Renal carcinoma cell lines inhibit natural killer activity via the CD94 receptor molecule

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Abstract MHC class I molecules protect normal and transformed cells from lysis by natural killer (NK) cells through recognition of receptors expressed on leukocytes. Defects in NK cell activity and lymphokine activated killer (LAK) cell generation have been previously demonstrated in patients with renal cell carcinoma (RCC). However, to date, the importance of NK receptor/MHC class I interactions for immune evasion by RCC cells has not been described. In this study, human RCC cell lines (HTB46, HTB47, ACHN, CRL 1933 and HTB44) were found to be susceptible to lysis by both NK cells and interleukin-15 (IL-15)-derived LAK cells from normal donors in vitro. However, when NK cells were co-cultured with RCC cells their expression of the CD94 NK receptor molecule was significantly increased and their cytolytic activity against RCC targets was reduced. The cytolytic activity of NK cells was restored by the addition of IL-15, which further augmented the expression of CD94 on CD56+ NK cells. Disruption of NK receptor-MHC class I interactions by the addition of blocking antibodies to CD94 had no effect on the lysis of K562 or HTB47 targets by NK cells. However, the sensitivity of HTB46 cells to NK-mediated lysis was increased by blocking the CD94 receptor molecule, but only when the NK cells had not been previously co-cultured with RCC cells. This was independent of the presence of IL-15. These results show that RCC cells can inhibit NK activity via CD94 and suggest that disruption of interactions between receptor and ligand on RCC cells in vivo may augment the immune response against tumours by innate effector cells.

Key words Renal cell carcinoma · Natural killer cells · CD94 · Interleukin-15

Introduction

Natural killer (NK) cells display cytotoxicity against malignant cells and virally infected cells without prior sensitisation [18, 24, 32], and contribute significantly to “immune surveillance” by recognising and destroying malignant tumour cells that evade antigen-specific detection by T lymphocytes. In murine models, a direct correlation between the level of NK activity and resistance to transplanted tumours has been demonstrated [16, 35].

Several studies have shown decreased NK cell function in patients with malignant tumours [14, 20]. RCC patients with advanced metastatic disease had reduced NK activity, whereas patients with localised disease had near-normal levels of NK activity [19]. Spontaneous regression of RCC occurs following nephrectomy, although rarely, suggesting removing tumour bulk can relieve immunological suppression in the host. The presence of NK and lymphokine activated killer (LAK) inhibiting factors in the plasma from RCC patients resulted in depressed LAK activity [11] and, following tumour removal, the inhibition was also removed. Augmentation of NK activity with exogenous cytokines, such as IL-2, resulted in antitumour immune responses in 16–35% of patients with RCC, but was associated with systemic toxicity [12, 33].

In humans, the cytotoxic activity of NK cells is regulated by NK receptors that belong to either the immunoglobulin (Ig) superfamily or the C-type lectin superfamily [3, 28], and inhibition is mediated by cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [36, 13]. Normal and transformed cells are protected from NK lysis through the interaction of NK receptors and MHC class I molecules. Well-characterised members of the Ig superfamily of NK receptors
include p58 (CD158) structures, which are known to bind HLA-C [27] and the 70 kDa NKBI receptor, which displays a high degree of homology with CD158 and recognises HLA-Bw44 [22]. Other examples of the Ig superfamily include p140, which is inhibitory upon HLA-A3 and HLA-A11 recognition [30], and BY55, which has a broad specificity for MHC class I molecules [23]. The C-type lectin superfamily of receptors includes NKR-P1A, CD94 and NKG2. NKR-P1A (CD161) is an 80 kDa glycoprotein and its HLA specificity is undefined [21]. CD94 is a 30 kDa molecule (p30) that forms a coherent heterodimer with p43 (A or B) or p39 (C, D and E) subunits, which belong to the NKG2 gene products [6, 8]. CD94/NKG2-A/B complexes correspond to the inhibitory form of the receptor, whereas CD94/NKG2-C/D/E are the activatory forms of the receptor [6, 21]. These receptor complexes recognise HLA-E molecules [5]. It is likely that a single NK cell clone possesses at least three different receptors, which function independently, giving mixed HLA class I recognition [22].

IL-15 has been shown to mediate the induction of CTLs and LAK cells [7], has a key role in the development of mature NK cells [9, 10, 15], promotes the survival of NK cells [10] and has been shown to induce NK cell proliferation in vitro [9]. IL-15 treatment induces protective antitumour responses in animal tumour models [29]. However, addition of IL-15 to cultures of early thymic precursors led to the development of NK cells that expressed CD94/NKG2-A as the only HLA class I specific inhibitory receptor, and the presence of this receptor inhibited lysis of HLA class I-positive autologous target cells [25]. It has also been demonstrated that IL-15 can induce the expression of CD94/NKG2-A on CD8-positive T cells in mixed lymphocyte cultures, which leads to an impairment of cytolytic activity. Cytolysis was restored by the addition of anti-CD94 monoclonal antibody [26]. Furthermore, anti-CD94 antibody has been shown to induce cytolytic activity in IL-2-stimulated NK cells against various tumour cell lines [1, 2]. It has been suggested that tumour cells may synthesise IL-15 [24], which is capable of inducing CD94/NKG2-A expression in NK cells, and this may represent a possible, but as yet unproven, mechanism of tumour escape.

We have investigated NK cell surveillance of tumours by examining the interaction between NK cells, renal tumour cells and the cytokine IL-15. Using an in vitro killing assay we have demonstrated inhibition of NK activity by renal tumour cells and that in some cases this is mediated via the receptor molecule CD94 in the presence of IL-15.

Materials and methods

Cell lines

The human renal cell carcinoma cell lines ACHN (ECACC, Porton Down, UK) HTB44, HTB46, HTB47 and CRL 1933 (ATCC, Rockville, MD, USA) were obtained and cultured according to the supplier’s instructions. K562, a human chronic myelogenous leukaemia cell line was obtained from (ATCC).

Tissue types were as follows. HTB46: HLA A23 [9], A24 [9], B44 [12], Cw4, DR7, DQ11 [5]; HTB47: HLA A1, A11, B8, B52 [5], Cw7, Cw12, DR3, DR11 [5]; CRL 1933: HLA A3, A24 [9], B7, Cw7, DR15 [2], DQ6 [1].

Mixed lymphocyte-tumour cultures (MLTCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of normal healthy donors collected into sodium heparin (10 U/ml) or from buffy coats, by centrifugation through Lymphoprep (Nycomed) and aliquots were stored in liquid nitrogen. Established renal cell lines were grown in RPMI-1640 with 2 mM glutamine (Gibco Life Technologies, Paisley, UK) containing 5% (v/v) foetal calf serum (Harlan-Seralab, Loughborough, UK), to 70-90% confluency and treated for 2 hours with 10 μg/ml mitomycin-C (Kyowa, Tokyo Japan) to arrest growth. Cells were then washed twice in media, harvested using EDTA (trypsin (Gibco Life Technologies) and aliquots stored in liquid nitrogen until required.

For the MLTC, growth-arrested renal cells were seeded at 5 × 105 cells/well in 6 well plates and incubated for 2 h at 37 °C, before the addition of 1 × 106 PBMC into a final volume of 5 ml. Where required, recombinant human IL-15 (R&D Systems, Abingdon, UK) (10 ng/ml) was also added and the cells cultured for 7 days in 5% CO2 in an humidified incubator at 37 °C. Following 7 days of co-culture, suspension cells (PBMCs) were harvested, washed, and viable cell counts determined using trypan blue exclusion. Cells were then used in a 51chromium-release cytotoxicity assay.

51Chromium-release cytotoxicity assay

The appropriate target renal cancer cells were harvested in log phase of growth, 2 × 104 cells were resuspended in 0.5 ml media, 150 μCi sodium 51chromate (Amersham Pharmacia Biotech, Little Chalfont, UK) added and incubated for 1 h at 37 °C, with regular gentle mixing. Following chromium labelling, target cells were thoroughly washed in media, and resuspended in fresh media to give a final concentration of 5 × 105 cells/100 μl. Dilutions of washed, co-cultured PBMCs were added to 96-well round-bottomed tissue culture plates, along with 5 × 105 target cells in 200 μl final volume, to produce a range of effector/target ratios. The cells were incubated for 4 h at 37 °C, pelleted by centrifugation at 444 × g for 5 min, and the radioactive content of 100 μl supernatant determined using a gamma-counter (LKB). Results were expressed as percentage specific release (percentage lysis):

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\text{% specific release} = \frac{(\text{experimental release (CCM)} - \text{spontaneous release (CCPM)})}{(\text{maximal release (CCPM)} - \text{spontaneous release (CCPM)})} \times 100
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Spontaneous release was measured by incubating labelled target cells with 100 μl tissue culture media and maximal release by incubating with 100 μl 0.1 M HCl. All samples were tested in triplicate, all controls in sextuplicate and spontaneous release was always less than 10% of the maximal release.

Where described, effector cell populations were depleted of NK cells using anti-CD56 monoclonal antibody (R&D Systems) and goat anti-mouse magnetic beads (Dynal, Oslo, Norway). Efficiency of the CD56 depletion was confirmed by flow cytometry with a PE-conjugated anti-CD56 (Serotec, Oxford, UK) monoclonal antibody of different epitope specificity. NK-depleted PBMCs were then used in the cytotoxicity assay as described.

Where indicated, 5 × 103 51chromium-labelled cells were pre-incubated with 2 μg/ml anti-CD94 monoclonal antibody (Pharmingen, San Diego, USA) for 30 min at room temperature. Target cells and antibody were then used as in the previously described cytotoxicity assay.