NK cells do not mediate renal injury in murine adriamycin nephropathy

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In adriamycin nephropathy (AN), a model of chronic proteinuric renal injury, the absence of functional B and T cells with residual natural killer (NK) cells, and macrophages in severe combined immunodeficient (SCID) mice results in more severe disease than in immunocompetent mice. We have recently shown expression of the stimulatory NK cell molecule NKG2D and its ligand RAE-1 in the adriamycin (ADR) kidney. Therefore, we sought to determine the role of NK cells in AN. We used anti-asialo GM1 NK cell depletion in immunocompetent BALB/c mice with AN, and also compared AN in immunodeficient SCID mice and immunodeficient nonobese diabetic (NOD)-SCID mice (that have impaired NK cell function). The number of NK cells was increased in AN in BALB/c mice compared with normal controls. NK cell depletion or reduction of NK function in NOD-SCID mice did not affect the severity of disease. In both wild type and immunodeficient models, ADR upregulated RAE-1 in the kidney. High levels of Class I major histocompatibility complex molecules were found in both models of AN. In conclusion, NK cells do not play a significant role in AN.

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Adriamycin (ADR)-induced nephropathy is a robust model of human focal segmental glomerulosclerosis.[1,2] The severity of renal damage and dysfunction in human renal disease and animal models correlates with the degree of interstitial infiltration by inflammatory cells, predominantly T lymphocytes and macrophages.[3–5] Previous studies in our laboratory showed that depletion of CD8+ T cells protects while depletion of CD4+ T cells aggravates adriamycin nephropathy (AN) in BALB/c mice.[6,7] However, the role of other effector cells such as natural killer (NK) cells is unknown. The greater sensitivity of the severe combined immunodeficient (SCID) mouse to ADR, where NK cells are the predominant immune effector cell besides macrophages, suggests either that the cognate immune cells provide protection from injury as suggested by our CD4 depletion data or that the NK population together with macrophages in these mice exacerbates the renal injury. In glomerulonephritis, a possible involvement of NK cells was suggested by finding that local production of fractalkine and the presence of CD16-positive cells, which include NK cells, in the interstitial lesions of human crescentic glomerulonephritis.[8] However, evidence for cytotoxic injury by NK cell was not found in rat antiglomerular membrane glomerulonephritis[9] and cell-mediated glomerulonephritis.[10]

NK cells, a pivotal component of the innate immune system, are derived from haematopoietic stem cells, which have been identified in sites such as bone marrow, thymus, spleen, omentum, and liver in adults.[11] Under normal conditions, NK cells are mostly confined to peripheral blood, spleen, and bone marrow, but they can migrate to inflamed tissues in response to different chemoattractants.[12] NK cells recognize other cells which lack major histocompatibility complex (MHC) Class I expression via 'the missing self hypothesis' using a variety of inhibitory receptors that bind to Class I molecules; this leads to NK responses to both tumors and virally infected cells that downregulate their Class I expression.[13] NK cells release both inflammatory cytokines and cytotoxic molecules including perforin, granzyme, and tumor necrosis factor-related apoptosis-inducing ligand causing rapid injury to tissues not recognized as self.[14] More recently, molecules that function as activating receptors have

been described on NK cells. Specifically, NKG2D binds to a co-receptor DAP12 on the NK cell and is activated by a range of Class Ib MHC molecules including RAE-1 and H-60 in mice and MICA in humans that are upregulated by stress or danger. It is unclear whether the activating signal received through these interactions can override the inhibitory signals generated by inhibitory receptors interacting with Class I MHC molecules. The crucial importance of this pathway is demonstrated by its preservation across species and the use of molecular homologues of ligands by viruses to avoid killing.

In order to test the role of NK cells we have used two approaches. We first performed NK cell depletion in the standard model of AN using an antibody to glycolipid asialo GM1 or ganglio-n-tetraosylceramide.[15] This has been shown to be effective for NK depletion in tumor and transplantation animal models.[16–18] Secondly, we examined NK cell effects in isolation by comparing AN in the SCID and nonobese diabetic (NOD)-SCID mouse. The severity of AN was assessed in both the antibody depletion model and in immunodeficient NOD-SCID mice, where the NK cells are present but functionally inactive. We also measured expression of NK cell activating ligand RAE-1 and inhibitory ligand H-2k^d and the presence of the activating receptor NKG2D.

RESULTS

NK cell depletion

Repeated injections of anti-asialo GM1 serum significantly depleted NK cells in kidney as well as spleen in BALB/c mice with AN (Figures 1–3). Renal NK cells appeared to be more numerous in BALB/c mice with AN than controls, but the difference was not significant when assessed by fluorescence-activated cell scan (FACScan) (0.52 ± 0.18 versus $0.30 \pm 0.14\%$, $P = 0.11$). However, the number of NK cells in kidney, estimated by multiplying total number of interstitial CD45+ cells isolated by the percentage of NK cells within the CD45+ population, was significantly higher in AN BALB/c mice than in normal control ($13\,534 \pm 2984$ versus 3460 ± 1937, $P < 0.05$) (Figure 1e). Anti-asialo GM1 serum significantly depleted NK cells from kidney of mice with AN (0.21 ± 0.08 versus $0.52 \pm 0.18\%$ by FACScan, $P < 0.01$, $13\,534 \pm 2984$ versus 3819 ± 1081 in total number of NK cells per kidney, $P < 0.05$) (Figure 1). Anti-asialo GM1 serum also depleted NK cells in spleen of mice with AN (4.28 ± 0.67 versus $10.93 \pm 1.09\%$ by FACScan, $P < 0.01$) (Figures 2 and 3).

Renal functional injury

As expected, mice with AN had significantly worse renal function at 5 weeks than control, as assessed by urine protein (3.88 ± 0.72 versus 1.05 ± 0.27 mg/16 h, $P < 0.05$) and creatinine clearance (21.7 ± 9.1 versus 42.2 ± 15.9 μl/min, $P < 0.05$). Anti-asialo GM1 depletion of NK cells had no effect on any renal function parameter in mice with AN (Table 1).

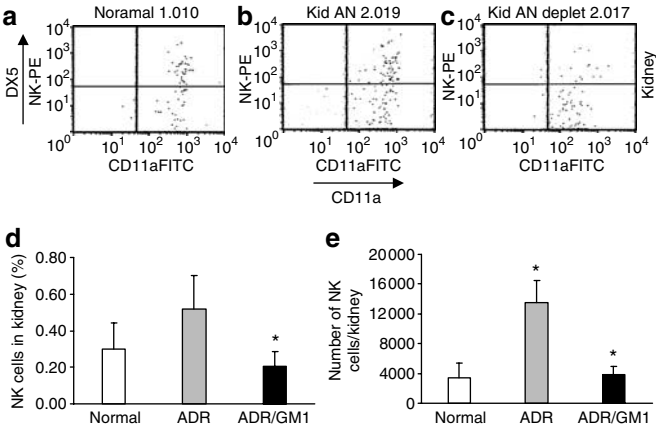

Figure 1 | NK cell depletion assessed by flow cytometry of kidney infiltrating NK cells. (**a–c**) Representative histograms of kidney infiltrating NK cells. (**d**) Percentage of NK cells in CD45+ gated population. (**a**) Normal BALB/c mice ($n = 4$), (**b**) AN mice ($n = 5$), and (**c**) AN mice with NK cells depleted by GM1 ($n = 5$). (**e**) Total number of NK cells per kidney estimated by multiplying total number of interstitial CD45+ cells isolated per kidney by the percentage of NK cells within the CD45+ population. *$P < 0.05$ versus ADR (analysis of variance).

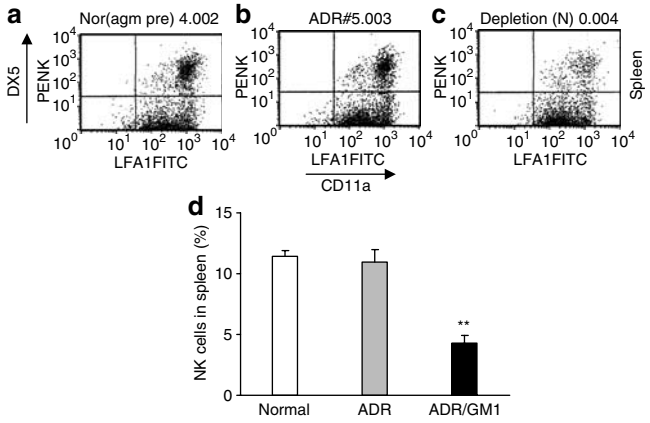

Figure 2 | NK cell depletion assessed by flow cytometry of splenic NK cells. (**a–c**) Representative histograms of splenic NK cells. (**d**) Percentage of NK cells in CD45+ gated population. (**a**) Normal BALB/c mice ($n = 4$), (**b**) AN mice ($n = 5$), and (**c**) AN mice with NK cells depleted by GM1 ($n = 5$). **$P < 0.01$ versus normal and ADR (analysis of variance).

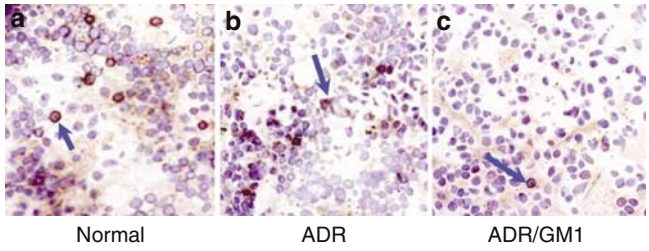

Figure 3 | (a–c) NK cell depletion assessed by immunohistochemical staining for NK cells in splenocytes. The arrows indicate NK cells located in the area of splenic pulp, where the membrane surfaces of NK cells are stained brown with GM1 antibody counterstained with hematoxylin. Original magnification $\times 400$.