

Adoptive Transfer of TRAIL-Expressing Natural Killer Cells Prevents Recurrence of Hepatocellular Carcinoma After Partial Hepatectomy

Masahiro Ohira, Hideki Ohdan, Hiroshi Mitsuta, Kohei Ishiyama, Yuka Tanaka, Yuka Igarashi, and Toshimasa Asahara



Background. Antitumor activity of the liver natural killer (NK) cells reportedly decreases after partial hepatectomy, suggesting that patients with such depressed immune status are susceptible to the recurrence of hepatocellular carcinoma (HCC). We hypothesize that adoptive immunotherapy using activated NK cells can be a novel strategy to improve the depressed immune status in patients with HCC after hepatectomy or partial liver transplantation. In the present study, we have tested this hypothesis by using a mouse model.

Methods. Intraportal injection of $1-5 \times 10^6$ Hepa1-6 cells (hepatoma cell line) did not result in liver metastases in untreated B6 mice, but led to the growth of liver metastases after extensive partial hepatectomy. Utilizing this murine HCC metastasis model, we investigated the antitumor activity of both remnant liver and exogenously transferred NK cells.

Results. The anti-HCC activity of liver NK cells significantly decreased after partial hepatectomy. The expression of CD69 and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) on liver NK cells was temporarily downregulated. The adoptive transfer of NK cells, including a TRAIL-expressing fraction, extracted from the liver perfusates of poly I:C-stimulated B6 mice inhibited the growth of liver metastasis in B6 or (B6 \times BALB/c) F1 (B6CF1) mice that underwent hepatectomy and received intraportal Hepa1-6 injection.

Conclusions. These findings indicate that adoptive immunotherapy using activated NK cells extracted from normal liver perfusates may be a novel technique for reconstituting the depressed immune status in cases of living donor liver transplantation involving HCC patients, recipients of a partial liver graft.

Keywords: NK cells, Hepatocellular carcinoma, Partial hepatectomy, Living donor liver transplantation.

(*Transplantation* 2006;82: 1712–1719)

Natural killer (NK) cells are believed to constitute the first line of defense against invading infectious microbes and neoplastic cells by exerting an effector function independent of priming (1, 2). Given the efficacy of NK cells in selectively killing abnormal cells, a variety of approaches have been considered when attempting selective augmentation of NK cell response to tumors (3–6).

We have recently determined the functional properties of peripheral blood (PB) NK cells and liver NK cells extracted

from liver perfusates of donors and recipients in clinical living donor liver transplantation (7). Donor liver NK cells showed maximum vigorous cytotoxicity against a hepatocellular carcinoma (HCC) cell line after in vitro interleukin (IL)-2 stimulation as compared to the donor PB NK cells and recipient liver NK cells. IL-2 stimulation increased tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) expression on liver NK cells; this was critical for mounting an NK cell-mediated antitumor response without affecting normal cells. These findings lead to a novel concept for preventing HCC recurrence after living donor liver transplantation, i.e., the adoptive transfer of IL-2-stimulated NK cells extracted from the donor liver graft into recipients sharing at least one haplotype.

Antitumor activity of liver NK cells reportedly decreases after partial hepatectomy, suggesting that patients with such depressed immune status are susceptible to HCC recurrence after partial hepatectomy or partial liver transplantation (8–10). Therefore, adoptive immunotherapy using activated NK cells probably has potential as a strategy to reconstitute the depressed immune status in HCC patients after partial liver transplantation. Here, using mice as a

This work was supported in part by a Grant-in-Aid for Scientific Research (B) (18390348) from the Japan Society for the Promotion of Science.

Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan.

Address correspondence to: Hideki Ohdan, M.D., Ph.D., Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.

E-mail: hohdan@hiroshima-u.ac.jp

Received 27 July 2006. Revision requested 24 August 2006.

Accepted 28 September 2006.

Copyright © 2006 by Lippincott Williams & Wilkins

ISSN 0041-1337/06/8212-1712

DOI: 10.1097/01.tp.0000250935.41034.2d

model, we investigated the influence of partial hepatectomy on NK cell activity against HCC and the potential of adoptively transferring activated NK cells to prevent HCC recurrence after partial hepatectomy.

Histological Assessment of Hepatocyte Proliferation

Bromodeoxyuridine (BrdU) immunostaining was performed as described previously (14). In brief, BrdU (Sigma Chemical Co., St Louis, MO) was administered intraperitoneally at a dose of 30 mg/kg, 60 min before sacrificing the mice to measure DNA synthesis in the regenerating liver. Deparaffinized liver sections were incubated with anti-BrdU antibody (Becton Dickinson Immunocytometry Systems, Mountain View, CA) for 60 min. Immunostaining for BrdU was performed by the avidin-biotin-immunoperoxidase method using a Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA).

Statistical Analysis

The results were analyzed by Student's *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

The Intensities of CD69 and TRAIL-Expression on Liver NK Cells Were Temporarily Downregulated After Extended Partial Hepatectomy

Resident leukocytes were extracted from the liver, spleen, and lung of B6 mice that were untreated or underwent partial hepatectomy. As shown in Figure 1, liver NK cells comprised cells that expressed CD69—one of the earliest expressed activating markers—at a greater frequency than spleen and lung NK cells. After three days of partial hepatectomy, the number of CD69-expressing NK cells was significantly reduced in the liver; however, the number remained constant in the spleen and lung. Thus, liver NK cells are naturally activated but extended hepatectomy probably deactivates them. Providing efficient cytotoxicity against trans-

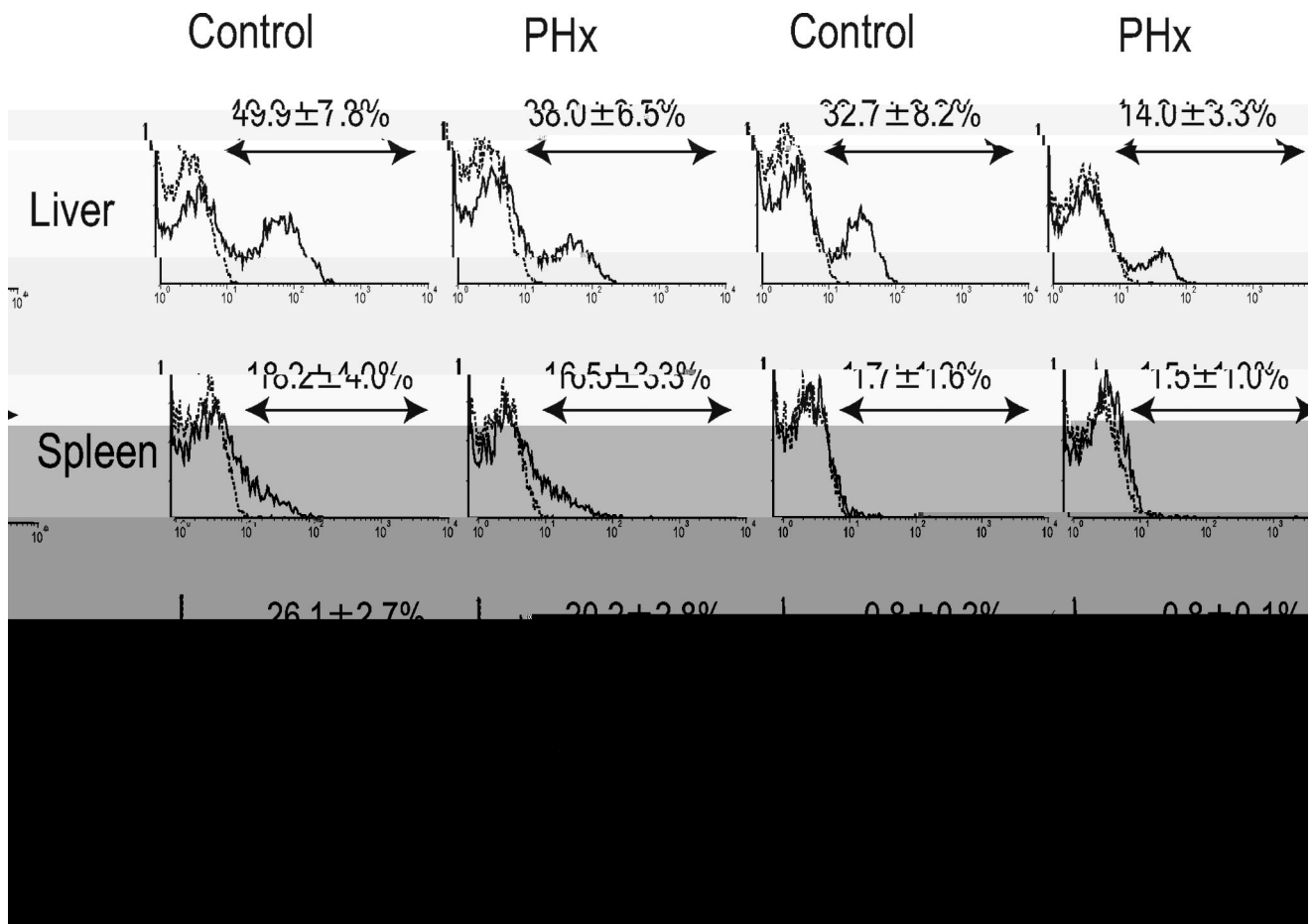


FIGURE 1. The intensities of CD69 and TRAIL-expression on liver NK cells were downregulated three days after partial hepatectomy. Liver, spleen, and lung leukocytes were isolated from untreated or -276.4(of)5-276.4(o450somy.)-281.9.7(liv6.4(o3(m

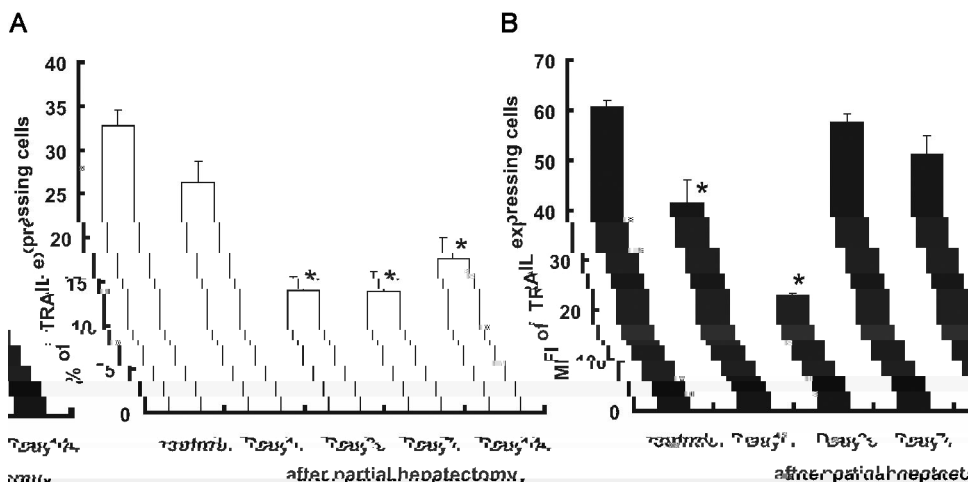


FIGURE 2. The kinetics of TRAIL-expression on liver NK cells after the hepatectomy. (A) The numbers represent the mean±SEM of the TRAIL⁺ cell populations in the NK1.1⁺TCRb⁻ NK cell subsets (n=7). *P<0.01 for Day three, Day seven, and Day 14 vs. control. (B) The numbers indicate the mean fluorescence intensity (MFI) of cells that stained positive for TRAIL (mean±SEM, n=7). *P<0.01 for Day one and Day three vs. control.

formed targets is a feature of activated NK cells—a property believed to be attributable to the elevated expression of TNF family members, including the Fas ligand (FasL), and the increased production of perforin, granzymes, and cytokines by these cells (15–19). Among the TNF family members, TRAIL has recently been shown to be critical for NK cell-mediated antitumor functions (20–22). Although FasL expression was not detected on any of the liver, spleen, and lung NK cells (data not shown), approximately 30–40% of the liver NK cells constitutively expressed TRAIL molecules unlike other

tissues (Fig. 1). It is noteworthy that the expression of TRAIL was markedly reduced on the liver NK cells after partial hepatectomy. The kinetics of TRAIL-expression on liver NK cells after the hepatectomy are shown in Figure 2. Both the proportion of TRAIL-expressing cells and the expression levels of TRAIL were progressively reduced until three days of hepatectomy (Fig. 2A and B). Thereafter, by two weeks after hepatectomy, these levels gradually improved. Such a change in the expression level of TRAIL on liver NK cells was well correlated with that in the cytotoxicity of liver NK cells against the Hepa1-6 hepatoma cell line (data not shown).

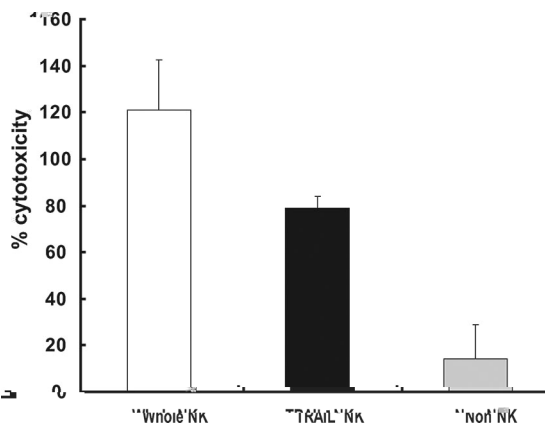


FIGURE 3. Cytotoxicity against the Hepa1-6 hepatoma cell line was mediated predominantly by TRAIL⁺ liver NK cells. Unlabeled NK cells and non-NK cells were isolated from the liver leukocytes of poly I:C-treated B6 mice (n=25) by negative selection using magnetic sorting as described in Materials and Methods. TRAIL⁻ NK cells were further sorted from the isolated liver NK cells. The isolated NK cell populations were used as effector cells in the assays for cytotoxicity against the indicated target cells at an E/T ratio of 20. Comparative cytotoxicity was calculated with the following formula: percent of cytotoxicity=(cytotoxicity of fractionated liver leukocytes)/(cytotoxicity of nonfractionated liver leukocytes)×100%. The data are represented as the average±SEM of values from triplicate samples.

TRAIL-Expressing Liver NK Cells Show Cytotoxicity Against the Hepatoma Cell Line

We attempted to determine whether TRAIL⁺ NK cells mediate cytotoxicity against the Hepa1-6 hepatoma cell line. By magnetic sorting, NK cells were isolated from the liver leukocytes of poly I:C-treated B6 mice. TRAIL⁺ and TRAIL⁻ NK cells were further sorted from the isolated liver NK cells, and the resulting populations were then analyzed for cytotoxicity against Hepa1-6. As shown in Figure 3, liver NK cells mediated significantly higher cytotoxicity against hepatoma cells when compared to the non-NK cell fraction. The liver NK cells comprising both TRAIL⁺ and TRAIL⁻ cells mediated a significantly higher antitumor activity than the TRAIL⁻ NK cells. This finding indicates that TRAIL⁺ liver NK cells efficiently mediate cytotoxicity against the hepatoma cell line.

The Remnant Liver After Extended Partial Hepatectomy Was Susceptible to the Growth of Hepatoma Metastases

Based on our results that the number of TRAIL-expressing NK cells that provided significant antihepatoma activity decreased after extended partial hepatectomy, we assumed that partial hepatectomy promotes susceptibility to the engraftment of intraportally injected hepatoma cells. To verify this, Hepa1-6 cells were intraportally injected in either

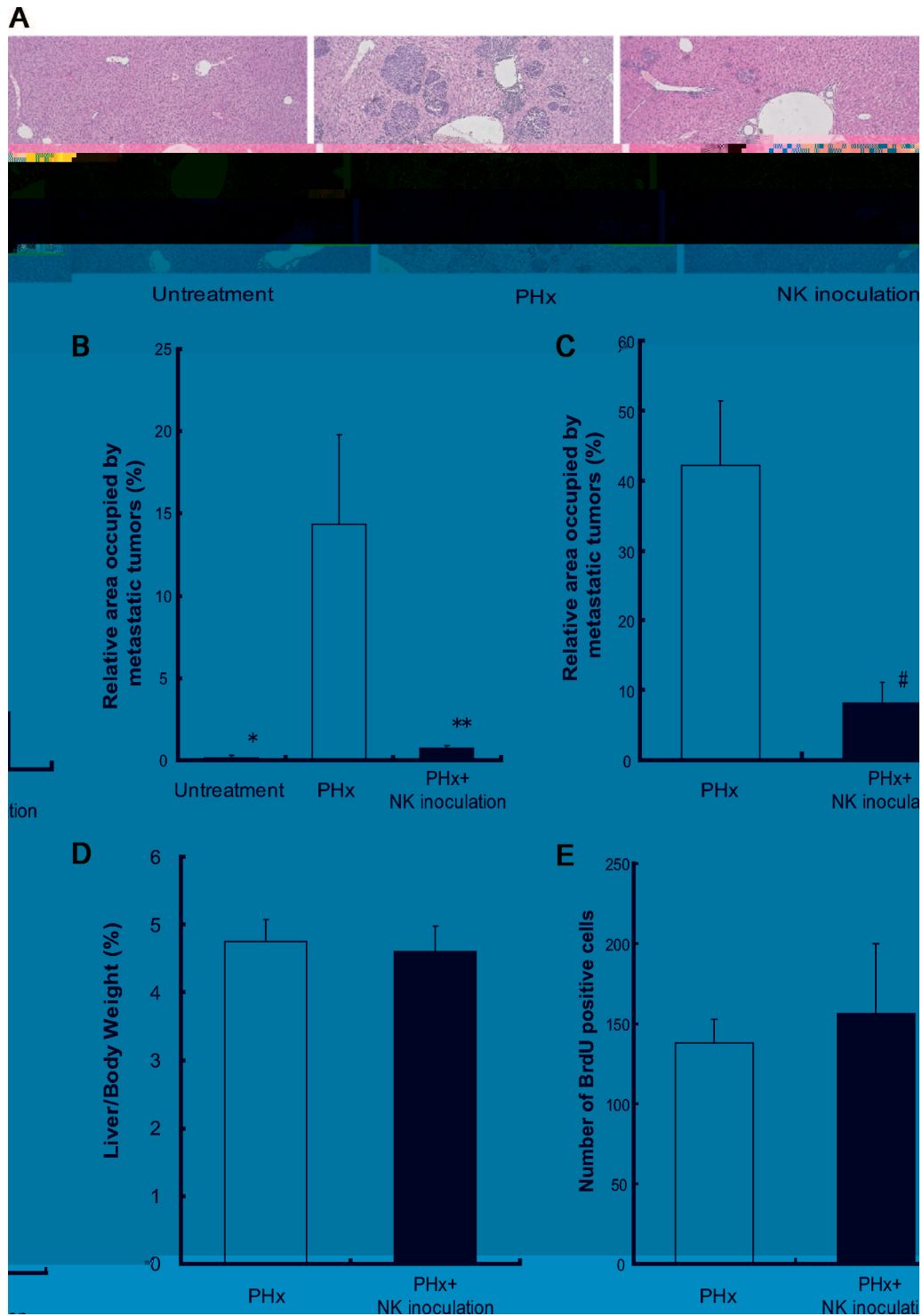


FIGURE 4. The adoptive transfer of activated liver NK cells inhibited the growth of liver metastasis induced by a portal venous injection of hepatoma cells in extensively hepatectomized mice. (A) Representative histopathological findings of the liver specimen (HE, $\times 4$ objective). Specimens from the untreated (left), partially hepatectomized (PHx; middle), and NK cell-receiving groups were inoculated after partial hepatectomy (right). (B) The adoptive transfer of activated B6 liver NK cells inhibited the growth of liver metastasis induced by a portal venous injection of hepatoma cells in extensively hepatectomized B6 mice. B6 mice were that either underwent or did not undergo partial hepatectomy and injected with tumor cells on Day 0. On Day three, the NK inoculation group was administered an intravenous injection of 5×10^5 B6 liver NK cells. On Day seven, the relative areas occupied by the tumors were calculated as the percentage of the total liver area. Data are represented as the mean \pm SEM of values from four untreated mice, 12 hepatectomized mice without NK-inoculation, and nine hepatectomized mice with NK-inoculation. * $P < 0.01$ for untreated mice vs. PHx mice; ** $P < 0.01$ for PHx mice vs. NK-inoculated mice. (C) The adoptive transfer of activated B6 liver NK cells inhibited the growth of liver metastasis induced

untreated mice or mice that underwent partial hepatectomy; the mice were then sacrificed seven days after the inoculation to evaluate metastatic growth in the liver. In the control untreated mice, limited numbers of metastatic lesions were detected in the liver, indicating their natural defensive activity against invading hepatoma cells. Even when 5×10^6 Hepa1-6 cells (10-fold higher than the number used in the present study) were intraportally injected into the untreated control mice, metastatic lesion were barely detected (data not shown). However, after extensive hepatectomy, significant growth of liver metastases was detected in the liver, suggesting the breakdown of such natural defensive activities (Fig. 4A and B).

The Adoptive Transfer of Activated Liver NK Cells Inhibited the Growth of Liver Metastasis Induced by Portal Venous Injection of Hepatoma Cells in Extensively Hepatectomized Mice

We next investigated whether the adoptive transfer of liver NK cells, including the TRAIL-expressing fraction, in the hepatectomized mice could reconstitute the defensive activities in intraportally injected hepatoma cells. After three days of administering the portal injection of Hepa1-6 cells in the hepatectomized B6 or B6CF1 mice, we intravenously administered NK cells extracted from the livers of poly I:C stimulated B6 mice (5×10^5 cells/mouse). Both B6 and B6CF1 mice receiving activated liver NK cells showed significantly suppressed levels of metastases seven days after the inoculation (Fig. 4B and C).

It has been previously speculated that the activity of liver NK cells is relevant to regenerating the activity of the liver (13, 23, 24). In the present study, however, NK cell inoculation influenced neither the liver mass to body weight

ratios nor the number of BrdU-positive cells in the remnant liver seven days after the partial hepatectomy (Fig. 4D and E). This indicates that the adoptive transfer of activated liver NK cells did not prevent the regeneration of the remnant liver after partial hepatectomy.

DISCUSSION

Liver transplantation is one of the few curative treatment modalities for patients with unresectable HCC (25, 26). Managing the prevention of graft rejection requires the use of immunosuppressive therapy after liver transplantation; however, this therapy poses a serious problem in that immunosuppressants increase the incidence of recurrence of HCC and induce HCC progression. It has been well accepted that the immunosuppressive regimen that is currently being used after liver transplantation, which uses tacrolimus/cyclosporine and methylprednisolone and reduces adaptive components of cellular immunity (predominantly T cell-mediated immune responses) while maintaining the innate components of cellular immunity (3, 27, 28). Because immune surveillance against tumors is mediated by both innate and adaptive components of cellular immunity, augmentation of NK cell responses to tumors, which has been believed to play a central role in innate immunity against tumors, might be a promising immunotherapy approach.

Previously published data redefine NK cells as potent constitutive immune effectors; these cells can utilize not only the perforin-mediated secretory/necrotic mechanism to kill rare leukemia cell targets but also the powerful TNF family ligand-mediated nonsecretory apoptotic mechanism to destroy most solid tumor cell targets (29). TRAIL is highly expressed on most NK cells after stimulation with IL-2, IFNs, or IL-15 in mice (21, 22, 30). Neutralization of TRAIL additively enhanced liver metastasis in perforin-deficient mice but not in IFN- γ -deficient mice (22). These findings clearly indicate that the two key cytotoxic effector pathways used by NK cells are those of perforin and TRAIL. Consistently, the liver TRAIL⁺ NK cells mediated cytotoxicity against hepatoma cells more efficiently than the liver TRAIL⁻ NK cells in the present study (Fig. 3). These TRAIL⁺ NK cells were temporarily but markedly reduced after extensive partial hepatectomy (Fig. 2). This might be one of the causative mechanisms for a previously reported fact that the level of cytotoxic activity against various tumor cells of liver NK cells decreased after partial hepatectomy (8–10).

The first step in the development of experimental liver metastases is believed to involve tumor cells mechanically trapped in the portal sinuses, where they adhere to sinusoidal endothelial cells before extravasation (31, 32). Since liver NK cells are located predominantly within the sinusoidal lumen in close contact with sinusoidal endothelial cells (33, 34), it is possible that intraportally injected hepatoma cells neighboring on the liver sinusoid are targeted by liver NK cell-mediated cytotoxicity. Although whether tumor cells come in contact with TRAIL⁺ NK cells before or after extravasation remains unknown, the results from the present study suggest that TRAIL⁺ NK cells play a critical role in preventing tumor cell invasion into the parenchyma.

It is a generally accepted fact that healthy cells are protected by their MHC class I molecules that bind to the corre-

FIGURE 4. Continued by a portal venous injection of hepatoma cells in extensively hepatectomized B6CF1 mice. B6CF1 mice were partially hepatectomized and injected with tumor cells on Day 0. On Day three, the NK-inoculation group was administered an intravenous injection of 5×10^5 B6 liver NK cells. On Day seven, the relative areas occupied by the tumors were calculated as the percentage of the total liver area. Data are represented as the mean \pm SEM of values from four mice without NK cell inoculation and five mice with NK cell inoculation. # $P < 0.01$ for PHx mice vs. NK-inoculated mice. (D) Comparison of liver weight to body weight ratio seven days after partial hepatectomy. These values were obtained from B6 mice partially hepatectomized on Day 0 ($n = 4$ in each group). On Day three, the NK inoculation group was administered an intravenous injection of 5×10^5 B6 liver NK cells. On Day seven, the body weight and liver weight were measured. Data are represented as the mean \pm SEM. There was no difference in the liver weight to body weight ratio ($P = 0.75$ for untreated mice vs. NK-inoculated mice). (E) For the bromodeoxyuridine (BrdU) incorporation assay, 60 min before sacrificing the B6 mice, BrdU was intraperitoneally injected at a concentration of 30 mg/kg of the body weight. The number of cells per field that stained positive was calculated seven days after partial hepatectomy ($n = 4$ in each group). Data are shown as mean \pm SEM. There was no difference in the number of BrdU-positive hepatocytes in either group ($P = 0.7$ for untreated mice vs. NK-inoculated mice).

sponding inhibitory receptors on NK cells; therefore, cells that lack or have altered MHC class I molecules are killed due to the absence of inhibitory mechanisms (35–38). The only requirement for NK cell receptor repertoire development appears to be that every NK cell should express at least one inhibitory receptor that is specific for autologous HLA class I, thereby ensuring tolerance against healthy cells that share one-haplotype MHC molecules (39, 40). It is well known that irradiated F1 hybrid mice reject bone marrow transplants from either inbred parent. This resistance is mediated by the host NK cells. We are not certain whether a similar hybrid resistance takes place during the engraftment of NK cells in one-haplotype mismatched living liver transplantation in humans. Since the liver is replaced by a donor liver graft after transplantation, the donor-type resident NK cells in the liver cannot reject the inoculated donor-type NK cells extracted from the donor liver grafts. Considering these facts together with the results of the present study, adoptive transfer of activated NK cells extracted from the donor liver graft into one-haplotype matched recipients suffering from HCC would hold potential as a strategy for preventing HCC recurrence.

Previously, we have reported that the majority of the TRAIL⁺ NK cells lack the expression of Ly-49 inhibitory receptors that are responsible for recognizing self-major histocompatibility complex (self-MHC) class I molecules, thereby indicating a propensity toward cells that share self-MHC class I (I3) Poly I:C treatment significantly upregulated the expression of Ly-49 receptors on the TRAIL⁻ NK cells. This might be a compensatory mechanism to protect the self-MHC class I-expressing cells from activated NK cell-mediated cytotoxicity. However, such a compensatory alteration in the TRAIL⁺ NK cell fraction was not observed at all. Thus, the liver TRAIL⁺ NK cells are less capable of self-recognition, and this might be involved in the NK cell-mediated self-hepatocyte toxicity. We assumed that a similar mechanism is involved in the TRAIL⁺ NK cell-mediated effects against Hepa1-6 cells, which expressed syngeneic H-2D^b (data not shown).

The mechanism by which the TRAIL expression of liver NK cells is down-regulated during the early phase following partial hepatectomy is currently unknown. Considering the previously reported finding that IL-12 induces TRAIL expression in the NK cells (22) as well as our unpublished finding that liver mRNA expression of IL-12 is significantly reduced after partial hepatectomy, it is possible that the IL-12 produced by different cells in the liver, such as dendritic cells, Kupffer cells or endothelial cells, plays a critical role in the regulation of TRAIL expression of liver NK cells. This hypothesis is currently being investigated.

ACKNOWLEDGMENTS

The authors thank Prof. Masao Kobayashi for his advice and encouragement and Yuko Ishida for her expert technical assistance.

REFERENCES

1. Trinchieri G. Biology of natural killer cells. *Adv Immunol* 1989; 47: 187.
2. Yokoyama WM. Natural killer cells. In: Paul EW, ed. *Fundamental Immunology*. Philadelphia: Lippincott-Raven, 1999: 575.
3. Miller JS, Soignier Y, Panoskaltis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; 105(8): 3051.
4. Klingemann HG, Martinson J. Ex vivo expansion of natural killer cells for clinical applications. *Cytotherapy* 2004; 6(1): 15.

5. Leung W, Iyengar R, Turner V, et al. Determinants of antileukemia effects of allogeneic NK cells. *J Immunol* 2004; 172(1): 644.
6. Meller B, Frohn C, Brand JM, et al. Monitoring of a new approach of immunotherapy with allogeneic (111)In-labelled NK cells in patients with renal cell carcinoma. *Eur J Nucl Med Mol Imaging* 2004; 31(3): 403.
7. Ishiyama K, Ohdan H, Ohira M, et al. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. *Hepatology* 2006; 43(2): 362.
8. Park SK, Brody JI, Wallace HA, Blakemore WS. Immunosuppressive effect of surgery. *Lancet* 1971; 1(7689): 53.
9. Morimoto H, Nio Y, Imai S, et al. Hepatectomy accelerates the growth of transplanted liver tumor in mice. *Cancer Detect Prev* 1992; 16(2): 137.
10. Minagawa M, Oya H, Yamamoto S, et al. Intensive expansion of natural killer T cells in the early phase of hepatocyte regeneration after partial hepatectomy in mice and its association with sympathetic nerve activation. *Hepatology* 2000; 31(4): 907.
11. Djeu JY, Heinbaugh JA, Holden HT, Herberman RB. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. *J Immunol* 1979; 122(1): 175.
12. Bouwens L, Remels L, Baekeland M, et al. Large granular lymphocytes or "pit cells" from rat liver: isolation, ultrastructural characterization and natural killer activity. *Eur J Immunol* 1987; 17(1): 37.
13. Ochi M, Ohdan H, Mitsuta H, et al. Liver NK cells expressing TRAIL are toxic against self-hepatocytes in mice. *Hepatology* 2004; 39(5): 1321.
14. Kimura T, Sakaida I, Terai S, et al. Inhibition of tumor necrosis factor- α production retards liver regeneration after partial hepatectomy in rats. *Biochem Biophys Res Commun* 1997; 231(3): 557.
15. Kagi D, Seiler P, Pavlovic J, et al. The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses. *Eur J Immunol* 1995; 25(12): 3256.
16. Schneider P, Holler N, Bodmer JL, et al. Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. *J Exp Med* 1998; 187(8): 1205.
17. Kashii Y, Giorda R, Herberman RB, et al. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J Immunol* 1999; 163(10): 5358.
18. Smyth MJ, Thia KY, Street SE, et al. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. *J Exp Med* 2000; 192(5): 755.
19. Street SE, Cretney E, Smyth MJ. Perforin and interferon- γ activities independently control tumor initiation, growth, and metastasis. *Blood* 2001; 97(1): 192.
20. Zamai L, Ahmad M, Bennett IM, et al. Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *J Exp Med* 1998; 188(12): 2375.
21. Kayagaki N, Yamaguchi N, Nakayama M, et al. Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells. *J Immunol* 1999; 163(4): 1906.
22. Smyth MJ, Cretney E, Takeda K, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) contributes to interferon- γ -dependent natural killer cell protection from tumor metastasis. *J Exp Med* 2001; 193(6): 661.
23. Sun R, Gao B. Negative regulation of liver regeneration by innate immunity (natural killer cells/interferon- γ). *Gastroenterology* 2004; 127(5): 1525.
24. Vujanovic NL, Polimeno L, Azzarone A, et al. Changes of liver-resident NK cells during liver regeneration in rats. *J Immunol* 1995; 154(12): 6324.
25. Wiesner RH, Freeman RB, Mulligan DC. Liver transplantation for hepatocellular cancer: the impact of the MELD allocation policy. *Gastroenterology* 2004; 127 (5 Suppl 1): S261.
26. Kulik L, Abecassis M. Living donor liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2004; 127 (5 Suppl 1): S277.
27. Hirata M, Kita Y, Saito S, et al. Increase in natural killer cell activity following living-related liver transplantation. *Transpl Int* 1998; 11 Suppl 1: S185.
28. Harada N, Shimada M, Okano S, et al. IL-12 gene therapy is an effective therapeutic strategy for hepatocellular carcinoma in immunosuppressed mice. *J Immunol* 2004; 173(11): 6635.
29. Vujanovic NL, Nagashima S, Herberman RB, Whiteside TL. Nonsecretory apoptotic killing by human NK cells. *J Immunol* 1996; 157(3): 1117.
30. Pitti RM, Marsters SA, Ruppert S, et al. Induction of apoptosis by

- Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 1996; 271(22): 12687.
31. Luzzi KJ, MacDonald IC, Schmidt EE, et al. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol* 1998; 153(3): 865.
 32. Naumov GN, Wilson SM, MacDonald IC, et al. Cellular expression of green fluorescent protein, coupled with high-resolution in vivo videomicroscopy, to monitor steps in tumor metastasis. *J Cell Sci* 1999; 112 (Pt 12): 1835.
 33. Wisse E, van't Noordende JM, van der Meulen J, Daems WT. The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. *Cell Tissue Res* 1976; 173(4): 423.
 34. Vermijlen D, Luo D, Robaye B, et al. Pit cells (Hepatic natural killer cells) of the rat induce apoptosis in colon carcinoma cells by the perforin/granzyme pathway. *Hepatology* 1999; 29(1): 51.
 35. Moretta A, Vitale M, Sivori S, et al. Human natural killer cell receptors for HLA-class I molecules. Evidence that the Kp43 (CD94) molecule functions as receptor for HLA-B alleles. *J Exp Med* 1994; 180(2): 545.
 36. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986; 319(6055): 675.
 37. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. *Immunol Today* 2000; 21(9): 447.
 38. Falk CS, Mach M, Schendel DJ, et al. NK cell activity during human cytomegalovirus infection is dominated by US2-11-mediated HLA class I down-regulation. *J Immunol* 2002; 169(6): 3257.
 39. Shilling HG, McQueen KL, Cheng NW, et al. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood* 2003; 101(9): 3730.
 40. Valiante NM, Uhrberg M, Shilling HG, et al. Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. *Immunity* 1997; 7(6): 739.