



The spleen-liver axis supports obesity-induced systemic and fatty liver inflammation via MDSC and NKT cell enrichment

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ABSTRACT

Obesity promotes adipose tissue inflammation and leads to impaired local but also systemic immune cell homeostasis. This chronic low-grade inflammation plays a significant role in the development of obesity-associated secondary diseases such as metabolic associated fatty liver disease or cancer. The spleen as the central organ of immune cell regulation is anatomically directly connected to the visceral adipose tissue and the liver via the portal vein circulation. However, the inter-organ crosstalk and linkage between obesity-induced systemic, hepatic and splenic immune cell dysregulation is not clearly outlined. In this study blood, spleen, and liver immune cells of non-obese wildtype vs. leptin deficient obese BTBR mice were isolated and analyzed in terms of leukocyte composition by flow cytometry. Significant differences between circulating, spleen- and liver-resident immune cell distribution revealed, that obesity-induced hepatic and systemic immune cell dysregulation is distinct from splenic immune cell reprogramming. Fatty liver inflammation was associated with splenic myeloid derived suppressor cell (MDSC) and natural killer T cell (NKT) enrichment whereas loss of hepatic T and B cells was not reflected by the splenic lymphocyte landscape. Correlation analysis confirmed a selective strong positive correlation between spleen and liver MDSC and NKT cell distribution indicating that the spleen-liver axis modulates obesity-induced immune dysregulation in a cell-specific manner. Similar results were observed in a diet-induced obesity mouse model. These data provide novel insights into the role of the spleen-liver axis in obesity-induced inflammation and foster the understanding of obesity-associated complications such as fatty liver disease and cancer.

1. Introduction

Obesity and obesity-associated secondary diseases such as non-alcoholic fatty liver disease (NAFLD, now also termed as metabolic associated fatty liver disease = MAFLD), diabetes mellitus type 2, cardiovascular diseases, and several types of cancer account for most deaths worldwide. Thus, obesity has been considered an emerging public health problem by the World Health Organization (WHO) already years ago

(WHO, 2023). Obesity is mainly caused by an energy-dense diet combined with sedentary behavior leading to an imbalance between calorie intake and consumption. This overnutrition results in lipid accumulation, dysfunction of adipocytes and increased release of hormones and cytokines that alter the local immunity towards adipose tissue and consequently chronic low-grade systemic inflammation (Stolarczyk, 2017; Han and Levings, 1950; Reilly and Saltiel, 2017).

Adipose tissue (AT) inflammation is characterized by infiltration of

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activated immune cells that overproduce proinflammatory cytokines leading to a loss of local immune regulation. Obesity-induced alterations of AT immune cells are well described and include both cells of the innate and adaptive immune system (Alwarawrah et al., 2018). In terms of innate immunity macrophages, the most abundant immune cell population in adipose tissue, play a center role (Weisberg et al., 2003; Boutens and Stienstra, 2016). Additionally, obesity induces the accumulation of neutrophils, mast cells and subsets of dendritic cells that are known to further promote macrophage infiltration (Bertola et al., 2012; Stefanovic-Racic et al., 2012; Huh et al., 2014). Besides cells of the innate immune system, adaptive immune cells support the proinflammatory microenvironment in obese AT. Both T and B lymphocytes expand and mainly exhibit proinflammatory phenotypes (Winer et al., 2009; Gerriets and MacIver, 2014). Other regulatory immune cells found in adipose tissue are natural killer T cells (NKT) that usually accumulate in lean adipose tissue and produce anti-inflammatory cytokines such as IL-4 or IL-10 (Huh et al., 2013; Park et al., 2018). In obese mice, numbers of NKT cells are significantly reduced contributing to the loss of immune regulation and consecutive AT inflammation (Lynch et al., 2009, 2012; Schipper et al., 2012). Taken together, an imbalance of the pro- and anti-inflammatory immune homeostasis results in systemic low-grade inflammation and promotes the development of obesity-associated secondary diseases.

While the obesity-induced shift in adipose tissue-resident immune cells has been the subject of extensive research over the past decades, obesity-mediated reprogramming of systemic immunity and its role in promoting obesity-associated secondary diseases such as cancer or fatty liver disease has come into focus only recently (Alwarawrah et al., 2018; Afify et al., 2022). NAFLD is the most common liver disease worldwide and one of the main causes for liver transplantation (Tilg et al., 2021). NAFLD ranges from simple fatty liver (steatosis) over non-alcoholic steatohepatitis (NASH) to liver fibrosis and cirrhosis and can lead to hepatocellular carcinoma (Parthasarathy et al., 2020). To prevent progression of NAFLD early diagnosis and understanding of the underlying molecular mechanisms is mandatory but still under investigation (Buzzetti et al., 2016). Current literature proclaims NAFLD development to be multifactorial (multi-hit hypothesis) with numerous contributing parameters including genetic predisposition, diet-induced metabolic dysregulation, altered gut microbiome and reprogramming of local immunity (Buzzetti et al., 2016; Behary et al., 2021). Thereby intrahepatic inflammation is regarded as a hallmark of the progression from NAFLD to NASH including immune dysregulation of innate and adaptive immune mechanisms (Tilg et al., 2021; Parthasarathy et al., 2020; Byun and Yi, 2017). While the recruitment of different immune cells into the liver in NAFLD is well described, underlying signals leading to this are still a topic of current research.

In this context, the role of the spleen-liver axis in NAFLD progression has been put into focus lately (Tarantino et al., 2021; Barrea et al., 2018). By clearance of exhausted or apoptotic circulating cells and differentiation as well as activation of lymphocytes the spleen plays an important role in regulating systemic immunity (Borek, 1986). Besides being the central organ of immune regulation, the spleen has direct anatomic connection to the liver and visceral adipose tissue via the portal vein circulation. However, its role in modulating obesity-induced systemic and hepatic inflammation remains unclear yet (Tarantino et al., 2021; Barrea et al., 2018).

In this study we compared immune cell distribution of three different leukocyte compartments (blood, spleen, and liver) in Black Tan and Brachyuric (BTBR) obese (ob/ob) vs. wildtype (WT) mice. Due to mutational-induced leptin-deficiency, BTBR ob/ob mice develop obesity, hyperglycaemia, and severe type 2 diabetes. Since obesity leads to chronic hyperleptinemia resulting in a leptin resistance, leptin-deficient BTBR mice mimic a well-established mouse model for obesity (Keller et al., 2023). Only lately, BTBR ob/ob mice have been shown to also represent an excellent experimental model to study obesity-associated secondary diseases such as NAFLD progression from

steatosis to steatohepatitis (Opazo-Rí et al., 2022). Our findings in this mouse model indicate that the spleen-liver axis might contribute to chronic obesity-induced systemic and hepatic inflammation by splenic enrichment of MSDC and NKT cells. Comparable results were obtained in C57BL/6 mice on a Western-type diet.

2. Material and methods

2.1. Experimental animal procedures

Immune cell distribution in blood, spleen and liver was analyzed in non-diabetic BTBR WT vs. leptin deficient BTBR ob/ob mice and C57BL/6 mice. Male BTBR WT and ob/ob mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed in a controlled environment at a pathogen-free facility with constant temperature under a 12-h light/dark cycle and free access to food and water. Mice were fed with standard chow for up to 24 weeks. Body weight and food consumption were recorded regularly. At 4, 8, 16, 20 and 24 weeks (n = 3–5/group) mice were euthanized by cervical dislocation followed by cardiac puncture. Blood samples, liver tissue and spleen tissue were collected and analyzed as pointed out in 2.2.

Male C57BL/6 mice were purchased from Charles River Laboratories (Sulzfeld, Germany) at 12 weeks of age and housed in a temperature-controlled room under a 12-h light–dark cycle with free access to food and water. After 2 weeks of acclimatization, mice were fed either with standard diet (control) or a NASH-inducing diet (ND), enriched with pork lard (15%), beef tallow (15%), palmitic acid (4%), stearic acid (4%), cholesterol (0.2%) and sucrose (30%) as described before (Dorn et al., 2014). Both chows were prepared by Ssniff (Soest, Germany). After 12 weeks of feeding, animals were killed by heart puncture under deep ketamine/xylazine anesthesia, and spleen and liver tissues were collected for further analyses as pointed out in 2.2. All procedures were approved by the local ethics committee at the University Hospital of Regensburg (No. 55.2-2532-2-1259, No. 54-2532.1-49/13).

2.2. Mouse tissue processing

Livers and spleens were minced into pieces in RPMI 1640 supplemented with 10% fetal calf serum (FCS), squeezed through with the plug of a 2 ml syringe, and centrifuged after filtration through a 100 µm (spleen) or 70 µm (liver) cell strainer. Erythrocytes in single cell suspensions of livers and spleens were lysed with ACK lysis buffer for 3 min at room temperature (RT). For blood samples, erythrocyte lysis with ACK buffer was performed for 5 min at RT after a washing step with flow cytometry buffer (phosphate buffered saline + 2% FCS). All samples were washed with flow cytometry buffer and passed through a 100 µm cell strainer. Cells were counted with a CASY TT Cell Counter (OLS). 1–3 × 10⁶ cells were used for immunostaining and analyzed by flow cytometry.

2.3. Flow cytometry

Flow cytometry was performed using a BD FACS Celesta or FACS Fortessa X20. Data was analyzed with the FlowJo software (v10.8.1, v10.9.1, Tree Star). The following antibodies were used for immunostaining according to the manufacturer's instruction: anti-CD45, anti-CD3, anti-CD4, anti-CD8, anti-CD11b, anti-CD11c, anti-Gr1, anti-F4/80, anti-NK1.1, anti-CD19, anti-CD44, anti-CD62L, anti-CD25 and anti-IA/IE (all BioLegend). After staining cells were incubated for 20 min at 37 °C and 5% CO₂, washed twice with flow cytometry buffer (PBS + 2% FCS) and subsequently analyzed by FACS. Myeloid cells were defined as CD45⁺CD11b⁺, MDSCs as CD45⁺CD11b⁺ Gr1⁺ cells, DCs as CD45⁺CD11b⁺ CD11c⁺, eosinophils as CD45⁺CD11b⁺IA/IE⁺ and macrophages as CD45⁺CD11b⁺F4/80⁺Gr1^{low}. B cells were defined as CD45⁺CD19⁺, NK cells as CD45⁺CD3⁺NK1.1⁺, NKT cells as CD45⁺CD3⁺NK1.1⁺. Cytotoxic T cells were defined as

CD45⁺CD3⁺CD4⁻CD8⁺ cells and conventional T helper cells were defined as CD45⁺CD3⁺CD8⁻CD4⁺ cells. Regulatory T cells were defined as CD45⁺CD3⁺CD8⁻CD4⁺FoxP3⁺ cells. T cell subsets were determined by anti-CD25, anti-CD44 and anti-CD62L; thereby CD44⁻CD62L⁺ represent naïve T cells, CD44⁺CD62L⁺ central memory T cells, CD44⁺CD62L⁻ effector memory T cells and CD25⁺ activated T cells.

2.4. Metabolic parameter measurement

For metabolite quantification blood serum was collected and analyzed as follows: Blood glucose was measured enzymatically using commercially available reagents from Roche and an ADVIA 1650 (Bayer). Plasma cholesterol and triglyceride levels were analyzed by biochemical platforms at the Department of clinical chemistry at the University Hospital of Regensburg using commercially available kits.

2.5. Visualization and statistical analysis

Statistical analysis was performed using GraphPad Prism (V8, V10, GraphPad Software, La Jolla, CA). Results represent $n = 3-5$ mice unless otherwise indicated and are shown as the mean plus the standard error of the mean (SEM). Obese and control mice were compared using Mann-Whitney U test and significance was indicated for $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). Correlation analysis was performed using Spearman correlation. Figures were created with GraphPad Prism (V8, V10, GraphPad Software, La Jolla, CA) or Microsoft Excel 2021 (Microsoft, Redmond, Washington). The graphical abstract was created with Biorender (<https://biorender.com>).

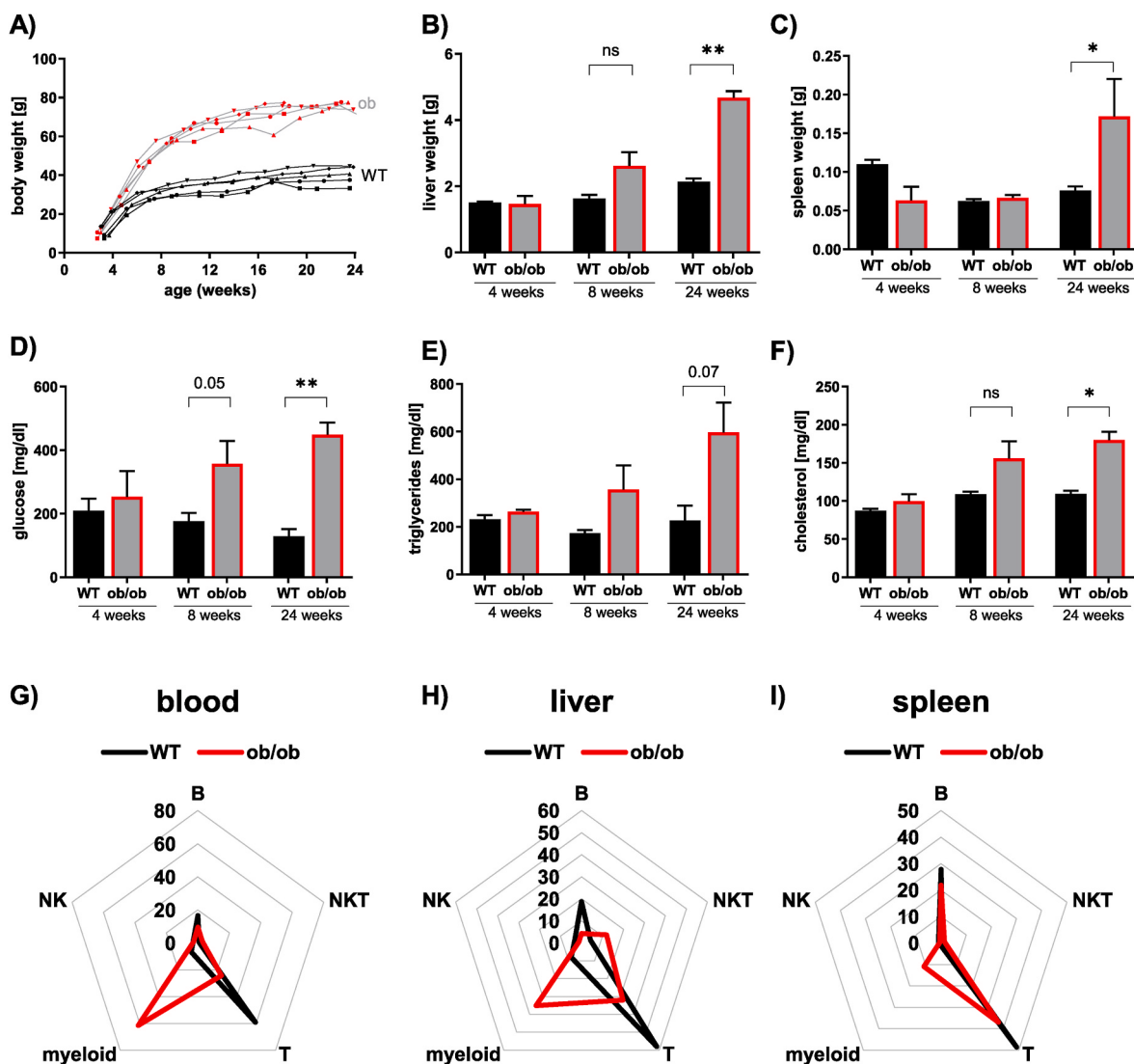


Fig. 1. Obesity-induced metabolic and immune landscape dysregulation. (A–F) Body composition and metabolic features of BTBR WT vs. ob/ob mice are shown. BTBR WT and ob/ob mice were fed a standard chow and analyzed in terms body (A), liver (B) and spleen (C) weight as well as metabolic serum parameters such as fasting glucose (D), triglycerides (E) and cholesterol (F) for 24 weeks. Results are shown as mean + SEM of $n = 3-5$. WT and OB mice were compared using Mann-Whitney U test. Significance is indicated for $p < 0.05$ (*), ns indicates no significant difference. (G–I) Immune cell landscape of obese vs. wildtype mice is depicted by spider net diagrams. Blood (G), liver (H) and spleen (I) immune cell distribution of BTBR WT and ob/ob mice was analyzed by flow cytometry at 24 weeks. Each cell fraction is plotted as proportion of total CD45⁺ cells and shown as the mean of $n = 3-5$ mice. BTBR wildtype (WT) mice are color-depicted in black, ob/ob (OB) mice in grey/red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Obesity-induced metabolic dysregulation alters systemic and local immune cell composition

To characterize onset of obesity and fatty liver disease in BTBR mice, wildtype (WT) vs. obese (ob/ob) mice were analyzed in terms of metabolic features. Mice were fed a standard chow for up to 24 weeks and changes in phenotype, body, liver, spleen weight and serum (blood glucose, cholesterol, triglycerides) metabolic parameters were monitored (Fig. 1 and Fig. S1). In line with previous reports, BTBR ob/ob mice could be differentiated from their WT littermates by phenotypic

changes (Fig. S1 A) and body weight gain (Fig. 1A and S1B) starting from week 8. In parallel increased liver weight (Fig. 1B) and hepatomegaly occurred (Fig. S1C), which is known to correlate with histopathological stigmata of steatosis (Opazo-Rí et al., 2022). Differences in spleen weight were found beyond 24 weeks (Fig. 1C). Body and liver weight changes of BTBR ob/ob mice were associated with enhanced fasting blood glucose levels from week 8 on (Fig. 1D). Between week 8 and 24 obese mice started to exhibit increased blood triglyceride (Fig. 1E) and cholesterol levels (Fig. 1F).

It is commonly known that obesity-induced metabolic dysregulation comes along with alterations in systemic and hepatic immune cell composition. However, data about obesity-induced splenic immune

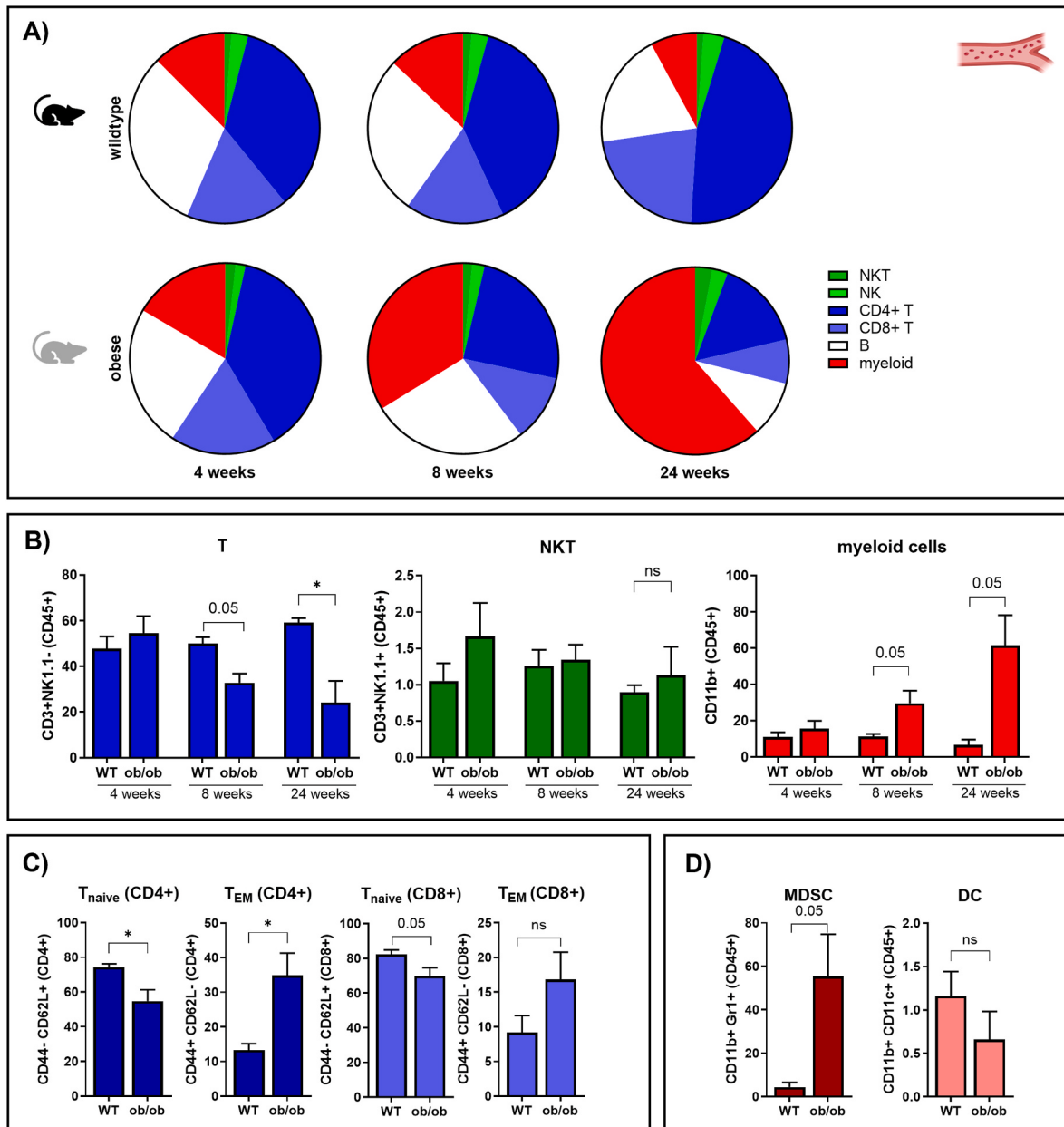


Fig. 2. Obesity-induced blood immune cell dysregulation. Peripheral blood mononuclear cells (PBMCs) of BTBR WT and ob/ob mice were isolated at 4, 8 and, respectively, 24 weeks and analyzed by flow cytometry. (A) Blood immune cell landscape of obese (ob/ob) vs. wildtype (WT) mice during aging (4–24 weeks) is depicted as parts of whole. Each cell fraction is given as proportion of CD45⁺ cells and plotted as the mean of $n = 3–5$ mice. (B) Kinetics of T, NKT and myeloid cells are shown at 4, 8 and 24 weeks. (C, D) Subsets of lymphoid (C) and myeloid (D) blood immune cells are shown at 24 weeks. Cells are shown as fraction of the total cells in brackets. The color of each column represents the corresponding cell fraction in (A). Each column represents the mean + SEM of $n = 3–5$ mice. WT and OB mice were compared using Mann-Whitney U test. Significance is indicated for $p < 0.05$ (*), ns indicates no significant difference. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dysregulation are rare up to now (Tarantino et al., 2021). To evaluate whether leptin-deficiency in BTBR ob/ob mice leads to immunomodulatory effects, we analyzed blood, liver and spleen leukocyte distribution by flow cytometry at 24 weeks (Fig. 1G–I). Spider net diagram analysis revealed significant obesity-induced alterations in all three compartments (Fig. 1G–I). While cell distribution in WT mice (black) showed similar, nearly symmetric immune profiles for blood (Fig. 1G), liver (Fig. 1H) and spleen (Fig. 1I), immunity shapes of ob/ob mice (red) significantly differed between the blood, spleen and liver compartment as well as in comparison to their WT littermates. These results strengthen the hypothesized role of the spleen-liver axis as modulator of obesity-induced immune dysregulation.

3.2. Obesity limits blood T cells but induces expansion of circulating MDSCs

To further analyze the kinetics of obesity-induced alterations in systemic immune cell homeostasis, peripheral leukocytes of BTBR ob/ob vs. WT mice were isolated and characterized by flow cytometry at 4, 8, 16, 20 and 24 weeks. Obesity modified both myeloid and lymphoid cell fractions in an age-dependent manner (Fig. 2A, data for 16- and 20-weeks old mice not shown). In WT mice aging reduced the percentage of myeloid and B cells and increased T cell fractions whereas NKT and NK cell composition did not change (Fig. 2B, Fig. S2A). In contrast, ob/ob mice displayed significantly increased myeloid cells and reduced T cells from week 8 on. Age-induced alterations in the NK, NKT cell (no difference) and B cell (slight but non-significant decrease) composition did not differ from WT mice (Fig. 2B, Fig. S2A).

More detailed quantification of obesity-induced T cell reprogramming showed that BTBR ob/ob mice revealed reduced numbers of circulating CD4⁺ and CD8⁺ T cells beyond 8 weeks of age (data not shown). However, within the activated T cell compartment (CD3⁺CD25⁺), obese mice displayed significantly higher fractions of CD4⁺(CD25⁺) but not CD8⁺(CD25⁺) T cells displaying an obesity-induced switch towards an enhanced CD4⁺/CD8⁺ ratio (Fig. S2B). Since no significant expansion of regulatory T cells (T_{reg}, CD4⁺FOXP3⁺) was observed (data not shown), the increased percentage of CD4⁺ T cells seemed to represent T helper cells. Characterization of T cell subsets, furthermore, indicated an obesity-induced switch from naïve (T_{naïve}, CD44⁺CD62L⁺) to central (T_{CM}, CD44⁺CD62L⁺) and effector memory (T_{EM}, CD44⁺CD62L[−]) phenotypes for both CD8⁺ and CD4⁺ T cells (Fig. 2C and Fig. S2B).

Dysregulation of T cells was associated with significant expansion of myeloid CD11b⁺ cells in BTBR ob/ob mice (Fig. 2B). Obesity-induced myeloid cells mainly represented myeloid-derived suppressor cells (MDSCs, CD11b⁺Gr1⁺, Fig. 2D). In contrast, no significant alteration in dendritic cells (DC, CD11b⁺CD11c⁺, Fig. 2D), non-myeloid DCs (CD11b[−]CD11c⁺, not shown) or eosinophils (CD11b⁺IA/IE⁺, Fig. S2C) was detected while circulating macrophages (F4/80⁺Gr1^{low}, Fig. S2C) tended to decrease.

All in all, obesity induced the expansion of circulating MDSCs while the percentage of T cells decreased. Within the T cell compartment obesity altered circulating T cell composition towards an enhanced ratio of activated CD4⁺/CD8⁺ T cells and a subset shift from naïve to central and effector memory T cells.

3.3. Fatty liver inflammation is only partly reflected in the blood immune cell landscape

Systemic but also local inflammation is known to contribute to the development of obesity-associated secondary diseases such as NAFLD. Opazo-Rios et al. have shown that BTBR ob/ob mice display histopathological stigmata of steatosis at 6 weeks and steatohepatitis at approximately 22 weeks of age, suggesting that BTBR mice are an excellent model for studying the transition from steatosis to steatohepatitis (Opazo-Ríos et al., 2022). To evaluate the correlation between

obesity-induced systemic and hepatic immune cell dysregulation, we analyzed liver immune cells at 4, 8 and 24 weeks (Fig. 3). Notably, obesity led to significant alterations in all hepatic cell fractions analyzed (Fig. 3A).

Similar to the obesity-induced shift in circulating immune cells, BTBR ob/ob mice displayed increased liver-resident myeloid and reduced T cell fractions in comparison to their WT littermates at 24 weeks (Fig. 3B, Fig. S3A). In contrast to blood immune cell composition, NKT cells were increased in fatty livers, while hepatic B and NK cells were reduced (Fig. 3B, Fig. S3A).

Within the T cell compartment, obesity decreased total numbers of CD8⁺ but not CD4⁺ T cells (data not shown) and increased the activated CD4⁺/CD8⁺ ratio (Fig. S3B). Subsets were shifted from naïve towards effector memory T cells (Fig. 3C, Fig. S3B). Detailed characterization of CD11b⁺ cells again revealed an expansion of MDSCs while macrophages, DCs and eosinophils decreased or remained constant (Fig. 3D, Fig. S3C).

Taken together, hepatic immune cell dysregulation in fatty liver disease was mainly characterized by the expansion of MDSC and NKT cells as well as concomitant dysregulation of T cells. While reprogramming of liver-resident T cells and MDSCs reflected obesity-induced alterations in circulating immune cells, the expanded NKT cell population was a special feature of fatty liver inflammation that could not be found in the blood compartment.

3.4. Obesity induces splenic enrichment of MDSCs and NKT cells but does not alter splenic T or B cell composition

The spleen is the central organ of immune cell regulation and is anatomically directly linked to the liver and visceral adipose tissue via the portal vein circulation. To investigate whether systemic and hepatic immune cell dysregulation was reflected in splenic immune cell composition we isolated splenic leukocytes and analyzed them by flow cytometry (Fig. 4A).

Interestingly, in contrast to the blood and liver immune cell landscape, no significant differences in B or T cell composition between WT and obese spleens were detected (Fig. 4B + C, Fig. S4A + B). However, obese mice exhibited significantly reduced NK cell fractions at 24 weeks whereas spleen resident DCs (Fig. S4A), MDSCs and NKT cells increased (Fig. 4B + D). Notably, splenic MDSCs and NKT cells in WT mice decreased during aging (Fig. 4B + D). Thus, obesity-induced splenic enrichment of NKT cells and MDSCs counteracts the age-associated decrease of both cell populations in WT littermates (Fig. 4B + D). While MDSC expansion was also reflected in the blood, the expanded NKT cell population could not be found in the blood but in the liver compartment.

3.5. Obesity-induced splenic NKT and MDSC enrichment correlates with fatty liver immune dysregulation

Distinct immune dysregulation in the blood, spleen and liver compartment indicated that the spleen-liver axis might play an important role in mediating obesity-induced inflammation. Thereby, comparison of blood, liver and spleen immune landscape of BTBR ob/ob in relation to WT mice suggested a positive spleen-liver correlation for macrophages, MDSCs, NK and NKT cells (Fig. 5A).

For analyzing the spleen-liver axis in more detail, net diagrams of hepatic and splenic immune cell distribution were generated (Fig. 5B + C). In WT mice (Fig. 5B), the splenic and hepatic immune cell distribution showed similar symmetric patterns. Overlay of spleen and liver immunity shapes in obese mice (Fig. 5C) confirmed an obesity-induced hepatic expansion of NKT and MDSCs which was accompanied by a parallel increase of splenic MDSCs and NKT cells. Vice versa, the reduction of hepatic macrophages and NK cells resulted in concomitant splenic reduction of these cell types. In contrast hepatic reprogramming of B, T and other myeloid cells was not reflected in spleen immune cell

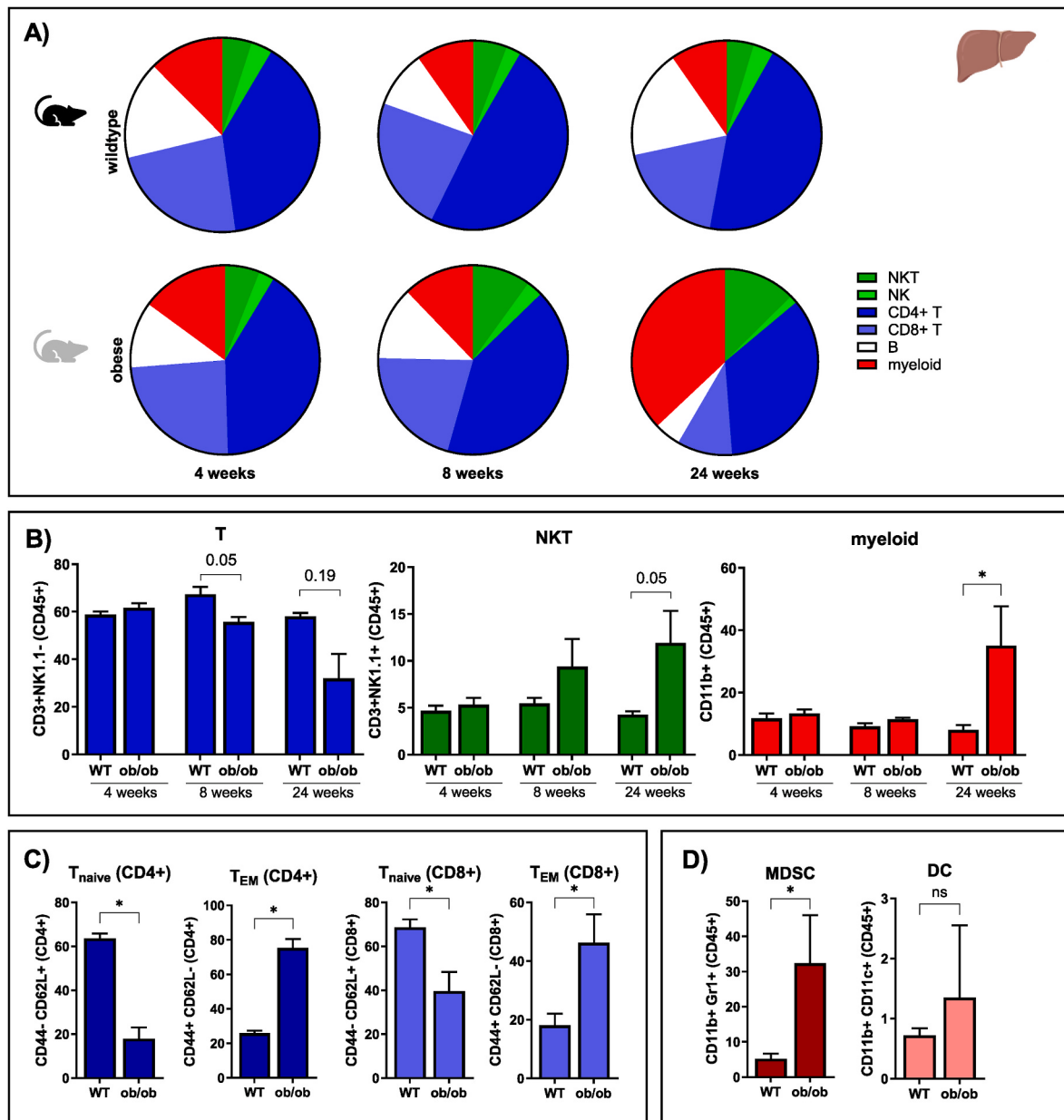


Fig. 3. Obesity-induced liver immune cell dysregulation. Liver immune cells of BTBR WT and ob/ob mice were isolated at 4, 8 and, respectively, 24 weeks and analyzed by flow cytometry. (A) Liver immune cell landscape of obese vs. wildtype mice during aging (4–24 weeks) is depicted as parts of whole. Each cell fraction is given as proportion of CD45⁺ cells and plotted as the mean of n = 3–5 mice. (B) Kinetics of T, NKT and myeloid cells are shown at 4, 8 and 24 weeks. (C, D) Subsets of lymphoid (C) and myeloid (D) liver immune cells are shown at 24 weeks. Cells are shown as fraction of the total cells in brackets. The color of each column represents the corresponding cell fraction in (A). Each column represents the mean ± SEM of n = 3–5 mice. WT and OB mice were compared using Mann-Whitney U test. Significance is indicated for p < 0.05 (*), ns indicates no significant difference. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

composition.

To further support our hypothesis about the spleen-liver axis, we next performed correlation analyses of spleen vs. liver immune cells (Fig. 5D). Spearman correlation confirmed a strong positive correlation between liver and spleen resident MDSCs and weak positive correlation for NKT cell accumulation (Fig. 5D). All in all, our results suggest that splenic enrichment of NKT and MDSC cells impacts obesity-induced immune dysregulation via the spleen-liver axis.

To test whether the results obtained in our genetically induced obesity model could be reproduced independently of the experimental setting, we next analyzed immune cell composition of spleens and livers in C57BL/6 mice fed either with standard or an obesity-inducing diet (SFig. 5). Similar to the results obtained in BTBR ob/ob mice, mice fed

with an Western type diet revealed obesity-induced hepatic and splenic NKT and MDSC accumulation by trend.

4. Discussion

Increasing evidence demonstrates that the interplay of metabolic dysregulation and chronic inflammation is one of the main drivers of obesity and its secondary diseases (Reilly and Saltiel, 2017; Gregor and Hotamisligil, 2011). Understanding the underlying mechanisms between immunologic and metabolic reprogramming is mandatory to prevent the development of and evolve therapeutic strategies for obesity-associated complications such as NAFLD, cardiovascular diseases, diabetes mellitus or cancer. Our study shows that obesity-induced

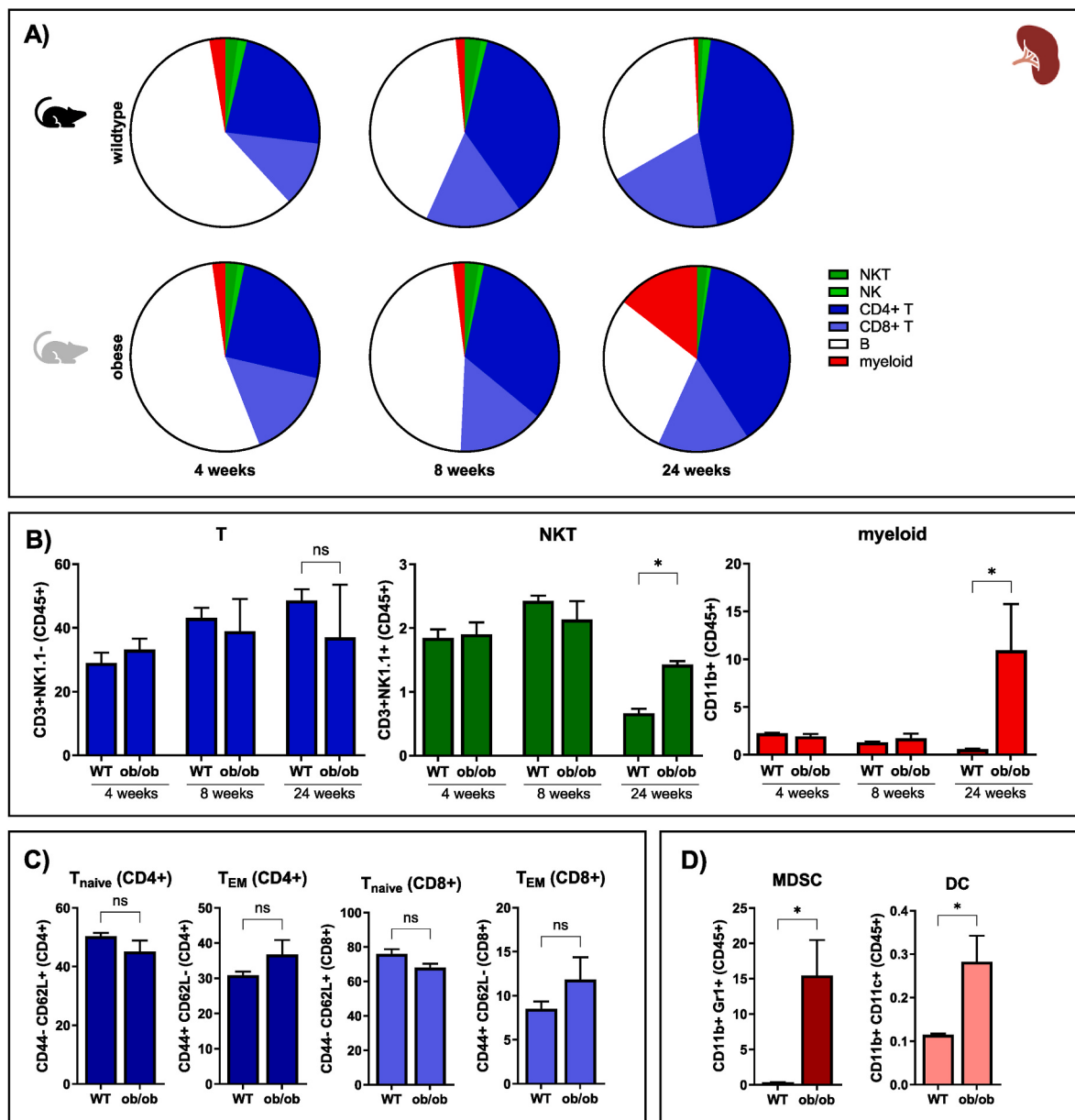


Fig. 4. Obesity-induced spleen immune cell dysregulation. Spleen immune cells of BTBR WT and ob/ob mice were isolated at 4, 8 and, respectively, 24 weeks and analyzed by flow cytometry. (A) Spleen immune cell landscape of obese vs. wildtype mice during aging (4–24 weeks) is depicted as parts of whole. Each cell fraction is given as proportion of CD45⁺ cells and plotted as the mean of n = 3–5 mice. (B) Kinetics of T, NKT and myeloid cells are shown at 4, 8 and 24 weeks. (C, D) Subsets of lymphoid (C) and myeloid (D) splenic immune cells are shown at 24 weeks. Cells are shown as fraction of the total cells in brackets. The color of each column represents the corresponding cell fraction in (A). Each column represents the mean + SEM of n = 3–5 mice. WT and OB mice were compared using Mann-Whitney U test. Significance is indicated for p < 0.05 (*), ns indicates no significant difference. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

inflammation is not only associated with systemic and hepatic immune dysregulation but also alterations of the splenic immune cell landscape. Notably, corresponding shifts in liver- and spleen-resident immune cell fractions of BTBR ob/ob mice are limited to selective leukocyte subtypes including NK, NKT, MDSC and macrophages, but are not observed in other myeloid cell populations or the B and T cell compartment. These data uncover new aspects of the spleen-liver axis which may play an important, cell-specific role in modulating obesity-induced immune dysregulation.

Already years ago Mito et al. demonstrated that NAFLD progression is associated with changes in splenic cytokine production and suggested that the crosstalk between the spleen and liver plays an important role in obesity-mediated immune dysregulation (Mito et al., 2000). Since then,

detailed studies on the spleen-liver axis investigating obesity-induced alterations of different immune cell subpopulations and their inter-organ crosstalk have been rare (Tarantino et al., 2021). In line with our results, Baccan et al. reported that a high-fat diet leads to a decrease in splenic NK cell activity (Baccan et al., 2013). Obesity-mediated effects on splenic regulatory T cells are contradictory and include reports about an increase (Deuiliis et al., 2011) or decrease (He et al., 2017) in obese rodents whereas in our study obesity had no impact on splenic regulatory T cells.

The role of MDSCs in obesity, a heterogeneous population of immature myeloid cells that suppress T cell immunity, was broadly discussed over the last years (Sanchez et al., 2021; Shibata et al., 2022; Ostrand and Rosenberg, 2018). Consistent with our results several

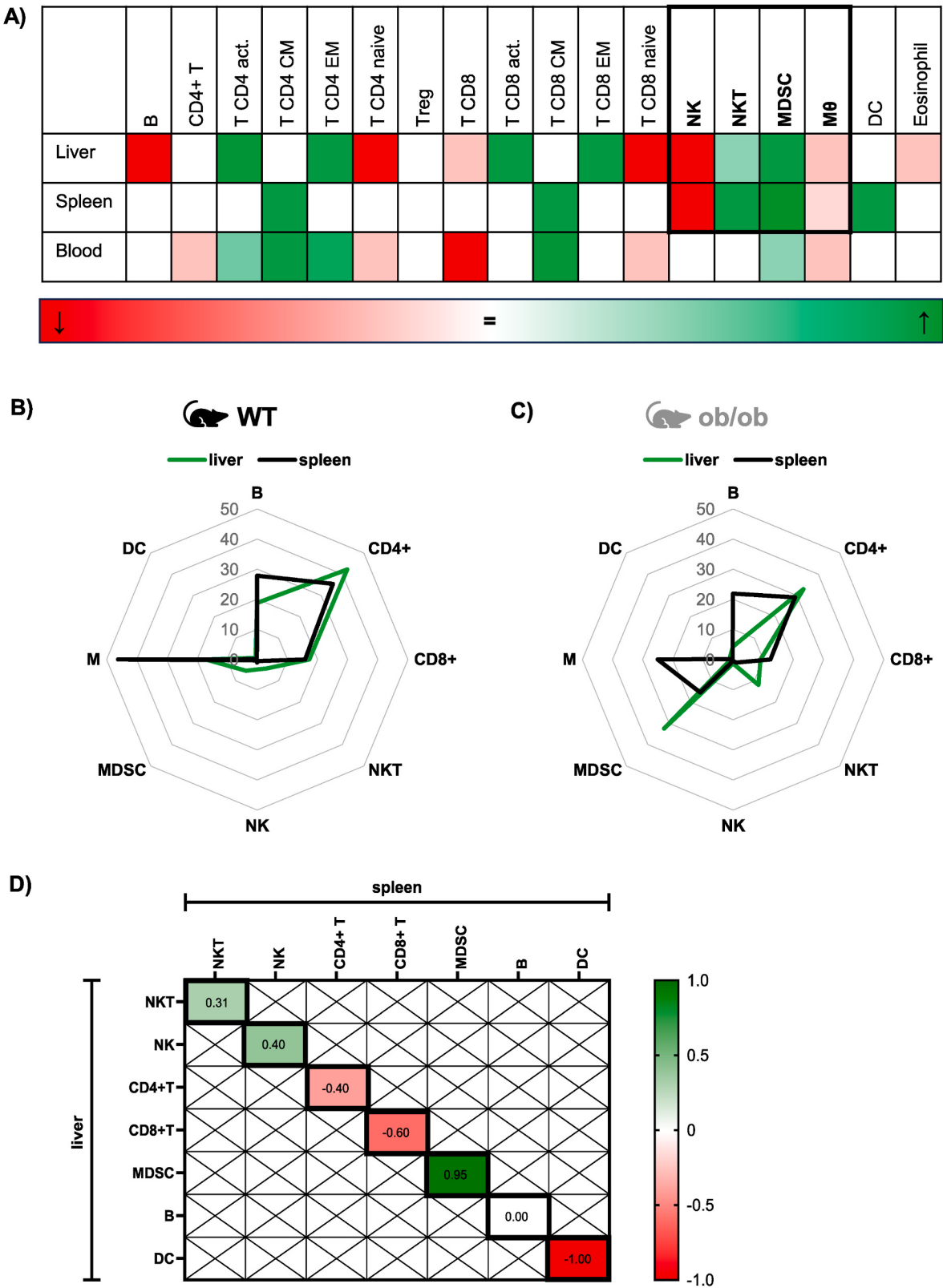


Fig. 5. Spleen-liver axis in obesity-induced immune dysregulation. A) Summary of raw data shown in Figs. 2–4. Significantly increased (↑) cell percentages in BTBR ob/ob mice in comparison to WT littermates at 24 weeks are color-depicted in green, decreased (↓) in red, white indicates no significant difference. B-C) Spider net diagrams of spleen and liver immune cell distribution indicate obesity-induced splenic accumulation for NKT and MDSC cells. D) Spleen-liver correlation matrix between different immune cells in BTBR ob/ob mice. Spearman correlation coefficients r are shown as color-depicted in the legend. Green indicates positive correlation, red negative correlation, white no correlation. Correlations not analyzed are depicted as [X]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

studies have reported that MDSCs accumulate in blood, livers as well as adipose tissue of obese mice (Hundertmark et al., 2018; Krenkel et al., 2020; Xia et al., 2011; Chen et al., 2015; Clements et al., 2018) and humans (Bao et al., 2015). We here show that obesity associated expansion of circulating MDSCs is not only associated with hepatic but also splenic MDSC expansion. Splenic MDSC enrichment in obese mice has been reported before (Hale et al., 2015; Turbitt et al., 2019). However, previous studies were conducted in tumor-bearing mice (renal/pancreatic cancer) and obesity-induced splenic MDSC accumulation has not been shown or discussed in the context of the spleen-liver axis and NAFLD development yet.

In obesity MDSCs can be regarded as a double-edged sword: On the one hand, MDSCs display protective effects since they counteract chronic inflammation, impair T cell activity, what is in line with our findings, and improve metabolic dysregulation (Xia et al., 2011; Clements et al., 2018). On the other hand, elevated MDSC levels eventually contribute to enhanced tumor progression, shown not only in the context of NAFLD/HCC (Sun et al., 2018), but also for several other cancer entities such as oral squamous cell (Peng et al., 2021) and renal carcinoma (Hale et al., 2015), pancreatic (Turbitt et al., 2019; Incio et al., 2016), oesophageal (Jianmin et al., 2023), ovarian (Yang et al., 2021), and breast cancer (Clements et al., 2018; Gibson et al., 2020). Underlying triggers of obesity-associated MDSC transition from protective towards harmful have been broadly discussed including different phenotypes as well metabolic, environmental and epigenetic influencing factors, but are still not fully understood (Sanchez-et al., 2021; Yao et al., 2016). In this context, Clement et al. proclaimed that obesity-associated MDSC enrichment is promoted by leptin (Clements et al., 2018). However, we observed obesity-induced MDSC expansion in a leptin-deficient mouse model indicating that leptin-independent mechanisms contribute to MDSC recruitment. By showing that obesity-induced hepatic accumulation of MDSCs strongly correlates with splenic MDSC enrichment, our results suggest that the spleen might modulate MDSC expansion in fatty livers. However, since splenic accumulation of MDSC was also associated with enhanced levels of circulating MDSCs, we cannot differentiate whether mainly spleen- or blood-derived MDSCs contribute to obesity-induced fatty liver inflammation. Additionally, a detailed analysis of MDSC subpopulations was not performed in this study. Historically, MDSCs have been characterized by the co-expression of Gr1 and CD11b, which are the markers used in our research (Bronte et al., 2016). Meanwhile, distinct MDSC subpopulations have been identified, including polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs, $CD11b^{+}Ly6C^{low}Ly6G^{+}$) and monocytic myeloid-derived suppressor cells (M-MDSCs, $CD11b^{+}Ly6C^{high}Ly6G^{-}$) (Bronte et al., 2016). The primary focus of this study was to examine obesity-induced immune dysregulation within the T cell compartment. As a result, we did not delve into the detailed investigation of MDSC subpopulations. However, this could be the objective of further studies.

Unlike MDSCs, splenic and hepatic enrichment of NKT cells was not reflected in the blood immune cell landscape. Regarding the direct anatomic linkage between spleen and liver via the portal vein circulation, this indicates that obesity induced NKT cells in fatty livers are mainly derived from the spleen. In line with our results, expansion of hepatic NKT cells in NAFLD has been shown before (Bhattacharjee et al., 2017; Wolf et al., 2014); however, to our knowledge, we are the first to report a correlation to obesity-induced splenic NKT enrichment. The role of NKT cells in obesity and fatty liver disease is complex and has been controversially discussed for years (Gu et al., 2022; Kumar, 2013; Satoh and Iwabuchi, 2018). NKT cells are a heterogeneous group of CD1d-restricted, lipid antigen-reactive immunoregulatory T lymphocytes that link innate and adaptive immunity by producing Th1 and Th2 type cytokines, mediate immune responses in inflammation, infection, autoimmunity and cancer and are known to be highly enriched in adipose tissue and the liver (Park et al., 2018; Lynch et al., 2012; Godfrey et al., 2010). Liver-resident NKT cells display distinct phenotypes with conflicting roles ranging from protective to pathogenic in different liver

diseases (Bandyopadhyay et al., 2016). In line with our results obesity-induced NKT enrichment has been associated with NASH progression before (Bhattacharjee et al., 2017; Wolf et al., 2014; Syn et al., 2010, 2012). In contrast, other studies reported NKT depletion during NAFLD development (Zheng et al., 2022; Liang et al., 2014; Li et al., 2005; Ma et al., 2008) and mice lacking NKT cells to be more susceptible to steatosis, liver inflammation and liver fibrosis (Miyagi et al., 2010; Kotas et al., 2011; Martin-Murphy et al., 2014). Furthermore, Tang et al. have shown that obesity-induced suppression of liver-resident NKT cells via excessive hepatic cholesterol accumulation and overexpression of sterol regulatory element-binding protein 2 (SREBP2) is associated with impaired anti-tumor immunosurveillance (Tang et al., 2022).

Taken together, the role of MDSCs and NKT cells in obesity and the liver microenvironment is still not fully understood. Possible explanations for opposing reports about obesity-induced alterations in these two cell populations include mouse model specific variations, dietary and environmental associated factors, contrary roles of MDSC and NKT phenotypes and analysis at different time points of NAFLD progression (Bhattacharjee et al., 2017; Gu et al., 2022; Bandyopadhyay et al., 2016; Zheng et al., 2022). In this context, it has to be emphasized that our data have been obtained in BTBR ob/ob mice, a genetic mouse model for obesity. To exclude that these results were limited to leptin-deficient mice we conducted complementary experiments in a Western type diet induced obesity mouse model (Dorn et al., 2014) and received similar results by trend.

In conclusion, the findings of this study uncover a potential role of the spleen-liver axis in modulating obesity-mediated systemic and fatty liver immune dysregulation by splenic accumulation of MDSCs and NKT cells. Thereby, our results might explain data on the impact of splenectomy in obese mice: Inoue et al. reported that splenectomy worsened dyslipidemia and accelerated hepatic inflammation (Inoue et al., 2012). In line, Wang and colleagues showed that splenectomy promoted NAFLD progression by downregulation of PTEN (Wang et al., 2015). Interestingly, Gotoh et al. have shown that splenectomy-induced progression of hepatic inflammation could be reversed by IL-10 (Gotoh et al., 2012). Since immunosuppressive effects of MDSCs have been associated with PTEN signaling cascade (Mei et al., 2015; Yang et al., 2018; Liu et al., 1950) and NKT subpopulations are known to produce IL-10 (LaMarche et al., 2020), splenic depletion of MDSC and NKT cells could well explain the reported effects of splenectomy in obese rodents. Both NKT and MDSC cells are innate-like leukocytes and splenic enrichment was exclusively restricted to those two cell populations. This indicates that the spleen might play a cell-specific role in supporting obesity-induced inflammation. Further studies analyzing MDSC and NKT subpopulations are needed to better understand the role of the spleen-liver axis in obesity and obesity-associated secondary diseases.

Besides fostering the immunologic understanding of obesity, our data are also relevant from a clinical point of view: Since the observed immune alterations in BTBR ob/ob mice correlated with splenomegaly, monitoring of spleen size in overweight patients, e.g. by ultrasound, might be a cheap, easily accessible and non-invasive tool to detect and prevent progression of obesity-associated secondary diseases such as metabolic-associated fatty liver disease (Mendes et al., 2012). However, this warrants further investigations in clinical trials.

CRedit authorship contribution statement

Christina Brummer: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Katrin Singer:** Writing – review & editing, Methodology, Data curation. **Kathrin Renner:** Writing – review & editing, Data curation. **Christina Bruss:** Writing – review & editing, Data curation. **Claus Hellerbrand:** Writing – review & editing, Data curation. **Christoph Dorn:** Writing – review & editing, Data curation. **Simone Reichelt-Wurm:** Writing – review & editing, Data curation. **Wolfram Gronwald:** Data curation, Writing – review & editing. **Tobias Pukrop:** Writing – review & editing,

Supervision. **Wolfgang Herr:** Writing – review & editing, Resources. **Miriam Banas:** Writing – review & editing, Data curation, Conceptualization. **Marina Kreutz:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mce.2025.112518>.

Data availability

Data will be made available on request.

References

- Afify, S.M., Hassan, G., Seno, A., Seno, M., 2022. Cancer-inducing niche: the force of chronic inflammation. *Br. J. Cancer* 127 (2), 193–201.
- Alwarawrah, Y., Kiernan, K., MacIver, N.J., 2018. Changes in nutritional status impact immune cell metabolism and function. *Front. Immunol.* 9, 1055.
- Baccan, G.C., Hernández, O., Díaz, L.E., Gheorghe, A., Pozo-Rubio, T., Marcos, A., et al., 2013. Changes in lymphocyte subsets and functions in spleen from mice with high fat diet-induced obesity. *Proc. Nutr. Soc.* 72 (OCE1), E63.
- Bandyopadhyay, K., Marrero, I., Kumar, V., 2016. NKT cell subsets as key participants in liver physiology and pathology. *Cell. Mol. Immunol.* 13 (3), 337–346.
- Bao, Y., Mo, J., Ruan, L., Li, G., 2015. Increased monocytic CD14⁺HLADR^{low}/− myeloid-derived suppressor cells in obesity. *Mol. Med. Rep.* 11 (3), 2322–2328.
- Barrea, L., Di Somma, C., Muscogiuri, G., Tarantino, G., Tenore, G.C., Orio, F., et al., 2018. Nutrition, inflammation and liver-spleen axis. *Crit. Rev. Food Sci. Nutr.* 58 (18), 3141–3158.
- Behary, J., Amorim, N., Jiang, X.T., Raposo, A., Gong, L., McGovern, E., et al., 2021. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat. Commun.* 12 (1), 187.
- Bertola, A., Ciucci, T., Rousseau, D., Bourlier, V., Duffaut, C., Bonnafous, S., et al., 2012. Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 61 (9), 2238–2247.
- Bhattacharjee, J., Kirby, M., Softic, S., Miles, L., Salazar-Gonzalez, R., Shivakumar, P., et al., 2017. Hepatic natural killer T-cell and CD8⁺ T-cell signatures in mice with nonalcoholic steatohepatitis. *Hepatol Commun.* 1 (4), 299–310.
- Borek, F., 1986. Previously unrecognized functions of the spleen: development and maintenance of immune competence and regulation. *Crit. Rev. Immunol.* 6 (4), 287–293.
- Boutens, L., Stienstra, R., 2016. Adipose tissue macrophages: going off track during obesity. *Diabetologia* 59 (5), 879–894.
- Bronte, V., Brandau, S., Chen, S.H., Colombo, M.P., Frey, A.B., Greten, T.F., et al., 2016. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* 7 (1), 12150.
- Buzzetti, E., Pinzani, M., Tsochatzidis, E.A., 2016. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 65 (8), 1038–1048.
- Byun, J.S., Yi, H.S., 2017. Hepatic immune microenvironment in alcoholic and nonalcoholic liver disease. *BioMed Res. Int.* 2017, 1–12.
- Chen, S., Akbar, S.M.F., Miyake, T., Abe, M., Al-Mahtab, M., Furukawa, S., et al., 2015. Diminished immune response to vaccinations in obesity: role of myeloid-derived suppressor and other myeloid cells. *Obes. Res. Clin. Pract.* 9 (1), 35–44.
- Clements, V.K., Long, T., Long, R., Figley, C., Smith, D.M.C., Ostrand-Rosenberg, S., 2018. Frontline Science: high fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. *J. Leukoc. Biol.* 103 (3), 395–407.
- Deiuliis, J., Shah, Z., Shah, N., Needleman, B., Mikami, D., Narula, V., et al., 2011. Visceral adipose inflammation in obesity is associated with critical alterations in regulatory cell numbers. *PLoS One* 6 (1), e16376.
- Dorn, C., Engelmann, J.C., Saugspier, M., Koch, A., Hartmann, A., Müller, M., et al., 2014. Increased expression of c-Jun in nonalcoholic fatty liver disease. *Lab. Invest.* 94 (4), 394–408.
- Gerriets, V.A., MacIver, N.J., 2014. Role of T Cells in malnutrition and obesity. *Front. Immunol. [Internet]* 5 [cited 2023 Nov 5] Available from: <http://journal.frontiersin.org/article/10.3389/fimmu.2014.00379/abstract>.
- Gibson, J.T., Orlandella, R.M., Turbitt, W.J., Behring, M., Manne, U., Sorge, R.E., et al., 2020. Obesity-associated myeloid-derived suppressor cells promote apoptosis of tumor-infiltrating CD8 T cells and immunotherapy resistance in breast cancer. *Front. Immunol.* 11, 590794.
- Godfrey, D.I., Stankovic, S., Baxter, A.G., 2010. Raising the NKT cell family. *Nat. Immunol.* 11 (3), 197–206.
- Gotoh, K., Inoue, M., Masaki, T., Chiba, S., Shimasaki, T., Ando, H., et al., 2012. A novel anti-inflammatory role for spleen-derived interleukin-10 in obesity-induced inflammation in white adipose tissue and liver. *Diabetes* 61 (8), 1994–2003.
- Gregor, M.F., Hotamisligil, G.S., 2011. Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* 29 (1), 415–445.
- Gu, X., Chu, Q., Ma, X., Wang, J., Chen, C., Guan, J., et al., 2022. New insights into iNKT cells and their roles in liver diseases. *Front. Immunol.* 13, 1035950.
- Hale, M., Itani, F., Buchta, C.M., Wald, G., Bing, M., Norian, L.A., 2015. Obesity triggers enhanced MDSC accumulation in murine renal tumors via elevated local production of CCL2. *PLoS One* 10 (3), e0118784.
- Han, J.M., Levings, M.K., 1950. Immune regulation in obesity-associated adipose inflammation. *J. Immunol. Baltim Md* 191 (2), 527–532.
- He, B., Wu, L., Xie, W., Shao, Y., Jiang, J., Zhao, Z., et al., 2017. The imbalance of Th17/Treg cells is involved in the progression of nonalcoholic fatty liver disease in mice. *BMC Immunol.* 18 (1), 33.
- Huh, J.Y., Kim, J.I., Park, Y.J., Hwang, I.J., Lee, Y.S., Sohn, J.H., et al., 2013. A novel function of adipocytes in lipid antigen presentation to iNKT cells. *Mol. Cell Biol.* 33 (2), 328–339.
- Huh, J.Y., Park, Y.J., Ham, M., Kim, J.B., 2014. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. *Mol. Cells* 37 (5), 365–371.
- Hundertmark, J., Krenkel, O., Tacke, F., 2018. Adapted immune responses of myeloid-derived cells in fatty liver disease. *Front. Immunol.* 9, 2418.
- Incio, J., Liu, H., Suboj, P., Chin, S.M., Chen, I.X., Pinter, M., et al., 2016. Obesity-induced inflammation and desmoplasia promote pancreatic cancer progression and resistance to chemotherapy. *Cancer Discov.* 6 (8), 852–869.
- Inoue, M., Gotoh, K., Seike, M., Masaki, T., Honda, K., Kakuma, T., et al., 2012. Role of the spleen in the development of steatohepatitis in high-fat-diet-induced obese rats. *Exp Biol Med Maywood NJ* 237 (4), 461–470.
- Jianmin, P., Qinchao, H., Chunyang, W., Jiayu, Z., Siyu, W., Li, W., et al., 2023. Depletion of Gr1⁺ myeloid cells attenuates high-fat-diet-aggravated esophageal squamous cell carcinoma in mice. *Carcinogenesis* 44 (7), 587–595.
- Keller, M.P., Hudkins, K.L., Shalev, A., Bhatnagar, S., Kebede, M.A., Merrins, M.J., et al., 2023. What the BTBR/J mouse has taught us about diabetes and diabetic complications. *iScience* 26 (7), 107036.
- Kotas, M.E., Lee, H.Y., Gillum, M.P., Annicelli, C., Guigni, B.A., Shulman, G.I., et al., 2011. Impact of CD1d deficiency on metabolism. In: Jayaseelan, S. (Ed.), *PLoS One* 6 (9), e25478.
- Krenkel, O., Hundertmark, J., Abdallah, A.T., Kohlhepp, M., Puengel, T., Roth, T., et al., 2020. Myeloid cells in liver and bone marrow acquire a functionally distinct inflammatory phenotype during obesity-related steatohepatitis. *Gut* 69 (3), 551–563.
- Kumar, V., 2013. NKT-cell subsets: promoters and protectors in inflammatory liver disease. *N. J. Hepatol.* 59 (3), 618–620.
- LaMarche, N.M., Kane, H., Kohlgruber, A.C., Dong, H., Lynch, L., Brenner, M.B., 2020. Distinct iNKT cell populations use IFN γ or ER stress-induced IL-10 to control adipose tissue homeostasis. *Cell Metab.* 32 (2), 243–258.e6.
- Li, Z., Soloski, M.J., Diehl, A.M., 2005. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatol Baltim Md* 42 (4), 880–885.
- Liang, S., Webb, T., Li, Z., 2014. Probiotic antigens stimulate hepatic natural killer T cells. *Immunology* 141 (2), 203–210.
- Liu, Y., Lai, L., Chen, Q., Song, Y., Xu, S., Ma, F., et al., 1950. MicroRNA-494 is required for the accumulation and functions of tumor-expanded myeloid-derived suppressor cells via targeting of PTEN. *J. Immunol. Baltim Md* 188 (11), 5500–5510.
- Lynch, L., O'Shea, D., Winter, D.C., Geoghegan, J., Doherty, D.G., O'Farrelly, C., 2009. Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur. J. Immunol.* 39 (7), 1893–1901.
- Lynch, L., Nowak, M., Varghese, B., Clark, J., Hogan, A.E., Toxavidis, V., et al., 2012. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* 37 (3), 574–587.
- Ma, X., Hua, J., Li, Z., 2008. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J. Hepatol.* 49 (5), 821–830.
- Martin-Murphy, B.V., You, Q., Wang, H., De La Houssaye, B.A., Reilly, T.P., Friedman, J.E., et al., 2014. Mice lacking natural killer T cells are more susceptible to metabolic alterations following high fat diet feeding. In: Ye, J. (Ed.), *PLoS One* 9 (1), e80949.
- Mei, S., Xin, J., Liu, Y., Zhang, Y., Liang, X., Su, X., et al., 2015. MicroRNA-200c promotes suppressive potential of myeloid-derived suppressor cells by modulating PTEN and FOG2 expression. *PLoS One* 10 (8), e0135867.
- Mendes, F.D., Suzuki, A., Sanderson, S.O., Lindor, K.D., Angulo, P., 2012. Prevalence and indicators of portal hypertension in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc.* 10 (9), 1028–1033.e2.
- Mito, N., Hosoda, T., Kato, C., Sato, K., 2000. Change of cytokine balance in diet-induced obese mice. *Metabolism* 49 (10), 1295–1300.

- Miyagi, T., Takehara, T., Uemura, A., Nishio, K., Shimizu, S., Kodama, T., et al., 2010. Absence of invariant natural killer T cells deteriorates liver inflammation and fibrosis in mice fed high-fat diet. *J. Gastroenterol.* 45 (12), 1247–1254.
- Opazo-Ríos, L., Soto-Catalán, M., Lázaro, I., Sala-Vila, A., Jiménez-Castilla, L., Orejudo, M., et al., 2022. Meta-inflammation and de novo lipogenesis markers are involved in metabolic associated fatty liver disease progression in BTBR ob/ob mice. *Int. J. Mol. Sci.* 23 (7), 3965.
- Ostrand-Rosenberg, S., 2018. Myeloid derived-suppressor cells: their role in cancer and obesity. *Curr. Opin. Immunol.* 51, 68–75.
- Park, Y.J., Park, J., Huh, J.Y., Hwang, I., Choe, S.S., Kim, J.B., 2018. Regulatory roles of invariant natural killer T cells in adipose tissue inflammation: defenders against obesity-induced metabolic complications. *Front. Immunol.* 9, 1311.
- Parthasarathy, G., Revelo, X., Malhi, H., 2020. Pathogenesis of nonalcoholic steatohepatitis: an overview. *Hepatol Commun* 4 (4), 478–492.
- Peng, J., Hu, Q., Chen, X., Wang, C., Zhang, J., Ren, X., et al., 2021. Diet-induced obesity accelerates oral carcinogenesis by recruitment and functional enhancement of myeloid-derived suppressor cells. *Cell Death Dis.* 12 (10), 946.
- Reilly, S.M., Saltiel, A.R., 2017. Adapting to obesity with adipose tissue inflammation. *Nat. Rev. Endocrinol.* 13 (11), 633–643.
- Sanchez-Pino, M.D., Dean, M.J., Ochoa, A.C., 2021. Myeloid-derived suppressor cells (MDSC): when good intentions go awry. *Cell. Immunol.* 362, 104302.
- Satoh, M., Iwabuchi, K., 2018. Role of natural killer T cells in the development of obesity and insulin resistance: insights from recent progress. *Front. Immunol.* 9, 1314.
- Schipper, H.S., Rakhshandehroo, M., van de Graaf, S.F.J., Venken, K., Koppen, A., Stienstra, R., et al., 2012. Natural killer T cells in adipose tissue prevent insulin resistance. *J. Clin. Investig.* 122 (9), 3343–3354.
- Shibata, M., Nanno, K., Yoshimori, D., Nakajima, T., Takada, M., Yazawa, T., et al., 2022. Myeloid-derived suppressor cells: cancer, autoimmune diseases, and more. *Oncotarget* 13, 1273–1285.
- Stefanovic-Racic, M., Yang, X., Turner, M.S., Mantell, B.S., Stolz, D.B., Sumpter, T.L., et al., 2012. Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes* 61 (9), 2330–2339.
- Stolarczyk, E., 2017. Adipose tissue inflammation in obesity: a metabolic or immune response? *Curr. Opin. Pharmacol.* 37, 35–40.
- Sun, H., Yang, W., Tian, Y., Zeng, X., Zhou, J., Mok, M.T.S., et al., 2018. An inflammatory-CCRk circuitry drives mTORC1-dependent metabolic and immunosuppressive reprogramming in obesity-associated hepatocellular carcinoma. *Nat. Commun.* 9 (1), 5214.
- Syn, W.K., Oo, Y.H., Pereira, T.A., Karaca, G.F., Jung, Y., Omenetti, A., et al., 2010. Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. *Hepatol Baltim Md* 51 (6), 1998–2007.
- Syn, W.K., Agboola, K.M., Swiderska, M., Michelotti, G.A., Liaskou, E., Pang, H., et al., 2012. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 61 (9), 1323–1329.
- Tang, W., Zhou, J., Yang, W., Feng, Y., Wu, H., Mok, M.T.S., et al., 2022. Aberrant cholesterol metabolic signaling impairs antitumor immunosurveillance through natural killer T cell dysfunction in obese liver. *Cell. Mol. Immunol.* 19 (7), 834–847.
- Tarantino, G., Citro, V., Balsano, C., 2021. Liver-spleen axis in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 15 (7), 759–769.
- Tilg, H., Adolph, T.E., Dudek, M., Knolle, P., 2021. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. *Nat. Metab.* 3 (12), 1596–1607.
- Turbitt, W.J., Collins, S.D., Meng, H., Rogers, C.J., 2019. Increased adiposity enhances the accumulation of MDSCs in the tumor microenvironment and adipose tissue of pancreatic tumor-bearing mice and in immune organs of tumor-free hosts. *Nutrients* 11 (12), 3012.
- Wang, Z., Li, N., Wang, B., Lin, J., 2015. Nonalcoholic fatty liver disease progression in rats is accelerated by splenic regulation of liver PTEN/AKT. *Saudi J. Gastroenterol.* 21 (4), 232.
- Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante, A.W., 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Investig.* 112 (12), 1796–1808.
- WHO, 2023. Obesity and overweight - WHO fact sheet. Available from: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.
- Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., et al., 2009. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 15 (8), 921–929.
- Wolf, M.J., Adili, A., Piotrowitz, K., Abdullah, Z., Boege, Y., Stemmer, K., et al., 2014. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 26 (4), 549–564.
- Xia, S., Sha, H., Yang, L., Ji, Y., Ostrand-Rosenberg, S., Qi, L., 2011. Gr-1+ CD11b+ myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. *J. Biol. Chem.* 286 (26), 23591–23599.
- Yang, R., Cai, T.T., Wu, X.J., Liu, Y.N., He, J., Zhang, X.S., et al., 2018. Tumour YAP1 and PTEN expression correlates with tumour-associated myeloid suppressor cell expansion and reduced survival in colorectal cancer. *Immunology* 155 (2), 263–272.
- Yang, Q., Yu, B., Kang, J., Li, A., Sun, J., 2021. Obesity promotes tumor immune evasion in ovarian cancer through increased production of myeloid-derived suppressor cells via IL-6. *Cancer Manag. Res.* 13, 7355–7363.
- Yao, L., Abe, M., Kawasaki, K., Akbar, S.M.F., Matsuuru, B., Onji, M., et al., 2016. Characterization of liver monocytic myeloid-derived suppressor cells and their role in a murine model of non-alcoholic fatty liver disease. *PLoS One* 11 (2), e0149948.
- Zheng, S., Yang, W., Yao, D., Tang, S., Hou, J., Chang, X., 2022. A comparative study on roles of natural killer T cells in two diet-induced non-alcoholic steatohepatitis-related fibrosis in mice. *Ann. Med.* 54 (1), 2233–2245.