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ORIGINAL ARTICLE

Diet-induced obesity, exogenous leptin-, and MADB106 tumor cell challenge affect tissue leukocyte distribution and serum levels of cytokines in F344 rats

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Abstract The adipocyte-derived catabolic protein leptin alters cell-mediated immunity and cytokine crosstalk. This may provide new insights into the altered immune response, seen in obese individuals. Therefore, we determined the tissue distribution of immune cells in diet-induced obese (dio) and normal weight F344 rats challenged with MADB106 tumor cells or leptin. Immune cell distribution in blood (by FACS analysis) and tissues (NK cells in spleen and liver, immunohistologically) as well as pro-inflammatory cytokines (IL-6, TNF-α; by flow cytometry) were investigated in 28 normal weight and 28 dio rats ($n = 4$–6/group). Pro-inflammatory cytokines were increased 3-fold for IL-6 and 7-fold for TNF-α in obese animals. Higher numbers of blood monocytes and NK cells were found in obese as compared to normal weight animals. In dio rats challenged with leptin and MADB106 tumor cells, monocyte numbers were decreased as compared to the obese control animals. Immunohistochemistry revealed an altered NK cell distribution in a compartment-, treatment-, and bodyweight-specific manner. In conclusion, our data reveal a distinct distribution pattern of monocytes and NK cells in dio rats as compared to normal weight littermates and an additional modulatory effect of a leptin- and MADB106 tumor cell challenge.

Keywords Obesity · Chronic inflammation · NK cells · Leptin · TNF-α · IL-6

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Introduction

Obesity is already a major but still growing health problem in developed countries and emerging nations [1]. In general, fat tissue is known to release various pro-inflammatory and anti-inflammatory adipokines, including adiponectin, resistin, and leptin as well as cytokines such as TNF-α, IL-1, IL-6, and chemokines such as monocytes chemoattractant protein-1 (MCP-1) and IL-8 [2]. It is well known that higher amounts of adipocytes lead to a misbalance in the immune system causing a status of low-grade chronic inflammation [2, 3]. Accordingly, overweight subjects are predestinated to develop severe chronic diseases such as type 2 diabetes, cardiovascular diseases, and hypertension. In addition, overweight serves as an independent risk factor for several types of cancer in both, rodents and humans [4, 5].

The adipokine leptin seems to play a crucial role in the development of obesity. Produced mainly by adipocytes, leptin normally exerts catabolic effects by binding to hypothalamic leptin-receptors (Ob-R). However, obese subjects develop a resistance to this mechanism, with

significantly higher levels of free leptin compared to lean subjects [6, 7]. Interestingly, Ob-R expression is not limited to the hypothalamus. Monocytes, T-lymphocytes [8], B-lymphocytes [9], and natural killer cells (NK cells) also express Ob-R [10], indicating a possible influence of leptin on leukocyte subsets. Phylogenetic studies of the 3D structure revealed that leptin is close to the cluster formed by interleukin-6 (IL-6) [11]. The Ob-R shares homology and sequence similarity to gp130 (IL-6R) [12]. Indeed, recent investigations underline a strong pro-inflammatory effect of leptin itself in innate as well as in adaptive immune responses [13, 14]. It has been shown to act on the cell cycle of lymphocytes [9], monocytes, and granulocytes [15], to increase the production of pro-inflammatory cytokines, as TNF-α and IL-6 [16] in particular, and to modulate the activation of NK cells, monocytes, and lymphocytes [10, 17]. Previous studies revealed that mice with a lack of leptin—ob/ob mice [18], as well as fasted wild-type mice are more susceptible to lipopolysaccharide-induced lethality, which could be partly reversed by leptin applications [19, 20]. Additionally, the same positive effect was found for infection with pneumococcal pneumonia [21]. Comparing diet-induced obese (dio) with lean animals, Mito et al. [22] showed a different cytokine response toward a leptin treatment in lean and dio mice and Nave et al. [23] demonstrated a lack of intracellular signaling events in NK cells after leptin treatment in dio rats. Recent data additionally revealed a very distinct migratory performance, especially of B- and T-lymphocytes, in dio mice challenged by different chemokines [24]. However, the effect of leptin on cell distribution and composition in lean and obese rodents has to be elucidated. Especially the evaluation of the influence of obesity on NK cells and monocytes may lead to a greater understanding of the mechanisms, contributing to the higher risk of obese individuals to develop certain types of cancer [4, 5].

In this study the effects of diet-induced obesity and leptin challenges on the leukocyte cell composition in the blood and tissues of F344 rats were systematically investigated. Since a challenge with MADB106 tumor cells has been shown to serve as a strong activator of NK cells [25], we additionally performed cell activation with this challenge alone or in combination with leptin. Furthermore, the levels of TNF-α and IL-6 in the sera of the experimental animals were measured.

Materials and methods

Animals

Eight-week-old Fischer 344 (F344) male rats ($n = 56$) were obtained from Charles River GmbH, Sulzfeld, Germany. All animals were group-housed (2 rats per cage) in plastic-based cages covered by standard cage-bedding in a specific pathogen-free, sound-proofed, and air-conditioned facility under a 12-h light–12-h dark cycle (lights on at 08:00 a.m.) at an ambient temperature of 24°C. Standard carbohydrate chow (50% carbohydrate, 19% protein, 12% water, 4% fat, and 2.1 kcal/g; Altromin 1234, Altromin, Lage, Germany) and water were available ad libitum. Half of the group ($n = 28$) was randomly selected to additionally receive a high-fat diet (34% carbohydrate, 17% protein, 4% water, 35% fat, and 5.2 kcal/g; Altromin C1057, Altromin). Animals were weighed and handled by the experimenters weekly. We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research.

After 8 weeks of high-fat diet, the animal experiments started with a significant higher weight of the obese littermates than their age-matched lean counterparts (392.5 ± 3 vs. 318.7 ± 2 g; $P < 0.05$).

Leptin and MADB106 tumor cells

Rats were challenged with 500 μg/kg human recombinant- (hrec-) leptin (Natutec, Frankfurt, Germany) dissolved in 0.2 ml of 0.9% saline. The dosage was determined in previous studies (e.g., [23]). The 9-10 dimethyl-1-2-benzanthracene-induced MADB106 mammary adenocarcinoma syngeneic tumor is a selected variant cell line obtained from a pulmonary metastasis in F344 rats [26]. Shingu et al. [25] demonstrated NK cell activation and a peak of the NK cell amount in the lung 15 min after intravenous (i.v.) injection of MADB106 cells. The adherent MADB106 tumor cells were cultured in 50 ml (25 cm^2) culture flasks with filter caps (Greiner Bio-one, Frickenhausen, Germany) at 37°C and 5% CO_2 as non-confluent monolayer cultures in RPMI 1640 medium (Gibco®, Invitrogen, Carlsbad, California, USA) supplemented with 10% heat-inactivated FCS, 45 U penicillin/ml, 0.045 mg streptomycin/ml, 2 mM L-glutamine, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate. For the assays, cells were detached at 80–90% confluency by removing the medium and rinsing two times with PBS. Finally, 5 ml PBS containing 0.25% trypsin was added and the cells were incubated at 37°C for 5–10 min until all cells were suspended. After transfer into 15-ml tubes, cells were washed twice in PBS and resuspended in PBS. Directly before the assay, tumor cells were labeled with 1.5 μM (final concentration) fluorescein derivate 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, Oregon, USA) at 37°C in PBS containing 0.1% BSA (Sigma-Aldrich). Ten minutes later five volumes of ice-cold medium were added and cells were incubated on ice for additional 5 min. After two final washes with PBS the

cells were re-suspended in RPMI medium. Finally, the cells were injected at a concentration of 1×10^6 cells/300 g [27].

Experimental design and intravenous cannulation

Experiments were conducted from 04:00 to 06:00 p.m. on four consecutive days. Under intramuscular (i.m.) ketamine hydrochloride- (10%, 0.35 ml; Gräub, Senden-Bösensell, Germany) and medetomidine hydrochloride- (0.1%, 0.05 ml; Pfizer, Karlsruhe, Germany) anesthesia a central i.v. catheter was introduced into the right external jugular vein of the animals. The cannula (total length 20 cm; inner diameter (i.d.): 0.5 mm; outer diameter (o.d.): 0.9 mm) was enclosed with a Teflon ring (i.d.: 0.9 mm; o.d.: 4 mm; height: 2 mm) approximately 1.8 cm from the distal tip. Thereafter the right external jugular vein was exposed through a 1.5 cm longitudinal incision in the skin of the neck 0.5 cm above the clavicle. Two ligatures (Sutupak 3-0, 0.7 metric, Ethicon, Norderstedt, Germany) were placed around the vein, approximately 0.5 cm apart. Under slight traction on the upper ligature, a small (2 mm) longitudinal incision was made with microsurgical scissors on the anterior surface of the vein. The catheter, previously filled with isotonic saline containing 50 U/ml heparin, was inserted until the Teflon ring touched the vein at the point of incision—with the tip of the catheter placed in the superior vena cava. The ligatures were tied around the vein and the catheter. Anesthesia was maintained over the whole experiment. Operations were performed on a warming blanket (surface temperature of 30°C) to avoid perioperative hypothermia [28]. Experiments started with a first i.v. application of either saline or hrec-leptin. Five minutes later animals received the second injection (saline or MADB106 tumor cells) and blood and tissue sampling was conducted 15 min thereafter. For details of group-assignment and treatment see Table 1.

Blood and tissue sampling

Fifteen minutes after the second injection the abdominal aorta was punctured and the spleen and the right medial lobe of the liver were removed. The organs were immediately frozen in liquid nitrogen and stored at −80°C for immunohistological analysis. Heparinized blood was either stored on crushed ice for immediate flow cytometric analysis or centrifuged at $400 \times g$ for 10 min and stored at −80°C for later cytokine measurement.

Cytokine levels analyses

TNF-α and IL-6 serum levels were measured in the blood of 4 lean and 4 obese control (NaCl/NaCl) animals. In brief, 50 µl serum was incubated at room temperature with a mixture of rat cytokine capture bead suspension and the PE detection reagent (BD Biosciences, Heidelberg, Germany). After 3 h, samples were washed and analyzed by Fluorescence Activated Cell Sorting (FACSAria, BD Biosciences) and by using BD FCAP array software. Rat cytokine standards provided with the kit were appropriately diluted and used in parallel for samples for preparation of standard curves.

Flow cytometry

Leukocyte numbers were determined using a Coulter counter. FACS analysis (FACS-Scan, Becton Dickinson, Heidelberg, Germany) was performed using the monoclonal mouse anti-rat antibodies (mAb) 10/78 (NK cells/NKR-P1A+/CD161bright; Serotec GmbH, Düsseldorf, Germany), R73 (α/β-T-cell receptor, CD3; Serotec), OX12 (rat immunoglobulin M, B-lymphocytes; Serotec), W3/25 (rat $CD4^+$-T-lymphocytes; Serotec), OX8 (rat $CD8^+$-T-lymphocytes; Serotec), and ED9 (180-kDa membrane antigen, monocytes; Serotec). For details of the FACS analyses see Bedoui et al. [29].

Table 1 Group assignment and treatment conditions

Group	Phenotype	First i.v. challenge[a]	Second i.v. challenge[a]	Number of F344 rats
1	Lean	NaCl	NaCl	$n = 6$
2	Obese (dio)	NaCl	NaCl	$n = 6$
3	Lean	hrec-leptin	NaCl	$n = 6$
4	Obese (dio)	hrec-leptin	NaCl	$n = 6$
5	Lean	NaCl	MADB106 cells	$n = 6$
6	Obese (dio)	NaCl	MADB106 cells	$n = 6$
7	Lean	hrec-leptin	MADB106 cells	$n = 6$
8	Obese (dio)	hrec-leptin	MADB106 cells	$n = 6$
9[b]	Lean	NaCl	NaCl	$n = 4$
10[b]	Obese (dio)	NaCl	NaCl	$n = 4$

i.v. intravenous

[a] The second challenge was conducted 5 min after the first one. Then, with a latency of 15 min, blood, liver, and spleen were sampled

[b] Groups 9 and 10 were used for TNF-α and IL-6 analysis only

Immunohistology

Cryostat sections (5–7 μm) were obtained and immunohistochemical characterization of NK cells was performed with the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Sections were immersion fixed with acetone for 10 min, rehydrated with tris-phosphatase buffered saline (TBS), and afterward incubated with the primary mouse mAb 10/78 (NKR-P1A+; 1:100) overnight. Binding of the primary mAbs was revealed by incubation with mouse IgG (1:50 in 5% rat serum in PBS for 30 min). To visualize the binding, the APAAP-complex (1:50 in TBS for 30 min; DakoCytomation, Hamburg, Germany) was utilized, followed by the repetition of the last two steps for 15 min. The sections were stained with Fast Red for 25 min, counterstained with hematoxylin (1:5 in PBS) for 90 s, and mounted in glycergel (DakoCytomation).

In situ quantification of NK cells in the liver and spleen

Each experimental group was composed of four to six animals. For the quantitative analysis in the liver, three sections were randomly selected and four visual fields were examined per section for each animal (resulting in 12 visual fields with a total area of 147 mm^2 being investigated per rat). In the spleen three sections were inspected with two visual fields in the transition zone between white and red pulp and two fields in the red pulp. Sections were examined with an Axioplan 2 imaging microscope (Carl Zeiss, Jena, Germany) by two observers blinded to the treatment conditions.

Statistics

Data are expressed as means ± standard error of the mean (SEM). To analyze the effect of the leptin and or tumor cell application and the body weight on the different parameters measured, two-way-ANOVA was applied. "Treatment" (leptin and/or MADB106 tumor cells was the first and "body weight" (dio and lean) the second factor, with the parameter measured (e.g., cell counts or cytokine levels) being the dependent variable. Fisher PLSD post hoc analysis was implemented to determine significant differences in the case of main treatment effects. Differences were considered significant if $P < 0.05$.

Results

Significantly higher TNF-α and IL-6 levels in dio rats

Results from arterial blood samples showed a 7-fold higher TNF-α (Fig. 1a) and 3-fold higher IL-6 (Fig. 1b) level in obese control compared to lean control animals.

Subset-specific alterations of blood leukocyte numbers in obese animals

The results of the FACS analysis are displayed in Fig. 2a–f. Blood leukocyte (Fig. 2a), T-lymphocyte (Fig. 2b), and granulocyte (data not shown) concentration showed no statistical significance between all investigated groups. Concerning the T-lymphocytes no statistically significant difference was observed, either in the $CD4^+$ or in the $CD8^+$ T-lymphocytes (data not shown). B-lymphocytes were significantly higher in lean leptin-treated animals (leptin/NaCl) compared to their controls (NaCl/NaCl; Fig. 2c). The treatment had no effect on the blood monocytes in lean animals (Fig. 2d). However, obese control animals (NaCl/NaCl) showed a significantly higher monocyte count compared to their lean littermates. Furthermore, leptin or MADB106 tumor cell challenge resulted in a significant lower number of monocytes in obese animals compared to obese controls (Figs. 2d, 3a, b). Several previous studies described a distinct monocyte subpopulation ($ED9^+W3/25^+10/78^+$) as activated

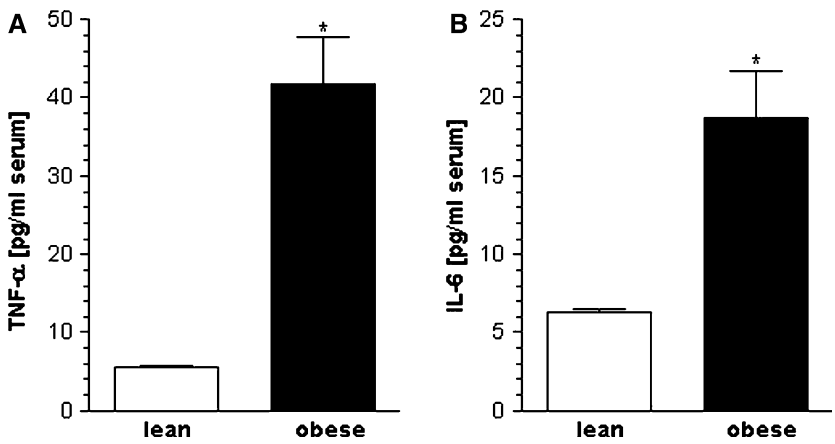

Fig. 1 Arterial TNF-α (**a**) and IL-6 (**b**) levels of anaesthetized lean and obese rats. Significant post hoc effects of the obese versus the lean animals are indicated by *asterisks*; * $P < 0.05$

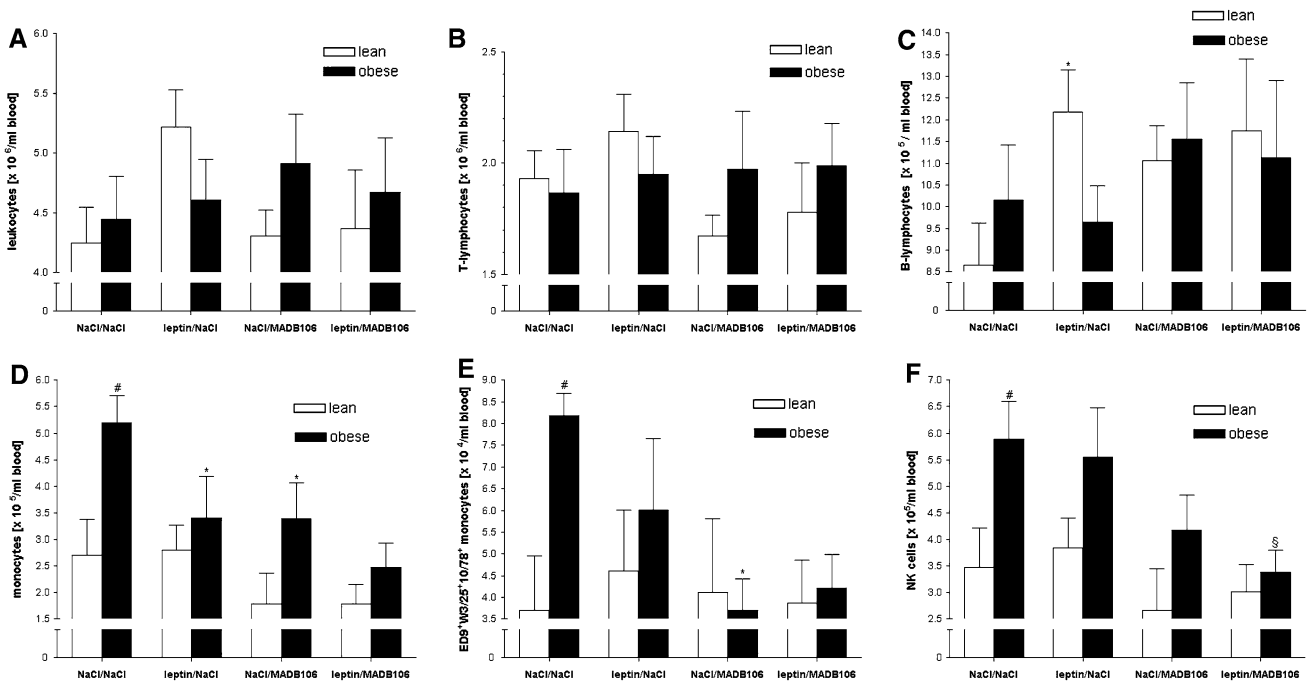

Fig. 2 Numbers of blood leukocytes (**a**), T-lymphocytes (**b**), B-lymphocytes (**c**), monocytes (**d**), activated monocytes ($ED9^+W3/25^+10/78^+$) (**e**) and NK cells (**f**) after leptin and/or MADB106 tumor cell treatment in lean and obese animals ($n = 4$–6). * $P < 0.05$, leptin/NaCl- or NaCl/MADB106-treatment versus NaCl/NaCl-treatment of similar weight animals; # $P < 0.05$, obese versus lean in one treatment group; § $P < 0.05$, leptin/MADB106-treatment versus leptin/NaCl-treatment of similar weight animals

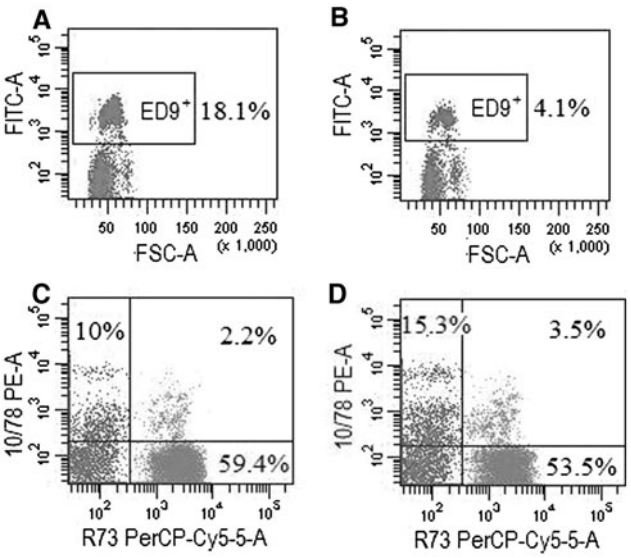

Fig. 3 Representative scatter-plots from flow cytometry histograms; monocytes were identified by high $ED9^+$ expression in NaCl/NaCl (**a**) and leptin/NaCl-treated (**b**) obese rats; NK cells are represented by $10/78^+$ and $R73^-$ expression in NaCl/NaCl lean (**c**) and NaCl/NaCl obese rats (**d**)

monocytes [30]. In this population, similar to monocytes, the obese control group showed significantly higher cell numbers than lean control animals. Furthermore, the concentration of activated monocytes tends to be lower in leptin or tumor cell-treated dio animals compared to obese controls. In contrast to the results of the monocyte counts this was only significant for MADB106 tumor cell-challenged animals (Fig. 2e).

Whereas no effects of leptin and/or tumor cells on blood NK cell counts were detected in lean rats, obese animals showed a significantly higher blood NK cell amount in the control group (NaCl/NaCl) compared to lean animals (Figs. 2f, 3c, d). The leptin application did not affect NK cells, whereas the cell amount was slightly decreased after a tumor cell challenge, and the combined application of leptin and MADB106 tumor cells significantly decreased the NK cell amount in the peripheral blood of obese animals compared to the leptin/NaCl-treated dio group. In lean as well as in obese animals no treatment effect was seen on NKT cells (data not shown).

Organ-, compartment-, and treatment-specific modulation of NK cell numbers in obese animals

Figure 4a–d shows representative images of examined tissue sections. The results of quantitative analysis are displayed in Fig. 5a–d. Obese control (NaCl + NaCl) and leptin-infused (leptin/NaCl) animals showed significantly lower hepatic NK cell numbers compared to the corresponding lean controls (Figs. 4a, b, 5a). The cell amount was lower in leptin, MADB106- or leptin/MADB106-treated lean animals

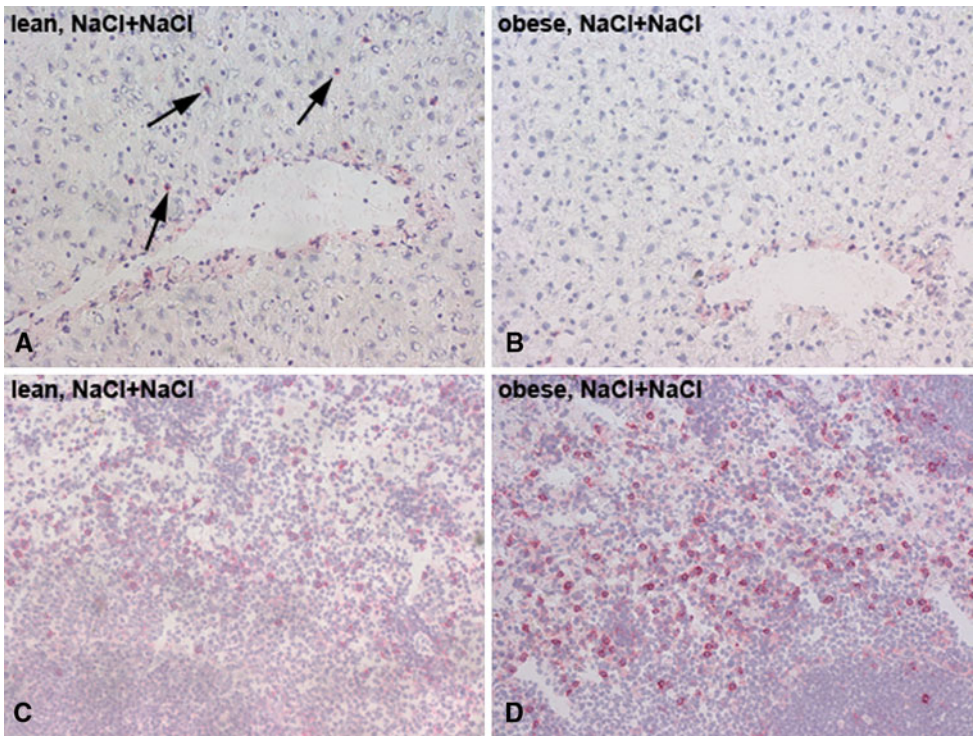

Fig. 4 Immunohistochemically stained NK cells in the liver of a NaCl/NaCl-treated lean (**a**) and an obese (**b**) rat. NK cells are indicated by *black arrows* (**a**). NK cells in the spleen of a NaCl/NaCl-treated lean (**c**) and an obese (**d**) rat

compared to their lean control group. Furthermore, the cell amount in leptin/MADB106-treated obese rats was significantly lower as compared to the solely leptin challenged obese animals.

In contrast to the results of the hepatic immunohistological quantifications, numbers of splenic NK cells were higher in control (Fig. 4c, d), MADB106-, and double-challenged obese animals compared to the corresponding lean littermates (Fig. 5b). NK cell numbers remained similar in all lean groups. However, a non-significant tendency to an increase in the concentration of NK cells could be observed in leptin-treated lean rats. Interestingly, the dramatic increased NK cell amount in control and the slightly increase in MADB106- and double-challenged obese animals could be traced back to distinct compartments in the spleen. Whereas the higher NK cell amount in the obese control animals compared to lean controls was caused by alterations in the red pulp and the transition zone, changes of the NK cell amount in MADB106- and double-challenged obese rats were caused by slightly higher NK cell levels solely in the transition zone (Fig. 5c, d).

Discussion

Fat tissue serves as an endocrine secretory organ producing a variety of so-called adipokines (e.g., leptin) as well as proinflammatory chemokines and cytokines such as IL-6, TNF-α, and MCP-1 [31]. Accordingly, obesity is characterized by a status of low-grade chronic inflammation leading to markedly elevated concentrations of proinflammatory cytokines in humans [2, 3]. Comparable to cytokine measurements in humans, this study shows a 3-fold higher serum concentration for IL-6 and a 7-fold higher concentration for TNF-α in obese F344 rats compared to their lean littermates. Interestingly, this is in contrast to the findings of Fenton et al. [32], revealing significantly higher IL-6, and significantly lower TNF-α concentrations in the sera of dio mice as compared to lean animals.

To date only little information is published concerning the body weight-dependent tissue distribution of different immune cells in dio rats. Therefore, this study was performed investigating the cell distribution in blood and tissues in lean and dio rats challenged with MADB106 tumor cells and leptin.

In lean F344 rats a leptin challenge lead to a significant higher B-cell amount as compared to the control group. Interestingly, this effect of leptin could not be observed in dio animals. However, in obese control animals the B-cell amount was slightly higher as compared to the corresponding lean rats. This supports the results of Papathanassoglou et al., demonstrating anti-apoptotic effects of leptin on B-cells in vitro [9]. Furthermore, it could be shown that B-cell proliferation could not be triggered by B-cell mitogens in dio animals [33].

Significantly higher monocyte numbers were found in the blood of NaCl/NaCl-treated obese compared to lean

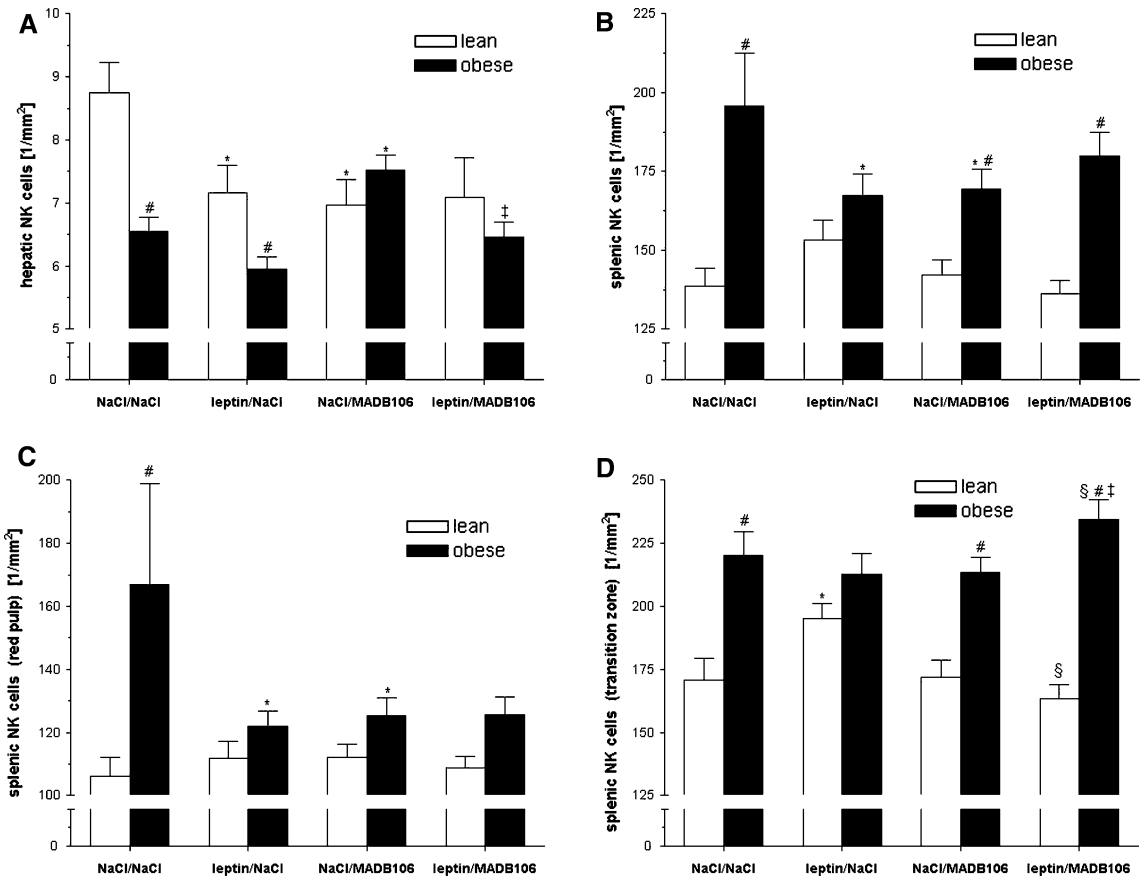

Fig. 5 NK cell numbers in the liver (**a**) after leptin and/or MADB106 tumor cell treatment; **b–d** NK cell numbers in the spleen (**b**), red pulp (**c**), and transition zone (**d**); $n = 4–6$, * $P < 0.05$, leptin/NaCl- or NaCl/MADB106-treatment versus NaCl/NaCl-treatment of similar weight animals; $^{\#}$ $P < 0.05$, obese versus lean in one treatment group; § $P < 0.05$, leptin/MADB106-treatment versus leptin/NaCl-treatment of similar weight animals; ‡ $P < 0.05$, leptin/MADB106-treatment versus NaCl/MADB106-treatment of similar weight animals

control rats. This supports recent investigations showing that an additional application of leptin abolished a suppressed myelopoesis in ob/ob mice [15]. Since leptin can regulate anti-apoptotic factors like Bcl-2 and Bcl-XL [34, 35], another possible explanation for the elevated monocyte numbers could be an anti-apoptotic effect of leptin via activation of NF-κB. Interestingly, we found a dramatically higher concentration of activated monocytes in obese compared to lean control rats. One explanation could be that endogenous leptin is capable of promoting Th1 cytokine production [16], which is essential for the activation of macrophages and monocytes. An additional stimulation with leptin and/or MADB106 tumor cells in the present study showed a significant reduction of the monocyte number in obese animals compared to their corresponding control group, whereas in lean rats only a tendency to reduced monocyte numbers was detectable. This indicates that monocytes in obese animals are more prone to respond to the treatment conditions. Bigorgne et al. [24] showed a hyperresponsiveness of T- and B-lymphocytes toward different chemokines in dio mice resulting in an enhanced migration into the liver. Our data indicate an exaggerated response of monocytes to the different treatment conditions in obese rats.

Our data show for the first time that a leptin and/or a MADB106 tumor cell challenge, a potent trigger of NK cell activation, modulate NK cell numbers in an organ-, treatment- and bodyweight-specific manner. The absolute number of blood and splenic NK cells was highly elevated in obese rats compared to their lean counterparts in all investigated experimental groups (unstimulated, single-, or double-challenged). These data are in line with previous findings showing a decrease in the number of blood and tissue NK cells (liver, spleen and lung) of leptin receptor deficient mice [36]. Since latest investigations delineate a significantly reduced intracellular signal transduction for NK cell cytotoxicity in NK cells of obese rats [23], the present findings can be interpreted as a compensatory proliferative reaction of the NK cells. Concerning the organ-specificity in the obese animals (elevated splenic NK cells and reduced hepatic NK cells), it is well established that NK cells are a heterogenous cell group which differs in

its expression of chemokine receptors and the reaction to chemotactical signals. Klemm et al. [37] even presumed a diverse cytokine production of rat NK cells in different lung compartments. Furthermore, human NK cells can be divided into $CD56^{dim}$ cells, mainly localized in the blood, and $CD56^{bright}$ cells, primarily detectable in secondary lymphoid organs. These two subsets do not only vary in their expression of CD56, but also express distinct chemokine receptors [38]. Another subpopulation are the $CD4^{+}$ NK cells also localized mainly in secondary lymphoid tissues. These cells are more susceptible to IL-16 induced chemotaxis [39]. Other studies report that human NK progenitor cells are able to change their receptor repertoire due to the influence of their microenvironment [40, 41]. Another study confirmed this finding in mice even in mature NK cells [42]. Thus, elevated adipokines, cytokines, and chemokines in obese subjects may change the cell surface receptor composition of NK cells and as a consequence the migratory performance is altered. As discussed for monocytes, another explanation for the altered distribution pattern of NK cells in dio rats of the present study could also be a general hyperresponsiveness to the treatments conditions. This was impressively shown by Bigorgne et al. [24] for B- and T-lymphocytes toward different chemokines in mice.

This study further revealed that a challenge with leptin or MADB106 tumor cells leads to a significant decrease of splenic NK cells in obese animals. Interestingly, the same tendency—although not significant—was observed in the blood. Thus, the question arises whether the loss of NK cells is due to apoptosis or migration in a different compartment. It is unlikely that the NK cells were forced to perform apoptosis, as the exposure time of the treatment was rather. The possible migration of NK cells to other tissues could not be confirmed by the examination of the liver. More likely, NK cells migrate to the marginal pool since Klonz et al. [43] proved that the marginal pool contains not only granulocytes, B-lymphocytes, T-lymphocytes, and monocytes but also NK cells. NK cells in the marginal pool are able to detach themselves and migrate into the peripheral blood or the tissue again. Since a migration toward the marginal pool can be characterized as a non-directional sign of activation, this strengthens the assumption of a hyperresponsive behavior of NK cells in dio rats.

In conclusion, this study indicates a major influence of obesity on the composition of leukocytes subsets. Furthermore, the treatment-specific distribution pattern of NK cells showed a drastically exaggerated response to tumor cell or leptin treatment in dio rats. The same tendency could be seen for monocytes. As a possible underlying mechanism, we consider the status of elevated cytokines and adipokines in obese subjects and herewith an altered metabolic microenvironment influencing immune cell activation and migration.

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