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The Lytic Potential of Human Liver NK Cells Is Restricted by Their Limited Expression of Inhibitory Killer Ig-Like Receptors

Bryan M. Burt,* George Plitas,* Zeguo Zhao,† Zubin M. Bamboat,* Hoang M. Nguyen,* Bo Dupont,† and Ronald P. DeMatteo2*

The human liver is enriched in NK cells which are potent effectors of the innate immune system. We have determined that liver NK cells freshly isolated from surgical specimens from patients with hepatic malignancy have less cytolytic activity than autologous blood NK cells. This difference was due to a higher proportion of CD16−NK cells in the liver and reduced cytotoxicity by CD16+ liver NK cells compared with their blood counterparts. CD16+ liver NK cells had similar expression of activating NK receptors and had similar intracellular granzyme B and perforin content compared with CD16+ blood NK cells. CD16+ liver NK cells contained a reduced fraction of cells with inhibitory killer Ig-like receptors specific for self-MHC class I (self-killer Ig-related receptor (KIR)) and an increased fraction of self-KIR−NKG2A+ and self-KIR+NKG2A−cells. Using single-cell analysis of intracellular IFN-γ production and cytotoxicity assays, we determined that CD16+ liver NK cells expressing self-KIR were more responsive to target cells than those cells that did not express self-KIR molecules. CD16+ liver NK cells gained cytolytic function when stimulated with IL-2 or cultured with LPS or poly(I:C)-activated autologous liver Kupffer cells. Thus, the human liver contains NK cell subsets which have reduced effector function, but under appropriate inflammatory conditions become potent killers. The Journal of Immunology, 2009, 183: 1789–1796.

The liver is continuously confronted with a large antigenic load that includes toxins, harmless dietary proteins, and antigenic elements from commensal intestinal organisms. The liver is also a common site of chronic viral and parasitic infection and is the most common location for distant metastatic disease in cancer. Therefore, the hepatic immune system must differentiate harmless foreign Ags from invasive threats to deliver appropriate immune responses. Although the local immune mechanisms that are required to direct immunogenic or tolerogenic responses to these varied antigenic challenges are largely unknown, the overall balance in hepatic immunity tends to favor tolerance. For example, HLA matching between donor and recipient does not affect the outcome of liver transplantation, whereas HLA-disparate heart, lung, and kidney transplants are frequently rejected (1). Furthermore, compared with other solid organ transplants, T cell-mediated rejection of liver allografts is uncommon (2). Additionally, the liver is believed to play a central role in oral tolerance, a phenomenon in which systemic immune responses to specific Ags are suppressed by prior administration of the Ag via the oral route or portal vein (3).

To cope with potentially toxic insults without launching harmful systemic immune responses, the liver may rely on its innate immune system. The lymphoid system of the liver is uniquely weighted with innate cells. Although peripheral blood lymphocytes are dominated by T and B cells that possess clonotypic Ag-specific receptors and are capable of mediating adaptive responses, the lymphoid system of the liver is comprised chiefly of innate immune cells such as NK cells, NKT cells, and y6 T cells which use invariant receptors and are capable of detecting and responding rapidly to pathogens and malignant cells (4).

NK cells are bone marrow-derived lymphocytes that are able to recognize and kill transformed and virus-infected cells without the need for prior sensitization. NK cells are a predominant lymphocyte population in the murine and human liver. Whereas NK cells account for <20% of all circulating lymphocytes, they may comprise up to 50% of the total lymphocyte pool in the human liver (4). In humans, two main functional subsets of NK cells exist based on their surface density of CD56 and CD16 (5). CD56dim CD16+ NK cells (referred to hereafter as CD16+ NK cells) comprise upward of 90% of NK cells in the periphery, contain an abundance of lytic granules, and have high cytotoxic potential. CD56bright CD16− NK cells (referred to hereafter as CD16− NK cells) constitute a minor population of circulating NK cells and have decreased cytotoxic ability, but are capable of producing copious amounts of cytokines (6).

The distribution of human NK cell subsets varies within solid organs, possibly reflecting a tissue-specific division of labor. Although rare in the blood, CD16− NK cells comprise ~75% of NK cells in lymph nodes and 50% of NK cells in the spleen (7, 8). NK cells in lymphoid tissues can be found in close proximity to dendritic cells (DCs) (9). Through production of IFN-γ, NK cells may act to polarize adaptive T cell responses (10, 11) and can limit EBV-triggered B cell transformation (12). In the uterus and placenta, a unique subset of NK cells with the CD16− phenotype

3 Abbreviations used in this paper: DC, dendritic cell; MHC-I, MHC class I; KIR, killer Ig-like receptor; LMNC, liver mononuclear cell; KC, Kupffer cell.

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predominates (13, 14). Although these NK cells do contain an abundance of cytolytic granules, they do not lyse the HLA-A- and HLA-B-negative extravillous trophoblasts that invade the decidua during pregnancy and, when freshly isolated, demonstrate weak cytolytic function (13). In the placenta, these tissue-resident NK cells appear to be specialized for regulating specific developmental processes at the fetal-maternal interface (15).

The effector functions of NK cells are regulated in part by their interaction with MHC class I (MHC-I) molecules. Ligation of NK cell inhibitory receptors by their specific MHC-I ligands on target cells results in inhibition of NK cell function. Therefore, targets with normal MHC-I expression are more resistant to killing than those lacking MHC-I and target cells lacking MHC-I are sensitive to NK cytotoxicity. Through a variety of inhibitory and activating receptors and their ligands on target cells, NK cells identify cells that have lost or altered MHC-I expression, as occurs in viral infection and malignant transformation. Recently, the “missing-self” paradigm (16) has been modified to accommodate the observation that a substantial subset of NK cells are lacking all MHC-I-specific receptors or inhibitory receptors for “self.” MHC-I NK cells require “licensing” or “education” by an inhibitory receptor that can interact with a self-MHC-I ligand to elicit strong responses to targets without self-MHC-I molecules, while NK cells without such receptors are hyporesponsive (17–23). The functional NK cell repertoire is therefore controlled by the inhibitory receptors that recognize self-MHC-I ligands. In humans, inhibitory NK receptors that recognize MHC-I include the polymorphic killer Ig-like receptors (KIRs) and the lectin-like CD94-NKG2A heterodimer (24). The inhibitory KIRs consist of KIR2DL1 which recognizes the group of HLA-C molecules with a Lys80 residue (HLA-C2 group), KIR2DL2/2DL3 which recognize the group of HLA-C with an Asn80 residue (HLA-C1 group), and KIR3DL1 which recognize Bw4-containing HLA-B alleles (HLA-Bw4 group). The CD94-NKG2A heterodimer recognizes a complex of the nonclassical MHC-I molecule HLA-E bound to peptides from the leader sequences of other MHC-I molecules. Using fresh surgical specimens from patients with malignancy involving the liver, we show that the human liver NK cell repertoire as based on inhibitory receptor status is substantially different from its circulating counterpart. We have characterized the phenotype and function of NK cells in paired autologous liver and blood samples. We have found that human liver NK CD16+ NK cells display a decreased functional response toward MHC-I-deficient target cells and that this is caused by a decrease in the proportion of NK cells with alicensed phenotype. Our findings emphasize the multitude of ways in which immune responses can be regulated in an organ-specific manner.

Materials and Methods

Cell isolation

Blood and matched liver samples were collected from 46 patients undergoing elective hepatectomy at Memorial Sloan-Kettering Cancer Center. The indications for operation included metastatic disease (n = 10), primary cancer (n = 10), and benign disease (n = 2). Detailed clinicopathologic data are shown in supplemental Table 1.4 Informed consent was obtained according to an institutional review board-approved protocol. Blood was drawn intraoperatively and PBMCs were isolated by density centrifugation over Ficoll-Paque Plus (GE Healthcare). Liver tissue was obtained according to an institutional review board-approved protocol. Blood was drawn intraoperatively and PBMCs were isolated by density centrifugation over Ficoll-Paque Plus (GE Healthcare). Liver tissue was obtained according to an institutional review board-approved protocol.

Flow cytometry

Immunophenotypic analysis of cells was performed with seven-color flow cytometry on a FACS Aria cytometer (BD Biosciences) using mAbs conjugated to FITC, PE, PerCP-Cy5.5, PE-Cy7, allophycocyanin, allophycocyanin-Cy7, and Alexa Fluor 700 (AF700). Abs used for cell analysis included FITC-CD16 (3G8), FITC-CD62L (Dreg56), FITC-CD94 (HP-1D9), PE-NKp30 (P10-15), PE-NKp44 (P44-81), PE-NKp46 (9E2), PE-TRAIL (R1-K2), PE-NKG2D (1D11), PerCP-Cy5.5-CD3 (SK7), PE-CD7-Cy63 (G8), allophycocyanin-Cy63 (DX12), allophycocyanin-Cy63-7HLA-DR (L243), allophycocyanin-Cy63-5HLA-C (L283), allophycocyanin-Cy63-6HLA-C (FN50), AF700-CD56 (B159) (all from BD Biosciences), PE-CD56 (AF1I-7H3), allophycocyanin-KIR2DL2/1D51S (11BP6), allophycocyanin-KIR2DL2/1D51L (DX27), allophycocyanin-KIR3DL1 (DX9) (all from Miltenyi Biotec), PE-NKG2A (Z199; Beckman Coulter), and PE-NKG2C (134591; R&D Systems). Intracellular perforin and granyme content was detected using FITC-perforin (d9F, eBiocience) and PE-granyme B (eBioGrB; eBiocience) following cell permeabilization (Cytotox/ Cytoperm; BD Biosciences). Ig isotype controls were used where appropriate. Flow cytometry data were analyzed with FlowJo software (Tree Star).

FACS

LMNCs or PBMCs were stained with the following Abs for cell separation: FITC-CD16, PE-CD56, PE-Cy7-CD11b (1CR-F44; BD Biosciences), allophycocyanin-KIR2DL2/1D52L, allophycocyanin-KIR2DL2/1D53L, allophycocyanin-KIR3DL1, allophycocyanin-CD3 (SK7), PE-CD56, allophycocyanin-Cy7-CD3 (SK7), PE-CD7, and PE-CD56-Cy7-CD14 (My9; BD Biosciences). Cell sorting was performed using a MoFlo (DakoCytomation) or BD FACS Aria (BD Biosciences) cell sorter and post sort purities were routinely >98%. NK cells were selected from the lymphocyte gate as CD56+CD3− cells and in some cases as CD56dim CD16+CD3− cells. Liver Kupffer cells (KC) were sorted as CD14+CD11b− cells contained within the monocye gate.

Cytotoxicity assay

Standard cytotoxicity assays were performed using freshly isolated NK cells and 51Cr-labeled MHC-I-negative target cells. The K562 cell line was obtained from the American Type Culture Collection and the LCL721.221 cell line was provided by P. Parham (Stanford University, Palo Alto, CA). Cell lines were maintained in complete RPMI 1640 containing 10% heat-inactivated FCS, 2 mM t-glutamine, 0.1% 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. Two million target cells were labeled with 100 μCi of Na51CrO4 (PerkinElmer Life and Analytical Sciences) at 37°C for 90 min and then washed with medium. Lysis assays were performed in triplicate for 4 h at the indicated E:T ratio. In some experiments, NK cells were treated with 100 ng/ml concanamycin A (ICN Biomedical) or a DMSO (Fischer Scientific) vehicle for 2 h at 37°C before the addition of targets. Chromium release was measured with a TopCount NXT microplate scintillation and luminescence counter (PerkinElmer) and percent specific lysis was calculated as (cpm experimental − cpm spontaneous release) × 100/cpm maximum release − cpm spontaneous release. In other experiments, freshly isolated NK cells were cultured overnight in medium alone, in medium containing 200 U/ml recombinant human IL-2 (Proleukin; Novartis), or with autologous liver KC at an NK:KC ratio of 5:1. Before coculture, KC were pulsed for 1 h with LPS (1 μg/ml from Escherichia coli 055:B5; Sigma-Aldrich) or poly(I:C) (50 μg/ml; InvivoGen) and then washed. Following the culture period, NK cells were recounted for use in cytotoxicity assays.

*The online version of this article contains supplemental material.*
Human liver NK cells are poorly cytolytic

Because of their activated phenotype, we hypothesized that resident liver NK cells would have greater cytolytic function than circulating NK cells. On the contrary, we found that freshly isolated blood NK cells were consistently more potent than autologous liver NK cells in their ability to kill K562 and MHC-I-deficient LCL721.221 targets (Fig. 2, A and B). To determine whether subset composition was merely responsible for the difference in lysis between blood and liver bulk NK cells, we compared the lytic function of blood and liver CD16⁺ NK cells. Strikingly, CD16⁺ liver NK cells demonstrated markedly less killing (Fig. 2, C and D). The low number of CD16⁺ NK cells within our limited number of paired blood and liver samples that were freshly isolated did not allow for further analysis. However, in agreement with previous reports (27), CD16⁺ blood NK cells were less cytolytic than CD16⁺ blood NK cells (data not shown) and in two patients where adequate numbers could be isolated to compare sorted liver and blood CD16⁺ NK cells, lysis of class I-negative targets was similar (liver: 30.9 ± 0.6% vs blood: 32.9 ± 1.3% at E:T 15:1 for K562 and liver: 12.8 ± 1.6% vs blood: 10.6 ± 0.2% at E:T 15:1 for LCL721.221). Therefore, human liver is enriched in NK cells with decreased cytotoxic function because of both a higher percentage of CD16⁺ NK cells and overall decreased cytolytic activity of liver CD16⁺ NK cells.

CD16⁺ liver NK cells express activating receptors and contain intracellular perforin and granzyme B

We next sought to determine the reason for decreased killing by CD16⁺ liver NK cells. It has become clear that activating receptors contribute substantially to NK cell specificity and may provide the critical threshold of signals needed to override the counterbalancing effects of inhibitory receptors to mount a
productive response (28). We did not find a difference in the expression of activating receptors between blood and liver CD16/H11001 NK cells that could account for their disparate cytotoxicity (supplemental Table II). Using concanamycin A to inhibit the granule exocytosis pathway (29), we found that both liver and blood CD16/H11001 NK cells killed K562 targets predominantly through granule release (Fig. 3A). Both liver and blood CD16+ NK cells contained intracellular perforin and granzyme B, although a trend toward decreased perforin content was observed in liver NK cells (Fig. 3, B–D). Therefore, CD16+ liver NK cells are armed for killing but have overall decreased cytotoxic activity.

A lower proportion of liver NK cells possesses inhibitory KIR receptors for self-MHC-I

NK cells that possess inhibitory receptors with specificity for self-MHC-I Ags are endowed with functional competence (i.e., licensed) and are highly responsive to tumor targets (17–23). Therefore, we investigated whether the expression of these inhibitory receptors was different on liver and blood NK cells. HLA-KIR ligand groups C1, C2, and Bw4 were assigned preoperatively to each patient by HLA genotyping and KIRs specific to self-MHC-I were identified (self-KIR). NK cell expression of self-KIR and NKG2A receptors was determined for eight patients (Fig. 4 and Table I).

The phenotype distribution of CD16+ NK cells was markedly different between liver and blood. CD16+ NK cells in the liver contained...
Inhibitory receptor expression on liver NK cells

<table>
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<tr>
<th></th>
<th>Self-KIR⁺NKG2A⁺</th>
<th>Self-KIR⁺NKG2A⁻</th>
<th>Self-KIR⁻NKG2A⁺</th>
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<td>Bulk NK cells</td>
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<td>0.009</td>
<td>0.002</td>
<td>&lt;0.0001</td>
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<td>CD16⁺ NK cells</td>
<td></td>
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<tr>
<td>Liver</td>
<td>2.9 ± 0.6</td>
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<td>63.6 ± 3.2</td>
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<tr>
<td>$p$</td>
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*Matched liver and blood samples are shown for eight individual patients.

The relative distribution of NK cell subpopulations in the liver and blood accounts for the differences in effector function in CD16⁺ NK cells

We investigated how the inhibitory receptor subset phenotype of liver CD16⁺ NK cells contributed to their functional capacity. We observed that liver CD16⁺ NK cells had modestly decreased intracellular IFN-γ responses to K562 target cells (Fig. 5, A and B) and CD16 cross-linking than their matched blood CD16⁺ NK cells (Fig. 5, C and D). We next compared the four subsets of NK cells defined by self-KIR and NKG2A and their IFN-γ production following stimulation with K562 (Fig. 5B) and CD16 cross-linking (Fig. 5D). There were no significant differences between the responses for each subset of NK cells in blood and liver. For example, the two self-KIR⁺ subsets (i.e., self-KIR⁺NKG2A⁺ and self-KIR⁺NKG2A⁻) had higher responses than the two self-KIR⁻ subsets in both blood and liver and displayed a similar hierarchy of responsiveness (Fig. 5, B and D). Cytolytic effector function of the self-KIR expressing liver CD16⁺ NK cells was compared with liver CD16⁺ NK cells that did not possess self-KIR using standard chromium-release assays. Consistent with the NK cell licensing hypothesis, self-KIR⁺ liver NK cells had higher cytolytic activity than self-KIR⁻ cells (Fig. 5E). We have not found the degree of CD107a membrane expression on liver NK cells to correlate reliably with classical chromium-based lysis assays (data not shown). Similar to a previously published comparison between licensed and unlicensed blood NK cells (19), we did not find a difference in perforin expression between self-KIR⁺ and self-KIR⁻ subsets of liver CD16⁺ NK cells that could account for the observed difference in killing (Fig. 5F). Taken together, the decreased frequency of self-KIR-expressing CD16⁺...
The cytolytic restriction on liver CD16+ NK cells is removed by IL-2 or an activated liver APC

IL-2 potentiates the cytotoxic capability of NK cells by up-regulating cytolytic machinery and increasing intracellular perforin (30). Moreover, human blood NK cells gain an equal level of cytotoxicity when cultured in IL-15 regardless of the expression of inhibitory receptors for MHC-I (20). To determine whether liver CD16+ NK cells were anergic or whether their cytolytic restriction could be overcome, they were cultured overnight in IL-2. NK cells became robust cytolytic effectors after activation with IL-2 with comparable potency to IL-2-cultured blood CD16+ NK cells (Fig. 6A). Similar results were obtained using IL-15 (data not shown).

Although a predominance of NK cells with weak resting cytolytic function may be advantageous to prevent hyperactivity in the potentially volatile, endotoxin-rich environment of the liver, their cytolytic function could be beneficial during an infectious or metastatic insult. Therefore, we asked whether an activated resident liver immune cell could provide the necessary signals to boost their killing potential. When freshly isolated liver CD16+ NK cells were cultured overnight with autologous Kupffer cells activated either by LPS or poly(I:C), they became more potent killers of K562 targets (Fig. 6B). Hence, although the liver contains an abundance of CD16+ NK cells with cytolytic potential that is limited to some degree by their inhibitory receptor expression, they are capable of mounting effective lytic responses in the appropriate inflammatory settings.

**Discussion**

The threshold for activation of innate immune responses varies among different organs. These activation profiles are controlled by different organ-specific regulatory mechanisms that are required to sustain immune homeostasis yet can result in immunopathology if disrupted (31). For example, the teeming microbial mass in the colon does not induce overt inflammation in the intestinal mucosa, suggesting the existence of specialized regulatory mechanisms that curtail initiation of inflammatory and bactericidal programs against the luminal microflora. Recent studies have suggested that intestinal epithelial cells may have evolved polarized signaling mechanisms to maintain colonic homeostasis and regulate tolerance and immunity. Although basolateral TLR9 stimulation mobilizes an inflammatory immune cascade, apical (luminal) TLR9 stimulation results in tolerogenic immune responses (32). Additionally, human intestinal macrophages do not express innate response receptors for LPS (CD14) and do not produce proinflammatory cytokines in response to a range of inflammatory stimuli (33). Similarly, human liver DCs promote CD4+ T cell hyporesponsiveness and favor the generation of regulatory T cells (34) and human Kupffer cells respond to LPS paradoxically by secreting the anti-inflammatory cytokine IL-10 (35). In the lung, administration of TLR ligands induces high levels of the negative immunoregulator IDO that can decrease Th2-driven experimental asthma (36).

The immune environment of the liver is unique because it is continually exposed to commensal-derived endotoxin that is present in the portal circulation and therefore lends itself to constitutive TLR4 ligation in the absence of an infectious threat. LPS is a powerful activator of both innate and adaptive immunity through the triggering of inflammatory cascades, secretion of proinflammatory cytokines, and maturation of specialized APCs. NK cells are present in abundant numbers in the liver and are responsible for hepatocyte death and liver inflammation during hepatitis B virus infection (37).

Furthermore, hepatocytes make up 60–80% of the cellular mass of the liver and the expression of MHC-I on human hepatocytes has been well documented to be low and even absent in vivo (38–40). This potentially MHC-I deficient environment lends itself to relief of the tonic inhibition on NK cells; however, NK cells do not appear to be hyperactive in the potentially hostile immune environment of the liver. Therefore, to prevent liver injury and compromise liver function, it is crucial that autoaggressive activation of NK cells be regulated.

It has been previously recognized that human liver NK cells are enriched in CD16+ NK cells (41) and that this subset of NK cells, through cross-talk with activated resident liver macrophages, will produce IFN-γ (42). We have determined that the subset composition of NK cells in the human liver is variable but on average is represented by equal percentages of CD16+ and CD16- NK cells. Furthermore, we and others have found that NK cells in the human liver display an activated phenotype (43) and we show here that this phenotype is accounted for predominantly by the CD16- subset of liver NK cells. Similar to CD16- lymph node NK cells, this subset of human liver NK cells express high levels of HLA-DR, Nkp44, and CD69 and low levels of CD62L (8, 44). Despite their activated phenotype, CD16- NK cells had poor cytotoxic capacity like their respective blood NK cell equivalents. However, CD16- NK cells that are regarded as the cytotoxic subset of NK cells in the liver were also poorly cytotoxic, suggesting one potential regulatory means by which liver NK cell autoreactivity could be quelled.

The isolation of immune cells from solid organs is more difficult than isolating circulating lymphocytes and requires processing of the tissues. Using propidium iodide and annexin V staining, we initially established that the method of isolation did not influence the viability or killing ability of the cells (data not shown). Furthermore, by subjecting whole blood to enzyme treatment and a mock isolation procedure identical to that used to isolate liver NK

**FIGURE 6.** Liver CD16+ NK cell cytolytic function is increased by IL-2 or activated KCs. CD16+ NK cells were purified from the blood or liver of the same patient and cultured overnight in either medium or IL-2 (200 IU/ml). The following day the lytic capacity of the cultured NK cells were assayed using chromium-labeled K562 targets. These data are representative of experiments from three individual patients. **A**, CD16+ liver NK cells were cultured overnight with autologous sort-purified KCs that were first pulsed with poly(I:C), LPS, or medium. The following day their lytic ability was tested using chromium-labeled K562 targets. These data are representative of experiments from three individual patients.
cells, we clarified that KIR and NKG2A expression were unaf-
fected by the isolation process and, importantly, did not find any dif-
ference in lysis of K562 cells (data not shown).

To avoid inadvertent destruction of normal tissues, the poten-
tive effector functions of NK cells are controlled through their inter-
actions with ubiquitously expressed MHC-I molecules. KIRs and
NKG2A are inhibitory NK cell receptors that recognize MHC-I and
play a major role in mediating the balance between self-tol-
erance and appropriately targeted effector responses. For example,
these inhibitory NK cell receptors fulfill the missing-self hypoth-
esis by inhibiting NK cell lysis and cytokine production when they
are engaged by their cognate ligands on normal tissues. Con-
versely, NK cell effector functions are released when these ligands
are missing from an infected or malignant target cell. It is now
evident that these same inhibitory receptors are required for a NK
cell to become a functionally competent effector. In other words, it
has been shown that to be strongly reactive to missing-self-targets,
a NK cell is required to have at least one inhibitory receptor that
can interact with a self-MHC-I ligand (17–23). This model for NK
cell licensing provides an explanation for the decreased lytic re-
sponses of liver CD16+ NK cells based upon their repertoire of
self-KIR-deficient NK cells.

The regulation of NK cell responses by inhibitory receptor phe-
notypes applies to the resting state and the requirement for self-
MHC recognition in establishing functional tolerance can be over-
come by cytokine stimulation (17, 20, 23). Along these lines, we
have found that CD16+ liver NK cells like blood CD16+ NK cells
would become potent effectors by short culture in IL-2 or IL-15.
Similarly, others have found that 4 days of culture in IL-2 will
activate bulk human liver NK cells to become more potent cyto-
lytic killers (45). Like human blood NK cells which can be acti-
ated by DCs (46–48) and macrophages (49), liver CD16− NK cells
can be activated by autologous liver KCs (42). We have simi-
larly found that the lytic capacity of liver CD16+ NK cells can be enhanced
by activated liver DCs (data not shown) or KCs. Taken

Acknowledgments

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Disclosures

The authors have no financial conflict of interest.

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Chemo (Chemotherapy within 3 months of surgery), Embo (embolization within 3 months of surgery), Cholangio (Cholangiocarcinoma), CRC (Colorectal cancer), GB (Gallbladder cancer), HCC (Hepatocellular carcinoma), GIST (Gastrointestinal stromal tumor), FNH (Focal nodular hyperplasia), M (Metastatic disease), P (Primary liver tumor), PV (Portal vein embolization), HA (Hepatic artery embolization).
Supplemental Table 2. Activating receptors on CD16\(^+\) liver and blood NK cells

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Percent positivity of various surface markers on CD16\(^+\) NK cells (mean ± SD) from matched liver and blood samples from 6 patients.