# Differential Function of Natural Killer Cells in the Liver Graft Perfusate of Korean Population

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ABSTRACT

Background. Liver perfusate (LP) lymphocytes show unique subsets compared with peripheral blood (PB) lymphocytes. LP natural killer (NK) and NKT cells may display unique cytotoxicity and cytokine production, thus leading to distinct roles in liver transplantation. In this study, we sought to evaluate the functions of graft perfusate NK and NKT cells in clinical liver transplantation.

Methods. The living donor right lobe graft was initially washed with 1 L of histidine-tryptophan-ketoglutarate solution to collect the perfusate. We also collected donor PB. Lymphocytes separated by the Ficoll-Hypaque density gradient method underwent immunophenotyping using multicolor flow cytometry. To assess cytokine secretion, we performed the enzyme-linked immunosorbent assay.

Results. There were more NK and NKT cells in LP confirming previous reports. In particular, $CD56^{bright}CD16^{low}$ NK cells accounted for approximately 50% of total NK cells compared with 5% to 10% among PB NK cells. In response to cytokine stimulation LP NK cells produce tumor necrosis factor–$\alpha$ at different levels and less interleukin-10 compared with PB NK cells. The major source of interferon-$\gamma$ production upon stimulation with liver caner cells were $CD56^{dim}$ NK cells and $CD56^{-}CD3^{-}$ cells rather than NKT or T cells. Unlike PB NK cells, LP $CD56^{bright}CD16^{low}$ NK cells along with $CD56^{dim}CD16^{high}$ NK cells and NKT cells were efficient killers against Korean liver cancer cells.

Conclusion. LP NK and NKT cells showed unique functions in cytotoxicity and cytokine production.

INTRAHEPATIC lymphocytes show distinct phenotypes.[1] The unique phenotypes in liver graft perfusates are similar to liver-resident lymphocytes.[2,3] As liver perfusate (LP) is an abundant source of lymphocytes, Ohdan and colleagues have used them for immunotherapy against hepatitis C virus infection, bacteremia, and hepatocellular carcinoma after liver transplantation.[4–6] An earlier study showed a much greater proportion of $CD56^{+}$ cells in LP compared with that in peripheral blood (PB).[2] The liver is known to contain more natural killer (NK) and NKT cells than PB. Interestingly, $CD3^{-}CD56^{+}CD16^{-}$ cells, which comprise more than 50% amongs NK and $CD4^{-}CD8^{+}$ T cells, are much more frequent than $CD4^{+}CD8^{-}$ T cells in LP. In the periphery, $CD3^{-}CD56^{+}CD16^{-}$ cells are typically less than 10% and $CD4^{+}CD8^{-}$ T cells, more than $CD4^{-}CD8^{+}$ T cells. $CD3^{+}CD56^{+}$ cells are categorized as a subset of NK cells, which are now called NKT cells. A recent report demonstrated more details about LP NK cells[3]; more of them express CD69, an activation marker, and NKp44 which is expressed only on activated NK cells. However, NKG2D is expressed only by 60% of PB NK cells, although it is normally on more than 90% of PB NK cells. NK cells play an essential role in innate immunity by virtue of their cytotoxicity against tumor and virus-infected cells and their production of an

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**Table 1. Demography of Healthy Donors for Liver Perfusate[a]**

| Healthy Donor (No) | Gender | Age (y) |
|---|---|---|
| 19 | Male | 25 ± 6.4 |
| 9 | Female | 35.3 ± 15.5 |

[a]All the donors were Korean.

**Table 2. Natural Killer and Natural Killer T cell Populations in Liver Perfusate (N = 5)**

| NK (CD3−CD56+) | NKT (CD3+CD56+) | $CD56^{bright}$ $CD16^{low}$NK/total NK | $CD56^{dim}CD16^{high}$ NK/total NK |
|---|---|---|---|
| 38.4 ± 11.1 | 22.9 ± 7.74 | 50.7 ± 9.40 | 49.3 ± 8.89 |

array of cytokines and chemokines, including interferon γ (IFN-γ) and tumor necrosis factor (TNF-α). In terms of LP NK cell functions they kill K562 cells, a standard target, as well as HepG2 hepatocellular carcinoma cells.[7] Their cytotoxic actions are even stronger than those of PB NK cells, possibly due to the active cytotoxicity of $CD56^{bright}$ NK cells.[3] We sought to investigate the characteristics of LP NK cells in the Korean population. We confirmed previous results on LP lypmhocytes, in particular NK cells, and suggest that they might have distinct roles in cytokine production.

## METHODS

### Subjects

Living donor right lobe grafts were initially washed with 1 L of histidine-tryptophan-ketoglutarate (HTK) solution to collect LP. The demography of the donors is shown in Table 1. In some experiments, PB was collected from healthy volunteers. The study was approved by our institutional Review Board (Approval No. 2012-0006). LP and PB mononuclear cells (MCs) were isolated using the Ficoll-Hypaque density gradient method (GE Healthcare Life Sciences, Waukesha, Wis, United States).

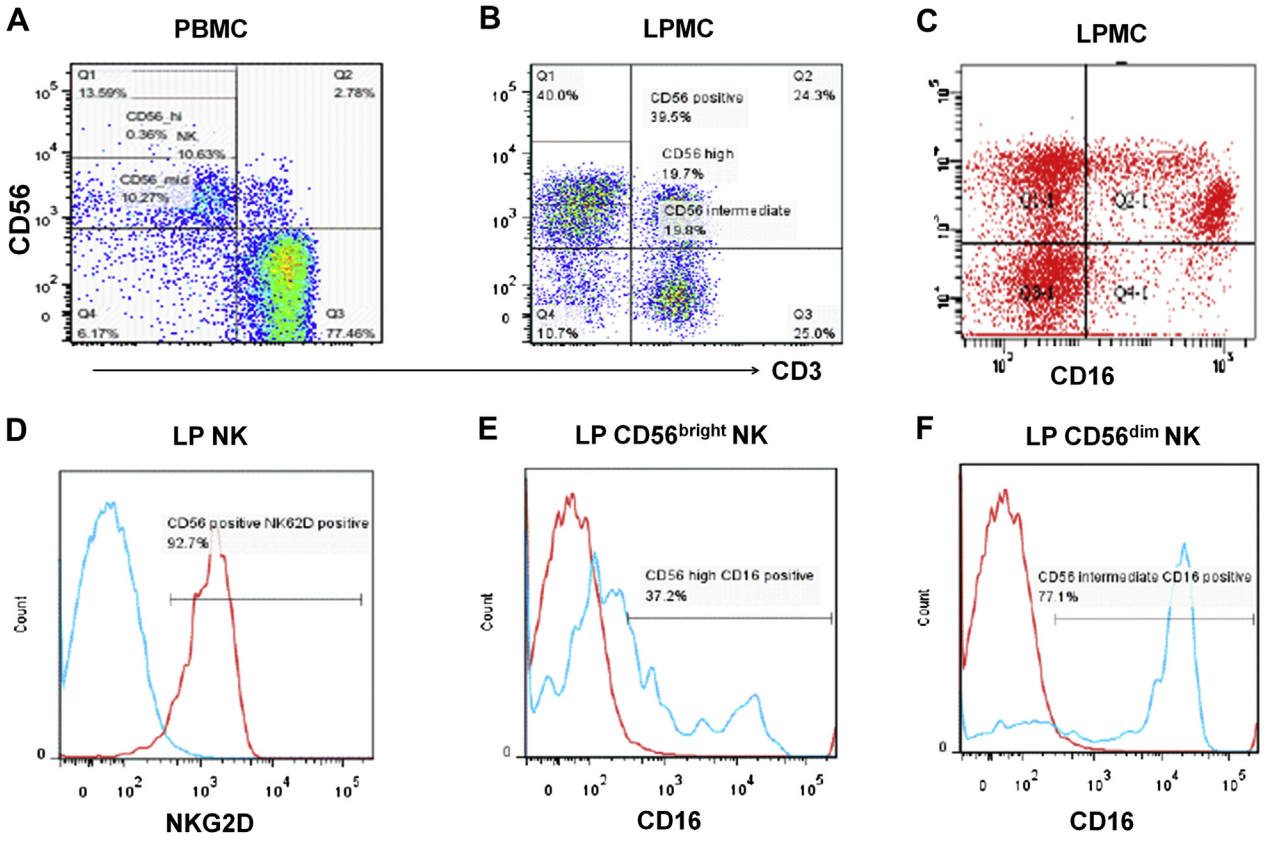


**Fig 1.** Liver perfusate lymphocytes are more similar to liver-resident lymphocyte population than PBMCs. LP was collected from healthy donors by perfusion with (HTK) solution. Lymphocytes were isolated by Ficoll-Hypaque gradient and stained with and anti-CD3, CD19, CD56, CD16, and NKG2D Abs. Flow cytometry was performed with BD FACScanto II. PB was collected from volunteers and processed as described above. NK cells were defined as $CD3^{-}CD19^{-}CD56^{+}$. Dot plots of PBMC (**A**), LPMC (**B, C**) are shown. LP NK cells occupied nearly 40% in total lymphocytes and comprised of 50% of $CD56^{bright}$ NK cells and 50% of $CD56^{dim}$ NK cells. In contrast, PB NK cells were approximately 10% of total lymphocytes and $CD56^{bright}$ NK cells were only less than 4% of total NK cells. NKT cell population in LP was also higher that of PB. (**D**) Histogram of NKG2D. Only NK cells were plotted. Most of LP NK cells expressed NKG2D as in PB. (**E**) Histogram of CD16. Only $CD56^{dim}$ NK cells were plotted. (**F**) Histogram of CD16 Only $CD56^{dim}$ NK cells were plotted. $CD16^{bright}$ NK cells in LP were $CD56^{low}$ and $CD56^{dim}$ NK cells were $CD16^{high}$ as shown in **C**.