Short-term and long-term leptin exposure differentially affect human natural killer cell immune functions

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Obesity and type 2 diabetes have developed into a global epidemic. In the US, two-thirds of the adult population is obese or overweight and 23.6 million have diabetes, resulting in ~237,199 excess deaths annually (21, 41). Recent epidemiological studies indicate that, in addition to the well-known risk for metabolic and cardiovascular diseases, obesity is also associated with certain types of cancer, e.g., esophageal adenocarcinoma, colon cancer, or postmenopausal breast cancer (11, 27, 28, 45, 54, 58). Leptin, the product of the ob gene, is one of these highly elevated adipocyte-derived hormones (59). Leptin acts as a major regulator of energy homeostasis and metabolism by decreasing food intake and increasing energy expenditure (1). Besides its metabolic functions, leptin has also been shown to affect immune functions mainly by acting as a proinflammatory regulator of T cells (29, 33, 53, 54). Several clinical studies have implicated a pathogenic role for leptin in autoimmune inflammatory diseases such as type 1 diabetes, rheumatoid arthritis, and chronic bowel disease (19, 44). Natural killer (NK) cells are an immune cell population important for mounting a successful antitumor response. NK cells are part of the innate immune system. They exhibit the ability to directly kill infected or transformed cells and coordinate the immune response by secreting various cytokines, such as interferon-γ (IFNγ) (30, 56). NK cells are found abundantly in adipose tissue. They can comprise up to 30% of the cells present in the stromal vascular fraction of epididymal adipose tissue in mice (13). A study in humans showed that they are more frequent in the visceral adipose tissue and express higher levels of proinflammatory IFNγ compared with the subcutaneous adipose tissue (13, 42). NK cells are also involved in the development of latent autoimmune diabetes in adults and diabetes in nonobese diabetic mice (2, 8) but have been suggested to be protective against the development of metabolic syndrome in obese humans (34). Studies using leptin receptor-deficient mice (db/db) have indicated that leptin is necessary for normal NK cell development (52). The human lymphoma NK cell lines NK-92 and YT can be stimulated by leptin, leading to increased NK cell cytotoxicity in vitro (61). In two murine tumor studies leptin treatment was beneficial, which was thought to be due to improved NK cell function (17, 37). Interestingly, our group demonstrated recently that NK cells from rats with diet-induced obesity were resistant to activation by leptin stimulation that was caused by an abrogated postreceptor JAK/STAT signaling (40). However, the effect of leptin on human NK cell function, especially in obesity, remains unknown. Therefore, the present study aims to investigate the effects of short-term and long-term treatment of NK cells isolated from human blood donors with human recombinant leptin in vitro. We found that, although short-term treatment with leptin had a stimulatory effect, long-term exposure to leptin significantly impaired central immune functions such as cytotoxicity, cytokine secretion, and cell proliferation, suggesting that leptin possibly mediates increased cancer sus-
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ceptibility in obesity by decreasing the NK cell antitumor response.

MATERIALS AND METHODS

Reagents. If not indicated otherwise, reagents were obtained from Sigma-Aldrich (Seelze, Germany). Recombinant human leptin was obtained from R & D Systems (Wiesbaden, Germany).

Isolation of human NK cells from blood donors and human subjects. Leukocyte filters from blood donors were obtained from the Institute for Transfusion Medicine, Hannover Medical School, Hanover, Germany, and reversely flushed with PBS, and the cell suspension was subjected to Ficoll gradient (Biocoll; Biochrom, Berlin, Germany) centrifugation. Either the peripheral blood mononuclear cells (PBMC) were collected from the interphase and cultured for further analysis or NK cells were isolated by magnetic-activated cell sorting using the negative NK cell isolation kit from Miltenyi Biotec (Bergisch Gladbach, Germany), following the manufacturer’s instructions. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the Hannover Medical School. Furthermore, whole blood samples were taken from four normal-weight (mean BMI: 20.28–24.51) and four obese (mean BMI: 35.06–42.53) humans. The blood samples were heparinized, and PBMC and NK cells were isolated in the same manner as described above.

Animal studies. Animal studies were approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center. Mice were kept under 14:10-h light-dark cycles at constant temperature (22°C) with free access to food and water.

Cell culture, NK cells, and 3T3-L1 cells. NK cells were cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 1 mM sodium pyruvate (Biochrom, Berlin, Germany) at 37°C in a humidified atmosphere with 5% CO2 and IL-2 (50 U/ml IL-2; Eurocetus, Amsterdam, The Netherlands) at a density of 1 × 10⁶/ml. NK cells were stimulated with recombinant human leptin (1–100 nM) or vehicle only as control and harvested at the indicated time points. In cases where intracellular staining for cytokines was performed, brefeldin A was added 1 h after the beginning of cell stimulation to inhibit cytokine secretion. 3T3-L1 cells were grown in DMEM-high glucose (Gibco-Invitrogen, Carlsbad, CA) with 10% bovine calf serum (Hyclone; Thermo Fisher Scientific, Waltham, MA). Two days after confluence, differentiation was induced with 800 nM insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine in DMEM-high glucose with 10% FBS (Atlas Biological, Fort Collins, CO) for 3 days, followed by 2 days with 800 nM insulin and 1 μM dexamethasone. Adipocyte culture supernatants were collected from mature adipocytes 7 days after induction, stored at −80°C, and sterile-filtered before use. Isolated PBMCs were incubated in regular cell culture media or adipocyte cell culture supernatants for 24 h and analyzed by flow cytometry.

Extracellular and intracellular staining for flow cytometric analysis. The following antibodies were used to detect extracellular surface marker: CD3 conjugated with phycoerythrin (CD3-PE; SK7), CD3 conjugated with phycoerythrin Cy7 (PE-Cy7; SK7), CD16 conjugated with CD16-peridinin chlorophyll protein complex-Cy5.5 (3G8), CD56 conjugated with allophycocyanin (APC; CD56-APC, NCAM16.2), and CD20 conjugated with APC-H7 (L27), CD69-PE (L78), CD107a-FITC (H4A3), CD178-biotin (NOK-1), anti-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-PE (R7K-2), PerCP-Cy5.5 streptavidin (BD Pharmingen, Heidelberg, Germany), and Ob-R conjugated with carboxyfluorescein diacetate succinimidyl ester (CFSE; Ob-R-CFSE) (clone no. 52263; R & D Systems). Cells were stained in 96-well plates, and 1 × 10⁵ PBMC and 250,000 NK cells/sample were used. For extracellular staining, cells were washed once with PBS-1% BSA, and Pentaglobin (Biotest, Pharma, Dreieich, Germany) was added as an Fc-blocker against nonspecific binding. Cells were labeled with the indicated antibodies at 4°C for 15 min, washed twice with PBS-1% BSA, resuspended in 250 μl of PBS-1% BSA, and analyzed in a BD FACSDuo Software (BD Biosciences, Heidelberg, Germany). For intracellular staining the following antibodies were used: granzyme A conjugated with fluorescein isothiocyanate (FITC; granzyme A-FITC) (CB9), perforin-PE (d69), and anti-interferon γ (IFNγ; PE-Cy7) (4S.B3) (BD Pharmingen). After staining for extracellular markers as described above, the cells were incubated in PBS-4% parafomaldehyde for 10 min at room temperature. Cells were washed with PBS-0.1% saponin-0.01 M HEPES and labeled with the indicated antibodies for 30 min at 4°C. After two washes with PBS-1% BSA, cells were resuspended in 250 μl PBS-1% BSA, and flow cytometry was performed as described above.

Quantitative RT-PCR analysis for Ob-Rb. Total RNA was prepared from isolated NK cells using the RNeasy Mini Kit (Qiagen, Düsseldorf, Germany). mRNA was reverse transcribed to cDNA using Superscript III First Strand Synthesis SuperMix (Invitrogen, Karlsruhe, Germany) according to the manufacturer’s instructions. cDNA was subjected to real-time quantitative PCR analysis using iQaq Supermix with ROX (Bio-Rad, Muenchen, Germany) in a Prism 7000 Sequence Detector (Applied Biosystems, Darmstadt, Germany). The primer and probe sequences for the Ob-Rb specific isoform were 5′-AGGTTATCGTGCCCATTCC-3′ and 5′-GCAAACCTGTCCTGGAGAACTCGTA-3′, respectively (probe: 5′-FAM-AGCCCCCTTGTATTACCGGACTAMRA-3′; MWG, Ebersberg, Germany). For detection of human β-actin, TaqMan Gene Expression Assay with ID Hs99999903_m1 (Applied Biosystems) was used. Each sample was run in triplicate, and expression levels of Ob-Rb were normalized to β-actin using the standard curve method. All values were expressed as ratio of Ob-Rb to β-actin expression.

Western blot analyses of NK cells. Freshly isolated NK cells (1 × 10⁶ cells) were stimulated at 37°C in vitro with 50 nM recombinant human leptin or 100 ng/ml lipopolysaccharide (LPS) for 30 min and lyzed using Laemmli buffer containing 5% β-mercaptoethanol. Protein was quantified using the Bradford assay (Bio-Rad, Muenchen, Germany). Eight micrograms of total protein per lane was separated on a NuPAGE 4–12% Bis-Tris gel (Invitrogen) and then transferred to a nitrocellulose membrane. Membranes were incubated overnight with antibodies to phospho-Jak2 (Tyr1007/1008) rabbit mAb, phospho-4E-BP1 (Erk1/2) (179F1), 4E-BP1, p53, or β-actin rabbit mAb (New England Biolabs, Frankfurt, Germany). A horseradish peroxidase-linked anti-rabbit IgG antibody was used as secondary antibody (New England Biolabs), and protein was detected by enhanced chemoluminescence (ECL plus; Amersham, GE Healthcare, Freiburg, Germany) according to the manufacturer’s instructions. Quantitative analysis of the Western blots was performed using ImageQuant TL software (GE Healthcare Life Sciences).

Quantification of IFNγ and leptin cell supernatants and mouse serum. Collected cell supernatants and mouse serum were stored at −80°C until analysis. Analysis was performed using a commercially available IFNγ ELISA from R & D Systems or mouse leptin ELISA from Chrystal Chem according to the manufacturer’s instructions.

Cell cytotoxicity assays. The LIVE/DEAD Cell-Mediated Cytotoxicity Kit (Invitrogen) was used to assess the cell cytotoxicity, following the manufacturer’s instructions. In brief, the human erythroleukemia cell line K562 served as target cells and was labeled with 3′,3′,5′-iododeoxyxarabinocytosine (DiO). Isolated human NK cells (effector cells) and K562 cells (target cells, 1 × 10⁶ cells/sample) were incubated in ratios of 10:1, 5:1, and 2.5:1 for 2 h in a cell incubator. Cells were labeled with PI to detect dead cells. The number of dead target cells (DiO+PI+id FL1+FL3+) was determined by flow cytometry. For the 5¹CrChromium-release (Amersham Biosciences) assay, a standard 4-h protocol was used as described previously (25). Lytic units (LU20/10⁷ cells) were calculated using the standard method of Bryant et al. (9).

Conjugate-forming assay. After stimulation, PBMC or NK cells were labeled with CD3-PE and CD56-APC antibodies and washed

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two times with PBS, the cell number was adjusted to $1 \times 10^6$/ml and 40,000 K562 cells ($1 \times 10^6$/ml) were added to 240,000 NK cells. The cell suspension was centrifuged at 100 g for 3 min, followed by a 15-min incubation in a cell incubator. Flow cytometry was performed, with gating on CD56+ CD3− NK cells. K562 cells were identified by their discrete autofluorescence. The percentage of conjugates was determined as the number of cell clusters simultaneously positive in FL1 and FL4. Additionally, the percentage of CD56bright or CD56dim cells forming conjugates was measured.

Cell proliferation assay and detection of dead and apoptotic cells. Isolated NK cells were washed with PBS-0.2% BSA, resuspended in 250 μl of PBS ($1 \times 10^6$/ml), and incubated with CFSE (final concentration 1.5 μM) for 7 min at 37°C in a cell incubator. Two volumes of cold FBS were added, followed by three washes with PBS. The cells were resuspended in 250 μl of cell culture medium ($1 \times 10^6$/ml) and treated with 50 nM leptin or vehicle only and incubated for 7 days. Flow cytometry was performed, and the percentage of proliferating cells was determined as the number of cells that displayed a distinctively lesser fluorescence in fluorescent channel 1 (FL1) than the original cell population.

Statistics. Data are expressed as means ± SE. Significance was assigned where $P < 0.05$. Data sets were analyzed using Student’s t-test or one-way ANOVA with the Tukey multiple comparison test for post hoc analysis. The software used was GraphPad Prism 5.0 (GraphPad Software).

RESULTS

Leptin receptor Ob-Rb is expressed on peripheral blood NK cells. Peripheral mononuclear blood cells were isolated from blood donors, labeled with various antibodies to mark lymphocyte subsets as well as an antibody against the extracellular domain of the leptin receptor (Ob-R), and analyzed by flow cytometry. We found that all lymphocyte subsets, T cells (CD3+CD56−), natural killer T (NKT) cells (CD3+CD56+), and NK cells (CD3−CD56+) as well as β-cells (CD3−CD20+) have Ob-R+ subpopulations (Fig. 1A). Approximately 5% of the NK cells were found to be Ob-R+ compared with isotype control (Supplemental Fig. S1A).

The Ob-R leptin receptor (Ob-R) has various isoforms [Ob-R(a–f)] due to alternative splicing. All Ob-Rs share a common extracellular domain, but only the long-form Ob-Rb contains the long intracellular domain. Although some signaling can occur through the short isoforms, such as Ob-Ra (7), only

Fig. 1. Human peripheral blood natural killer (NK) cells express the long isoform of the leptin receptor (Ob-Rb). A: flow cytometric analysis of peripheral mononuclear blood cells (PBMC) isolated from lymphocyte filters from blood donors (BD) stained with antibodies for CD3, CD56, CD20, and Ob-R. Nos. of Ob-R+ NK cells (CD56+CD3−ObR+), T cells (CD3−CD56−), and natural killer T (NKT) cells (CD3+CD56+) are shown as percentages of lymphocytes as means ± SE. B: quantitative PCR analysis of Ob-Rb mRNA expression in NK cells isolated from BD normalized to β-actin (ACTB), shown as means ± SE. C: no. of CD56dim and CD56bright NK cells and Ob-R+ NK cells, shown as means ± SE. D: no. of CD16+ NK cells and Ob-R+ NK cells shown as means ± SE. E: effect of leptin stimulation on the number of Ob-R+ NK cells. Isolated human NK cells were stimulated with 50 nM leptin for 18 h. Nos. of positive cells are shown as %NK cells, shown as means ± SE. n.s., No statistically significant difference. *$P < 0.05$, compared with vehicle.
Ob-Rb is able to activate the intracellular JAK-STAT signaling pathway (5, 22) and is believed to account for virtually all of leptin’s known actions (55). The expression of the functional isoform (Ob-Rb) in isolated NK cells was confirmed with TaqMan qPCR using an isoform-specific probe (Fig. 1B). Depending on their levels of CD56, NK cells can be divided into two distinct subpopulations: CD56dim and CD56bright (24). The CD56dim cells are involved mainly in killing abnormal cells through a mechanism called cell-dependent cytotoxicity. The CD56bright NK cells display a higher capacity to secrete cytokines, thereby regulating immune responses. The Ob-R+ subpopulation showed the same expression pattern of CD56dim and CD56bright as the total NK cell population (Fig. 1C). However, the expression of CD16, a cell surface receptor mediating antibody-dependent activation, is significantly lower in the Ob-R+ NK cell population (Fig. 1D). Stimulation with leptin increased the expression of Ob-R (Fig. 1E) but did not affect the expression of CD56dim, CD56bright, or CD16 (Supplemental Fig. S1, B–D).

Effect of leptin stimulation on cellular cytotoxicity of human NK cells. Short-term incubation of isolated NK cells with recombinant leptin for 20 min ≤ 24 h revealed a dose-dependent stimulatory effect on the cell-dependent cytotoxicity against the erythroleukemia cell line K562 (Fig. 2, A and B). The early increase in cytotoxicity was accompanied by an improved ability of the NK cells to form conjugates with their target tumor cells, although no subpopulation-specific effects on CD56dim or CD56bright NK cells were observed (Fig. 2C). In addition, the expression of TRAIL was increased after leptin stimulation. TRAIL is a cell surface molecule that induces apoptosis of target cells by binding its corresponding receptor (Fig. 2D). Other effector mechanisms for cytotoxicity, such as expression of CD178 or CD107a (Supplemental Fig. S2, A and B), as well as the production of the cytotoxic enzymes granzyme A and perforin (Supplemental Fig. S2, C and D), were not altered. In contrast, incubation of leptin for longer than 4 days significantly inhibited cell cytotoxicity (Fig. 2E).

Effect of leptin stimulation on cytokine production of human NK cells. Similarly, a short-term stimulation of isolated NK cells with leptin for 18 h significantly increased the secretion of IFNγ (Fig. 3A). This increase was associated with an early increase in the expression of the activation marker CD69 after 4.5 h (Fig. 3B) before the intracellular production of IFNγ was significantly elevated (Fig. 3C). Consistent with the effect on cytotoxicity, long-term incubation with leptin for > 72 h significantly decreased the production of IFNγ (Fig. 3D).
Effect of leptin stimulation on cell proliferation of human NK cells. The ability to proliferate and expand the cell population in response to stimulation is a key factor in effective immune responses. To address the effect of leptin to stimulate cell proliferation, we labeled NK cells isolated from blood donors with CFSE to track proliferating cell populations with flow cytometry and incubated NK cells with leptin or vehicle for only 7 days. Leptin decreased cell proliferation rates significantly (Fig. 4A). To exclude the possibility that the reduced cell proliferation is due to increased cell death, we assessed the number of dead (propidium iodide-positive) and apoptotic (annexin V-positive) cells after leptin stimulation and did not observe any significant differences (Fig. 4, B and C).

Stimulation of isolated human PBMC with recombinant human leptin results in an activation of the intracellular signaling cascade of the leptin receptor Ob-Rb in normal-weight but not in obese individuals. PBMC isolated from normal-weight (BMI: 20.28–24.51) and obese (BMI 35.06–42.53) humans were stimulated with recombinant human leptin for 30 min, and phosphorylation of Jak-2, a downstream target of the intracellular signaling cascade of the Ob-Rb receptor, was evaluated by Western blot. A representative Western blot comparing two individuals is shown in Fig. 5. The time point of 30-min leptin stimulation was chosen after a time course experiment was performed (Supplemental Fig. S3). The phosphorylation of Jak-2 in PBMC from obese individuals was greatly diminished compared with normal-weight individuals (Fig. 5, A and C). In contrast, after stimulation with LPS for 30 min, equal phosphorylation of extracellular kinase 1/2 (Erk1/2) in PBMC from normal-weight and obese subjects was observed (Fig. 5, B and D).

Effect of adipocyte cell culture supernatant on human NK cell immune functions. Apart from leptin, adipocytes secrete a variety of other adipokines, such as adiponectin, retinol-binding protein 4, and proinflammatory cytokines (e.g., IL-6 or IL-1β) during obesity (48). 3T3-L1 adipocytes are a well-established in vitro model to study adipogenesis and adipocyte biology because they display similar transcription profiles as primary adipocytes. But they express only minimal amounts of leptin (Supplemental Fig. S4) (35). Therefore, to evaluate the

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**Fig. 3.** Effect of leptin stimulation on cytokine production by human NK cells. A: isolated human NK cells were stimulated with 50 nM leptin for 18 h, and IFNγ was measured in the cell culture supernatant with ELISA. Values are shown as means ± SE. B and C: isolated human NK cells were stimulated with 50 nM leptin for 4.5 h, and the number of CD69+ (B) and IFNγ + NK cells (C) was analyzed with flow cytometry. Values are shown as means ± SE. D: isolated human NK cells were stimulated with leptin for 72 h, and IFNγ was measured in the cell culture supernatant with ELISA. Values are shown as means ± SE. *P < 0.05 compared with vehicle.

**Fig. 4.** Effect of leptin stimulation on cell proliferation of human NK cells. A: isolated human carboxyfluorescein diacetate succinimidyl ester-stained NK cells were incubated with 50 nM leptin for 7 days, and the number of proliferating NK cells was assessed with flow cytometry. Values are shown as means ± SE. B and C: nos. of apoptotic/annexin V + NK cells (C) and dead/propidium iodide + NK cells are shown as means ± SE. *P < 0.05 compared with vehicle.
Leptin, a hormone produced by adipocytes, is a major regulator of metabolism and has been shown to modulate immunity. However, its role in regulating human NK cell functions is largely unknown. Here, we describe the expression of the leptin receptor, including its functional long isoform for intracellular signaling Ob-Rb, on a subpopulation of isolated primary human NK cells. The size of this Ob-R+ NK cell subpopulation is comparable with the size of other immune cell subpopulations, such as peripheral blood Ob-R+ T cells or adipose tissue CD4+ Foxp3+ T regulatory (Treg) cells, which play an important physiological role in the modulation of starvation-induced immune suppression and insulin resistance in obesity, respectively (20, 33).

The Ob-R+ subpopulation displayed the same expression of the NK cell markers CD56* and CD56bright as the total NK cell population. However, the expression of CD16, an Fc receptor expressed on the cell surface, was decreased. Short-term stimulation with leptin increased IFNγ secretion as well as expression of the activation marker CD69 and cell-dependent cytotoxic lysis of tumor cells. This was associated with improved formation of conjugates between the NK cells and the tumor cells and with increased expression of TRAIL, a surface molecule that upon binding its corresponding receptor on its target cells induces apoptosis. Although a stimulatory effect of short-term leptin exposure on NK cell function had been described in the human NK cell lymphoma cell lines NK-92 and YT (61), our study is the first detailed examination of the effects of leptin on primary human NK cells. Tian et al. (52) found that a short-term leptin stimulation of NK cells from wild-type but not from db/db mice resulted in an increase in cytotoxicity and CD69 expression. This seems to be a leptin-specific effect since other NK cell stimulants such as IL-15 or poly I:C were able to activate NK cells from db/db mice. The stimulatory effect of leptin is in line with earlier findings demonstrating an in vitro activation (e.g., increased proinflammatory cytokine production) of immune cells, such as monocytes, hepatic stellate cells, neutrophils, and T cells, following a short-term stimulation with leptin (3, 10, 36, 60).

Ob/ob mice lacking the leptin gene and the db/db mice lacking a functional leptin receptor are prone to developing several immune defects, especially in the adaptive immune response (29, 33). Ob/ob mice display a higher susceptibility to viral myocarditis with lower cytotoxicity of spleen NK cells (26), and administration of leptin ameliorates the course of disease and the immune deficiency (51). Ob/ob mice also show a reduced spleen weight, the peripheral tissue that contains most NK cells besides blood, and a lower NK cell cytotoxicity after immunization (14). Db/db mice have reduced numbers of NK cells in blood, spleen, liver, and lung and reduced expressions of...
sion of the activation marker CD69 (52). In prediabetic db/db mice, the abnormal NK cell development in the bone marrow was found to be due to an increase in apoptosis of NK cells during differentiation (32). In humans with congenital leptin deficiencies, various immune defects, mainly in the T cell response, have been reported, and treatment with leptin improved the rate of infections and autoimmune disorders (18, 23). Nevertheless, there was no focus on NK cell immunity in these investigations. Taken together, our findings are in line with the notion that leptin is a critical regulator not only in NK cell development but also in NK cell activation in the acute immune response.

In contrast to short-term leptin stimulation, we found that long-term leptin exposure inhibits important NK cell functions, including cytotoxicity, production of IFNγ, and cell proliferation. A state of long-term, highly elevated leptin exposure is found in obesity. Mice with diet-induced obesity display a higher susceptibility to influenza with decreased proinflammatory intrapulmonary cytokine production and significant reduction in NK cell cytotoxicity (49). The number of NK cells in epididymal fat pads in diet-induced obese mice was decreased compared with normal-weight controls (13). In mice on a high-fat diet, fewer NK cells migrate into the epididymal fat pad compared with lean controls, but ob/ob mice have normal numbers of NK cells in that fat pad, suggesting leptin to be the cause (12).

Clinical studies in humans have found that obesity impairs NK cell immunity and leaves the NK cells more susceptible to various stressors (16, 38, 43). Interestingly, obese subjects with no metabolic syndrome display significantly higher levels of NK cells than the ones with metabolic syndrome, with higher expression of the activation marker CD69 and lower expression of inhibitory receptors (34). Studies in patients before and after bariatric surgery demonstrated normalization of NK cell immunity 6–12 mo after surgery (15, 39). This is consistent with studies showing impaired leptin-mediated NK cell activation in obese rats (40), and that this altered phenotype of NK cells can be normalized by transfer into lean animals, supporting the involvement of a secreted factor in the pathogenesis of impaired NK cell immunity in obesity (31). Interestingly, when we stimulated the cells with supernatants from mature 3T3-L1 adipocytes, which produce most of the adipokines but not leptin, we observed a response that was much weaker and not sufficient because leptin is also involved in NK cell activation in the acute immune response.

Epidemiological studies have shown an association of hyperleptinemia with prostate, colorectal, and breast cancer. Usually, leptin’s action as a mitogen or its interaction with receptors for growth factors (e.g., with estrogen receptors in breast cancer) was used to explain the mechanisms for this association (46, 57). However, Mori et al. (37) recently demonstrated that leptin (ob/ob) or leptin receptor-deficient (db/db) mice develop more severe pulmonary metastasis than wild-type mice when injected with B16-BL6 melanoma and Lewis lung carcinoma cells. This was due to decreased NK cell functions, which could be improved by injecting ob/ob mice with leptin.

In a murine model for hepatocellular carcinoma, the administration of leptin also suppressed tumor growth by inducing NK cell activation (17). Enhanced tumor growth in adiponectin knockout mice with different cancer models was found to be due to its effect on reducing macrophage infiltration into the tumor rather than its angiogenic effects (50).

Taken together, these findings show that adipokines can promote a successful immune response against cancer. To investigate the specific contribution of leptin on NK cell dysfunction in obesity, an inducible leptin knockout mouse would be required. The use of global ob/ob knockout mice is not sufficient because leptin is also involved in NK cell development (14, 52).

NK cells represent an immune cell population crucial for mounting an effective antitumor response. In the present study, we show that after long-term leptin exposure, similar to the situation in obesity with pathologically high leptin serum levels, integral parts of NK cell immune functions, such as cytotoxic lysis of tumor cells, IFNγ secretion, and cell proliferation, are significantly impaired, possibly linking leptin to increased cancer susceptibility in obesity.

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

C.D.W., R.J., L.G., and H.N. analyzed the data; C.D.W., T.L., L.H., S.K., R.J., and H.N. interpreted the results of the experiments; C.D.W., T.L., and L.H. prepared the figures; C.D.W. drafted the manuscript; C.D.W., R.J., L.G., and H.N. edited and revised the manuscript; C.D.W. and H.N. approved the final version of the manuscript.

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