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The Microenvironment of Visceral Adipose Tissue and Liver Alter Natural Killer Cell Viability and Function

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JLB

Brief Conclusive Report

The microenvironment of visceral adipose tissue and liver alter natural killer cell viability and function

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ABSTRACT

The role of NK cells in visceral adipose tissue (VAT) and liver inflammation in obesity is not fully understood. This study investigated the frequency, cytokine expression, chemokine receptor, and cytotoxicity receptor profile of NK cells in the blood, omentum, and liver of patients with the obesity-associated cancer, oesophageal adenocarcinoma (OAC). The effect of chronically inflamed tissue microenvironments on NK cell viability and function was also examined. We identified significantly lower NK cell frequencies in the liver of OAC patients compared with healthy controls and within the omentum and liver of OAC patients compared with blood, whereas IL-10-producing populations were significantly higher. Interestingly, our data suggest that reduced frequencies of NK cells in omentum and liver of OAC patients are not a result of impaired NK cell chemotaxis to these tissues. In fact, our functional data revealed that secreted factors from omentum and liver of OAC patients induce significant levels of NK cell death and lead to reduced percentages of TNF-α^+ and NKP46$^+$ NK cells and higher frequencies of IL-10-producing NK cells. Together, these data suggest that the omental and hepatic microenvironments of OAC patients alter the NK cell phenotype to a more anti-inflammatory homeostatic role. *J. Leukoc. Biol.* 100: 1435–1442; 2016.

Introduction

The burgeoning obesity epidemic is associated with increased prevalence of both cancer and nonalcoholic fatty liver disease and is attributable for ~3.5 million deaths every year [1, 2]. We [3] and others [4] have previously identified macrophages and T cells as key players in adipose tissue inflammation, but the role of NK cells has not been fully defined in human obesity. Furthermore, whereas previous studies have revealed intriguing alterations in NK cell numbers and phenotypes within the VAT, they have not uncovered why NK cell frequencies are altered in obesity nor elucidated the reciprocal effects of the inflamed VAT microenvironment on their function, particularly in obesity-associated cancer [5, 6]. Moreover, whereas NK cells have been studied extensively in viral hepatitis and have already been shown to be protective in liver fibrosis, their role in the inflamed fatty liver has not been identified [7, 8].

This study uniquely examined the frequencies, cytokine expression, chemokine receptor expression, and NCR profiles of NK cells in the omentum and liver of patients with the obesity-associated malignancy, OAC [1], and addressed the influence of such tissue microenvironments on NK cell viability and function. We report herein that NK cell frequencies are lower in the liver of OAC patients compared with noncancer controls and within the inflamed omentum and liver of OAC patients compared with blood. In addition, we demonstrate that secreted factors from these tissue microenvironments can induce significant changes in NK cell viability, cytokine expression, and NCR profiles.

MATERIALS AND METHODS

Subjects

Fifteen consecutive OAC patients, attending the National Oesophageal and Gastric Centre at St. James's Hospital (Dublin, Ireland) were enrolled in this study. The group included 12 men and 3 women (with an average age of 63.9 yr), and 73.3% had received neo-adjuvant chemoradiotherapy, and 93.3% were overweight or obese with a mean BMI of 28.02 kg/m^2 and mean VFA of 169.5 cm^2 [9]. There were no metastases in the omentum or liver of this cohort.

Abbreviations: ACM = adipose conditioned media, APC = allophycocyanin, BMI = body mass index, HCV = hepatitis C virus, LCM = liver conditioned media, M199 = medium 199, NCR = natural cytotoxicity receptor, OAC = oesophageal adenocarcinoma, PI = propidium iodide, VAT = visceral adipose tissue, VFA = visceral fat area

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

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Blood and omentum were taken from noncancer control subjects attending St. James's Hospital. Noncancer control liver was taken from donor livers at the Liver Transplant Unit, St. Vincent's University Hospital (Dublin, Ireland). Patients provided informed consent for sample acquisition, and the study received ethical approval from the St. James's Hospital Ethics Review Board and St. Vincent's University Hospital Ethics Committee. BMI, VFA, and anthropometric variables were measured, as described previously [3, 9].

Sample preparation

PBMCs were isolated by density centrifugation. Omental samples were prepared as described previously but with a shorter collagenase treatment time of 15 min to prevent loss of surface CD56 [3]. Liver samples were processed, as described in Curry et al. [10], with modifications to replace the density centrifugation step with the use of a red cell lysis step. Healthy donor livers were perfused, and hepatic mononuclear cells were isolated, as described previously [11]. ACM was prepared as described previously [3]. LCM was prepared by adding 350 μl serum-free M199 media/0.1 g minced liver and incubating at 37°C, 5% CO_2, for 72 h. The media was filtered, tissue discarded, and the remaining supernatant was used as LCM for subsequent experiments.

Quantification of pro- and anti-inflammatory NK cells and phenotypical analysis of NCR and chemokine receptor profiles

Single-cell suspensions from blood, omentum, and liver were stained with CD3-APC-Cy7 (BioLegend, San Diego, CA, USA) and CD56-APC, CD56-FITC, NKP46-PE, NKP30-APC, NKG2D-APC, CCR5-FITC, CXCR3-PE, CCR1-APC, CCR2-APC, CXCR2-FITC, CCR3-PE, and CCR6-APC (Miltenyi Biotec, Bergisch Gladbach, Germany). NK cells were quantified as $CD56^+CD3^-$ cells within the lymphocyte gate. For cytokine profiling and degranulation assay, cells were stimulated with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 1 h. PE-conjugated anti-CD107a (BD Biosciences UK, Oxford, United Kingdom) was added at this time to detect degranulation, followed by the addition of brefeldin A (10 μg/ml) for a further 3 h. Subsequent staining with IFN-γ-V500, TNF-α-APC, and IL-10-PE (BD Biosciences UK) was performed. Cells were acquired using a CyAn ADP flow cytometer (Beckman Coulter, Brea, CA, USA) and analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

NK cell chemotaxis assays

Chemotaxis was assessed using a 5 μm pore Transwell filter system. NK cells from the blood of 6 OAC patients and 3 noncancer controls were added to the top chamber at a density of 0.2×10^6 cells/100 μl RPMI and incubated for 2 h at 37°C. CountBright Beads were used to enumerate the number of migrated NK cells.

Culture of NK cells in ACM and LCM

PBMCs from 8 healthy donors were cultured in 500 μl RPMI and supplemented with 500 μl serum-free M199 or ACM or LCM for 48 h at 37°C. Following incubation, flow cytometric analysis of CD56, CD3, NKP46, NKP30, NKG2D, IFN-γ, TNF-α, and IL-10 expression was performed. Staining with Annexin V-FITC (IQ Products, Groningen, Netherlands) and PI (Thermo Fisher Scientific, Waltham, MA, USA) was carried out to detect apoptotic and necrotic cells.

NK cell-mediated cytotoxicity assay

Natural and IL-2-induced cytotoxicity by PBMCs from 3 healthy donors against the myeloid erythroleukemia-derived cell line K562 cells was measured using the Cell-Mediated Cytotoxicity Assay (ImmunoChemistry Technologies, Bloomington, MN, USA) following culture in 500 μl complete RPMI, supplemented with 500 μl serum-free M199 or ACM or LCM, in the presence or absence of 100 U/ml IL-2 at 37°C for 24 h. The cytotoxic capabilities were evaluated at E:T ratios of 50:1.

Statistical analyses

Statistical analysis was carried out using Prism GraphPad Version 5.0 (GraphPad Software, La Jolla, CA, USA). Differences between groups were assessed using the paired (Wilcoxon signed-rank) or unpaired (Mann-Whitney U) t test where appropriate. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

It is well-established that the omentum and liver of obese individuals are sites of excessive inflammation, fueled in part by an abundance of proinflammatory Th1 cells and M1 macrophages [12, 13]. More recently, NK cells have also been identified as mediators of adipose tissue inflammation and insulin resistance [6, 14]. However, to date, no studies have examined the effects of these inflamed tissue microenvironments on NK cells. Herein, we have addressed the prevalence and inflammatory profiles of NK cells in the omentum and liver of patients with OAC, an exemplar model of an obesity-associated cancer, together with the effects of these microenvironments on NK cell viability and function.

Significantly lower frequencies of NK cells in the omentum and liver of OAC patients compared with patient-matched blood

To ascertain whether NK cell numbers differed between the circulation, the omentum, and liver of OAC patients and noncancer control subjects, the frequencies of $CD56^+CD3^-$ (NK) cells were quantified as a percentage of lymphocytes in the blood, omentum, and liver of 14 OAC patients, in the blood of 7, and in the omentum and liver of 3 noncancer control patients (**Fig. 1A**). This analysis identified significantly fewer NK cells in OAC omentum and liver, relative to matched blood: Blood vs. Omentum (12.42% vs. 2.24%; $P < 0.0001$) and Blood vs. Liver (12.42% vs. 3.73%; $P = 0.009$; Fig. 1B). Comparisons between noncancer controls and OAC patients revealed that the NK cell frequencies in OAC liver are significantly lower than those of noncancer controls; however, the peripheral blood and omental proportions were similar between patient cohorts, which may be more reflective of the physiological state at these sites: Noncancer Liver vs. OAC Liver (29.18% vs. 3.73%; $P = 0.009$; Fig. 1C). Interestingly, the proportions of $CD56^{BRIGHT}$ NK cells were significantly higher in OAC blood compared with noncancer controls, whereas the proportions of $CD56^{DIM}$ NK cells were significantly lower, suggesting that the frequencies of circulating cytokine-producing NK cells are expanded in the cancer cohort: Noncancer Blood vs. OAC Blood ($CD56^{BRIGHT}$: 3.15% vs. 6.7%, $P = 0.008$; $CD56^{DIM}$: 96.85% vs. 93.3%, $P = 0.009$; Fig. 1D). However, no significant differences were observed in the proportions of $CD56^{BRIGHT}$ and $CD56^{DIM}$ NK cells in the omental or liver tissues (Fig. 1D).

These data suggest that the underlying malignancy somehow perpetuates the loss of NK cells from the liver. Reductions in NK cell frequencies have previously been reported in the tumor regions of liver in patients with hepatocellular carcinoma [15]. However, the livers used in our study were tumor free. Previous studies in viral hepatitis report observations that NK cell frequencies are altered in patients with liver inflammation, and

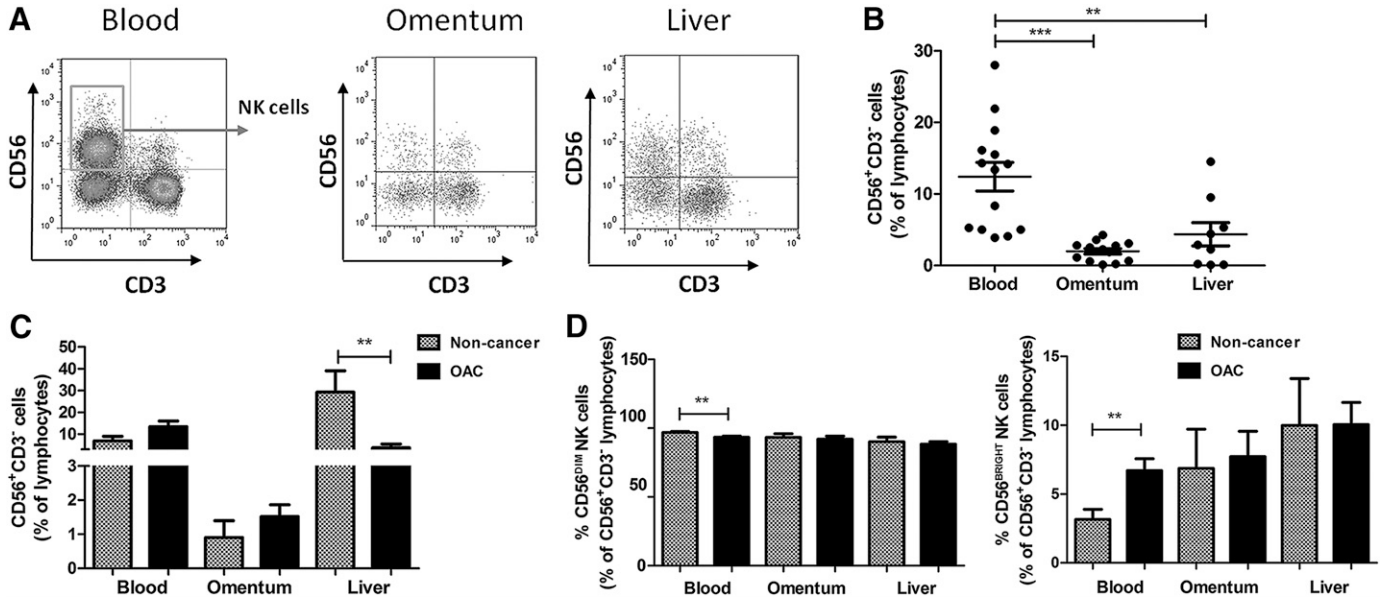

Figure 1. Significantly lower frequencies of NK cells in the omentum and liver of OAC patients compared with matched blood. (A) Representative dot plots of NK cell gating as $CD56^+CD3^-$ lymphocytes in blood, omentum, and liver. (B) The frequencies of $CD56^+CD3^-$ (NK) cells as a percentage of total lymphocytes in the blood, omentum, and liver of 14 OAC patients ($^{**}P < 0.005$, $^{***}P < 0.0005$ by paired t test). (C) The frequencies of $CD56^+CD3^-$ (NK) cells as a percentage of total lymphocytes in the blood of 7 noncancer controls and omentum and liver of 3 noncancer controls (patterned bars) and 14 OAC patients (black bars; $^{**}P < 0.005$ by unpaired t test). (D) The frequencies of $CD56^{DIM}$ and $CD56^{BRIGHT}$ NK cells as a percentage of total NK cells in the blood of 7 noncancer controls and omentum and liver of 3 noncancer controls (patterned bars) and 14 OAC patients (black bars; $^{**}P < 0.005$ by unpaired t test).

we propose that depletions of NK cells in OAC liver compared with both matched blood and noncancer control liver might represent a consequence of hepatic inflammation in obesity-associated cancer [16, 17].

Significantly higher proportions of IL-10-producing NK cells are found in OAC omentum and liver relative to matched blood

To examine whether the observed differences in NK cell frequencies among blood, omentum, and liver were accompanied by differences in NK cell inflammatory profile, the frequencies of IFN-γ-, TNF-α-, and IL-10-producing NK cells were assessed in the blood, omentum, and liver of 7 OAC patients. Significantly greater frequencies of IL-10-producing NK cells were observed in the omentum and liver compared with matched blood in the cancer cohort: Blood vs. Omentum (2.1% vs. 12.4%; $P = 0.02$) and Blood vs. Liver (2.1% vs. 19.8%; $P = 0.04$; **Fig. 2A**). However, whereas there was an abundance of IFN-γ- and TNF-α-producing NK cells in OAC omentum and liver, their frequencies were not significantly different than that in the matched blood: IFN-γ, Blood versus Omentum (21.7% vs. 39.3%; $P = 0.1$) and Blood vs. Liver (21.7% vs. 43.9%; $P = 0.2$); and TNF-α, Blood vs. Omentum (8.3% vs. 18.8%; $P = 0.2$) and Blood vs. Liver (8.3% vs. 13.8%; $P = 0.2$; Fig. 2A). The IL-10-producing capabilities of NK cells have been previously implicated in limiting immune-mediated damage in viral hepatitis and within the uterine microenvironment, and in our cohort, they may serve to limit pathological obesity-associated inflammation [17, 18].

Significantly lower $NKP46^+$ compartments in the NK cell population within OAC omentum and liver relative to matched blood

As there appeared to be an enrichment of anti-inflammatory NK cells in the omentum and liver of OAC patients, their NCR expression was also examined in ex vivo tissues to identify any alterations in NK cell activation. This study revealed significantly lower frequencies of $NKP46^+$ NK cells in the omentum and liver of OAC patients compared with blood: Blood vs. Omentum (71.7% vs. 19.7%; $P = 0.03$) and Blood vs. Liver (71.7% vs. 35.3%; $P = 0.04$; Fig. 2B and C). However, frequencies of $NKP30^+$ and $NKG2D^+$ NK cells were not significantly different: NKP30, Blood vs. Omentum (16.7% vs. 16.7%; $P = 0.6$) and Blood vs. Liver (16.7% vs. 9.4%; $P = 0.4$); NKG2D, Blood vs. Omentum (15.2% vs. 13.1%; $P = 0.9$) and Blood vs. Liver (15.2% vs. 19.4%; $P = 0.6$; Fig. 2B). These data suggest that the prevalence of NK cells, particularly the $NKP46^+$ compartment, is low in OAC omentum and liver but provide no indication of whether they are depleted in these tissues or whether their trafficking toward these sites is impaired.

Migration of OAC-derived NK cells to the adipose tissue and liver-conditioned media of OAC patients is not significantly different to that of noncancer, control-derived NK cells, whereas $CCR5^+$ NK cells are significantly enriched in OAC omentum

Ex vivo chemotaxis assays were performed to address whether the lower NK cell numbers in OAC omentum and liver were a

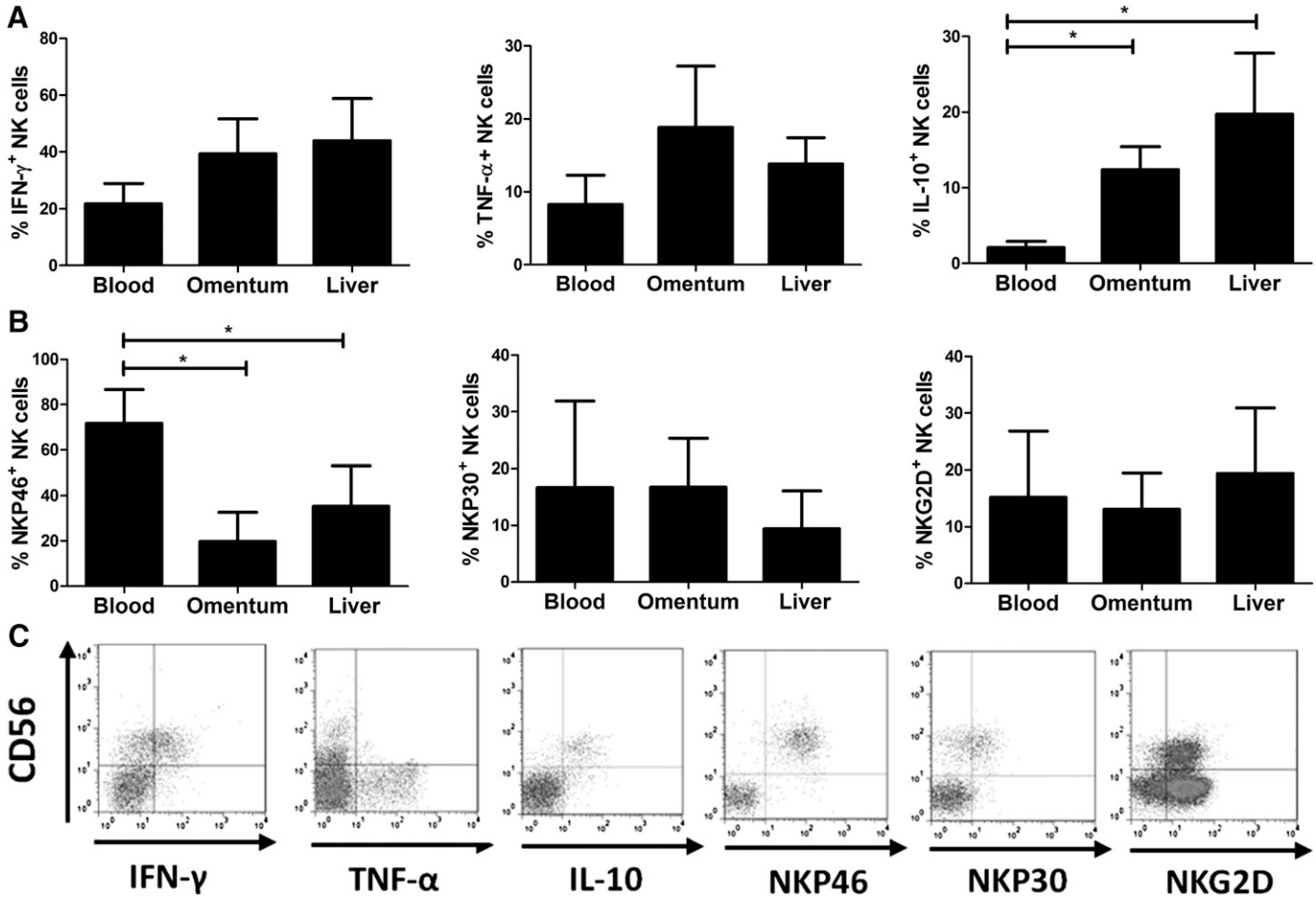

Figure 2. Significantly higher percentages of anti-inflammatory NK cells in OAC omentum and liver relative to matched blood, whereas NKP46$^+$ NK cell frequencies are significantly lower. (A) The frequencies of IFN-γ-, TNF-α-, and IL-10-expressing NK cells as a percentage of total NK cells in the blood, omentum, and liver of 7 OAC patients (*$P < 0.05$). (B) The frequencies of NKP46$^+$, NKP30$^+$, and NKG2D$^+$ NK cells as a percentage of total NK cells in the blood, omentum, and liver of 5 OAC patients (*$P < 0.05$). (C) Representative dot plot showing gating of IFN-γ-, TNF-α-, IL-10-, NKP46-, NKP30-, and NKG2D-expressing CD56$^+$ cells, previously gated on the total lymphocyte population.

result of reduced NK cell migration toward these tissues. Our data revealed that NK cells isolated from the blood of OAC patients preferentially migrate toward ACM and LCM generated from 6 OAC patients when compared with M199 media alone. This suggests that NK cell chemotaxis to secreted factors from omentum and liver is not impaired in OAC; fold-change migration to M199 vs. ACM (1 vs. 8.2; $P = 0.1$) and M199 vs. LCM (1 vs. 4.4; $P = 0.04$; **Fig. 3A**). Moreover, noncancer control blood-derived NK cells migrated to ACM and LCM at lower numbers than OAC-derived NK cells, further indicating that NK cell migratory capacity is not impaired in OAC: Noncancer vs. OAC migration to ACM (4.3 vs. 8.2; $P = 0.2$) and Noncancer vs. OAC migration to LCM (2.6 vs. 4.4; $P = 0.1$; Fig. 3A). Phenotypic analysis of chemokine receptor expression by NK cells in the blood, omentum, and liver revealed that CCR5$^+$ NK cell frequencies were significantly higher in OAC omentum and substantially higher in OAC liver compared with blood: Blood vs. Omentum (32.8 vs. 58.7; $P = 0.03$) and Blood vs. Liver (32.8% vs. 51.2%; $P = 0.4$; Fig. 3B). These data suggest that NK cells can effectively migrate to omentum and liver in OAC, and it is likely that CCR5 ligands are among the chemokines that play a key role in such chemotaxis. However, further work is needed to confirm the specific chemokine pathways guiding NK cells to these tissues.

Significantly higher levels of NK cell death were observed following culture in adipose tissue and liver conditioned media

As it appeared that NK cell migration to omentum and liver is not impaired in OAC, it was proposed that reduced NK cell frequencies in these tissues may be, at least in part, a result of enhanced cell death. To assess the effects of the omental and hepatic microenvironment on NK cell viability, PBMCs from 4 healthy donors were cultured in ACM or LCM from OAC patients for 48 h and subsequently stained with Annexin V and PI. These studies revealed that soluble factors in OAC omentum and liver resulted in significantly increased NK cell but not T cell death (Annexin V$^+$ PI$^+$ cells). Therefore, the depletion of NK cells observed in the omentum and liver of OAC patients is, in part, a result of secreted factors from these tissues. T cells

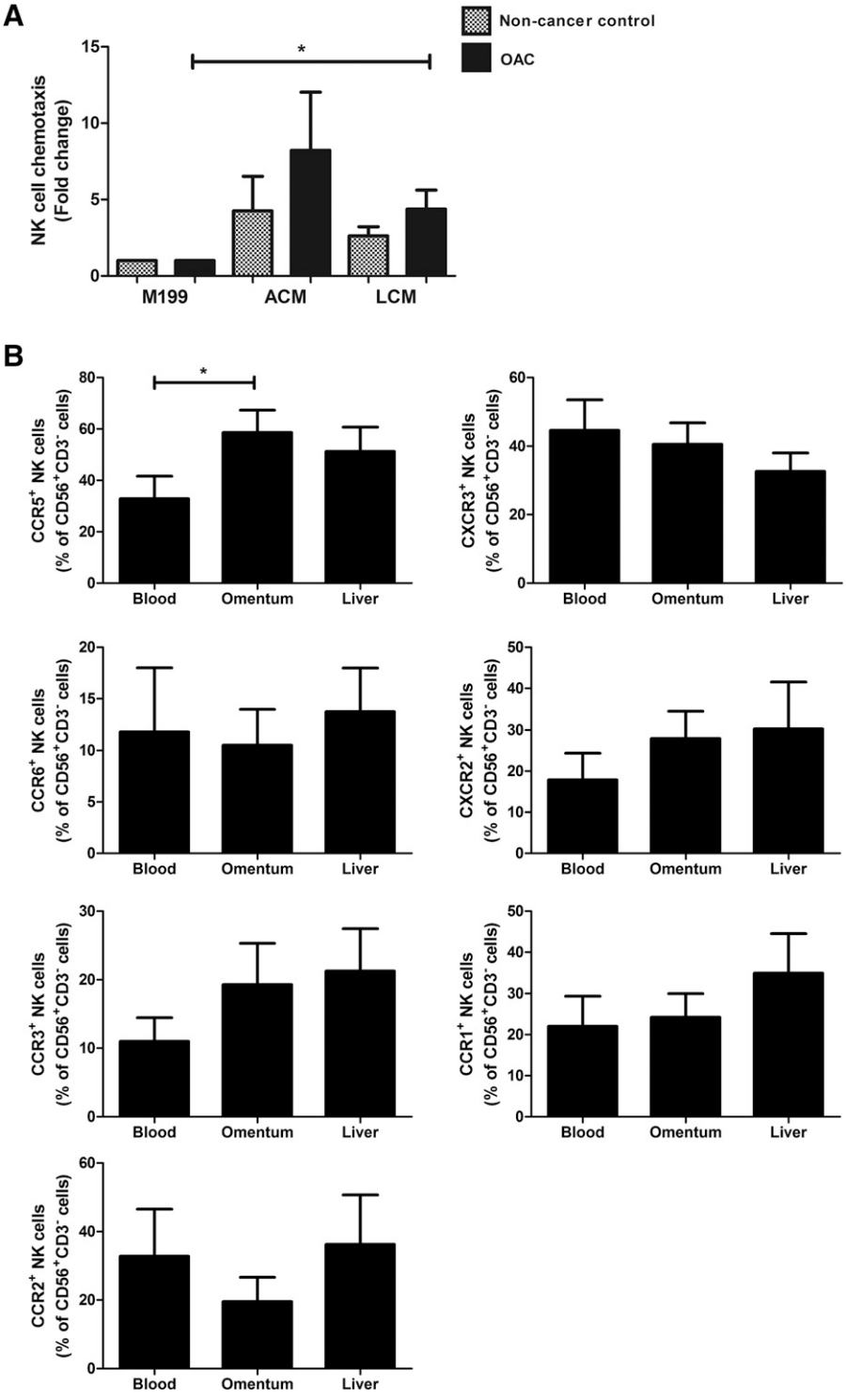

Figure 3. NK cell chemotaxis to secreted factors from omentum and liver is not impaired in OAC. (A) Bar chart showing the fold-change migration of noncancer control blood-derived (patterned bars) and OAC patient blood-derived (black bars) NK cells to M199 media alone (M199), ACM, and LCM generated from 6 obese OAC patients (*$P < 0.05$ by paired t test). (B) Bar charts showing the frequencies of CCR5, CXCR3, CCR6, CXCR2, CCR3, CCR1, and CCR2 expressing NK cells in the blood, omentum, and liver of 10 OAC patients. Frequencies are shown as a percentage of the total $CD56^+CD3^-$ (NK) cell population (*$P < 0.05$ by paired t test).

cultured with OAC-derived ACM and LCM were not, however, susceptible to such cell death, which is in line with their prevalence in these tissues [3, 4]: NK cells, M199 vs. ACM (2.49% vs. 15.27%; $P = 0.05$) and M199 vs. LCM (2.49% vs. 25.15%; $P = 0.02$); T cells, M199 vs. ACM (3.5% vs. 3.6%; $P = 0.7$) and M199 vs. LCM (3.5% vs. 4.4%; $P = 0.6$; **Fig. 4A** and **B**). Further investigations revealed that noncancer control-derived ACM induced substantial but nonsignificant levels of NK cell death, whereas noncancer, control-derived LCM induced significant levels of NK cell death, suggesting that these changes in NK cell viability are not limited to the omental and hepatic microenvironments of OAC patients and perhaps represent a normal physiological function of these tissues: % Late apoptotic NK cells, M199 vs. control ACM (4.6% vs. 22.6%; $P = 0.06$) and M199 vs.

control LCM (4.6% vs. 15.9%; $P = 0.02$); T cells, M199 vs. control ACM (1.6% vs. 8.9%; $P = 0.2$) and M199 vs. control LCM (1.6% vs. 21.6%; $P = 0.3$; Fig. 4A). However, it must be noted that the apoptosis-inducing capabilities of OAC-derived LCM are higher, albeit nonsignificant, than that of the noncancer-derived LCM, suggesting that NK cells from OAC patients may be more susceptible to apoptotic signals from the liver (Control LCM vs. OAC LCM: 15.9% vs. 25.15%; $P = 0.4$). It is also interesting to note that T cell apoptosis is lower following culture with conditioned media from the liver and omentum of OAC patients compared with noncancer controls. This may lead to an accumulation of inflammatory T cells at these sites in OAC, but this requires further investigation.

Significantly lower frequencies of TNF-α-producing and NCR$^+$ NK cells following culture in OAC-derived LCM compared with media alone, whereas IL-10-producing NK cells are significantly higher

As soluble factors in the omental and hepatic microenvironment appeared to induce NK cell death, their effects on the pro- and anti-inflammatory compartments of NK cells were also examined. The cytokine profiles of the remaining viable NK cells were analyzed using flow cytometry following 48 h culture in ACM and LCM. Interestingly, significant increases in IL-10-producing NK cells were observed: M199 vs. ACM (1.1% vs. 6.3%; $P = 0.02$) and M199 vs. LCM (1.1% vs. 5.6%; $P = 0.008$; Fig. 4C). However, of most significance was the reduction in TNF-α-expressing NK cells following culture in LCM: M199 vs. ACM (31.4% vs. 25.1%; $P = 0.2$), M199 vs. LCM (31.4% vs. 9.4%; $P = 0.0004$), and ACM vs. LCM (25.1% vs. 9.4%; $P = 0.02$; Fig. 4C). In contrast to this, the frequencies of IFN-γ-expressing NK cells were not altered significantly: M199 vs. ACM (53.9% vs. 45.3%; $P = 0.2$) and M199 vs. LCM (53.9% vs. 44.1%; $P = 0.2$; Fig. 4C). Interestingly, further investigations revealed substantial increases in IL-10-producing NK cell frequencies when cultured in the presence of ACM or LCM from noncancer control subjects (Fig. 4C). Furthermore, TNF-α- and IFN-γ-producing NK cells were significantly less abundant following culture in noncancer control-derived ACM ($P = 0.01$), whereas these trends toward lower frequencies of TNF-α and IFN-γ producers were also observed following culture

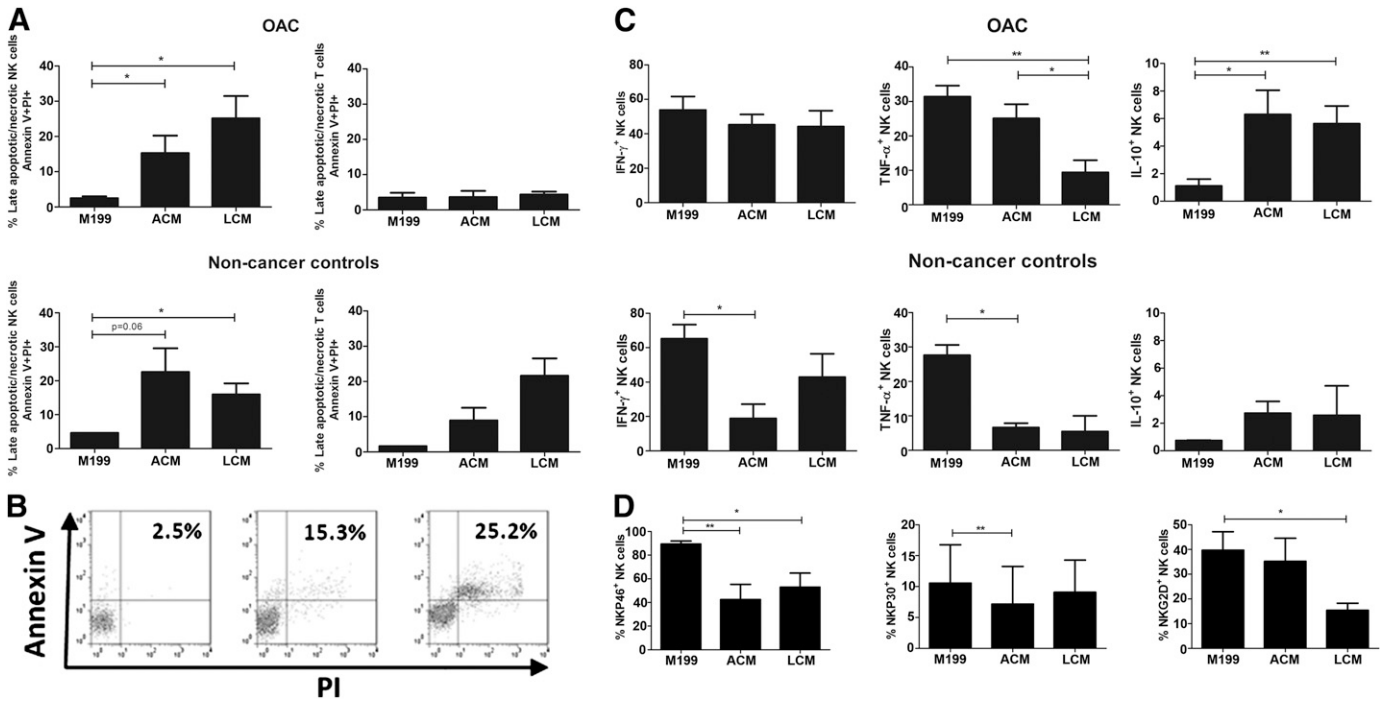

Figure 4. Significantly higher levels of NK cell death and significant reductions in NCR$^+$ and TNF-α$^+$ proportions following culture in LCM. (A, upper) The frequencies of Annexin V$^+$ PI$^+$ NK cells as a percentage of total NK cells (left) and Annexin V$^+$ PI$^+$ T cells as a percentage of total T cells (right) from the blood of 4 healthy donors following 48 h of culture in M199 media, OAC-derived ACM, and OAC-derived LCM (*$P < 0.05$ by paired t test). (Lower) The frequencies of Annexin V$^+$ PI$^+$ NK cells as a percentage of total NK cells (left) and Annexin V$^+$ PI$^+$ T cells as a percentage of total T cells (right) from the blood of 4 healthy donors following 48 h of culture in M199 media, noncancer control-derived ACM, and noncancer control-derived LCM (*$P < 0.05$ by paired t test). (B) Representative dot plots of Annexin V$^+$ PI$^+$ NK cells, previously gated on CD56$^+$CD3$^-$ lymphocytes following 48 h of culture in M199 media, ACM, and LCM. (C, upper) The frequencies of IFN-γ-, TNF-α-, and IL-10-expressing NK cells as a percentage of total NK cells from the blood of 8 healthy donors following 48 h of culture in M199 media, OAC-derived ACM, and OAC-derived LCM (*$P < 0.05$, **$P < 0.005$, by paired t test). (C, lower) The frequencies of IFN-γ-, TNF-α-, and IL-10-expressing NK cells as a percentage of total NK cells from the blood of 8 healthy donors following 48 h of culture in M199 media, noncancer control-derived ACM, and noncancer control-derived LCM (*$P < 0.05$, by paired t test). (D) The frequencies of NKP46$^+$, NKP30$^+$, and NKG2D$^+$ NK cells as a percentage of total NK cells from the blood of 5 healthy donors following 48 h of culture in M199 media, OAC-derived ACM, and OAC-derived LCM (*$P < 0.05$, **$P < 0.005$, by paired t test).

in LCM, but this was not statistically significant (TNF-α, $P = 0.1$; IFN-γ, $P = 0.06$; Fig. 4C). Together with our observations in the cancer patient cohort, these data suggest that proinflammatory NK cell frequencies are naturally controlled in liver and omentum, perhaps to maintain a more homeostatic state, reflective of the tolerogenic microenvironment of the liver. Our data support a previous study that demonstrated a phenotypic switch in NK cells from a proinflammatory to an anti-inflammatory phenotype in response to systemic inflammation in toxoplasmosis [19]. In the context of obesity, proinflammatory cytokine-producing NK cells have recently been implicated in adipose tissue stress and insulin resistance [6]. Therefore, it is possible that the depletions in TNF-α-producing NK cells and increases in IL-10-producing NK cells reported here are a means of limiting inflammation and maintaining immune homeostasis in the tissue microenvironments of omentum and liver. However, further studies are needed to elucidate fully the mechanisms through which this might occur.

Frequencies of NCR^+ NK cells of 5 healthy subjects were also quantified following 48 h culture in ACM and LCM. $NKP46^+$ NK cell frequencies were reduced significantly following 48 h of culture in ACM and LCM: M199 vs. ACM (89.3% vs. 42.4%; $P = 0.007$) and M199 vs. LCM (89.3% vs. 52.9%; $P = 0.02$; Fig. 4D). $NKG2D^+$ NK cell frequencies were significantly reduced following 48 h of culture in LCM: M199 vs. ACM (39.7% vs. 35.2%; $P = 0.6$) and M199 vs. LCM (39.7% vs. 15.4%; $P = 0.02$), whereas $NKP30^+$ NK cell frequencies were significantly reduced following 48 h of culture in ACM: M199 vs. ACM (10.6% vs. 7.2%; $P = 0.005$) and M199 vs. LCM (10.6% vs. 9.1%; $P = 0.4$; Fig. 4D). Similar trends were observed with mean fluorescence intensity values for these markers (data not shown). These data indicate that NCR surface expression is reduced by soluble factors in ACM and LCM, suggesting that the omental and hepatic tissue microenvironments can influence NK cell activation. Whereas others have reported that cytotoxicity of intrahepatic NK cells is impaired in the inflamed liver of patients with chronic HCV, and our data indicate significant alterations in NCR expression in OAC omentum and liver, our supplemental data suggest that NK cell cytotoxicity against K562 cells is unaffected by secreted factors in ACM and LCM (Supplemental Fig. 1) [20]. As $NKP46^+$ NK cells previously have been shown to mediate M1 polarization and adipose tissue inflammation and contribute to insulin resistance, it is possible that their depletion in our cohort is another means of controlling not just inflammation but also metabolic dysfunction [6]. Furthermore, NKP46 expression by NK cells has been associated with liver inflammation and immune-mediated liver damage in HCV, and therefore, its modulation in OAC patient liver might also be a means of limiting pathology in the inflamed hepatic microenvironment [21].

Concluding remarks

For the first time, we have shown depletions in inflammatory NK cell frequencies in the omentum and liver of patients with the obesity-associated malignancy, OAC. Our mechanistic studies indicate that this may be, at least in part, a result of increased NK cell death. These data suggest that the microenvironments of omentum and liver promote an anti-inflammatory NK cell cytokine profile with a reduction in proinflammatory $NKP46^+$ NK cell populations. In light of these data and previous studies that identified proinflammatory NK cells as mediators of insulin resistance, we propose that an enhancement of NK cell bias toward an anti-inflammatory phenotype may have potential as an immunotherapy to alleviate pathologic inflammation in obesity and liver disease.

AUTHORSHIP

M.J.C. performed the acquisition, analysis, and interpretation of data; drafted the manuscript; and completed the statistical analysis and study concept and design. J.L. obtained Health Research Board funding and technical and material support and performed a critical revision of the manuscript for intellectual content. V.F., S.L.D., and S.C. acquired data. N.R., N.G., and Z.U. provided material support. C.O. provided intellectual support. J.V.R. revised the manuscript for intellectual content and provided material support.

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DISCLOSURES

The authors declare no conflicts of interest.

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