Brief Definitive Report

HIGH HEPATIC NATURAL KILLER CELL ACTIVITY IN MURINE LUPUS

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NK cells are a heterogeneous group of lymphocytes operationally defined by their ability to lyse, without prior sensitization, specific tumor and virally infected cells (1). In addition to the role of NK in infection and tumor surveillance, these cells probably have an important function in the regulation of lymphocyte proliferation by means of suppression of dendritic cells that have interacted with antigens (2–4). The liver is the major organ responsible for the clearance and degradation of antigens. Because the liver provides a rich source of NK (5) and multiple abnormalities in immune regulation have been reported in murine lupus, we have examined NK activity of freshly isolated, unstimulated hepatic nonparenchymal cells (NPC) from MRL/*lpr* and (NZB × NZW)F_1 mice. We have found remarkably high levels of NK activity in the liver of these autoimmune strains that are not reflected in the spleen or peripheral blood.

Materials and Methods

Mice. C3H/HeN, BALB/c, and DBA/2J female mice, 1–8 mo old, were purchased from The Jackson Laboratory, Bar Harbor, ME. (NZB × NZW)F_1, 1–8 mo old, MRL/MpJ-*lpr/lpr* (MRL/*lpr*) and MRL/MpJ-+/+ (MRL+/+) female mice, 0.5–4.5 mo old, were obtained from colonies maintained at the National Institutes of Health, Bethesda, MD or from The Jackson Laboratories.

Irradiation. Cells were irradiated from a ^{137}Cs source at a rate of 170 rad/min.

Reagents and Cells. Collagenase IV and Metrizamide were purchased from Sigma Chemical Co., St. Louis, MO. *Corynebacterium parvum* was purchased from Burroughs Wellcome Co., Research Triangle Park, NC; 1.4 mg was injected intravenously 1–7 d before killing. Polyinosinic-polycytidylic acid (poly I:C) was purchased from Sigma Chemical Co.; 100 μg was injected intraperitoneally 24 h before killing. A mixture of lyophilized mouse IFN-α and IFN-β derived from cultures of mouse L929 cells stimulated with poly(I:C) was purchased from Lee Biomolecular Research Laboratories, Inc., San Diego, CA; 25,000 U were injected intraperitoneally 18 h before mice were killed.

Anti-asialo GM_1 antiserum (anti-AGM_1) was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Monoclonal anti-Thy-1, rat antibody designated AT83A, was a generous gift of Dr. Frank W. Fitch, The University of Chicago, Chicago, IL. Anti-Ly-1.1 and anti-Ly-2.1 mAbs and low-tox-M rabbit complement (C′) were purchased from Accurate Chemical & Scientific Corp., Westbury, NY. 10^7 cells/ml suspended in RPMI

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1640 (M.A. Bioproducts, Walkersville, MD) without FCS were treated with anti-AGM_1 at a final dilution of 1:100, anti-Thy-1 (1:10), anti-Ly-1.1 (1:20), or anti-Ly-2.1 (1:20) plus complement (1:10) for 1 h at 37°C; cells were washed twice after treatment. YAC-1 cells, originally derived from a Moloney virus–induced lymphoma in an A/Sn mouse and used widely to assay cytotoxicity of murine NK cells (6) were grown continuously.

Hepatic NPC Isolation. Under sterile conditions, NPC were purified as previously described (7) by collagenase perfusion of the portal vein and Metrizamide density centrifugation. Total recovery ranged from 2–8 × 10^6 NPC per liver. Contamination by hepatocytes, as determined by counting 200 cells from the preparation, was <1%. In selected experiments the NPC were adhered to 100-mm Petri dish (No. 3003; Falcon Labware, Oxnard, CA) for 90 min at 37°C (5% CO_2, 100% humidity) or treated with carbonyl iron particles by mixing 10^7 NPCs in 10 ml RPMI 1640 plus 5% FCS with 100 mg of carbonyl iron particles. The mixture was poured into 100-mm Petri dish. After incubation for 1 h at 37°C, the carbonyl iron particles were moved to one side of the dish with the magnet; and the nonadherent cells were then washed twice with fresh medium.

Spleen Cells and Peripheral Blood Leukocytes (PBL). Spleen cell suspensions were prepared by gently pressing spleen through a 150-mesh stainless steel sieve into RPMI 1640 plus 10% FCS followed by gravity sedimentation. PBL were obtained by phlebotomy of the retro-orbital plexus. Spleen cells and PBL were each separated on Ficoll-Hypaque gradients, washed twice, and resuspended in RPMI 1640 plus 10% FCS.

Assay for NK Activity. YAC-1 target cells (10^6/100 μl) were labeled with 100 μCi ^{51}Cr (New England Nuclear, Boston, MA) during 1 h of incubation at 37°C in 5% CO_2. After incubation the labeled YAC-1 cells were washed three times and resuspended in RPMI 1640 medium with 10% FCS. NPC in 100 μl RPMI plus 10% FCS were serially diluted in V-bottomed 96-well microtiter trays (Dynatech Laboratories, Inc., Alexandria, VA) and mixed with 5 × 10^3 ^{51}Cr-labeled YAC-1 cells in 100 μl medium. Plates were spun at 650 *g* for 3 min. After a 4-h incubation at 37°C, 100 μl of each supernatant were measured for radioactivity with a gamma counter. The percent lysis was calculated using the following formula: specific lysis = 100 × [(experimental release − spontaneous release)/(maximum release − spontaneous release)]. Spontaneous release was always <15% of the maximum release. Maximum release was calculated by mixing 50 μl ^{51}Cr-labeled target cells with 150 μl of a 1:20 dilution of Hematall LA lysing agent (Fisher Scientific Co., Itasca, IL) and then multiplying the count by two. Maximum release was 100% of the incorporated count. Experimental release was the average of duplicate assays.

Results

High Hepatic NK Cell Activity in MRL/lpr and (NZB × NZW)F_1 Mice. Hepatic NPC NK cell activity, as measured by cytotoxicity to ^{51}Cr-labeled YAC-1 target cells after a 4-h incubation, was always <16% lysis at an E/T ratio of 50:1 in C3H/HeN, BALB/c, DBA/2J, and MRL+/+ female mice at all ages examined and in MRL/*lpr* female mice <8 wk old. In MRL/*lpr* mice >8 wk old, cytotoxicity increased from 37% (age 10 wk) to 63% (age 18 wk) (Fig. 1*A*). In (NZB × NZW)F_1 mice <3 mo old, NK activity in the younger mice was similar to the nonautoimmune strains; however, the older mice (>3.5 mo) had higher NK activity (Fig. 1*B*).

NK Cell Activity: Comparison of Liver, Spleen, and Peripheral Blood. The markedly high NK cell activity of the liver in MRL/*lpr* mice was not found in the spleen or peripheral blood mononuclear cells isolated from the same animals (Fig. 2). In fact, the splenic NK activity of 4-mo-old MRL/*lpr* mice was slightly lower than C3H/HeN mice of the same age. However, this latter observation may be secondary to a dilutional effect; i.e., an increased number of $Thy\text{-}1^+$, $L3T4^-$, $Ly2^-$ lymphocytes (8).

Characteristics of Hepatic NK Cells in MRL/lpr Mice. The population of NPC

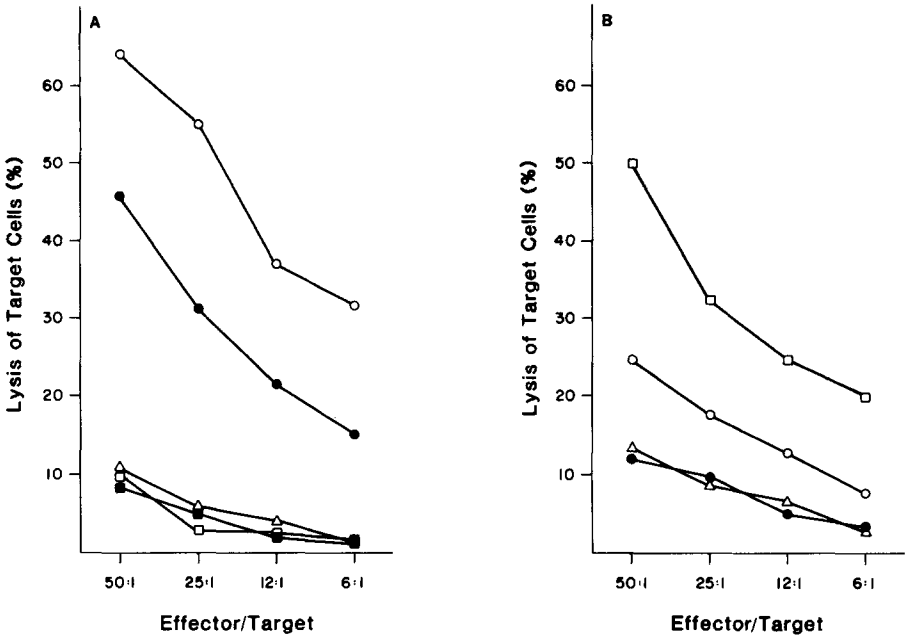

FIGURE 1. Hepatic NK cell activity assayed by cytotoxicity against ^{51}Cr-labeled YAC-1 target cells. (*A*) High NK activity noted in 2-mo (●) and 4-mo (○) MRL/*lpr* female mice as opposed to 2-mo (■) and 4-mo (□) MRL/++, and 3-mo-old C3H/HeN (△) female mice. (*B*) High hepatic NK activity also noted in older (NZB × NZW)F_1 mice at 3.5 mo (○) and 6.5 mo (□), but not at 2.5 mo (●). (△) NPC from C3H/HeN mice at 4 mo.

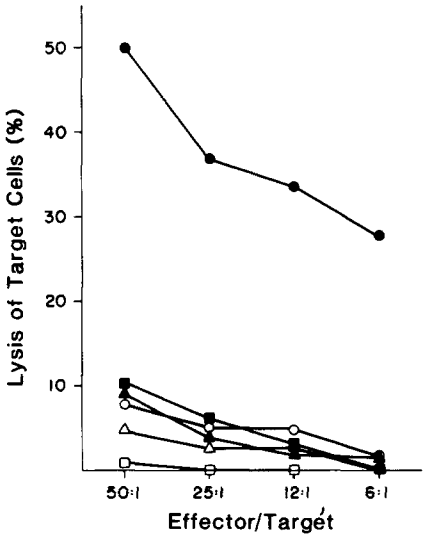

FIGURE 2. High NK activity in liver of 4-mo-old MRL/*lpr* female mice (●) is not reflected in the spleen (■) or peripheral blood (▲). Neither the liver (○), spleen (□), nor peripheral blood (△) of 4 mo MRL/++ have high NK activity.

that demonstrated cytotoxicity to YAC-1 targets was found to be resistant to irradiation (Fig. 3*A*) and not removed by adherence to plastic or carbonyl iron particles (Fig. 3*B*). 14–18% of the NPC were AGM_1^+ as determined by FACS analysis. The nonadherent cell population contained <3% macrophages (as measured by phagocytosis of latex beads or positive staining for nonspecific esterase), 25% large granular lymphocytes, and 25–40% large agranular lym-

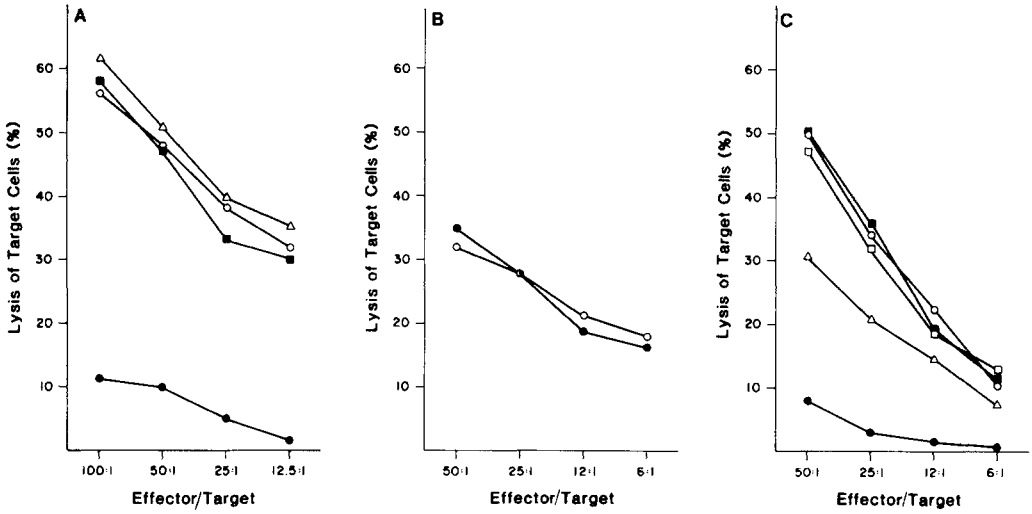

FIGURE 3. Characterization of hepatic NK cells assayed for cytotoxicity against ^{51}Cr-labeled YAC-1 target cells. (*A*) A nonadherent NPC population accounts for the high NK activity in MRL/*lpr* mice. 4-mo-old female MRL/*lpr* NPC were depleted of adherent cells by adherence to plastic (○) or carbonyl Fe treatment (△). Untreated NPC from 4-mo-old MRL/*lpr* (■) and MRL/++ (●) female mice were controls. (*B*) The NPC population with high NK activity is radioresistant. 2.5-mo-old female MRL/*lpr* were irradiated with 600 rad (○) or untreated (●) 18 h before harvesting NPC. (*C*) Hepatic NK in MRL/*lpr* mice are AGM_1^+ Ly-1$^-$, and Ly-2$^-$. NPC in medium only (■), anti-Ly-1 plus C′ (□), and anti Ly-2 plus C′ (○) retain high NK activity. Anti Thy-1 + C′ (△) partially and anti AGM_1 + C′ (●) almost completely abolish NK activity.

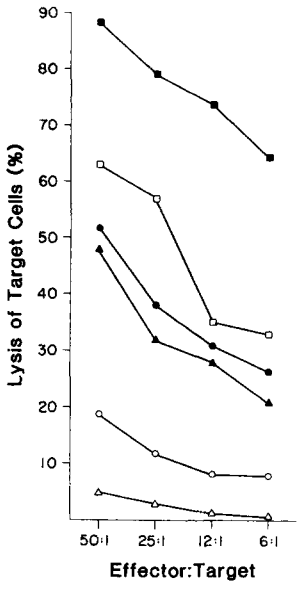

FIGURE 4. Effect of biologic response modifiers on hepatic NK activity. 1.4 mg *C. parvum* injected intravenously 24 h before isolation of NPC-augmented NK activity in not only 4-mo-old C3H/HeN (●) (○, untreated) and 4-mo-old MRL/++ (▲) (△, untreated), but also 4-mo-old MRL/*lpr* female mice (■) (□, untreated).

phocytes. In vitro treatment with anti-AGM_1 and complement eradicated NK activity. Although a mild decrease was noted after treatment with anti-Thy-1 and complement, anti-Ly1 and/or anti-Ly2 and complement did not reduce NK activity (Fig. 3*C*).

Hepatic NK Cell Activity: Effect of Biologic Response Modifiers. A single injection of *C. parvum* (1.4 mg i.v. 1–20 d before killed) augmented hepatic and splenic NK activity in nonautoimmune mice (Fig. 4). Despite high hepatic NK activity in the untreated MRL/*lpr* mouse, further enhancement was also noted with *C. parvum* (Fig. 4). Intraperitoneal injection of poly(I:C) and IFN-α/β also resulted in increased NK activity in the MRL/*lpr* and nonautoimmune strains. A similar response to *C. parvum* was noted in the (NZB × NZW)F_1 female mice, although less change was noted in the older (6.5-mo-old) mice (data not shown).

Discussion

This paper provides evidence for extremely high hepatic natural killer cell activity in the autoimmune MRL/*lpr* and (NZB × NZW)F_1 female mice. Although more extreme in the MRL/*lpr* mice, hepatic NK activity increases with age in both strains and is elevated before the development of autoantibodies or evidence of clinical disease. Moreover, this marked elevation in these autoimmune strains is not reflected in the spleen or peripheral blood. As is the case with the nonautoimmune strains (5), NK activity can be further augmented by in vivo administration of *C. parvum*, poly(I:C), and IFN-α/β.

Preliminary characterization of these hepatic NK cells indicates no differences from those isolated from the nonautoimmune strains. They are AGM_1^+, Ly-1^-, and Ly-2^-, radioresistant, nonadherent, and not removed by carbonyl iron. The fact that anti-Thy + C′ treatment of NPC decreases, but does not eradicate, cytotoxicity to YAC-1 cells suggests that hepatic NK represent a heterogeneous population of cells.

The role that high hepatic NK activity may play in the autoimmune process is conjectural. Our findings may only reflect the sequelae of endogenous Kupffer cell activation of MRL/*lpr* and (NZB × NZW)F_1 mice (9) and, therefore, have no functional significance per se. However, activated splenic NK have been shown (4) to regulate T lymphocyte proliferation, as measured by MLR, by eliminating accessory cells that have combined with antigen. Moreover, decreased allogeneic MLR responses, using spleen and lymph node cells from older MRL/*lpr* mice as stimulators, have been reported (10). Indeed, we have observed a markedly diminished allogeneic MLR using adherent hepatic NPC from both young and old MRL/*lpr* mice as stimulators (Magilavy, D. B., and S. L. Latta, manuscript in preparation).

This finding of high hepatic NK activity in these autoimmune strains appears to conflict with numerous reports of defective or diminished NK activity of peripheral blood from patients with active rheumatoid arthritis or systemic lupus erythematosus (11, 12). However, the striking compartmental differences in NK activity among the liver, spleen, and peripheral blood of the MRL/*lpr* mice that we observed may explain the paradox. There are clearly differences among not only the percentage of NK cells in the liver, spleen, and peripheral blood but also the microenvironment of these organs. Because the liver is the major organ responsible for the clearance of soluble IgG immune complexes and foreign antigens, it would not be surprising that hepatic NK from the autoimmune mice are selectively exposed to several secretory products which would result in either activation or recruitment to the liver of NK from other organs.

Summary

This study demonstrates a profound elevation of NK activity, as measured by cytotoxicity to YAC-1 targets in a 4-h incubation ^{51}Cr-release assay, of freshly isolated hepatic NPC from both MRL/*lpr* and (NZB × NZW)F_1 mice. This marked increase was not observed in splenic or peripheral blood NK. The hepatic NK were nonadherent, radioresistant, Ly-1^-,2^-, and AGM_1^+. Furthermore, biologic response modifiers can further augment hepatic NK activity in these autoimmune strains.

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