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

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Am J Physiol Endocrinol Metab 302: E108–E116, 2012. First published September 27, 2011; doi:10.1152/ajpendo.00057.2011.—Epidemiological studies have indicated that obesity is associated with a higher risk for certain cancers caused by elevated levels of adipocyte-derived hormones. Leptin, one such hormone produced by adipocytes, is a major regulator of metabolism and has also been shown to modulate immunity. However, its role in regulating human natural killer (NK) cell functions is largely unknown. Here, we show that the leptin receptor (Ob-R) is expressed on 5% of NK cells isolated from blood donors, as measured with flow cytometry, and expression of the signal-transducing long form of the leptin receptor Ob-Rb was confirmed with quantitative PCR. The Ob-R+ subpopulation displayed a lower expression of CD16, a cell surface receptor mediating antibody-dependent activation. Short-term stimulation with leptin increased IFNγ secretion, CD69 activation marker expression, and cytotoxic lysis of tumor cells; this was mediated by an improved conjugate forming between NK cells and tumor cells as well as higher expression of tumor necrosis factor-related apoptosis-inducing ligand. On the contrary, long-term incubation with leptin significantly impaired these NK cell immune functions and decreased cell proliferation. In addition, phosphorylation of Jak-2 after leptin stimulation was reduced in peripheral mononuclear blood cells from obese humans compared with normal-weight controls. NK cells represent an immune cell population that is crucial for an effective antitumor response. Here, we show that long-term exposure to leptin, similarly to the situation in obese individuals with elevated serum leptin levels, significantly impairs integral parts of NK cell immune functions, possibly linking leptin to increased cancer susceptibility in obesity.

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supernatants were collected from mature adipocytes 7 days after Systems). Cells were stained in 96-well plates, and 1 cinimidyl ester (CFSE; Ob-R-CFSE) (clone no. 52263; R & D many), and Ob-R conjugated with carboxyfluorescein diacetate suc- surface marker: CD3 conjugated with phycoerythrin (CD3-PE; SK7), analysis.

Cell culture supernatants for 24 h and analyzed by flow cytometry. For the 51Chromium-release (Amersham Biosci -ces) assay, a standard 4-h protocol was used as described previously

Quantitative RT-PCR analysis for Ob-Rb. Total RNA was prepared from isolated NK cells using the RNAspray Mini Kit (Qiagen, Düssel- dorf, 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 iTaq 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′-AGGGTATCGTGCCCATTCC-3′ and 5′-GCAACTGTCTGGAGAACTCTGA-3′, respectively (probe: 5′-FAM-AGGCCCTT-GTTTATCCGGAC-TAMRA-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 x 10^6 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 Laemml 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- 

Conjugate-forming assay. After stimulation, PBMC or NK cells were labeled with CD3-PE and CD56-APC antibodies and washed at 4°C for 15 min, washed twice with PBS-0.1% BSA, resuspended in 250 μl of PBS-1% BSA, and analyzed in a BD FACSDiva 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 (IFNy; PE-Cy7) (4S.B3) (BD Pharmingen). After staining for extracellular markers as described above, the cells were incubated in PBS-4% paraformaldehyde for 10 min at room temperature. Cells were washed with PBS-0.1% saponine-0.01 M HEPES and labeled with the indi- cated 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.

**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, Han- nover, Germany, and reversely flushed with PBS, and the cell sus- pension 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 x 10^6/ml. NK cells were stimulated with recombinant human interleukin 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, Carls- bad, CA) with 10% bovine calf serum (Hyclone; Thermo Fisher Scientific, Waltham, MA). Two days after confluence, differentiation was induced with 800 μM insulin, 1 μM dexamethasone, and 0.5 mM 3-isobuty-1-1-methylxanthine in DMEM-high glucose with 10% FBS (Atlas Biological, Fort Collins, CO) for 3 days, followed by 3 days with 800 μM 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 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 3.5 (3G8), CD56 conjugated with allophycocyanin (APC; CD56-APC, NCAM16.2), and CD20 conjugated with APC-7 (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 suc- cinimidyl ester (CFSE; Ob-R-CFSE) (clone no. 52263; R & D Systems). Cells were stained in 96-well plates, and 1 x 10^6 PBMC and 250,000 NK cells/sample were used. For extracellular staining the 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
two times with PBS, the cell number was adjusted to 1 X 10^6/ml, and 40,000 K562 cells (1 X 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 X 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 X 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 distinctly lesser fluorescence in fluorescent channel 1 (FL1) than the original cell population. For detection of dead or apoptotic NK cells, annexin V-FITC was added, following the manufacturer’s instructions. Flow cytometry was performed and the percentage of dead cells was determined as the number of annexin-FITC+ cells in FL1.

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–Ob-R+), 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. 2A, 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).

![Fig. 2. Effect of leptin stimulation on cytotoxicity of human NK cells.](http://ajpendo.physiology.org/)

![Fig. 3. Effect of leptin stimulation on cytokine production of human NK cells.](http://ajpendo.physiology.org/)
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

**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.
effect of adipokines other than leptin on NK cell immunity, we cocultured PBMC isolated from blood donors, with cell culture supernatant harvested from mature 3T3-L1 adipocytes or regular cell culture media. Treatment with adipocyte-conditioned media did not compromise the number of NK cells, T cells, or NKT cells (Supplemental Fig. S5A) or the normal ratio between CD56dim and CD56bright NK cells (Supplemental Fig. S5B). In contrast to our finding that leptin stimulation increased the expression of Ob-R (Fig. 1E), coculture with adipocyte-conditioned media did not change Ob-R expression (Supplemental Fig. S5C). Similarly, conjugate formation between NK cells and the tumor cell line K562 as target cells was not significantly altered by adipocyte-conditioned media (Supplemental Fig. S5D), and no alteration in the expression of TRAIL was observed (Supplemental Fig. S5E). However, leptin stimulation increased the formation of conjugates as well as the expression of TRAIL (Fig. 3, C and D). Interestingly, the number of NK cells and CD56dim NK cells expressing the cytotoxic enzyme granzyme A was significantly reduced after adipocyte coculture compared with the media/control (Supplemental Fig. S5F). The number of IFNγ+ CD56dim NK cells was significantly increased after treatment, whereas the CD56bright NK cells showed a tendency toward a lower IFNγ expression (Supplemental Fig. S5G). Taken together, stimulation with adipocyte-conditioned media led to NK cell population-specific changes that are distinct from the changes observed with leptin treatment.

**DISCUSSION**

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 CD56dim 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 expres-
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 distinct from the changes caused by treatment with recombinant leptin protein. This could indicate that, at least regarding the NK cell immune functions, leptin may have a contribution to the immune suppression in obesity that is distinct from effects of a general overexposure of adipokines.

Peripheral leptin resistance could explain the lack of leptin responsiveness in NK cells after long-term exposure. The phenomenon of central leptin resistance in obesity (the lack of reduction in food intake despite highly elevated serum leptin levels) has been widely acknowledged, and several mechanisms explaining this have been proposed (4, 6). An earlier study by our group suggested that impaired Jak-2 signaling could be the mechanism responsible for NK cell dysfunction in rats with diet-induced obesity (40). This is in line with our observation that in obese humans leptin stimulation evoked less phosphorylation in PBMC than in normal-weight individuals.

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. did the conception and design of the research; C.D.W., T.L., L.H., and S.K. performed the experiments; 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.

REFERENCES

2. Akesson C, Uvebrant K, Oderup C, Lynch K, Harris RA, Lernmark A, Agardh CD, Cilio CM. Altered natural killer (NK) cell frequency and
36. O’Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, Maizlin II, Joe BA, Roberts CT Jr, Slika MK, Marks DL. Depot-specific differences in inflammatory mediators and a role for NK cells and


