs to be interpreted with caution.

Frequencies of regulatory T cells were significantly higher in patients with obesity and comorbid depression when compared to obese controls, but not compared to healthy controls. To this day, the contribution of regulatory T cells to pathophysiological changes of the immune system in MDD remains unclear. While several studies have investigated the abundance of regulatory T cells, the results remain inconclusive. Two studies detected higher abundance of regulatory T cells by means of flow cytometry[48,49], while another study has reported decreased numbers of regulatory T cells, that showed an inverse correlation with the abundance of inflammatory monocytes[60].

Studies investigating the role of regulatory T cells in obese patients also yielded conflicting results. An immunophenotyping study including obese patients detected lower frequencies of activated regulatory T cells in both peripheral blood and increased presence of regulatory T cells in visceral adipose tissue samples[74]. Both observations significantly correlated with BMI. Thus, the decrease of circulating regulatory T cells might be explained by their migration to visceral adipose tissue in order to counteract the pro-inflammatory activity at this location[74].

Another study of the T cell composition in the peripheral blood of morbidly obese patients detected increased numbers of regulatory T cells[75]. These conflicting results concerning the abundance of regulatory T cells in both obesity and MDD could be, at least in part, attributed to alternative strategies employed for the identification of

these cells by flow cytometry (e.g. usage of FoxP3 antibody). The increased numbers of regulatory T cells in patients with obesity and comorbid depression compared to obese controls detected in this study neither correlated with PHQ-9 scores nor WHR, which renders our results difficult to interpret. In summary, the contribution of regulatory T cells to inflammatory processes observed in obesity and MDD warrants further investigation. In order to do this, the abundance of these cells both in the peripheral blood and visceral adipose tissue would need to be quantified, in addition to functional assays to detect potential dysfunction of these cell types.

Limitations of our study include small sample sizes per group and the purely descriptive approach of immune phenotyping, which precludes any information concerning the functional state of the cells in question. Moreover, no structured clinical interview was performed to diagnose depression, instead, participants were assigned to the group with comorbid depression if they scored ≥ 10 points on the PHQ-9. Waist to hip ratio measurements were used for correlation analyses and while they are considered useful for the risk assessment of conditions associated with obesity (since they assess the amount of visceral adipose tissue)[15], waist and hip circumference measurements are prone to errors in patients with morbid obesity and furthermore dependent on the expertise of examiner. Additionally, a control group of lean MDD patients is missing in this study, which complicates the identification of obesity-specific and MDD-specific effects on the immune phenotype.

Due to the cross-sectional design of the study, differentiation of cause and effect between immune phenotype and depressive symptoms is impossible. To deepen our understanding of the interplay between immunological markers and depression, interventional and/or longitudinal studies are necessary. Also, the effect of lifestyle behaviours - including smoking, exercise, and diet for example - should not be

underestimated. A direct association of poor lifestyle and depressive symptoms was found in patients with coronary heart disease[76]. The influence of personality traits and facets on the co-occurrence of mood disorders and obesity has been highlighted by a recent study, where personality traits were found to overlap between obesity and mood disorders and not, as previously assumed, between obesity and addiction[77].

Obesity negatively impacts the treatment response in MDD, since higher BMI is associated with reduced improvement of depressive symptoms during antidepressant treatment[78]. MDD patients with higher BMI also tend to exhibit a symptom profile resembling the atypical subtype, including less vegetative symptoms and HPA axis impairment[78]. A preliminary study on physically healthy MDD patients also reported poorer clinical response to treatment with selective serotonin reuptake inhibitors (SSRIs) in patients with metabolic dysregulation (as defined by the presence of risk factors for metabolic syndrome)[79]. The less favourable treatment response of overweight/obese MDD patients with an atypical symptom profile to standard antidepressant treatment might be in part explained by a distinct underlying pathobiology, including inflammatory processes. In accordance with that, a recent study found robust associations of poorer metabolic health and inflammation with atypical depression symptom profiles[80]. Together, these findings suggest that screening for patients with immuno-metabolic depression and subjecting them to alternative treatment strategies, potentially including anti-inflammatory agents, could lead to an improved outcome and probably even remission in this subset of patients.

Since this study was of an exploratory and cross-sectional nature and performed on a small sample size, the next step would be to perform similar analyses in a bigger clinical cohort of patients with MDD and comorbid obesity, including closely matched healthy controls and a control group of lean MDD patients. In addition to

immunophenotyping, functional assessments of natural killer cells, T cell subsets, dendritic cells and potentially other cell populations could shed light on their role in pathophysiological inflammatory processes described in both obesity and MDD. Methods to assess the functional state of these cells include metabolic (Seahorse) and proliferation assays, measurement of cytokine production (hight-sensitivity ELISA) as well as metabolomic profiling and RNA sequencing analyses. In addition, a flow cytometry-based method for a “metabolic screening” of key proteins and enzymes of several metabolic pathways (including glycolysis, citric acid cycle, mitochondrial respiration, fatty acid oxidation and synthesis), termed “Met-Flow” has recently been published[81]. This elegant method allows an estimation of metabolic activity by measuring mean fluorescence intensities of key metabolic enzymes and may be used as a starting point for further, more detailed analyses of the respective metabolic pathways. Since the topic of immunometabolism has gained increased research interest in recent times, additional metabolic analyses would optimally complement phenotypic ones. Moreover, within the scope of longitudinal studies, the application of anti-inflammatory treatments in MDD (for example statins or biologicals like IL-6 blocking agents) and their potential effects on observed altered immune phenotypes could be examined. Ultimately, further comprehensive measurements of immune cell phenotypes and function using sophisticated flow cytometry approaches might lead to the discovery of biomarkers to help characterize depression subtypes on a biological level, since the current subtype definitions based solely on symptom profiles and measuring CRP, IL-6 and TNF-α, is simply not enough. Identifying patients affected by immuno-metabolic depression could lead to the development of alternative and optimized treatment strategies in the future. In the context of translational research, the findings obtained in this study may provide a starting point towards biomarker discovery for MDD with comorbid obesity.

1.7. References

1. Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., Mohr, D. C. & Schatzberg, A. F. Major depressive disorder. *Nat. Rev. Dis. Prim.* **2**, 1–20 (2016).
2. Kennedy, S. H. Core symptoms of major depressive disorder: Relevance to diagnosis and treatment. *Dialogues Clin. Neurosci.* **10**, 271–277 (2008).
3. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. (2013).
4. APA. *American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.)*. *American Journal of Psychiatry* (2013).
5. Thase, M. E. Recognition and diagnosis of atypical depression. *J. Clin. Psychiatry* **68**, 11–16 (2007).
6. Penninx, B. W. J. H., Milaneschi, Y., Lamers, F. & Vogelzangs, N. Understanding the somatic consequences of depression: Biological mechanisms and the role of depression symptom profile. *BMC Med.* **11**, 1–14 (2013).
7. Gold, S. M., Moss-, R., Mehnert, A., Miranda, J., Bullinger, M., Steptoe, A., Whooley, M. A. & Otte, C. Comorbid depression in medical diseases. *Nat. Rev. Dis. Prim.* **6**, 1–22 (2020).
8. Pan, An; Keum, Nana; Okereke, Olivia I.; Sun, Qi; Kivimaki, Mika; Rubin, Richard R.; Hu, F. B. Bidirectional Association Between Depression and Metabolic Syndrome. *Diabetes Care* **35**, 1171–1180 (2012).
9. Gregory, J. M., Rosenblat, J. D. & McIntyre, R. S. Deconstructing Diabetes and Depression: Clinical Context, Treatment Strategies, and New Directions. *Focus (Madison).* **14**, 184–193 (2016).
10. González-Muniesa, P., Mártinez-González, M. A., Hu, F. B., Després, J. P., Matsuzawa, Y., Loos, R. J. F., Moreno, L. A., Bray, G. A. & Martinez, J. A. Obesity. *Nat. Rev. Dis. Prim.* **3**, (2017).
11. M Haftenberger, GBM Mensink, B Herzog, A Kluttig, KH Greiser, B Merz, U Nöthlings, S Schlesinger, S Vogt, B Thorand, A Peters, T Ittermann, H Völzke, S Schipf, J Neamat-Allah, T Kühn, R Kaaks, H Boeing, U Bachlechner, C Scheidt-Nave and A SchienkiewitzChanges in body weight and obesity status in German adults: Results of seven population-based prospective studies. *Eur. J. Clin. Nutr.* **70**, 300–305 (2016).

12. Bhupathiraju, S. N. & Hu, F. B. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. *Circ. Res.* **118**, 1723–35 (2016).
13. Calle, E. E. & Kaaks, R. Overweight, obesity and cancer: Epidemiological evidence and proposed mechanisms. *Nat. Rev. Cancer* **4**, 579–591 (2004).
14. Mansur, R. B., Brietzke, E. & McIntyre, R. S. Is there a 'metabolic-mood syndrome'? A review of the relationship between obesity and mood disorders. *Neurosci. Biobehav. Rev.* **52**, 89–104 (2015).
15. Akpinar, E., Bashan, I., Bozdemir, N. & Saatci, E. Which is the best anthropometric technique to identify obesity: Body mass index, waist circumference or waist-hip ratio? *Coll. Antropol.* **31**, 387–393 (2007).
16. Lamas, O., Marti, A. & Martínez, J. A. Obesity and immunocompetence. *Eur. J. Clin. Nutr.* **56**, S42–S45 (2002).
17. Rodríguez, C. P., González, M. C., Aguilar-Salinas, C. A. & Nájera-Medina, O. Peripheral lymphocytes, obesity, and metabolic syndrome in young adults: An immunometabolism study. *Metab. Syndr. Relat. Disord.* **16**, 342–349 (2018).
18. Floriana S. Luppino, Leonore M. de Wit, Paul F. Bouvy, T. S. & Pim Cuijpers, Brenda W. J. H. Penninx, F. G. Z. Overweight, Obesity, and Depression. *Arch. Gen. Psychiatry* **67**, 220–229 (2010).
19. Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., Charlson, F. J., Norman, R. E., Flaxman, A. D., Johns, N., Burstein, R., Murray, C. J. L. & Vos, T. Global burden of disease attributable to mental and substance use disorders: Findings from the Global Burden of Disease Study 2010. *Lancet* **382**, 575–86 (2013).
20. Peeters, A., Bonneux, L., Nusselder, W. J., De Laet, C. & Barendregt, J. J. Adult obesity and the burden of disability throughout life. *Obes. Res.* **12**, 1145–1151 (2004).
21. Kelly, T., Yang, W., Chen, C. S., Reynolds, K. & He, J. Global burden of obesity in 2005 and projections to 2030. *Int. J. Obes.* **32**, 1431–1437 (2008).
22. Huet, L., Delgado, I., Dexpert, S., Sauvant, J., Aouizerate, B., Beau, C., Forestier, D., Ledaguenel, P., Magne, E. & Capuron, L. Relationship between body mass index and neuropsychiatric symptoms: Evidence and inflammatory correlates. *Brain. Behav. Immun.* **94**, 104–110 (2021).
23. Hruby, A., Manson, J. A. E., Qi, L., Malik, V. S., Rimm, E. B., Sun, Q., Willett, W. C. & Hu, F. B. Determinants and consequences of obesity. *Am. J. Public*

Health **106**, 1656–1662 (2016).

24. Mariska Bot, Yuri Milaneschi, Tahani Al-Shehri, Najaf Amin, Sanzhima Garmaeva, Gerrit L.J. Onderwater, Rene Pool, Carisha S. Thesing, Lisanne S. Vijfhuizen, Nicole Vogelzangs, Ilja C.W. Arts, Ayse Demirkan, Cornelia van Duijn, Marleen van Greevenbroek, Carla J.H. van der Kallen, Sebastian Köhler, Lannie Ligthart, Arn M.J.M. van den Maagdenberg, Dennis O. Mook-Kanamori, Renée de Mutsert, Henning Tiemeier, Miranda T. Schram, Coen D.A. Stehouwer, Gisela M. Terwindt, Ko Willems van Dijk, Jingyuan Fu, Alexandra Zhernakova, Marian Beekman, P. Eline Slagboom, Dorret I. Boomsma, and Brenda W.J.H. Penninx, for the BBMRI-NL Metabolomics Consortium Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biol. Psychiatry* **87**, 409–418 (2020).
25. Soczynska, J. K., Kennedy, S. H., Woldeyohannes, H. O., Yim, C. Y. & Mcintyre, R. S. Mood Disorders and Obesity : Understanding Inflammation as a Pathophysiological Nexus. **1960**, 93–116 (2011).
26. Kenny L. Chan, Flurin Cathomas, S. J. R. Central and peripheral inflammation link metabolic syndrome and major depressive disorder. *Am. Physiol. Soc.* **34**, 123–133 (2018).
27. Milaneschi, Y., Lamers, F., Berk, M. & Penninx, B. W. Depression heterogeneity and its biological underpinnings: towards immuno-metabolic depression. *Biol. Psychiatry* **88**, 369–380 (2020).
28. Mikolaj Ogrodnik, Yi Zhu, Larissa G.P. Langhi, Tamar Tchkonia, Patrick Krüger, Edward Fielder, Stella Victorelli, Rifqha A. Ruswhandi, Nino Giorgadze, Tamar Pirtskhalava, Oleg Podgorni, Grigori Enikolopov, Kurt O. Johnson, Ming Xu, Christine Inman, Allyson K. Palmer, Marissa Schafer, Moritz Weigl, Yuji Ikeno,Terry C. Burns, João F. Passos, Thomas von Zglinicki, James L. Kirkland, and Diana Jurk. Obesity-Induced Cellular Senescence Drives Anxiety and Impairs Neurogenesis. *Cell Metab.* **29**, 1061–1077 (2019).
29. Yuri Milaneschi, PhD; Femke Lamers, PhD;Wouter J. Peyrot, MD, PhD; Bernhard T. Baune, MD, PhD, MPH, FRANZCP; Gerome Breen, PhD; Abbas Dehghan, MD, PhD; Andreas J. Forstner, MD; Hans J. Grabe, MD; Georg Homuth, PhD; Carol Kan, MA, MBBS, MRCPsych; Cathryn Lewis, PhD; Niamh Mullins, PhD; Matthias Nauck, MD; Giorgio Pistis, PhD; Martin Preisig, MD,

MPH; Margarita Rivera, PhD; Marcella Rietschel, MD; Fabian Streit, MD; Jana Strohmaier, PhD; Alexander Teumer, PhD; Sandra Van der Auwera, PhD; Naomi R. Wray, PhD; Dorret I. Boomsma, PhD; BrendaW. J. H. Penninx, PhD; for the CHARGE InflammationWorking Group and the Major Depressive DisorderWorking Group of the Psychiatric Genomics Consortium. Genetic association of major depression with a typical features and obesity-related immunometabolic dysregulations. *JAMA Psychiatry* **74**, 1214–1225 (2017).

30. de Kluiver, H., Jansen, R., Milaneschi, Y. & Penninx, B. W. J. H. Involvement of inflammatory gene expression pathways in depressed patients with hyperphagia. *Transl. Psychiatry* **9**, (2019).
31. Guo, H., Callaway, J. B. & Ting, J. P. Y. Inflammasomes: Mechanism of action, role in disease, and therapeutics. *Nat. Med.* **21**, 677–687 (2015).
32. Howren, M. B., Lamkin, D. M. & Suls, J. Associations of depression with c-reactive protein, IL-1, and IL-6: A meta-analysis. *Psychosom. Med.* **71**, 171–186 (2009).
33. Park, H. S., Park, J. Y. & Yu, R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. *Diabetes Res. Clin. Pract.* **69**, 29–35 (2005).
34. McLaughlin, A. P., Nikkheslat, N., Hastings, C., Nettis, M. A., Kose, M., Worrell, C., Zajkowska, Z., Mariani, N., Enache, D., Lombardo, G., Pointon, L., Cowen, P., Cavanagh, J., Harrison, N., Bullmore, E., Pariante, C. M. & Mondelli, V. The influence of comorbid depression and overweight status on peripheral inflammation and cortisol levels. *Psychol. Med.* (2021) doi:10.1017/S0033291721000088.
35. Reichenberg, A., Gorman, J. M. & Dieterich, D. T. Interferon-induced depression and cognitive impairment in hepatitis C virus patients: A 72 week prospective study. *Aids* **19**, 174–178 (2005).
36. Charles L. Raison, Marina Demetrashvili, Lucile Capuron, and A. H. M. Neuropsychiatric Adverse Effects of Interferon-α: **8**, 1385–1395 (2014).
37. Capuron, L. & Miller, A. H. Immune system to brain signaling: Neuropsychopharmacological implications. *Pharmacol. Ther.* **130**, 226–238 (2011).
38. Lee, A. H. & Dixit, V. D. Dietary Regulation of Immunity. *Immunity* **53**, 510–523 (2020).

39. Lacourt, T. E., Vichaya, E. G., Chiu, G. S., Dantzer, R. & Heijnen, C. J. The high costs of low-grade inflammation: Persistent fatigue as a consequence of reduced cellular-energy availability and non-adaptive energy expenditure. *Front. Behav. Neurosci.* **12**, 1–20 (2018).
40. Tilg, H. & Moschen, A. R. Adipocytokines: Mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.* **6**, 772–783 (2006).
41. Matarese, G., Procaccini, C. & De Rosa, V. At the crossroad of T cells, adipose tissue, and diabetes. *Immunol. Rev.* **249**, 116–134 (2012).
42. Naylor, C. & Petri, W. A. Leptin regulation of immune responses. *Trends Mol. Med.* **22**, 88–98 (2016).
43. Milaneschi, Y., Lamers, F., Bot, M., Drent, M. L. & Penninx, B. W. J. H. Leptin Dysregulation Is Specifically Associated With Major Depression With Atypical Features: Evidence for a Mechanism Connecting Obesity and Depression. *Biol. Psychiatry* **81**, 807–814 (2017).
44. Murakami, M. & Hirano, T. The molecular mechanisms of chronic inflammation development. *Front. Immunol.* **3**, 1–2 (2012).
45. Maecker, H. T., Mccoy, J. P. & Nussenblatt, R. Standardizing immunophenotyping for the Human Immunology Project. **12**, (2012).
46. Lynall, M. E., Turner, L., Bhatti, J., Cavanagh, J., de Boer, P., Mondelli, V., Jones, D., Drevets, W. C., Cowen, P., Harrison, N. A., Pariante, C. M., Pointon, L., Clatworthy, M. R. & Bullmore, E. Peripheral Blood Cell–Stratified Subgroups of Inflamed Depression. *Biol. Psychiatry* **88**, 185–196 (2019).
47. Hasselmann, H., Gamradt, S., Taenzer, A., Nowacki, J., Zain, R., Patas, K., Ramien, C., Paul, F., Wingenfeld, K. & Sada, I. Pro-inflammatory Monocyte Phenotype and Cell-Specific Steroid Signaling Alterations in Unmedicated Patients With Major Depressive Disorder. *Front. Immunol.* **9**, 1–9 (2018).
48. Suzuki, H., Savitz, J., Kent Teague, T., Gandhapudi, S. K., Tan, C., Misaki, M., McKinney, B. A., Irwin, M. R., Drevets, W. C. & Bodurka, J. Altered populations of natural killer cells, cytotoxic T lymphocytes, and regulatory T cells in major depressive disorder: Association with sleep disturbance. *Brain. Behav. Immun.* **66**, 193–200 (2017).
49. Patas, K., Willing, A., Demiralay, C., Engler, J. B., Lupu, A., Ramien, C., Schäfer, T., Gach, C., Stumm, L., Chan, K., Vignali, M., Arck, P. C., Friese, M. A., Pless, O., Wiedemann, K., Agorastos, A. & Gold, S. M. T cell phenotype

and T cell receptor repertoire in patients with major depressive disorder. *Front. Immunol.* **9**, 1–12 (2018).

50. Kroenke, K., Spitzer, R. L. & Williams, J. B. W. The PHQ-9: Validity of a brief depression severity measure. *J. Gen. Intern. Med.* **16**, 606–613 (2001).
51. Manea, L., Gilbody, S., Hewitt, C., North, A., Plummer, F., Richardson, R., Thombs, B. D., Williams, B. & McMillan, D. Identifying depression with the PHQ-2: A diagnostic meta-analysis. *J. Affect. Disord.* **203**, 382–395 (2016).
52. Gräfe, K., Zipfel, S., Herzog, W. & Löwe, B. Screening psychischer Störungen mit dem "Gesundheitsfragebogen für Patienten (PHQ-D)". *Diagnostica* **50**, 171–181 (2004).
53. Spitzer, R. L., Kroenke, K., Williams, J. B. W. & Löwe, B. A brief measure for assessing generalized anxiety disorder: The GAD-7. *Arch. Intern. Med.* **166**, 1092–1097 (2006).
54. Löwe, B., Decker, O., Müller, S., Brähler, E., Schellberg, D., Herzog, W., Herzberg, P. Y., Care, S. M., Mar, N., Lowe, B., Decker, O., Miiller, S. & Brdhler, E. Validation and Standardization of the Generalized Anxiety Disorder Screener (GAD-7) in the General Population. *Med. Care* **46**, 266–274 (2017).
55. Stiglbauer, V., Gamradt, S., Scherzer, M., Brasanac, J., Otte, C., Rose, M., Hofmann, T., Hinkelmann, K. & Gold, S. M. Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study. *Cell Biochem. Funct.* 1–9 (2021) doi:10.1002/cbf.3608.
56. Maecker, H. T., McCoy, J. P. & Nussenblatt, R. Standardizing immunophenotyping for the Human Immunology Project. *Nat. Rev. Immunol.* **12**, 191–200 (2012).
57. Duhen, T., Duhen, R., Lanzavecchia, A., Sallusto, F. & Campbell, D. J. Functionally distinct subsets of human FOXP3+ treg cells that phenotypically mirror effector Th cells (Blood (2012) 119, 19 (4430-4440)). *Blood* **120**, 4447 (2012).
58. Zorrilla, E. P., Luborsky, L., McKay, J. R., Rosenthal, R., Houldin, A., Tax, A., McCorkle, R., Seligman, D. A. & Schmidt, K. The relationship of depression and stressors to immunological assays: A meta-analytic review. *Brain. Behav. Immun.* **15**, 199–226 (2001).
59. Evans, D. L., Folds, J. D., Petitto, J. M., Golden, R. N., Pedersen, C. A., Corrigan, M., Gilmore, J. H., Silva, S. G., Quade, D. & Ozer, H. Circulating

Natural Killer Cell Phenotypes in Men and Women with Major Depression: Relation to Cytotoxic Activity and Severity of Depression. *Arch. Gen. Psychiatry* **49**, 388–395 (1992).

60. Grosse, L., Hoogenboezem, T., Ambrée, O., Bellingrath, S., Jörgens, S., de Wit, H. J., Wijkhuijs, A. M., Arolt, V. & Drexhage, H. A. Deficiencies of the T and natural killer cell system in major depressive disorder. T regulatory cell defects are associated with inflammatory monocyte activation. *Brain. Behav. Immun.* **54**, 38–44 (2016).
61. R Jansen, BWJH Penninx, V Madar, K Xia, Y Milaneschi, JJ Hottenga, AR Hammerschlag, A Beekman, N van der Wee, JH Smit, AI Brooks, J Tischfield, D Posthuma, R Schoevers, G van Grootheest, G Willemsen, EJ de Geus, DI Boomsma, FA Wright, F Zou, W Sun and PF Sullivan. Gene expression in major depressive disorder. *Mol. Psychiatry* **21**, 339–347 (2016).
62. O'Shea, D. & Hogan, A. E. Dysregulation of natural killer cells in obesity. *Cancers (Basel).* **11**, 1–12 (2019).
63. Lynch, L. A., O'Connell, J. M., Kwasnik, A. K., Cawood, T. J., O'Farrelly, C. & O'Shea, D. B. Are natural killer cells protecting the metabolically healthy obese patient? *Obesity* **17**, 601–605 (2009).
64. Ciaramella, A., Salani, F., Bizzoni, F., Orfei, M. D., Caltagirone, C., Spalletta, G. & Bossù, P. Myeloid dendritic cells are decreased in peripheral blood of Alzheimer's disease patients in association with disease progression and severity of depressive symptoms. *J. Neuroinflammation* **13**, 1–11 (2016).
65. Knijff, E. M., Ruwhof, C., De Wit, H. J., Kupka, R. W., Vonk, R., Akkerhuis, G. W., Nolen, W. A. & Drexhage, H. A. Monocyte-derived dendritic cells in bipolar disorder. *Biol. Psychiatry* **59**, 317–326 (2006).
66. O'Shea, D., Corrigan, M., Dunne, M. R., Jackson, R., Woods, C., Gaoatswe, G., Moynagh, P. N., O'Connell, J. & Hogan, A. E. Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. *Int. J. Obes.* **37**, 1510–1513 (2013).
67. Robinson, S. P., Patterson, S., English, N., Davies, D., Knight, S. C. & Reid, C. D. L. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur. J. Immunol.* **29**, 2769–2778 (1999).
68. Zhong, G. S., Yang, R., Cai, R. Y., Xu, Z. S., Li, J. Y., Li, T. & Wu, M. N. Flow cytometric analysis of peripheral blood related immune cells and cytokines in

patients with depression. *Int. J. Clin. Exp. Med.* **11**, 2812–2820 (2018).

69. Toben, C. & Baune, B. T. An Act of Balance Between Adaptive and Maladaptive Immunity in Depression: a Role for T Lymphocytes. *J. Neuroimmune Pharmacol.* **10**, 595–609 (2015).
70. Miller, A. H. Depression and immunity: A role for T cells? *Brain. Behav. Immun.* **24**, 1–8 (2010).
71. Farber, D. L., Yudanin, N. A. & Restifo, N. P. Human memory T cells: Generation, compartmentalization and homeostasis. *Nat. Rev. Immunol.* **14**, 24–35 (2014).
72. Wang, X., Zhang, L., Lei, Y., Liu, X., Zhou, X., Liu, Y., Wang, M., Yang, L., Zhang, L., Fan, S. & Xie, P. Meta-analysis of infectious agents and depression. *Sci. Rep.* **4**, 1–10 (2014).
73. Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T. & Nagai, R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* **15**, 914–920 (2009).
74. Donninelli, G., Cornò, M. Del, Pierdominici, M., Scazzocchio, B., Varì, R., Varano, B., Pacella, I., Piconese, S., Barnaba, V., D'Archivio, M., Masella, R., Conti, L. & Gessani, S. Distinct blood and visceral adipose tissue regulatory T cell and innate lymphocyte profiles characterize obesity and colorectal cancer. *Front. Immunol.* **8**, 1–11 (2017).
75. Van Der Weerd, K., Dik, W. A., Schrijver, B., Schweitzer, D. H., Langerak, A. W., Drexhage, H. A., Kiewiet, R. M., Van Aken, M. O., Van Huisstede, A., Van Dongen, J. J. M., Van Der Lelij, A. J., Staal, F. J. T. & Van Hagen, P. M. Morbidly obese human subjects have increased peripheral blood CD4 + T cells with skewing toward a Treg- and Th2-dominated phenotype. *Diabetes* **61**, 401–408 (2012).
76. Sin, N. L., Kumar, A. D., Gehi, A. K. & Whooley, M. A. Direction of Association Between Depressive Symptoms and Lifestyle Behaviors in Patients with Coronary Heart Disease: the Heart and Soul Study. *Ann. Behav. Med.* **50**, 523–532 (2016).
77. Vainik, U., Misic, B., Zeighami, Y., Michaud, A., Mõttus, R. & Dagher, A. Obesity has limited behavioural overlap with addiction and psychiatric phenotypes. *Nat. Hum. Behav.* **4**, 27–35 (2020).

78. Kloiber, S., Ising, M., Reppermund, S., Horstmann, S., Dose, T., Majer, M., Zihl, J., Pfister, H., Unschuld, P. G., Holsboer, F. & Lucae, S. Overweight and Obesity Affect Treatment Response in Major Depression. *Biol. Psychiatry* **62**, 321–326 (2007).
79. Hough, C. M., Saverio Bersan, F., Mellon, S. H., Morford, A. E., Lindqvist, D., Reus, V. I., Epel, E. S., Wolkowitz, O. M., L., M., R., R., H., B., S., M., O., W. & V., R. Pre-treatment allostatic load and metabolic dysregulation predict antidepressant response in major depressive disorder. *Biol. Psychiatry* **81**, S406–S407 (2017).
80. Lamers, F., Milaneschi, Y., Vinkers, C. H., Schoevers, R. A., Giltay, E. J. & Penninx, B. W. J. H. Depression profilers and immuno-metabolic dysregulation: Longitudinal results from the NESDA study. *Brain. Behav. Immun.* **88**, 174–183 (2020).
81. Ahl, P. J., Hopkins, R. A., Xiang, W. W., Au, B., Kaliaperumal, N., Fairhurst, A.-M. & Connolly, J. E. A novel strategy for single-cell metabolic analysis highlights dynamic changes in immune subpopulations. *Commnications Biol.* **3**, 1–15 (2020).

1.8. Supplementary Figures

Nine-symptom Checklist

Name _____ Date _____

Over the *last 2 weeks*, how often have you been bothered by any of the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

(For office coding: Total Score _____ = _____ + _____ + _____)

If you checked off *any* problems, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

From the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PRIME-MD PHQ). The PHQ was developed by Drs. Robert L. Spitzer, Janet BW Williams, Kurt Kroenke, and colleagues. For research information, contact Dr. Spitzer at *rls8@columbia.edu*. PRIME-MD is a trademark of Pfizer Inc. Copyright 1999 Pfizer Inc. All rights reserved. Reproduced with permission

Figure S1 | Structure of the PHQ-9 questionnaire

by Kroenke K, Spitzer RL,Williams JBW. The PHQ-9: Validity of a brief depression severity measure.

J Gen Intern Med. 2001;16(9):606-613.

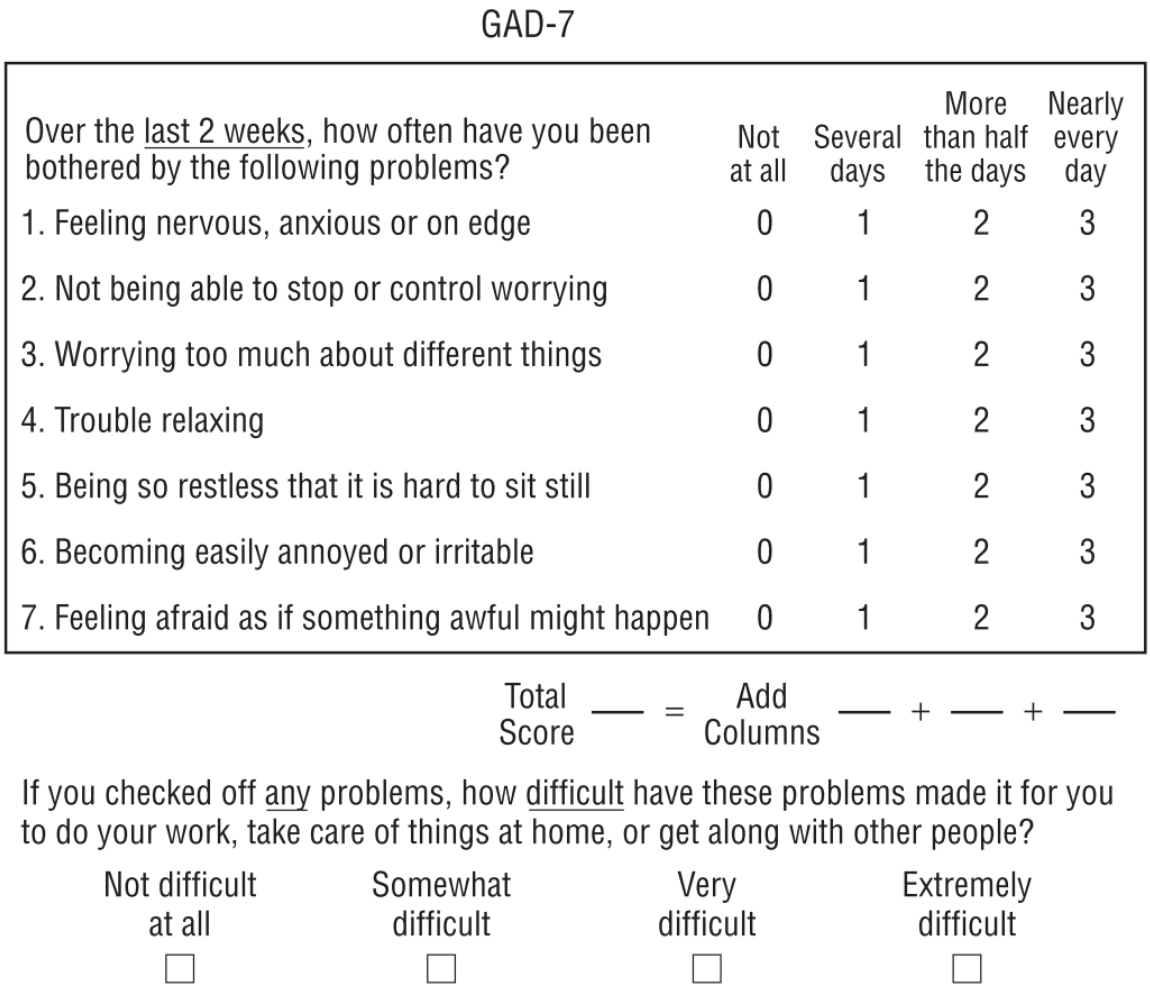

Figure S2 | Structure of the GAD-7 scale

by Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: The GAD-7. *Arch Intern Med*. 2006;166(10):1092-1097.

S3

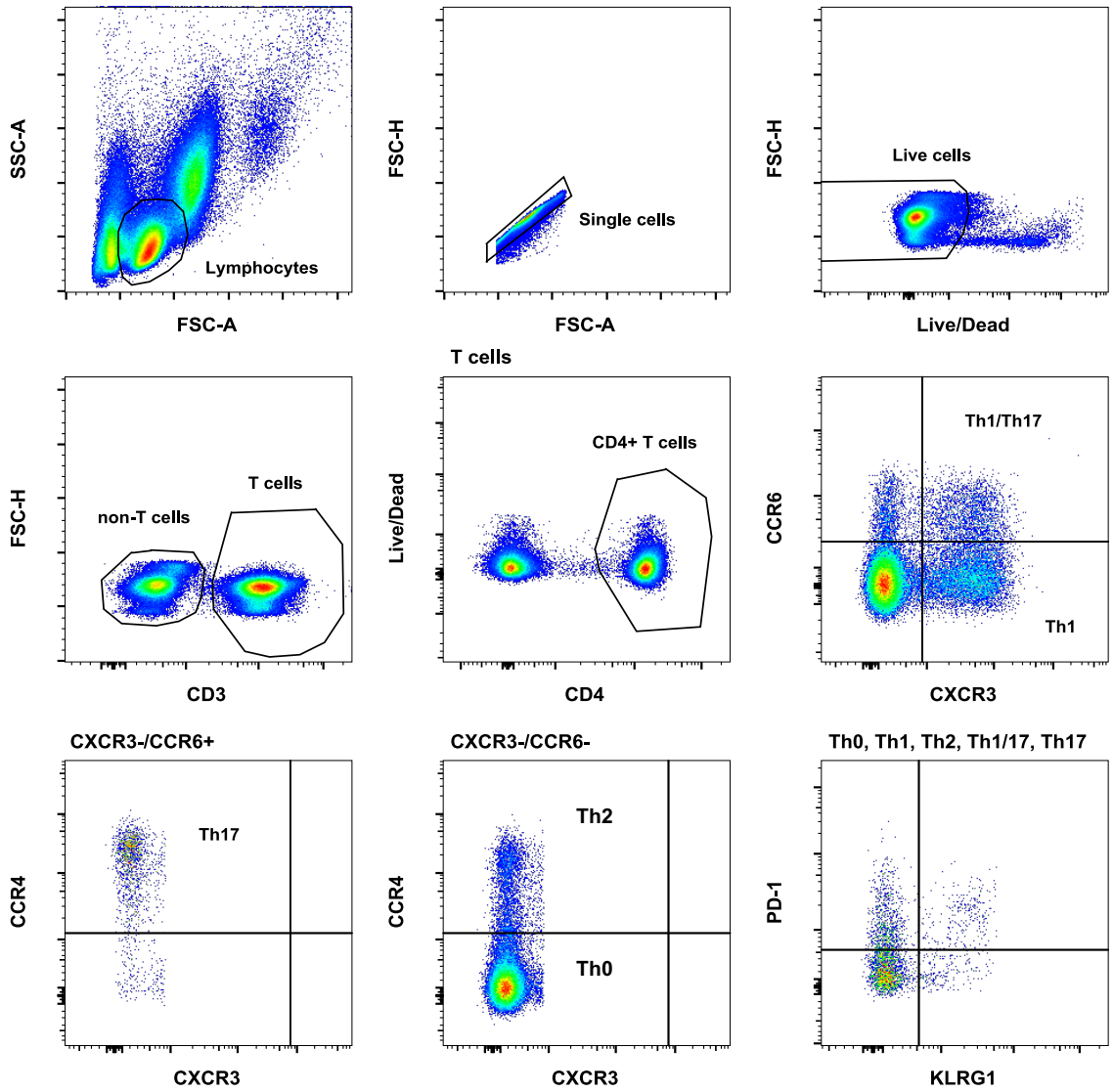

Figure S3 | Gating strategy for identification of Helper T cell subsets
Lymphocytes were identified according to FSC-A and SSC-A properties. Doublets and dead cells were excluded, and T cells separated from non-T cells by gating for CD3 positivity. After selection for CD4, T helper subsets were identified by gating for CCR6, CXCR3 and CCR4 expression. Th1 cells were identified as $CXCR3^{+}/CCR6^{-}$, Th17 cells as $CXCR3^{-}/CCR6^{+}/CCR4^{+}$, Th2 cells as $CXCR3^{-}/CCR6^{-}/CCR4^{+}$ and Th0 cells as $CXCR3^{-}/CCR6^{-}/CCR4^{-}$. All 4 populations were analyzed for their expression of PD-1 and KLRG1.

2. Eidesstattliche Versicherung

„Ich, Victoria Stiglbauer, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Immunological alterations in severely obese patients with comorbid depression“ / „Immunologische Veränderungen in einer Kohorte hochgradig adipöser Patienten mit komorbider Depression“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

3. Anteilserklärung an den erfolgten Publikationen

Victoria Stiglbauer hatte folgenden Anteil an den folgenden Publikationen:
Stiglbauer V, Gamradt S, Scherzer M, Brasanac J, Otte C, Rose M, Hofmann T, Hinkelmann K, Gold SM. Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study. Cell Biochem Funct. 2021 Apr;39(3):423-431. doi: 10.1002/cbf.3608. Epub 2021 Jan 5. PMID: 33401342.

Beitrag im Einzelnen:
Auswahl der in die Studie inkludierten Teilnehmer aus einer größeren Studienkohorte, Organisation des Probentransportes, Etablierung der Immunphänotypisierungspanels als Assistenz von Frau Dr. rer. nat. Stefanie Gamradt, praktische Durchführung aller Durchflusszytometrie-Experimente, sowie deren manuelle Auswertung mit FlowJo Software und anschließende statistische Analysen. Durchführung von Korrelationsanalysen der Ergebnisse mit klinischen Daten, u.a. WHR, PHQ, GAD, RR. Erstellung aller Grafiken und Tabellen für die Publikation (Ausnahme: Heatmaps Fig. 1C, Fig. 2C, Fig. 3C) und Verfassen des Manuskripts, sowie Bearbeitung der Revision für die Publikation.

_____
Unterschrift, Datum und Stempel des erstbetreuenden Hochschullehrers

_____
Unterschrift des Doktoranden/der Doktorandin

4. Auszug aus der Journal Summary List 2019

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI Selected Categories: **"BIOCHEMISTRY and MOLECULAR BIOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 297 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	CELL	258,178	38.637	0.564970
2	NATURE MEDICINE	85,220	36.130	0.168730
3	Annual Review of Biochemistry	20,499	25.787	0.024820
4	MOLECULAR CELL	69,148	15.584	0.166260
5	Molecular Cancer	15,448	15.302	0.023990
6	PROGRESS IN LIPID RESEARCH	6,139	15.083	0.005730
7	TRENDS IN BIOCHEMICAL SCIENCES	18,416	14.732	0.032060
8	TRENDS IN MICROBIOLOGY	13,604	13.546	0.022780
9	Signal Transduction and Targeted Therapy	1,182	13.493	0.003380
10	Nature Chemical Biology	22,084	12.587	0.060130
11	MOLECULAR PSYCHIATRY	22,227	12.384	0.054730
12	Molecular Plant	11,432	12.084	0.028530
13	NATURAL PRODUCT REPORTS	11,239	12.000	0.013610
14	NATURE STRUCTURAL & MOLECULAR BIOLOGY	27,178	11.980	0.056800
15	NUCLEIC ACIDS RESEARCH	201,649	11.501	0.403470
16	TRENDS IN MOLECULAR MEDICINE	10,618	11.099	0.018720
17	GENOME RESEARCH	41,755	11.093	0.076940
18	MOLECULAR BIOLOGY AND EVOLUTION	50,486	11.062	0.084810
19	CELL DEATH AND DIFFERENTIATION	21,095	10.717	0.029600
20	Redox Biology	10,157	9.986	0.023810

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
21	EMBO JOURNAL	64,724	9.889	0.059690
22	CURRENT OPINION IN CHEMICAL BIOLOGY	10,968	9.689	0.017770
23	PLANT CELL	54,927	9.618	0.048640
24	CURRENT BIOLOGY	63,256	9.601	0.133170
25	MOLECULAR ASPECTS OF MEDICINE	6,207	9.577	0.005750
26	Molecular Systems Biology	8,914	8.991	0.017390
27	Cell Systems	3,822	8.673	0.029290
28	MATRIX BIOLOGY	6,878	8.572	0.011920
29	ONCOGENE	66,303	7.971	0.068320
30	Cell Chemical Biology	3,326	7.739	0.015770
31	CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY	3,675	7.634	0.006380
32	EMBO REPORTS	14,976	7.497	0.030290
33	BIOCHIMICA ET BIOPHYSICA ACTA-REVIEWS ON CANCER	5,650	7.365	0.007800
34	PLOS BIOLOGY	31,650	7.076	0.060300
35	Essays in Biochemistry	2,383	6.966	0.005060
36	CURRENT OPINION IN STRUCTURAL BIOLOGY	11,035	6.908	0.021890
37	CELLULAR AND MOLECULAR LIFE SCIENCES	26,128	6.496	0.037010
38	Science Signaling	12,736	6.467	0.026590
39	ANTIOXIDANTS & REDOX SIGNALING	21,119	6.323	0.024660
40	Molecular Ecology Resources	10,868	6.286	0.019630
41	FREE RADICAL BIOLOGY AND MEDICINE	42,665	6.170	0.036960
42	BIOMACROMOLECULES	38,863	6.092	0.031320

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
43	Computational and Structural Biotechnology Journal	1,954	6.018	0.004980
44	CYTOKINE & GROWTH FACTOR REVIEWS	5,935	5.982	0.007380
45	Advances in Carbohydrate Chemistry and Biochemistry	634	5.800	0.000340
46	EXPERIMENTAL AND MOLECULAR MEDICINE	5,536	5.418	0.010300
47	AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY	12,243	5.373	0.016040
48	RNA Biology	6,589	5.350	0.015820
49	Acta Crystallographica Section D-Structural Biology	21,750	5.266	0.018220
50	MOLECULAR ECOLOGY	38,951	5.163	0.050800
51	INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES	47,121	5.162	0.057240
52	BIOCHEMICAL SOCIETY TRANSACTIONS	12,651	5.160	0.016140
53	HUMAN MOLECULAR GENETICS	39,652	5.100	0.064170
54	Journal of Genetics and Genomics	2,271	5.065	0.004310
55	Cell and Bioscience	1,898	5.026	0.004210
56	Antioxidants	2,568	5.014	0.004170
57	FASEB JOURNAL	43,126	4.966	0.043730
58	International Review of Cell and Molecular Biology	2,167	4.934	0.004350
59	Open Biology	2,886	4.931	0.009590
60	Journal of Integrative Plant Biology	5,005	4.885	0.006830
61	Advances in Microbial Physiology	1,227	4.875	0.000960
61	Nucleic Acid Therapeutics	1,030	4.875	0.003610

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
63	JOURNAL OF NUTRITIONAL BIOCHEMISTRY	11,460	4.873	0.011150
64	STRUCTURE	15,145	4.862	0.026940
65	International Journal of Biological Sciences	6,262	4.858	0.009710
66	BIOORGANIC CHEMISTRY	5,712	4.831	0.006730
67	Genes & Diseases	1,081	4.803	0.003310
68	JOURNAL OF MOLECULAR BIOLOGY	56,952	4.760	0.040330
69	BIOFACTORS	3,769	4.734	0.002930
70	BIOELECTROCHEMISTRY	4,944	4.722	0.004950
71	Reviews of Physiology Biochemistry and Pharmacology	805	4.700	0.000670
72	JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY	5,415	4.673	0.005420
73	BIOESSAYS	10,189	4.627	0.016560
74	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	77,286	4.556	0.143760
75	APOPTOSIS	6,539	4.543	0.005880
76	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR AND CELL BIOLOGY OF LIPIDS	10,266	4.519	0.016350
77	ACS Chemical Neuroscience	6,881	4.486	0.015300
78	JOURNAL OF LIPID RESEARCH	24,223	4.483	0.022420
79	ACS Chemical Biology	12,884	4.434	0.035490
80	FEBS Journal	18,845	4.392	0.025250
81	JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B-BIOLOGY	12,794	4.383	0.013640
82	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE	15,965	4.352	0.024200

Selected JCR Year: 2019; Selected Categories: “BIOCHEMISTRY and MOLECULAR BIOLOGY”

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
83	AMYLOID-JOURNAL OF PROTEIN FOLDING DISORDERS	1,486	4.323	0.002920
84	RNA	13,160	4.320	0.022400
85	CURRENT OPINION IN LIPIDOLOGY	4,151	4.254	0.005450
86	Epigenetics	5,512	4.251	0.009770
87	JOURNAL OF BIOLOGICAL CHEMISTRY	349,091	4.238	0.200770
88	JOURNAL OF CELLULAR BIOCHEMISTRY	22,080	4.237	0.019780
89	Frontiers in Molecular Biosciences	1,590	4.188	0.006180
90	CURRENT MEDICINAL CHEMISTRY	17,243	4.184	0.012960
91	Food & Function	12,239	4.171	0.020970
92	GENE THERAPY	6,795	4.128	0.005520
93	ADDICTION BIOLOGY	4,329	4.121	0.008280
94	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR CELL RESEARCH	17,307	4.105	0.023080
95	BIOCHEMICAL JOURNAL	45,579	4.097	0.025700
95	Metabolites	1,886	4.097	0.003710
97	MOLECULAR MEDICINE	4,955	4.096	0.004600
98	Biomolecules	3,498	4.082	0.009390
99	MOLECULES AND CELLS	5,064	4.081	0.007340
100	Progress in Molecular Biology and Translational Science	2,888	4.074	0.005900
101	JOURNAL OF NEUROCHEMISTRY	34,378	4.066	0.021840
102	GLYCOBIOLOGY	7,465	4.060	0.008010
103	BIOCONJUGATE CHEMISTRY	15,877	4.031	0.020310

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
104	NEUROCHEMISTRY INTERNATIONAL	8,928	3.881	0.008010
105	PROTEIN SCIENCE	13,592	3.876	0.017610
106	EXPERT REVIEWS IN MOLECULAR MEDICINE	1,827	3.875	0.001180
107	INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY	8,162	3.827	0.007670
108	MOLECULAR CARCINOGENESIS	5,947	3.825	0.008130
109	JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY	10,957	3.813	0.012900
110	METHODS	23,124	3.812	0.025210
111	Metallomics	5,237	3.796	0.009120
112	Current Topics in Membranes	768	3.744	0.002170
113	CHEMICO-BIOLOGICAL INTERACTIONS	12,153	3.723	0.011710
114	Biomedical Journal	991	3.697	0.002410
115	MOLECULAR PLANT-MICROBE INTERACTIONS	11,599	3.696	0.008300
116	INTERNATIONAL JOURNAL OF BIOCHEMISTRY & CELL BIOLOGY	16,037	3.673	0.013460
117	EUROPEAN JOURNAL OF HUMAN GENETICS	10,250	3.657	0.020500
118	MOLECULAR IMMUNOLOGY	11,488	3.641	0.013840
119	MOLECULAR AND CELLULAR BIOLOGY	52,658	3.611	0.023360
120	JOURNAL OF BIOCHEMICAL AND MOLECULAR TOXICOLOGY	2,430	3.606	0.002460
121	PLANT SCIENCE	16,280	3.591	0.014260
122	Biochimica et Biophysica Acta-Gene Regulatory Mechanisms	7,544	3.510	0.012190
123	MOLECULAR PHYLOGENETICS AND EVOLUTION	19,367	3.496	0.024610

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
124	Expert Opinion on Drug Metabolism & Toxicology	3,991	3.470	0.005900
125	BIOCHIMICA ET BIOPHYSICA ACTA-BIOENERGETICS	13,089	3.465	0.014710
126	CELLULAR & MOLECULAR BIOLOGY LETTERS	1,510	3.451	0.001590
127	CHROMOSOMA	3,259	3.442	0.004220
128	BIOCHIMICA ET BIOPHYSICA ACTA-GENERAL SUBJECTS	15,011	3.422	0.019730
129	MOLECULAR MICROBIOLOGY	34,588	3.418	0.024150
130	MACROMOLECULAR BIOSCIENCE	7,056	3.416	0.006020
131	BIOCHIMIE	11,006	3.413	0.012410
131	CHROMOSOME RESEARCH	2,237	3.413	0.002160
133	BIOCHIMICA ET BIOPHYSICA ACTA-BIOMEMBRANES	18,219	3.411	0.020320
134	ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS	21,796	3.391	0.013440
135	Molecular BioSystems	7,412	3.336	0.011880
136	NITRIC OXIDE-BIOLOGY AND CHEMISTRY	4,226	3.311	0.004760
137	PLANT MOLECULAR BIOLOGY	15,159	3.302	0.008710
138	Current Molecular Pharmacology	767	3.283	0.000980
139	BIOINORGANIC CHEMISTRY AND APPLICATIONS	947	3.273	0.000840
140	BIOLOGICAL CHEMISTRY	6,185	3.270	0.006890
141	MOLECULES	53,982	3.267	0.075300
142	JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY	9,581	3.255	0.011520
143	PROTEOMICS	14,205	3.254	0.017630
144	JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY	4,151	3.246	0.004820

Selected JCR Year: 2019; Selected Categories: “BIOCHEMISTRY and MOLECULAR BIOLOGY”

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
145	JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY	4,182	3.245	0.005770
146	IUBMB LIFE	5,876	3.244	0.005200
147	JOURNAL OF INORGANIC BIOCHEMISTRY	11,612	3.212	0.008050
148	DNA AND CELL BIOLOGY	3,602	3.191	0.004100
149	BMB Reports	2,771	3.167	0.004800
150	YEAST	4,533	3.143	0.002720
151	BIOORGANIC & MEDICINAL CHEMISTRY	30,067	3.073	0.024500
152	JOURNAL OF STRUCTURAL BIOLOGY	10,073	3.071	0.025320
153	AMINO ACIDS	9,918	3.063	0.008900
154	FEBS LETTERS	48,353	3.057	0.030350
155	PHYTOCHEMISTRY	31,929	3.044	0.010380
156	NEUROCHEMICAL RESEARCH	9,819	3.038	0.011300
157	Advances in Protein Chemistry and Structural Biology	733	3.014	0.001290
158	Comparative Biochemistry and Physiology D-Genomics & Proteomics	1,181	3.011	0.001600
159	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS	85,844	2.985	0.075090
160	CURRENT DRUG METABOLISM	3,749	2.960	0.003080
161	CYTOKINE	10,665	2.952	0.014160
161	JOURNAL OF PHYSIOLOGY AND BIOCHEMISTRY	1,854	2.952	0.002340
161	PROCESS BIOCHEMISTRY	17,861	2.952	0.010340
164	BIOSCIENCE REPORTS	5,711	2.942	0.009590
165	PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS	3,909	2.932	0.003540

Selected JCR Year: 2019; Selected Categories: “BIOCHEMISTRY and MOLECULAR BIOLOGY”

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
166	Lipids in Health and Disease	5,918	2.906	0.008830
167	COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY C-TOXICOLOGY & PHARMACOLOGY	6,711	2.892	0.003190
168	ANALYTICAL BIOCHEMISTRY	37,978	2.877	0.012300
169	BIOCHEMISTRY	70,613	2.865	0.046220
170	PEPTIDES	10,405	2.843	0.007000
171	FREE RADICAL RESEARCH	7,281	2.839	0.004740
172	ACTA BIOCHIMICA ET BIOPHYSICA SINICA	3,743	2.836	0.004840
173	PHOTOCHEMICAL & PHOTOBIOLOGICAL SCIENCES	6,997	2.831	0.005900
174	PROTEINS-STRUCTURE FUNCTION AND BIOINFORMATICS	15,177	2.828	0.011090
175	MOLECULAR REPRODUCTION AND DEVELOPMENT	5,496	2.823	0.004810
176	MedChemComm	4,365	2.807	0.007660
177	MOLECULAR GENETICS AND GENOMICS	4,740	2.797	0.006230
178	REDOX REPORT	1,666	2.753	0.001280
179	PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY	5,930	2.751	0.005660
180	Frontiers in Bioscience-Landmark	8,356	2.747	0.004350
181	PHOTOCHEMISTRY AND PHOTOBIOLOGY	10,220	2.721	0.004880
182	CURRENT ISSUES IN MOLECULAR BIOLOGY	735	2.695	0.000860
183	JOURNAL OF MOLECULAR NEUROSCIENCE	5,770	2.678	0.007150
184	BIOLOGICAL TRACE ELEMENT RESEARCH	9,470	2.639	0.007660
185	JOURNAL OF BIOMOLECULAR NMR	4,793	2.634	0.004360

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
186	CELL BIOCHEMISTRY AND FUNCTION	2,430	2.632	0.002110
187	CURRENT GENOMICS	2,190	2.630	0.002070
188	CHEMBIOCHEM	11,492	2.576	0.016820
189	Vitamins and Hormones	1,853	2.557	0.001810
190	Chemical Biology & Drug Design	4,549	2.548	0.005240
191	JOURNAL OF COMPUTER-AIDED MOLECULAR DESIGN	4,602	2.546	0.004100
192	INSECT MOLECULAR BIOLOGY	3,392	2.533	0.002930
193	CURRENT PROTEIN & PEPTIDE SCIENCE	2,795	2.520	0.003030
194	MOLECULAR MEMBRANE BIOLOGY	993	2.500	0.000490
195	BIOMETALS	4,360	2.479	0.003020
196	JOURNAL OF BIOCHEMISTRY	7,477	2.476	0.004430
197	EXTREMOPHILES	3,164	2.462	0.002890
198	Biochemistry and Cell Biology	2,773	2.460	0.002630
199	JOURNAL OF LIPOSOME RESEARCH	1,183	2.455	0.000790
200	International Journal of Genomics	947	2.414	0.002470
201	BIOCHIMICA ET BIOPHYSICA ACTA-PROTEINS AND PROTEOMICS	7,900	2.371	0.008600
202	JOURNAL OF CHEMICAL NEUROANATOMY	2,375	2.353	0.002250
203	Organogenesis	1,045	2.321	0.001160
204	Channels	1,246	2.311	0.002290
205	Innate Immunity	1,438	2.298	0.002520
206	MAMMALIAN GENOME	2,693	2.287	0.003490

Selected JCR Year: 2019; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Received: 3 October 2020 | Revised: 20 November 2020 | Accepted: 6 December 2020

DOI: 10.1002/cbf.3608

RESEARCH ARTICLE

CELL BIOCHEMISTRY & FUNCTION WILEY

Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study

Victoria Stiglbauer[1] | Stefanie Gamradt[1] | Marie Scherzer[2] | Jelena Brasanac[1,3] | Christian Otte[1] | Matthias Rose[2] | Tobias Hofmann[2] | Kim Hinkelmann[2] | Stefan M. Gold[1,2,4]

[1]Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt Universität zu Berlin, Berlin Institute of Health (BIH), Klinik für Psychiatrie und Psychotherapie, Campus Benjamin Franklin, Berlin, Germany

[2]Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt Universität zu Berlin, Berlin Institute of Health (BIH), Med. Klinik m.S. Psychosomatik, Campus Benjamin Franklin, Berlin, Germany

[3]Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt Universität zu Berlin, Berlin Institute of Health (BIH), NeuroCure Clinical Research Center (NCRC), Berlin, Germany

[4]Institut für Neuroimmunologie und MS (INIMS), Zentrum für Molekulare Neurobiologie, Universitätklinikum Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Stefan M. Gold, Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt Universität zu Berlin, Berlin Institute of Health (BIH), Klinik für Psychiatrie und Psychotherapie, Campus Benjamin Franklin, Berlin, Germany.
Email: stefan.gold@charite.de

Funding information
National MS Society, Grant/Award Number: MB-1707-28359

In this pilot study, we explored the immune phenotype of patients with severe obesity and comorbid depressive symptoms compared to non-depressed patients with obesity and normal-weight controls. Immune cell subsets were analysed by flow cytometry and depressive symptoms assessed using the Patient Health Questionnaire (PHQ-9). Cell frequencies were correlated with depressive symptom scores and waist-to-hip ratio (WHR). Patients with obesity and comorbid depression showed significantly lower numbers of circulating cytotoxic natural killer cells, dendritic cells and $CD8^+$ effector memory T cells, compared to normal-weight controls. Regulatory T cells and $CD4^+$ central memory T cells were increased compared to non-depressed patients with obesity and compared to normal-weight controls, respectively. Frequencies of cytotoxic natural killer cells and $CD4^+$ central memory T cells significantly correlated with PHQ-9 scores, but not with WHR. Reduced numbers of dendritic cells were observed in both patient groups with obesity and correlated with PHQ-9 scores and WHR. These findings provide evidence for an altered immune composition in comorbid obesity and depression, supporting a pathobiological overlap between the two disorders.

KEYWORDS
depression, immunophenotyping, inflammation, obesity

1 | INTRODUCTION

Major depressive disorder (MDD) is a common psychiatric disorder with a life-time prevalence of approximately 20%.[1] MDD is associated with increased mortality,[2,3] and causes substantial burden of disease.[4,5] Importantly, MDD is frequently associated with medical comorbidities,[3,6] including metabolic disorders such as type 2 diabetes and metabolic syndrome.[7,8] In particular, a strong bidirectional epidemiological link has been established between obesity and MDD, with both conditions increasing the risk of developing the other by approximately 50%.[9] Intriguingly, a recent analysis revealed that the link between depression and obesity may drive the association with numerous other medical comorbidities of

Kim Hinkelmann and Stefan M. Gold contributed equally to this study.

MDD.[10] Furthermore, this may extend to sub-clinical levels as a metabolomics study in MDD patients detected significant dyslipidaemia, characterized by increased very low-density lipoprotein (VLDL) and triglycerides as well as reduced high-density lipoprotein (HDL).[11] These findings were independent of sex, age or body mass index (BMI), suggesting that they may be early manifestations of metabolic dysfunction in MDD.[11]

Emerging evidence supports the notion that comorbid obesity and depression may—at least in part—be due to converging biological pathways.[12] For example, work in animal models has shown that obesity induces accumulation of senescent glial cells,[13] which, in turn, directly drive the occurrence of anxiety/depression-like behaviour.[13] Clearance of these pro-inflammatory cells rescued the behavioural phenotype. Similarly, inflammatory mechanisms have been implicated in comorbid depression and obesity in humans, as genetic risk factors overlap between these disorders.[14] This association was particularly obvious in patients with "atypical depression"—characterized by hyperphagia/weight gain, hypersomnia and fatigue—who were also more likely to carry genetic variants implicated in higher BMI and increased levels of C-reactive protein (CRP) and leptin.[14]

Indeed, chronic, low-grade inflammation has been described in both obesity and MDD with increased levels of peripheral markers such as CRP and cytokines, including Interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α).[15,16] The "immuno-metabolic" model of depression thus describes how a chronic pro-inflammatory state might induce symptoms like fatigue, changes in appetite/weight, hypersomnia/hyposomnia and depressed mood.[12,17] Adipose tissue also directly influences the function of the immune system through production of pro-inflammatory cytokines.[18,19] Of note, changes in frequencies of circulating lymphocytes, monocytes and natural killer cells have been described in both obesity[20-22] and MDD.[23-26]

Taken together, these findings support the idea that obesity-induced immune dysfunction might be one pathophysiological mechanism that is shared by obesity and MDD. Studying immune alterations in patients with severe obesity and comorbid depression could provide further insights into the potential role of the immune system in linking these two disorders.

2 | MATERIAL AND METHODS

2.1 | Participants

Three groups of participants were included in this study: lean healthy controls, participants with severe obesity and patients with severe obesity and comorbid depressive symptoms. The study was approved by the local ethics committee (Charité Universitätsmedizin Berlin, Campus Mitte, EA1/063/16). The study was conducted in accordance with the ethical standards concerning clinical studies involving human participants as stated in the Declaration of Helsinki. Written consent was provided by all participants prior to enrolment.

Healthy controls were recruited via online advertisements and bulletins and were free of relevant medical disorders (hypertension, insulin resistance or diabetes, hypothyroidism or coronary heart disease), or psychiatric illnesses, as assessed by taking their detailed medical history, weight and blood pressure measurements, and standard laboratory diagnostics (including count of leukocytes and quantification of haemoglobin, cholesterol, triglycerides, HDL, LDL and HbA1c).

Participants with obesity were recruited from the Department of Psychosomatic Medicine (Charité), at the beginning of a 6-month multimodal (psychosocial, nutritional and exercise) outpatient treatment programme specifically designed for patients with severe obesity.

2.2 | Clinical examination and questionnaires

Depressive symptoms were assessed using the Patient Health Questionnaire (PHQ-9)[27-30] and caseness defined by a score of ≥10. The Generalized Anxiety Disorder 7-item (GAD-7)[31,32] scale was used to assess the level of anxiety. Clinical parameters (blood pressure, height, weight, waist and hip circumference) were measured by trained medical staff.

2.3 | Blood collection and isolation of peripheral mononuclear blood cells

Whole blood was collected in heparinized tubes (BD, Germany), diluted in phosphate-buffered saline (PBS, 1:1; Gibco, ThermoFisher Scientific, Germany) and layered on top of density medium (Biocoll, Merck, Germany) for gradient centrifugation. PBMCs were collected from the interphase and after two washing steps with PBS, cells were aliquoted in RPMI 1640 + GlutaMax medium (Gibco, Thermofisher Scientific, USA) containing 25% foetal calf serum (FCS; Merck, Germany) and 10% dimethyl sulfoxide (DMSO; AppliChem, USA) at 1×10^7 cells/mL. Aliquots were stored in a freezing container filled with isopropyl alcohol (Mr. Frosty, Merck, Germany) at −80°C for 24 hours and then transferred to liquid nitrogen for long-term storage.

2.4 | Flow cytometry

Immunophenotyping of cryopreserved PBMC samples was performed by flow cytometry. Four antibody panels were established and applied for identification of several T cell subsets (including regulatory T cells, $CD4^+$ and $CD8^+$ naive/memory T cells), B cells, natural killer cells (NK), monocyte subsets (classical, non-classical and intermediate) and dendritic cells (DCs). A detailed description of all antibody panels is presented in Table S1. Experiments were carried out on a FACSCanto II flow cytometer (BD, Germany). Instrument set-up and compensation were performed prior to the experiments using the same protocol. Antibody staining mixes were freshly prepared in staining buffer (PBS (Sigma Aldrich, USA), +0.5% bovine serum albumin (Miltenyi Biotec, Germany) + 0.5 M EDTA (Promega, Germany)).

Two vials of cryopreserved PBMCs were thawed per donor. After removal from liquid nitrogen, PBMC vials were transferred to a 37°C water bath for 1 to 2 minutes. Cells were then slowly transferred

into a 12 mL centrifuge tube containing 10 mL pre-warmed RPMI 1640 + Glutamax medium (Gibco, Thermofisher Scientific, USA) + 10% FCS. Cells were centrifuged at 250×*g* for 6 minutes at room temperature (RT) and re-suspended in media. The samples were filtered using cell strainer 70 μm filters (Sarstedt, Germany) to obtain single-cell suspensions, washed three times with PBS (4°C) and then evenly distributed to four 5 mL flow cytometry tubes. Following a centrifugation step, PBMCs were incubated with 50 μL of a live/dead (L/D) marker in PBS. For the naive/memory T cell panel, CCR7 APC antibody was also added to the cell suspension. After a 15 minutes incubation time, antibody premixes were added to the respective tubes and incubated for an additional 15 minutes. Following incubation, 1 mL of staining buffer was added to each tube and samples were centrifuged at 350×*g* for 5 minutes. PBMCs were re-suspended in FACS buffer and tubes were kept on ice until measurement.

The gating strategies for the identification of PBMC sub-populations are depicted in Figures S1 and S2.

2.5 | Analysis and statistics

Analysis of flow cytometry data was carried out using FlowJo software version 10.1 (Trestar Inc., USA). PBMC sub-population frequencies were expressed as percentages among a suitable reference population. Due to varying sample quality, we only included cell populations of samples with at least 100 events in the respective parent gate. This explains diverging sample sizes for analyses of different cell populations. Sample sizes for each analysis are listed in the figure legends.

Statistical analyses were computed in GraphPad Prism Version 7 software (GraphPad Software Inc., USA). For this exploratory study, we employed a stepwise statistical approach: First, for each immune cell subset analysed, we performed an omnibus Kruskal-Wallis test, with adjusted pairwise post hoc comparisons (Dunn's multiple comparison test) in case of an overall effect in the omnibus test. Finally, for those markers that showed robust group differences in the post hoc test, we also ran correlation analyses (Spearman's correlation) to explore the strength of association with psychiatric (GAD-7 scores, PHQ-9 total scores and individual item scores, the latter are reported both without and with adjustments for multiple testing using Holm's correction) and metabolic (WHR, systolic and diastolic blood pressure) variables of interest. For display purposes, we show the Spearman rank correlation data as scatter plots of raw data.

3 | RESULTS

3.1 | Patient characteristics

Clinical and demographic characteristics of healthy controls (HCs), patients with obesity (Ob) and patients with obesity and comorbid depression (Ob + D) are shown in Table 1. Age and sex distributions were similar across groups. The two cohorts of people with severe obesity did not differ in BMI or waist circumference. PHQ-9 scores in the depressed group indicated moderate to severe depression. As expected, patients with obesity and comorbid depressive symptoms also reported higher scores on the GAD-7 scale.

3.2 | $CD4^+$ and $CD8^+$ T cell subsets

Overall, significant differences were observed regarding the frequency of $CD4^+$ central memory T cells (TCM) (KW $P = .018$) (Figure 1A). The post hoc test was significant for the comparison between Ob + D and HC. Frequencies of circulating $CD4^+$ TCM were significantly correlated with depression severity ($r = 0.3711$, $P = .0087$), but not WHR measurements, GAD-7 scores or blood pressure measurements (Figure 1B). Furthermore, as an explorative post hoc analysis, the following PHQ item scores were significantly correlated with $CD4^+$ TCM frequencies: Loss of interest and/or pleasure (PHQ 9_1, $r = 0.2986$, $P = .0415$), difficulty sleeping (PHQ_3, $r = 0.2909$, $P = .0473$), changes in appetite (PHQ_5, $r = 0.3471$, $P = .0168$) and difficulty concentrating (PHQ_7, $r = 0.3687$, $P = .0108$) (Figure 1C). However, no associations exceeded the threshold for significance after adjusting for multiple testing. No significant differences in population frequencies of naive, effector memory or effector memory $CD4^+$ T cells re-expressing CD45RA were detected (Figure S4).

The frequency of circulating regulatory T cells (Treg, defined as $CD4^+CD25^+CD127^-$cells) was significantly different across groups. (KW $P = .023$). Specifically, frequencies were increased in patients with obesity and comorbid depressive symptoms (Ob + D) when compared to non-depressed patients with obesity (Ob) (Figure S3A). No group differences in relative frequencies of naive and memory Treg sub-populations were detected (Figure S3B). The expression of the inhibitory marker PD-1 on Treg was significantly increased in patients with obesity compared to HCs (Figure S3C). However, numbers of regulatory T cells did not correlate with PHQ-9 scores or WHR.

In the $CD8^+$ T cell compartment, significant reductions of effector memory T cells ($CD8^+$ TEM) were observed in Ob compared to HC (KW $P = .03$) (Figure S5C). The amount of circulating $CD8^+$ TEM did not correlate with PHQ-9 scores or WHR. No changes in relative frequencies of naive $CD8^+$ T cells (Figure S5A), $CD8^+$ central memory (Figure S5B) or $CD8^+$ effector memory T cells re-expressing CD45RA (Figure S5D) were detected.

3.3 | Natural killer cell subsets

The relative frequency of circulating cytotoxic natural killer cells (NKc) showed significant group differences (KW $P = .006$, Figure 2A), with post hoc test confirming lower levels in Ob + D compared to HC. Neither frequency of total natural killer cells (NK) nor regulatory NK cells (NKreg) differed between groups (Figure 2A). Frequencies of NKc were inversely correlated with PHQ-9 scores ($r = -0.3862$, $P = .01$, Spearman's test), but not significantly associated with WHR (Figure 2B). The exploratory post hoc analysis showed a significant correlation of the following PHQ item scores with relative NKc

TABLE 1 Demographic and clinical characteristics

	HC (n = 20)	Ob (n = 20)	Ob + D (n = 15)	P value[a]	Post hoc statistic[b]
Age (years)	36.6 ± 10.9	47.7 ± 11.5	40.9 ± 13.8	.0217	—
Height (cm)	171.8 ± 8.8	165.6 ± 13.2	169.8 ± 11.2	.4483	—
Weight (kg)	67.7 ± 8.3	128.3 ± 23	130.1 ± 26.1	<.0001	HC vs Ob: P < .0001 HC vs Ob + D: P < .0001 Ob vs Ob + D: P > .9999
BMI (kg/m^2)	22.9 ± 1.6	46.1 ± 5.3	44.6 ± 5.3	<.0001	HC vs Ob: P < .0001 HC vs Ob + D: P = .0001 Ob vs Ob + D: P > .9999
Waist circumference (cm)	76.9 ± 6	128.5 ± 13.6	126.6 ± 15.7	<.0001	HC vs Ob: P < .0001 HC vs Ob + D: P < .0001 Ob vs Ob + D: P > .9999
Waist-to-hip ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	.0005	HC vs Ob: P = .0009 HC vs Ob + D: P = .0105 Ob vs Ob + D: P > .9999
Sys. blood pressure (mmHg)	113.4 ± 12.7	130.8 ± 15.3	130.9 ± 8	.0002	HC vs Ob: P = .0016 HC vs Ob + D: P = .0009 Ob vs Ob + D: P > .9999
Dias. blood pressure (mmHg)	75.1 ± 10.6	83.7 ± 12	83.27 ± 10.6	.0575	—
% Current smokers	20	11.76	20	.7707	—
% Females	75	70	73.3	.8	—
PHQ-9	3.1 ± 1.5	6.4 ± 2.5	14.7 ± 3.3	<.0001	HC vs Ob: P = .0131 HC vs Ob + D: P < .0001 Ob vs Ob + D: P = .0006
GAD-7 sum	2.7 ± 1.9	4.1 ± 3	8.4 ± 2.7	<.0001	HC vs Ob: P = .8728 HC vs Ob + D: P < .0001 Ob vs Ob + D: P = .0037
% Hypertension	0	65	33.3	<.0001	—
% Insulin resistance/diabetes	0	55	26.7	.0005	—
% Hypothyroidism	0	40	40	.0047	—
% Metabolic disorder	0	42.1	36.3	.0282	—

Note: Unless specified otherwise, values represent the mean ± standard deviation.
Abbreviations: BMI, body mass index; GAD-7, Generalizied Anxiety Disorder Scale-7; HC, healthy controls; Ob, patients with obesity; Ob + D, patients with obesity and comorbid depression; PHQ-9, Patient Health Questionnaire-9.
[a]Kruskal-Wallis test was used for continuous and chi-square test for dichotomous variables.
[b]Post hoc comparisons were calculated using Dunn's multiple comparisons.

frequencies: sadness/depression (PHQ_2, r = –0.409, P = .0065), negative self-perception (PHQ_6, r = –0.3639, P = .0164) and psychomotor symptoms (PHQ_8, r = –0.4126, P = .006) (Figure 2C). However, none of the correlations with PHQ items remained significant after correcting for multiple testing.

3.4 | Dendritic cells, monocyte and B cells

Frequencies of dendritic cells (DCs) showed significant group differences (KW P = .001, Figure 3A), driven by lower levels in both Ob and Ob + D compared to HC. Frequencies of circulating DCs inversely correlated with both depression severity (r = –0.4167, P = .0016, Spearman's test) and WHR (r = –0.4022, P = .0026) (Figure 3B). In addition, we found significant correlations of the following PHQ item scores: low energy level (PHQ_4, r = –0.3622, P = .0066), changes in appetite (PHQ_5, r = –0.3485, P = .0091), negative self-perception (PHQ_6, r = –0.3154, P = .019), difficulty concentrating (PHQ_7, r = –0.2912, P = .031) and psychomotor symptoms (PHQ_8, r = –0.3478, P = .0093) (Figure 3C). Again, none of the PHQ item correlations remained significant after correcting for multiple comparisons. No significant group differences concerning the relative frequencies of classical, intermediate or non-classical monocytes (Figure S6A) or B cells (Figure S6B) were found.

4 | DISCUSSION

In this exploratory study, we observed alterations in several innate and adaptive immune cell subsets between HCs, patients with obesity and patients with obesity and comorbid depression. Our most robust

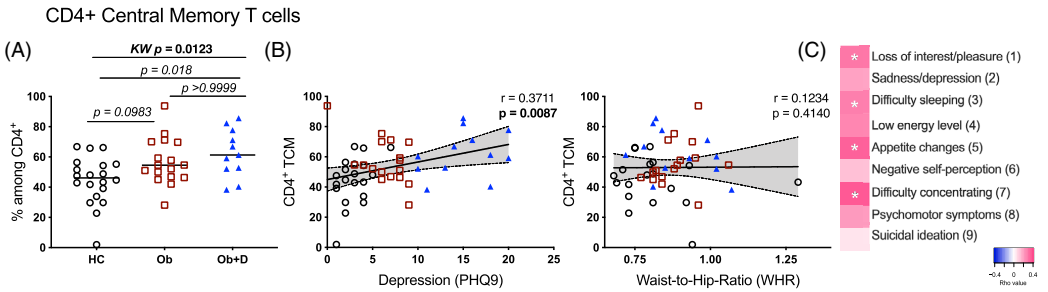

FIGURE 1 T cell sub-populations in comorbid depression and obesity: Relative frequencies of $CD4^+$ TCM, median shown (A). Correlation analysis of $CD4^+$ TCM relative frequencies with WHR and PHQ-9 scores in the total sample (B). Correlation of PHQ sub-items with relative frequencies of $CD4^+$ TCM. Rho value depicts the strength of the correlation, * represents unadjusted *P* value <.05 (C). *n* (HC) = 19; *n* (Ob) = 17; *n* (Ob + D) = 11. HC, healthy controls; Ob, patients with obesity; Ob + D, patients with obesity and comorbid depression; PHQ-9, Patient Health Questionnaire 9; TCM, central memory T cells; WHR, waist-to-hip ratio

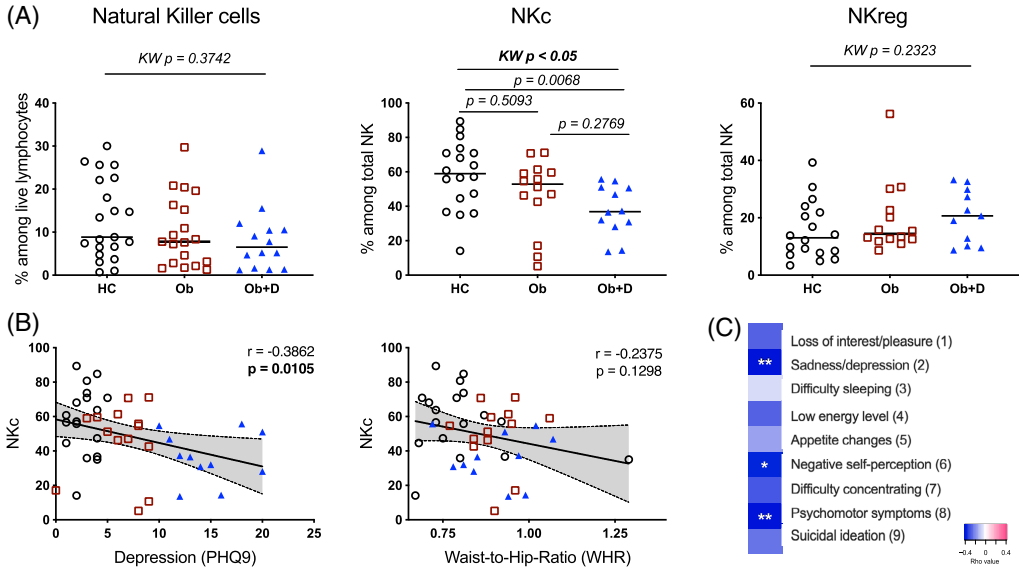

FIGURE 2 NK cell sub-populations in comorbid depression and obesity: Relative frequencies of NK cells among live lymphocytes and NKc and NKreg subsets among total NK cells, median shown (A). Spearman's correlation of NKc frequencies with PHQ-9 scores and WHR (B). Correlation of PHQ sub-items with relative frequencies of NKc. Rho value depicts the strength of the correlation, * represents unadjusted *P* value <.05; ** represents unadjusted *P* < .001 (C). NK frequency: *n* (HC) = 18; *n* (Ob) = 19; *n* (Ob + D) = 14; NKc and NKreg: *n* (HC) = 18; *n* (Ob) = 14; *n* (Ob + D) = 12. HC, healthy controls; NK, natural killer cells; NKc, cytotoxic natural killer cells; NKreg, regulatory natural killer cells; Ob, patients with obesity; Ob + D, patients with obesity and comorbid depression; PHQ-9, Patient Health Questionnaire 9; WHR, waist-to-hip ratio

findings included lower frequencies of NKc and higher levels of CD4 TCM cells in patients with comorbid obesity and depressive symptoms when compared to lean HCs. These findings also showed a significant bivariate association with depression severity but not WHR. This indicates potentially depression-specific immune dysregulations in patients with severe obesity.

In addition, numbers of dendritic cells were significantly reduced in patients with obesity as well as patients with obesity and comorbid depression compared to HCs. Importantly, the number of dendritic cells correlated with both WHR and PHQ scores. Our results thus suggest that dendritic cell frequency may be an immunological correlate that is shared between obesity and depression.

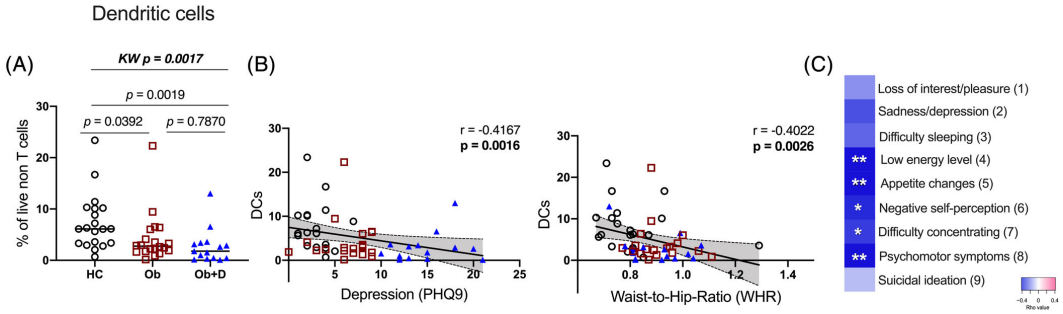

FIGURE 3 Dendritic cells in comorbid depression and obesity: Relative frequencies of DCs among live non-T cells, median shown (A). Spearman's correlation of DC frequencies with PHQ-9 scores and WHR (B). Correlation of PHQ sub-items with relative frequencies of DCs. Rho value depicts the strength of the correlation, * represents unadjusted *P* value <.05; ** represents unadjusted *P* < .001 (C). *n* (HC) = 20; *n* (Ob) = 20; *n* (Ob + D) = 15. DCs, dendritic cells; HC, healthy controls; Ob, patients with obesity; Ob + D, patients with obesity and comorbid depression; PHQ-9, Patient Health Questionnaire 9; WHR, waist-to-hip ratio

Decreased function[33-35] and lower numbers of NK cells[24,34,36,37] in MDD have long been reported in the literature, although not all studies have found this.[26] Gene expression analyses of PBMCs obtained from depressed patients revealed significant down-regulation of genes contributing to NK cell activation.[38] The findings of our study—lower numbers of circulating NKc in patients with obesity and comorbid depression—are in line with most previous findings in MDD. In obesity, dysregulation of NK cells also has pathophysiological implications.[39] Since reduced frequency of NKc significantly correlated with depression severity, but not waist-to-hip ratio, this might indicate some specificity of this association with depression, at least in comorbid patient cohorts like ours.

The role of dendritic cells in MDD is less clear. We observed significantly reduced numbers of DCs in patients with obesity as well as in patients with obesity and comorbid depression compared to HCs. Furthermore, the amount of circulating DCs correlated with both depression severity and WHR in the total sample. One study examining patients with Alzheimer's disease found that reduced numbers of circulating dendritic cells were significantly associated with depression severity.[40] A study in patients suffering from bipolar disorder also identified impaired function of DCs, which was improved upon *in vitro* treatment with lithium.[41] Reduced numbers of dendritic cells and their functional impairment have previously been reported in patients with severe obesity,[42] which is also in accordance with our findings. Given that number of dendritic cells correlated with both PHQ scores and WHR, altered dendritic cell number and function might be involved in a shared pathophysiological mechanism of obesity and MDD.

In our study, we defined dendritic cells as $CD20^{-}CD14^{-}/CD56^{-}/HLA\text{-}DR^{+}$ of the $CD3^{-}$ non-T cell population.[43] This identification strategy has obvious limitations, since we did not stain for markers specific for dendritic cells such as CD11c and CD123, which also allow for the differentiation of myeloid and plasmacytoid DCs.[43,44] In spite of these shortcomings, we believe that our findings could serve to encourage further research on dendritic cells in MDD and obesity, as altered phenotypes of other cells belonging to the myeloid lineage have been described in both MDD[23] and obesity.[20,22]

We detected increased frequencies of $CD4^{+}$ central memory T cells in patients with obesity and comorbid depression compared to HC, which also correlated with depression severity (but not WHR). While there are, to this date, not many studies that have specifically investigated the role of memory T cells in MDD,[24] there is a body of evidence that suggests a role of altered T cell phenotypes in MDD.[24,33,36,37] One possible explanation for the higher numbers of $CD4^{+}$ central memory T cells could be the increased vulnerability to infection in patients with depression.[45] A significant association of depression and herpes simplex-1 infection, among others, has been confirmed in a meta-analysis.[46] Higher numbers of $CD4^{+}$ TCM could be caused by more frequent and/or persistent infections in MDD patients compared to the general population, especially in those with comorbid obesity, given that obesity itself poses a risk factor for increased susceptibility to viral infection.[42]

Concerning correlations with individual PHQ items, in the case of dendritic cells, their abundance was correlated with appetite changes and fatigue (defined as "low energy level"), two symptom domains that are considered central to the atypical/"immuno-metabolic" depression profile.[1,12] Concerning $CD4^{+}$ TCM, significant correlations were observed in the domains of appetite changes and changes in sleeping patterns, also associated with this subtype of depression. In contrast, the PHQ sub-items, which were significantly correlated with the abundance of NKc, could be cautiously interpreted to be more reminiscent of a melancholic depression subtype.[1] However, following adjustment for multiple testing, none of the correlations with PHQ sub-items remained below a significance level of 0.05. Since these post hoc analyses were explorative in nature, these results need to be interpreted with great caution and should be followed-up in larger samples.

In our study, we found significantly increased frequencies of regulatory T cells in patients with obesity and comorbid depression

compared to controls with obesity but not compared to HCs. Previous studies on regulatory T cells in obesity are conflicting: One study reported lower frequencies in individuals with obesity,[20] but another study reported an increase of regulatory T cells.[21] These inconsistencies might be, in part, caused by different marker combinations for identification of regulatory T cells using flow cytometry. Since changes in the abundance of regulatory T cells in the depressed group did not correlate with PHQ, our results should be interpreted with caution. Generally speaking, the role of regulatory T cells in MDD and obesity is still being debated, with some studies reporting higher abundance[24,37] and others reduced numbers.[33]

It should be noted that we did not observe changes in the abundance of monocyte populations. Our findings are thus in contrast with previous reports, where an increase in non-classical and inflammatory monocytes has been reported for both obesity and MDD.[22,23,26] Similarly, monocyte-derived microglia-like cells have been shown to be correlated with suicidal ideation in psychiatric patients.[47] The discrepancies between our findings and the current literature could be due to the small sample size investigated in this study and warrant further clarification in the form of well-controlled, large-scale studies. While our study might encourage further research to be focused on the subsets we identified, monocytes and monocyte-derived cells remain important candidates to be investigated for their potential pathophysiological role in mood disorders with and without comorbid obesity.

Several potential limitations of our study need to be considered. First, our sample was comparatively small. Depression was defined by a PHQ-9 score ≥10 and not diagnosed by means of a structured clinical interview conducted by a trained psychiatrist. Our analysis of the immune phenotype was purely descriptive and does not include any functional readouts. We used measures of waist-to-hip ratio instead of BMI for correlation analyses, given the known shortcomings of BMI for assessing risk factors associated with obesity.[48] However, measuring waist and hip circumference can prove difficult in patients with morbid obesity. We refrained from performing correlation analyses of PHQ-9 scores with BMI and waist circumference given their strong bimodal distribution in our sample. Importantly, while some of our findings suggest potential additive associations with obesity and depressive symptoms, our study did not include a group of lean participants with depressive symptoms. Thus, these results should be interpreted with caution.

The cross-sectional nature of our study precludes any interpretations of cause and effect between depressive symptoms and immunological markers. Longitudinal or interventional studies would be needed to better understand the relationship between them. Moreover, additional factors, such as health behaviours (diet, exercise, smoking, etc.) may play a role. For example, a study on patients with coronary heart disease uncovered a direct association of depressive symptoms and poor lifestyle behaviours, including higher BMI and waist-to-hip ratio.[49] Another recent study identified a strong behavioural overlap between obesity and depression.[50]

Obesity affects treatment outcome in MDD, since patients with higher BMI tend to show an unfavourable treatment response to conventional treatment strategies.[51] Therefore, identifying patients with "immuno-metabolic" depression may ultimately help to optimize treatment strategies. In summary, our results indicate a potential role of cytotoxic NK cells, dendritic cells and certain T cell subsets in obesity with comorbid depression. These findings are in line with the notion that the immune system might play a role in the shared pathophysiology between MDD and obesity.

ACKNOWLEDGEMENTS

This research was in part supported by a mentor-based postdoctoral fellowship program from the National MS Society (MB-1707-28359). Open Access funding enabled and organized by ProjektDEAL.

CONFLICT OF INTEREST

Dr. Otte reports personal fees from Allergan, Ferring, Fortbildungskolleg, Limes Klinikgruppe, Lundbeck, MedOnline, Medical Tribune, Neuraxpharm, SAGE Therapeutics and Stillachhaus outside the submitted work and reports research support from the German Research Foundation (DFG), German Ministry of Education and Research (BMBF), the European Commission (Innovative Medicines Initiative) and the Brain & Behavior Foundation (NARSAD). Dr. Gold reports, personal fees from Almirall S.A., grants from Biogen, personal fees from Mylan GmbH, personal fees from Celgene, personal fees from FomF, grants from German Research Foundation (DFG), grants from German Ministry of Education and Research (BMBF), grants from German Ministry of Health (BMG), grants from the National MS Society (NMSS), grants from the European Commission (Innovative Medicines Initiative). The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data displayed in this publication, including supplementary materials, are available from the corresponding author upon request.

ORCID

Victoria Stiglbauer https://orcid.org/0000-0001-8179-3759
Jelena Brasanac https://orcid.org/0000-0001-9786-7310
Christian Otte https://orcid.org/0000-0002-4051-997X
Stefan M. Gold https://orcid.org/0000-0001-5188-4799

REFERENCES

1. Otte C, Gold SM, Penninx BW, et al. Major depressive disorder. *Nat Rev Dis Prim*. 2016;2(1):1-20.
2. Machado MO, Veronese N, Sanches M, et al. The association of depression and all-cause and cause-specific mortality: an umbrella review of systematic reviews and meta-analyses. *BMC Med*. 2018;16(112):1-13.
3. Gold SM, Moss-Morris R, Mehnert A, et al. Comorbid depression in medical diseases. *Nat Rev Dis Prim*. 2020;6(69):1-22.
4. McLaughlin KA. The public health impact of major depression: a call for interdisciplinary prevention efforts. *Prev Sci*. 2011;12(4):361-371.
5. Chiu M, Lebenbaum M, Cheng J, De Oliveira C, Kurdyak P. The direct healthcare costs associated with psychological distress and major depression: a population-based cohort study in Ontario, Canada. *PLoS One*. 2017;12(9):1-13.

6. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. *BMC Med*. 2013;11(1):1-14.
7. Vancampfort D, Mitchell AJ, De Hert M, et al. Type 2 diabetes in patients with major depressive disorder: a meta-analysis of prevalence estimates and predictors. *Depress Anxiety*. 2015;32(10):763-773.
8. Pan A, Keum N, Okereke OI, et al. Bidirectional association between depression and metabolic syndrome. *Diabetes Care*. 2012;35(5):1171-1180.
9. Luppino FS, de Wit LM, Bouvy PF, et al. Overweight, obesity, and depression. *Arch Gen Psychiatry*. 2010;67(3):220-229.
10. Marx P, Antal P, Bolgar B, Bagdy G, Deakin B, Juhasz G. Comorbidities in the diseasome are more apparent than real: what Bayesian filtering reveals about the comorbidities of depression. *PLoS Comput Biol*. 2017;13(6):1-23.
11. Bot M, Milaneschi Y, Al-Shehri T, et al. Metabolomics profile in depression: a pooled analysis of 230 metabolic markers in 5283 cases with depression and 10,145 controls. *Biol Psychiatry*. 2020;87(5):409-418.
12. Milaneschi Y, Lamers F, Berk M, Penninx BW. Depression heterogeneity and its biological underpinnings: towards immuno-metabolic depression. *Biol Psychiatry*. 2020;88(5):369-380.
13. Ogrodnik M, Zhu Y, Langhi LGP, et al. Obesity-induced cellular senescence drives anxiety and impairs neurogenesis. *Cell Metab*. 2019;29(5):1061-1077.
14. Milaneschi Y, Lamers F, Peyrot WJ, et al. Genetic association of major depression with a typical features and obesity-related immunometabolic dysregulations. *JAMA Psychiatry*. 2017;74(12):1214-1225.
15. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain Behav Immun*. 2020;87:901-909.
16. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes Rev*. 2013;14(3):232-244.
17. Kappelmann N, Arloth J, Georgakis MK, et al. Dissecting the association between inflammation, metabolic dysregulation, and specific depressive symptoms. *JAMA Psychiatry*. 2020. http://dx.doi.org/10.1001/jamapsychiatry.2020.3436. Online ahead of print
18. Matarese G, Procaccini C, De Rosa V. At the crossroad of T cells, adipose tissue, and diabetes. *Immunol Rev*. 2012;249(1):116-134.
19. Huh JY, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. *Mol Cells*. 2014;37(5):365-371.
20. Rodríguez CP, González MC, Aguilar-Salinas CA, Nájera-Medina O. Peripheral lymphocytes, obesity, and metabolic syndrome in young adults: an immunometabolism study. *Metab Syndr Relat Disord*. 2018;16(7):342-349.
21. Van Der Weerd K, Dik WA, Schrijver B, et al. Morbidly obese human subjects have increased peripheral blood CD4+ T cells with skewing toward a Treg- and Th2-dominated phenotype. *Diabetes*. 2012;61(2):401-408.
22. Devêvre EF, Renovato-Martins M, Clément K, Sautès-Fridman C, Cremer I, Poitou C. Profiling of the three circulating monocyte subpopulations in human obesity. *J Immunol*. 2015;194(8):3917-3923.
23. Hasselmann H, Gamradt S, Taenzer A, et al. Pro-inflammatory monocyte phenotype and cell-specific steroid signaling alterations in unmedicated patients with major depressive disorder. *Front Immunol*. 2018;9:1-9.
24. Suzuki H, Savitz J, Kent Teague T, et al. Altered populations of natural killer cells, cytotoxic T lymphocytes, and regulatory T cells in major depressive disorder: association with sleep disturbance. *Brain Behav Immun*. 2017;66:193-200.
25. Schiweck C, Valles-Colomer M, Arolt V, et al. Depression and suicidality: a link to premature T helper cell aging and increased Th17 cells. *Brain Behav Immun*. 2020;87:603-609.
26. Lynall ME, Turner L, Bhatti J, et al. Peripheral blood cell–stratified subgroups of inflamed depression. *Biol Psychiatry*. 2019;88(2):185-196.
27. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606-613.
28. Levis B, Sun Y, He C, et al. Accuracy of the PHQ-2 alone and in combination with the PHQ-9 for screening to detect major depression: systematic review and meta-analysis. *JAMA*. 2020;323(22):2290-2300.
29. Gräfe K, Zipfel S, Herzog W, Löwe B. Screening psychischer Störungen mit dem "Gesundheitsfragebogen für Patienten (PHQ-D)". *Diagnostica*. 2004;50(4):171-181.
30. Manea L, Gilbody S, Hewitt C, et al. Identifying depression with the PHQ-2: a diagnostic meta-analysis. *J Affect Disord*. 2016;203:382-395.
31. Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006;166(10):1092-1097.
32. Löwe B, Decker O, Müller S, et al. Validation and standardization of the generalized anxiety disorder screener (GAD-7) in the general population. *Med Care*. 2017;46(3):266-274.
33. Grosse L, Hoogenboezem T, Ambrée O, et al. Deficiencies of the T and natural killer cell system in major depressive disorder. T regulatory cell defects are associated with inflammatory monocyte activation. *Brain Behav Immun*. 2016;54:38-44.
34. Evans DL, Folds JD, Petitto JM, et al. Circulating natural killer cell phenotypes in men and women with major depression: relation to cytotoxic activity and severity of depression. *Arch Gen Psychiatry*. 1992;49(5):388-395.
35. Zorrilla EP, Luborsky L, McKay JR, et al. The relationship of depression and stressors to immunological assays: a meta-analytic review. *Brain Behav Immun*. 2001;15(3):199-226.
36. Zhong GS, Yang R, Cai RY, et al. Flow cytometric analysis of peripheral blood related immune cells and cytokines in patients with depression. *Int J Clin Exp Med*. 2018;11(3):2812-2820.
37. Patas K, Willing A, Demiralay C, et al. T cell phenotype and T cell receptor repertoire in patients with major depressive disorder. *Front Immunol*. 2018;9:1-12.
38. Jansen R, Penninx BWJH, Madar V, et al. Gene expression in major depressive disorder. *Mol Psychiatry*. 2016;21(3):339-347.
39. O'Shea D, Hogan AE. Dysregulation of natural killer cells in obesity. *Cancers (Basel)*. 2019;11(4):1-12.
40. Ciaramella A, Salani F, Bizzoni F, et al. Myeloid dendritic cells are decreased in peripheral blood of Alzheimer's disease patients in association with disease progression and severity of depressive symptoms. *J Neuroinflammation*. 2016;13(1):1-11.
41. Knijff EM, Ruwhof C, De Wit HJ, et al. Monocyte-derived dendritic cells in bipolar disorder. *Biol Psychiatry*. 2006;59(4):317-326.
42. O'Shea D, Corrigan M, Dunne MR, et al. Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. *Int J Obes*. 2013;37(11):1510-1513.
43. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immunol*. 2012;12(3):191-200.
44. Robinson SP, Patterson S, English N, Davies D, Knight SC, Reid CDL. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol*. 1999;29(9):2769-2778.
45. Andersson NW, Goodwin RD, Okkels N, et al. Depression and the risk of severe infections: prospective analyses on a nationwide representative sample. *Int J Epidemiol*. 2016;45(1):131-139.
46. Wang X, Zhang L, Lei Y, et al. Meta-analysis of infectious agents and depression. *Sci Rep*. 2014;4(4530):1-10.
47. Suzuki H, Ohgidani M, Kuwano N, et al. Suicide and microglia: recent findings and future perspectives based on human studies. *Front Cell Neurosci*. 2019;13(31):1-10.

48. Akpinar E, Bashan I, Bozdemir N, Saatci E. Which is the best anthropometric technique to identify obesity: body mass index, waist circumference or waist-hip ratio? *Coll Antropol*. 2007;31(2):387-393.
49. Sin NL, Kumar AD, Gehi AK, Whooley MA. Direction of association between depressive symptoms and lifestyle behaviors in patients with coronary heart disease: the heart and soul study. *Ann Behav Med*. 2016;50(4):523-532.
50. Vainik U, Misic B, Zeighami Y, Michaud A, Mõttus R, Dagher A. Obesity has limited behavioural overlap with addiction and psychiatric phenotypes. *Nat Hum Behav*. 2020;4(1):27-35.
51. Kloiber S, Ising M, Reppermund S, et al. Overweight and obesity affect treatment response in major depression. *Biol Psychiatry*. 2007;62(4):321-326.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Stiglbauer V, Gamradt S, Scherzer M, et al. Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study. *Cell Biochem Funct*. 2021;39:423–431. https://doi.org/10.1002/cbf.3608

Supplementary Material "**Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study"**

by Stiglbauer *et.al*

Antibody Panels

Fluorochrome	Regulatory T cells	Naïve/memory T cells	T cell subsets	Non-T cells
AF 488*/FITC$	KLRG1* (13F12F2) *Thermofisher*	KLRG1* (13F12F2) *Thermofisher*	KLRG1* (13F12F2) *Thermofisher*	HLA-DR (LN3)$ *Biolegend*
PE	PD-1 (EH12.2H7) *Biolegend*	PD-1 (EH12.2H7) *Biolegend*	PD-1 (EH12.2H7) *Biolegend*	CD56 (HCD56) *Biolegend*
PerCP-Cy5.5	CD4 (RPA-T4) *Biolegend*	CD4 (RPA-T4) *Biolegend*	CD4 (RPA-T4) *Biolegend*	CD4 (RPA-T4) *Biolegend*
PE-Cy7	CD45RA (HI100) *Biolegend*	CD45RA (HI100) *Biolegend*	CCR6 (G034E) *Biolegend*	CD20 (2H7) *Biolegend*
APC	CD127 (A019D5) *Biolegend*	CCR7 (G043H7) *Biolegend*	CCR4 (L291H4) *Biolegend*	CD16 (3G8) *Biolegend*
BV421	CD25 (M-A251) *Biolegend*	CD3 (UCHT1) *Biolegend*	CXCR3 (G025H7) *Biolegend*	CD14 (HCD14) *Biolegend*
BV510	CD3 (UCHT1) *Biolegend*	CD8 (RPA-T8) *Biolegend*	CD3 (UCHT1) *Biolegend*	CD3 (UCHT1) *Biolegend*
	Zombie NIR *Biolegend*	Zombie NIR *Biolegend*	Zombie NIR *Biolegend*	Zombie NIR *Biolegend*

Table 1 | Antibody panels for the analysis of PBMC subsets: regulatory T cells (Treg), naive and memory T cell subsets, helper T cell subsets based on the expression of chemokine receptors and non-T cell subsets. Antibody clones are indicated in brackets, company name in *italics*.

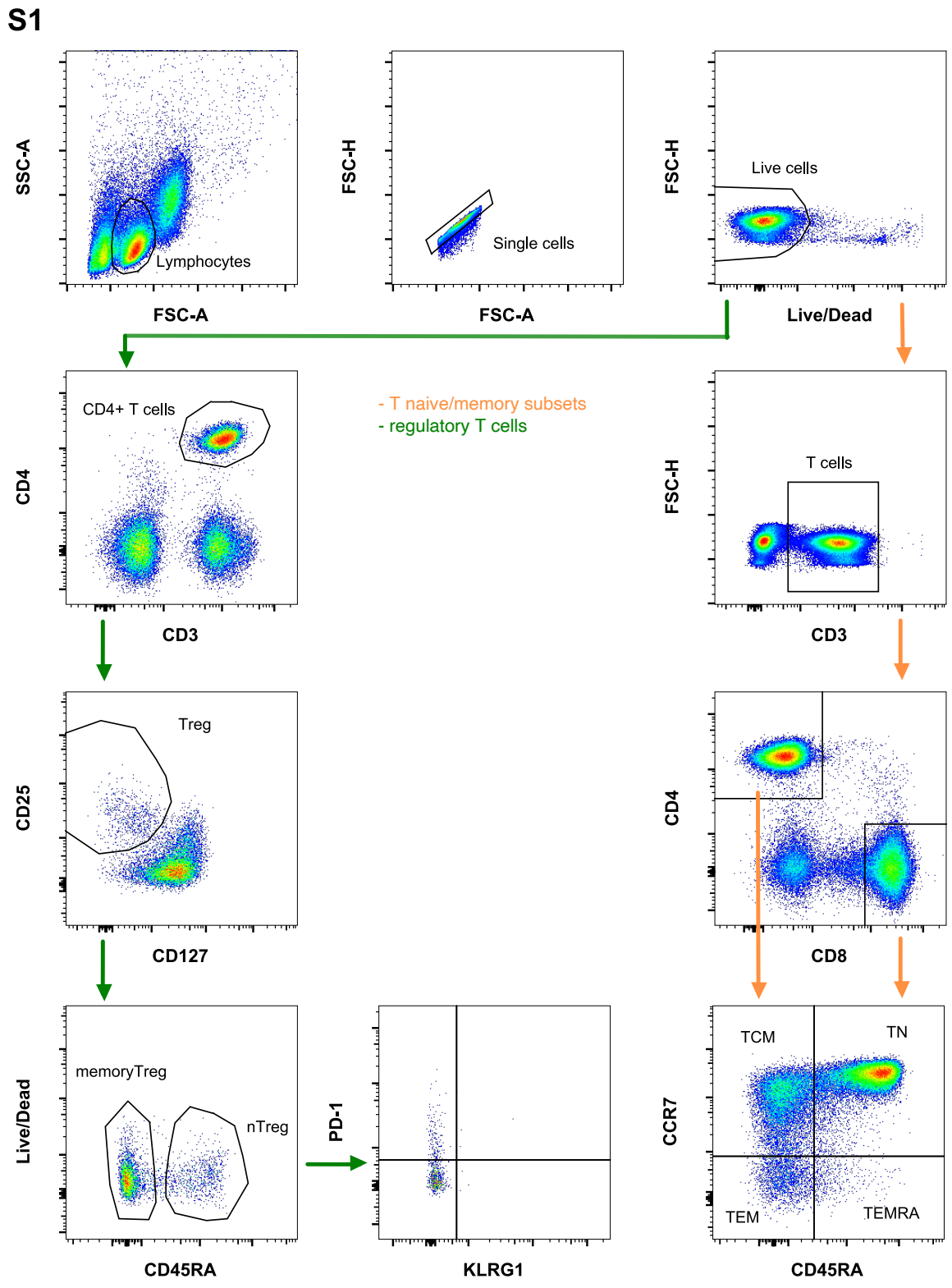

Figure S1 | Flow cytometry gating strategy for the identification of naïve and memory subsets in helper T and cytotoxic T cells and regulatory T cells (Treg). Lymphocytes were identified by forward scatter area (FSC-A) and sideward scatter (SSC-A) properties. Doublets and dead cells were then excluded. Next, the $CD3^{+}CD4^{+}$ population was gated on and Treg identified according to CD25 and CD127 surface expression ($CD25^{+}CD127^{-/low}$). Naïve and memory Treg subsets were further subclassified according to their expression of CD45RA. Tregs were also analysed for their expression

of PD-1 and KLRG1. Helper T cells and cytotoxic T cells were discriminated by their differential expression of $CD4^+$ (helper T cells) and $CD8^+$ (cytotoxic T cells). By gating for CCR7 and CD45RA, the $CD4^+$ and $CD8^+$ populations were split into naïve T cells ($CCR7^+/CD45RA^+$), central memory cells T cells ($CCR7^+/CD45RA^-$), effector memory T cells ($CCR7^-/CD45RA^-$) and effector memory T cells re-expressing CD45RA ($CCR7^-/CD45RA^+$)

S2

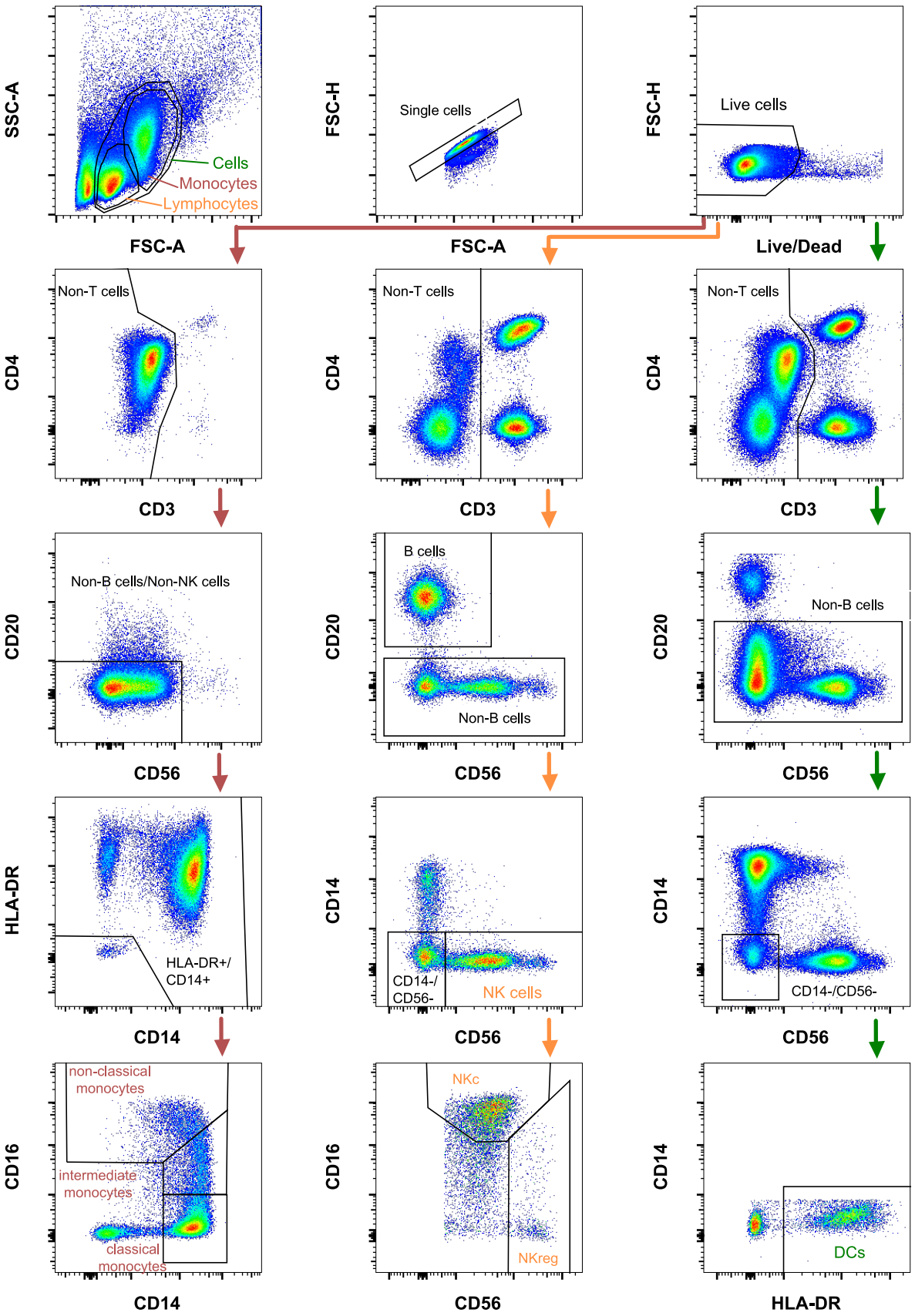
SSC-A
FSC-A
Cells
Monocytes
Lymphocytes
FSC-H
FSC-A
Single cells
FSC-H
Live/Dead
Live cells
CD4
CD3
Non-T cells
Non-T cells
Non-T cells
CD20
CD56
Non-B cells/Non-NK cells
B cells
Non-B cells
Non-B cells
HLA-DR
CD14
HLA-DR+/
CD14+
CD14
CD56
CD14-/
CD56-
NK cells
CD14
CD56
CD14-/CD56-
CD16
CD14
non-classical
monocytes
intermediate
monocytes
classical
monocytes
CD16
CD56
NKc
NKreg
CD14
HLA-DR
DCs

Figure S2 | Flow cytometry gating strategy for the identification of classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes, B cells, Natural killer (NK) cells and dendritic cells (DCs). Monocytes were identified by FSC-A and SSC-A properties, doublets and dead cells excluded. Remaining $CD3^{+}$ T cells, B cells ($CD20^{+}$) and NK cells ($CD56^{+}$) as well as $CD14^{-}$HLA-DR^{-} cells were excluded. Monocyte populations were then classified according to their expression of CD14 and CD16. B cells were defined according to positivity for CD20 and NK cells defined as $CD20^{-}CD14^{-}CD56^{+}$ starting from the lymphocyte gate. Cytotoxic NK cells (NKc) were defined as $CD56^{+}$ and $CD16^{++}$, whereas regulatory NK cells (NKreg) were classified as $CD56^{++}CD16^{-/+}$. Dendritic cells were defined as HLA-DR^{+} after the exclusion of monocytes, B cells and NK cells from $CD3^{-}$ non-T cell population gated from all cells.

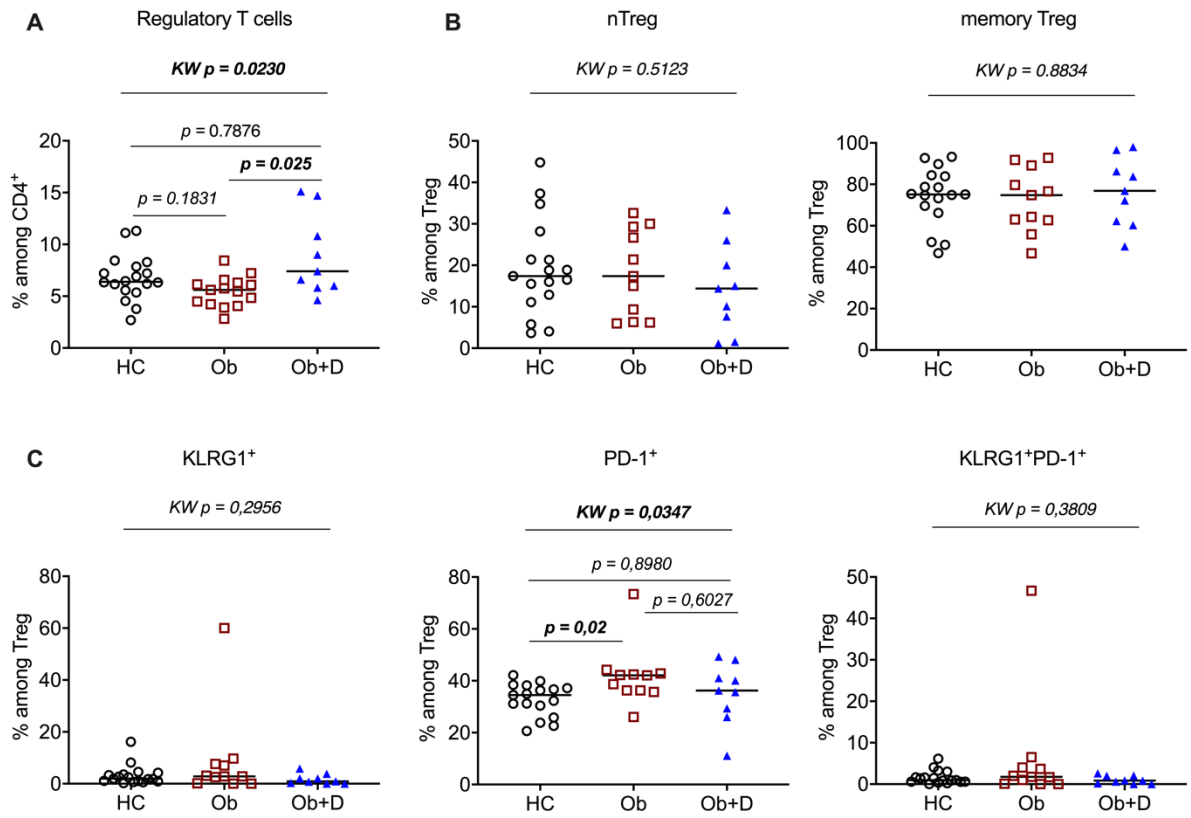

Figure S3 | Immune phenotype of regulatory T cells ($CD4^+/CD127^-/CD25^+$), including naïve and memory subpopulations in healthy controls, patients with obesity and patients with obesity and comorbid depression. Relative frequencies of regulatory T cells **(A)** and naïve and memory subsets **(B)** and expression of KLRG1 and PD-1 on Treg **(C)**, median shown. Gating strategies for these $CD4^+$ T cell subpopulations are depicted in **Figure S1**. Regulatory T cells: n (HC)= 18; n (Ob)= 15; n (Ob+D)= 9; nTreg and memory T reg: n (HC)= 17; n (Ob)= 11; n (Ob+D)= 9; HC, Healthy controls; Ob, Patients with obesity; Ob+D, Patients with obesity and comorbid depression; Treg, regulatory T cells; nTreg, naïve regulatory T cells.

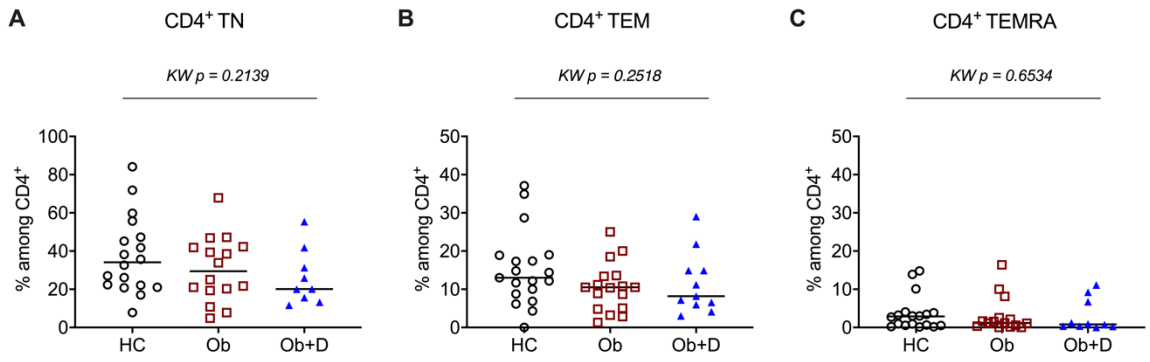

Figure S4 | Immune phenotype of naïve and memory CD4+ T cell subpopulations in healthy controls, patients with obesity and patients with obesity and comorbid depression. Relative frequencies of naïve T cells **(A)**, effector memory T cells **(B)** and effector memory T cells re-expressing CD45RA **(C)**, median shown. Gating strategies for these CD4+ T cell subpopulations are depicted in **Figure S1**. n (HC)= 19; n (Ob)= 17; n (Ob+D)= 11; HC, Healthy controls; Ob, Ob, Patients with obesity; Ob+D, Patients with obesity and comorbid depression; TN, naïve T cells; TEM, effector memory T cells; TEMRA, effector memory T cells re-expressing CD45RA

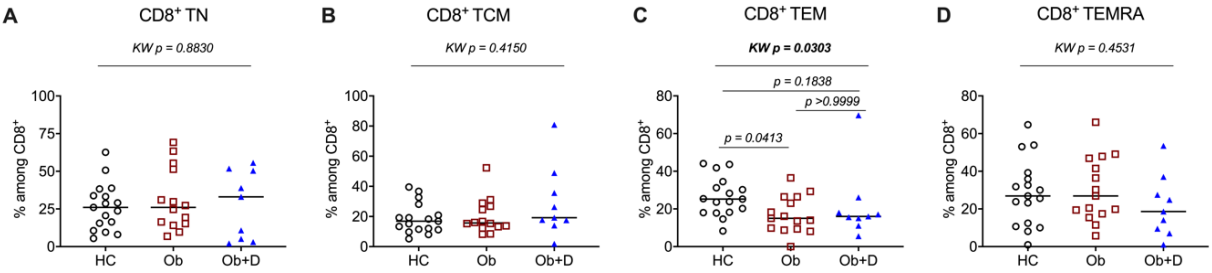

Figure S5 | Immune phenotype of naïve and memory CD8+ T cell subpopulations in healthy controls, patients with obesity and patients with obesity and comorbid depression. Relative frequencies of naïve T cells **(A)**, central memory T cells **(B)**, effector memory T cells **(C)** and effector memory T cells re-expressing CD45RA **(D)**, median shown. Gating strategy for these CD8+ T cell subpopulations is depicted in **Figure S1**. n (HC)= 17; n (Ob)= 14; n (Ob+D)= 9; HC, Healthy controls; Ob, Patients with obesity; Ob+D, Patients with obesity and comorbid depression; TN, naïve T cells; TCM, central memory T cells; TEM, effector memory T cells; TEMRA, effector memory T cells re-expressing CD45RA

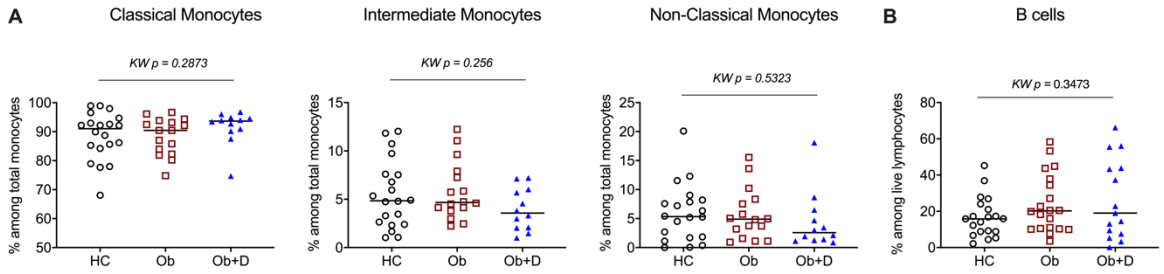

Figure S6 | Relative amount of monocyte subpopulations and B cells in healthy controls, patients with obesity and patients with obesity and comorbid depression. Relative frequencies of classical ($CD14^{++}/CD16^{-}$), intermediate ($CD14^{++}/CD16^{+}$) and non-classical ($CD14^{+}/CD16^{++}$) monocytes, median shown **(A)**. Relative B cell ($CD3^{-}/CD20^{+}$) frequencies, median shown **(B)**. Gating strategies for monocytes and B cells are shown in **Figure S2**. Monocytes: n (HC)= 20; n (Ob)= 16; n (Ob+D)= 12; B cells: n (HC)= 20; n (Ob)= 20; n (Ob+D)= 15; HC, Healthy controls; Ob, Patients with obesity; Ob+D, Patients with obesity and comorbid depression.

6. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

7. Publikationsliste

Publikation	**Impact factor** (Erscheinungsjahr)
Stiglbauer V, Hotka M, Ruiß M, Hilber K, Boehm S, Kubista H. Ca_v 1.3 channels play a crucial role in the formation of paroxysmal depolarization shifts in cultured hippocampal neurons. Epilepsia. 2017 May; 58(5):858-871.	5.482
Stiglbauer V, Gamradt S, Scherzer M, Brasanac J, Otte C, Rose M, Hofmann T, Hinkelmann K, Gold SM. Immunological substrates of depressive symptoms in patients with severe obesity: An exploratory study. Cell Biochem Funct. 2021 Apr;39(3):423-431.	3.685
Lennard Ostendorf, Philipp Dittert, Robert Biesen, Ankelien Duchow, Victoria Stiglbauer, Klemens Ruprecht, Judith Bellmann-Strobl, Dominik Seelow, Werner Stenzel, Raluca A. Niesner, Anja E. Hauser, Friedemann Paul, Helena Radbruch. SIGLEC1 (CD169): a marker of active neuroinflammation in the brain but not in the blood of multiple sclerosis patients. Scientific Reports | (2021) 11:10299	4.379
Gamradt Stefanie, Hasselmann Helge, Tänzer Aline, Brasanac Jelena, Stiglbauer Victoria, Sattler Arne, Max Sajitz-Hermstein, Sylwia Kierszniowska, Caren Ramien, Jan Nowacki, Lea Mascarell-Maricic, Katja Wingenfeld, Dominique Piber, Andreas Ströhle, Katja Kotsch, Friedemann Paul, Christian Otte, Stefan M. Gold. Reduced mitochondrials respiration in T cells of patients with major depressive disorder. iScience 2021; 24(11):103312.	5.08
Dannemann M, Milaneschi Y, Yermakovich D, Stiglbauer V, Kariis HM, Krebs K, Friese MA, Otte C; Estonian Biobank Research Team; Lehto K, Penninx BWJH, Kelso J, Gold SM. Neandertal introgression partitions the genetic landscape of neuropsychiatric disorders and associated behavioral phenotypes. Transl Psychiatry. 2022 Oct 5;12(1):433.	7.973

8. Danksagung

An dieser Stelle möchte ich mich herzlich bei allen an der Entstehung dieser Dissertation beteiligten Personen bedanken.

Mein besonderer Dank gilt meinem Doktorvater und Mentor Prof. Dr. Stefan Gold, der mir die Durchführung der Arbeit ermöglicht hat, meine Begeisterung für die biologische Psychiatrie nicht nur gefördert, sondern geradezu katalysiert hat und mir stets unterstützend zur Seite gestanden ist. Des Weiteren möchte ich mich herzlich bei Frau Dr.rer.nat. Stefanie Gamradt bedanken, dank ihrer ausführlichen, geduldigen und wertschätzenden Supervision konnte ich meine praktischen Fähigkeiten weiterentwickeln und Kenntnisse auf dem Gebiet der Immunologie vertiefen. Mein Dank gebührt auch meiner geschätzten Kollegin Frau Jelena Brasanac, die mit ihrer Begeisterung für die Forschung und ihrem wissenschaftlichen Talent eine unermüdliche Quelle der Inspiration darstellt.

Ich möchte mich auch bei allen Koautoren bedanken, welche mir wertvolles Feedback beim Verfassen des Manuskriptes gegeben haben, allen voran bei meinem Zweitbetreuer Prof. Dr. Christian Otte, der mit seinem Blick für das Wesentliche und klinisch relevante das Manuskript zu einem besseren gemacht hat.

Mein Dank gilt auch allen weiteren Mitgliedern der AG Gold, ich schätze mich sehr glücklich Teil eines so vielseitigen, kompetenten und wundervollen Teams sein zu dürfen.

Außerdem möchte ich bei PD Dr.med. Henrik Alle bedanken, der mir auf meinem wissenschaftlichen und medizinischen Werdegang seit Jahren mit Rat und Anregungen zur Seite steht.

Mein Dank gebührt auch meinem Mentor Dr. Erich Ritter, der stets eine Vorbildfunktion für mich innehatte und dessen unerschütterlicher Glaube an meine wissenschaftlichen Fähigkeiten mich auch über seinen Tod hinaus begleitet.

Meiner Familie, allen voran Gabriele und Rudolf Stiglbauer, möchte ich ebenso meinen tiefsten Dank aussprechen, dafür dass sie mir diese Ausbildung ermöglichen und ich von ihrer Seite stets Zuspruch erhalte.

Ich danke meinen Freunden in Berlin und Wien für ihre Unterstützung in Zeiten der Mutlosigkeit.

Besonderer Dank gilt auch meinem Partner Hilmar Magnússon, der mir geduldig zur Seite steht und meinen Blick für die wirklich wichtigen Dinge schärft.