Immunology 1988 **63** 225–231

Chondroitin sulphate proteoglycan production by NK cells and T cells: effects of xylosides on proliferation and cytotoxic function

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Accepted for publication 10 September 1987

SUMMARY

Cultured human NK cells and T cells grown in the presence of IL-2 and phytohaemagglutinin incorporate ^{35}S sulphate into two distinct macromolecular species. The larger molecule was identified as a chondroitin-4-sulphate proteoglycan and was present in both cell-associated and secreted material. The smaller component was identified as free glycosaminoglycan and was present only in the cell-associated material. The sulphated macromolecules synthesized by NK cells were smaller than those produced by T cells. Growth in the presence of β-D-xyloside led to a decrease in proteoglycan production, together with an increase in the synthesis of free glycosaminoglycan. The latter molecule was found in the secreted as well as the cell-associated fraction. In all instances, growth of T cells was inhibited by xyloside in a dose-dependent fashion. However, growth of NK cells from 3/7 donors was stimulated at low concentrations of xyloside (0·25 and 0·5 mM). Growth of NK cells in xyloside had no effect on their lytic activity, and the 'NK-like' cytolytic capacity of cultured T cells was similarly unaffected. Both NK cells and T cells grown in xyloside at a concentration resulting in a 50% inhibition of intact proteoglycan synthesis did not show increased susceptibility to autolysis in the presence of NK-cell targets. These findings suggest that optimal production of the intact proteoglycan molecule may not be essential for NK-cell lytic function or protection of effector cells *in vitro*.

INTRODUCTION

Human natural killer (NK) cells exert cytolytic activity against certain tumour cell targets and can also function as immunoregulatory cells *in vitro* (Herberman & Ortaldo, 1981). The cells responsible for NK activity are large granular lymphocytes (LGL) which comprise of a morphologically homogeneous population of peripheral blood mononuclear cells with low buoyant density and azurophilic cytoplasmic granules (Timonen, Ortaldo & Herberman, 1981). While the precise mechanism of lysis by NK cells remains uncertain, there is much evidence to suggest that a secretory mechanism operates in the post-binding phase of target-cell lysis (Henkart & Henkart, 1982). Soluble cytotoxic molecules, NK cytotoxic factors (NKCF), released by NK cells and specifically cytotoxic for NK targets, have been described (Wright & Bonavida, 1982; Farram & Targan, 1983).

Abbrevations: EDTA, ethylenediamine tetraacetic acid; LGL, large granular lymphocytes; NEM, *N*-ethyl-melaimide; NK, natural killer; NKCF, natural killer cytotoxic factor; PBS, phosphate-buffered saline; PMSF, phenyl methyl sulphonyl fluoride.

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These are presumed to be derived from LGL granules, but extracts from rat LGL leukaemia cell granules are toxic for NK-resistant as well as -sensitive targets (Henkart *et al.*, 1984). This lack of selectivity of LGL granule extracts would suggest that there is specificity at the level of release and action of cytotoxic factors by intact effector cells, although it is unclear whether non-leukaemic NK-cell granule extracts show a similar non-selectivity. Morphological studies have identified characteristic ring-like lesions in target-cell membranes (Podack & Dennert, 1983). It has been proposed that these are a result of insertion of NK-cell derived 'perforins', but the relationship of these molecules to NKCF is not clear.

Glycosaminoglycans are a group of negatively charged polysaccharides comprising of disaccharide-repeat sequences (uronate:hexosamine) which may be extensively sulphated. They are coupled to proteins to form proteoglycans, via a (glucuronic acid–galactose–galactose–xylose) linkage sequence which is covalently bound to the protein core through serine residues. Biosynthesis of these molecules involves the initial attachment of xylose to serine (Lindahl & Roden, 1964) before chain elongation occurs via the addition of single sugars in the Golgi complex (Roden & Schwartz, 1975). β-D-xylosides can

act in competition with xylose-substituted protein cores as acceptors for glycosaminoglycan-chain synthesis (Lohmander, Madsen & Minek, 1978). In the presence of β-D-xylosides, the formation of free glycosaminoglycan chains normally predominates over that of proteoglycans, and the activity of the glycosyl transferase system (responsible for the polysaccharide chain elongation) is increased (Lohmander, Hascall & Caplan, 1979). The addition of xylosides to several *in vitro* and *in vivo* systems has been shown to modify cell growth and development in embryonic and adult tissues, suggesting the importance of proteoglycan metabolism to cell function (Kinoshita & Saiga, 1979; Morriss-Kay & Crutch, 1982; Thompson & Spooner, 1983; Spooncer *et al.*, 1983; Gallagher & Hampson, 1984).

It has been reported recently that NK cells release a chondroitin sulphate proteoglycan during target cell lysis (Schmidt *et al.*, 1985) and that this molecule is sequestered within LGL granules prior to contact with target cells (MacDermott *et al.*, 1985). This highly acidic molecule is thought not to be the lytic moiety of NKCF, but a role in the delivery of the cytotoxic molecule to the target-cell membrane or in protection of the NK cell from autolysis has been suggested (MacDermott *et al.*, 1985).

We have studied the proteoglycans synthesized and secreted by highly purified cultured human T cells and NK cells. These cell types produce distinct molecular species of proteoglycan and also differ in their response to xylosides. While greatly inhibiting intact proteoglycan synthesis by cultured T cells and NK cells, growth in the presence of xyloside failed to inhibit subsequent target-cell killing. Further, NK cells grown in the presence of concentrations of xyloside that greatly inhibited intact proteoglycan synthesis were no more susceptible to autolysis when incubated with NK-sensitive target cells than were untreated effector cells.

MATERIALS AND METHODS

Cell lines

The human B lymphoblastoid cell line BSM (van de Griend *et al.*, 1984) was maintained in RPMI-1640 + 10% heat-inactivated fetal calf serum supplemented with 100 μg/ml streptomycin and 200 μg/ml ampicillin (RPMI-CS). The human erythroleukaemic cell line K562 was maintained in the same medium but with newborn in place of fetal calf serum. Cell lines were routinely tested for the presence of mycoplasma and found to be negative.

Cell purification

Peripheral blood was obtained from a panel of normal donors by venepuncture. Mononuclear cells were isolated by layering heparinized blood onto lymphocyte separation medium (Flow Laboratories, Irvine, Ayrshire and centrifuging at 400 ***g*** for 20 min. Cells accumulating at the interface were washed twice with phosphate-buffered saline (PBS) prior to use. Unseparated mononuclear cells were used as feeders, whereas cells to be sorted were incubated in plastic flasks for 2 hr at 37° in an atmosphere of 5% CO_2, 95% air, in order to remove the bulk of the monocytes. Non-adherent cells were then sorted into NK-cell enriched and T-cell enriched populations as described previously (Roberts & Moore, 1985). Briefly, cells were labelled with the B73.1 (CD16) monoclonal antibody, which recognizes the Fcγ receptor on peripheral NK cells and granulocytes (Perussia *et al.*, 1983). After washing twice with PBS at 4°, cells were labelled with fluorescein-conjugated rabbit anti-mouse immunoglobulin antiserum (Dakopatts, Copenhagen, Denmark) for 30 min at 4°. After further washing, cells were sorted into $B73.1^+$ and $B73.1^-$-enriched populations using a FACS IV (Becton-Dickinson, Mountain View, CA). Purities of the $B73.1^-$ (T cell)-enriched populations were routinely >99% while $B73.1^+$ cells were normally 95–98% pure. The latter were therefore resorted, resulting in $B73.1^+$ cells which were >99% pure.

Cell growth

Growth of $B73.1^+$ and $B73.1^-$ cell populations was initiated in 96-well round-bottomed plates in RPMI-1640 plus 10% autologous plasma. The growth medium was supplemented with: 10^5 BSM/ml and 2×10^5 autologous or allogeneic peripheral blood mononuclear cells/ml (both given 50 Gy gamma irradiation), 1 μg/ml indomethacin, 1 μg/ml phytohaemagglutinin (PHA; Wellcome Laboratories, Beckenham, Kent) and IL-2. The latter was added in the form of culture supernatant from the MLA 144 cell line (Roberts & Moore, 1985) or recombinant material (Biogen), and in both cases the concentration used was that resulting in almost maximal proliferation of a T-cell line. Cultures were initiated at 10–100 cells/well and after 7–10 days cells were taken up into fresh feeder mixture, as above, in the presence of the xyloside *p*-nitrophenyl-β-D-xylopyranoside (Koch-Light Laboratories Ltd., Colnbrook, Berks) at concentrations ranging from 0–2 mM. Cells were grown under these conditions for 10–14 days, refeeding every 3–4 days, prior to investigation.

Cytotoxicity assays

Effector cells were washed and prior to assay incubated overnight in RPMI-CS with or without xyloside. Cytotoxic activity against K562 target cells was performed as described previously (Roberts & Moore, 1985). Briefly, K562 cells were labelled with $Na_2{}^{51}CrO_4$ (Amersham International, Amersham, Bucks), washed thoroughly in PBS and resuspended at a concentration of 5×10^4/ml in RPMI-CS. Target cell suspension, 0·2 ml, was added to LP3 tubes and effector cells were added in 0·2 ml RPMI-CS at an effector:target (E:T) ratio of 2·5–20:1. Assays were performed in triplicate and tubes were centrifuged at 100 ***g*** for 10 min and incubated for 4 hr at 37°. The assay was terminated by removing 0·2 ml supernatant and this and the cell pellet plus remaining supernatant were counted using a gamma counter. Spontaneous ^{51}Cr release was measured using target cells in 0·4 ml RPMI-CS alone, and maximum release was measured by suspending target cells in 1% Triton X-100. Specific ^{51}Cr release was measured as follows:

$$\% \text{ specific cytotoxicity} = \frac{\text{test } {}^{51}\text{Cr release} - \text{spontaneous } {}^{51}\text{Cr release}}{\text{maximum } {}^{51}\text{Cr release} - \text{spontaneous } {}^{51}\text{Cr release}} \times 100.$$

Spontaneous release was always <20% and maximum release was between 90% and 110%.

In some experiments effector cells were labelled with ^{51}Cr in the same manner in order to investigate the autolytic capacity of cells grown in the presence or absence of xyloside. Spontaneous isotope release was measured by incubating effector cells in RPMI-CS alone at a concentration of 10^5 cells/ml and test

release was that observed with the addition of unlabelled K562 target cells at E:T ratios of 10:1 (T cells) or 2·5:1 (NK cells). In all other respects the assays were performed as above.

Radiolabelling of cells and extraction of proteoglycans

Cultured B73.1$^+$ and B73.1$^-$ cells were biosynthetically radiolabelled with 30 μCi of Na_2 $^{35}SO_4$ (specific radioactivity 25–40 Ci/mg; Amersham) per ml of culture medium over 48 hr. For some experiments [^{3}H]glucosamine (specific radioactivity 2·6 Ci/mmol), 10 μCi/ml, was added to the medium. The cell suspensions were then centrifuged at 1000 *g* for 5 min and the culture media removed. The cell pellets were washed with PBS and were subsequently solubilized in 1 M NaCl containing 1% Triton X-100 and protease inhibitors (0·1 M 6-amino hexanoic acid, 5 mM EDTA, 0·25 mM PMSF). Solubilized pellets and media were then dialysed into PBS (containing 0·1% Triton X-100 and protease inhibitors) prior to ion-exchange chromatography on columns (1 × 5 cm) of DEAE–cellulose (Whatman Chemical Separation, Maidstone, Kent). Bound radioactivity was eluted with a linear gradient of 0·15–0·5 M NaCl/20 mM sodium phosphate buffer, pH 7·5, and aliquots of radioactivity were counted on a Beckman series 750 scintillation counter. The elution profiles were plotted and fractions corresponding to the peaks of radioactivity (representing the proteoglycans) were pooled, dialysed into water and lyophilized.

Analysis of proteoglycans

Analysis of proteoglycans obtained from ion-exchange chromatography was performed using gel-filtration chromatography on Sepharose CL-4B and CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden) columns (55 × 1·5 cm). Elution was with guanidine hydrochloride, pH 6·0, containing 0·1% v/v Triton X-100 at a flow rate of 6 ml/hr, and 0·5-ml fractions were collected. In order to demonstrate the proteoglycan nature of the material, samples were chromatographed after digestion with Pronase (Boehringer, Mannheim, FRG), at an enzyme concentration of approximately 1 mg/ml sample, in 0·1 M Tris-HCl, 5 mM calcium acetate, pH 7·8, for 48 hr at 40°. Polysaccharide chains were released from proteoglycans by treatment with alkaline borohydride (50 mM sodium hydroxide, 1 M sodium borohydride) at 38° for 48 hr and subsequently neutralized with 10% acetic acid.

Analysis of glycosaminoglycans

The glycosaminoglycan nature of the isolated proteoglycans was analysed by specific enzymatic digestion of the intact molecules obtained from ion-exchange chromatography. Chondroitinase ABC (Seikagaku Kogyo Co., Tokyo, Japan) was used at a concentration of 0·1 U/ml sample in 50 mM Tris-HCl/50 mM NaCl buffer, pH 8·0 (with 0·1 mg/ml bovine serum albumin), and chondroitinase AC (Seikagaku Kogyo Co.) was used at a concentration of 0·2 U/ml sample in 0·06 M Tris-HCl buffer, pH 7·5 (with 100 μg/ml bovine serum albumin). Enzymatic digestion was carried out for 18 hr at 40°. Assessment of the digests was made using gel-filtration chromatography on a Biogel P-6 (Bio-Rad Labs, Watford, Herts) column (55 × 1cm) using 0·25 M ammonium hydrogen carbonate as the eluent.

The degree of sulphation of chondroitin sulphate constituent disaccharides was analysed using high voltage paper electrophoresis of chondroitinase ABC digests of the material as described by Gallagher & Walker (1985). On some occasions chondroitinase digests were treated further with chondro-4-sulphatase (0·2 U/ml) in the same buffer.

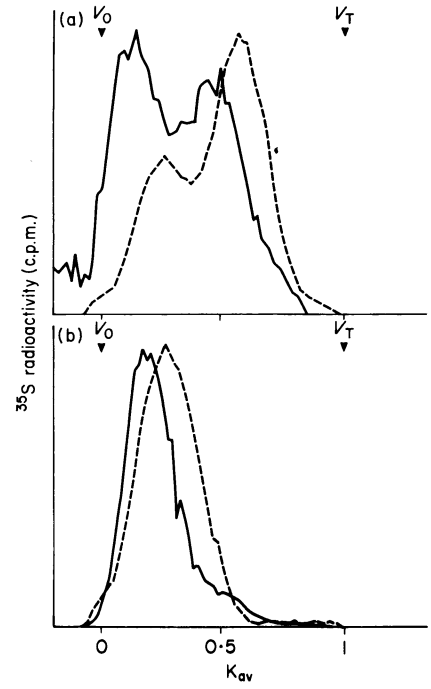

Figure 1. Profiles from CL-6B gel filtration chromatography using 4 M guanidine hydrochloride (pH 6·0) as eluent at flow rate of 6 ml/hr. Fractions (0·5 ml) were collected and radioactivity in each counted. The void volume (V_O) was marked with dextran blue and the total volume (V_T) with ^{35}S. Profile (a) represents cell-associated material, (——) T cell; (– – –) NK cell; and (b) represents medium material (——) T cell; (– – –) NK cell.

RESULTS

Proteoglycan production by cultured NK and T cells

Both NK and T cells incorporated $^{35}SO_4$ label into proteoglycans which were found in cell-associated and secreted (medium) fractions. With both cell types the majority of the proteoglycans were recovered from the medium (approximately 60% in NK cells and 85% in T cells after 48 hr). Profiles from Sepharose CL-6B gel-filtration chromatography of sulphated macromolecules derived from NK- and T-cell pellets were heterogenous, with two distinct species being identified. In both cell types, the elution position of the larger molecule was identical to that of the material derived from the medium of the same cells (K_{AV} for NK cell 0·26 and for T cell 0·18). The smaller molecule was not secreted into the medium in either cell type (Fig. 1). The proteoglycan nature of the medium-derived material and of the early eluting peak from the cell pellet was confirmed by the later elution following alkali treatment (to K_{AV} of 0·55 in NK cells and 0·5 in T cells). No change in the elution position of the later eluting peak from the cell-pellet derived material was seen after alkali treatment, suggesting it to be a free glycosaminoglycan.

Pronase digestion of intact proteoglycans derived from both

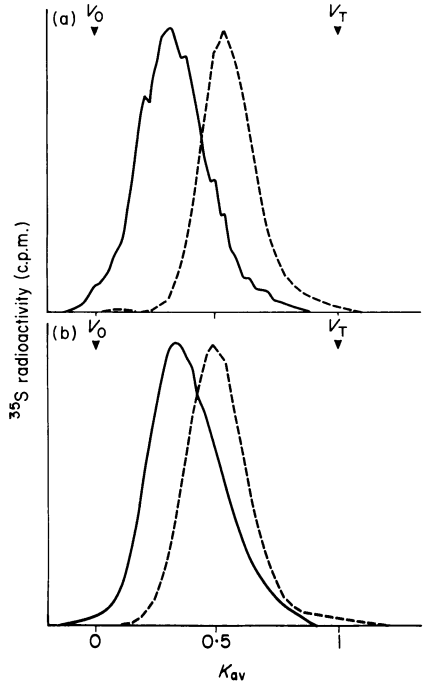

Figure 2. Profiles from CL-6B gel-filtration chromatography of medium-derived material from (a) NK cells and (b) T cells, illustrating the relative Pronase resistance of the proteoglycans. Profiles after digestion with Pronase (——) and treatment with alkaline borohydride (- - -) are shown.

cell types failed to significantly alter the elution profiles (Fig. 2), suggesting that the polysaccharide chains were arranged in a dense cluster along the protein core.

Glycosaminoglycan structure

Glycosaminoglycans obtained by alkali cleavage eluted as single peaks on Sepharose CL-4B and CL-6B. Media-derived and cell-associated glycosaminoglycans from each cell type eluted with similar K_{AV} values (0·69 on CL-4B, 0·5 on CL-6B for T cells and 0·7 on CL-4B, 0·55 on CL-6B for NK cells). Interpolation of Sepharose CL-6B elution positions using the calibration curve published by Wasteson (1971) would suggest a molecular weight for the glycosaminoglycans of approximately 22,000 for T cells and 15,000 for NK cells. The glycosaminoglycans were completely degraded by chondroitinase AC or ABC, indicating that chondroitin sulphate was the only polysaccharide component. The disaccharides produced by chondroitinase ABC migrated solely as monosulphated components on high-voltage electrophoresis. These were identified as 4-O-sulphated derivatives on the basis of their sensitivity to chondro-4-sulphatase.

Effect of culturing cells in the presence of xyloside

NK and T cells were cultured and radiolabelled in the presence of differing concentrations (0,0·25,0·5,1·0 and 2·0 mM) of para-nitrophenyl-β-D-xylopyranoside. In both cell types, incorporation of $^{35}SO_4$ into the medium fraction increased, with the maximal increase being at 0·5 mM xyloside concentrations.

Table 1. Incorporation of ^{35}S-sulphate into glycosaminoglycans and proteoglycans secreted into medium of NK and T cells cultured in different concentrations of para-nitrophenyl-β-D-xyloside

	Concentration xyloside (mM)	^{35}S c.p.m. 10^5 cells
NK cells	0	130
	0·25	280
	0·5	600
	1	680
	2	815
T cells	0	55
	0·25	150
	0·5	440
	1	335
	2	135

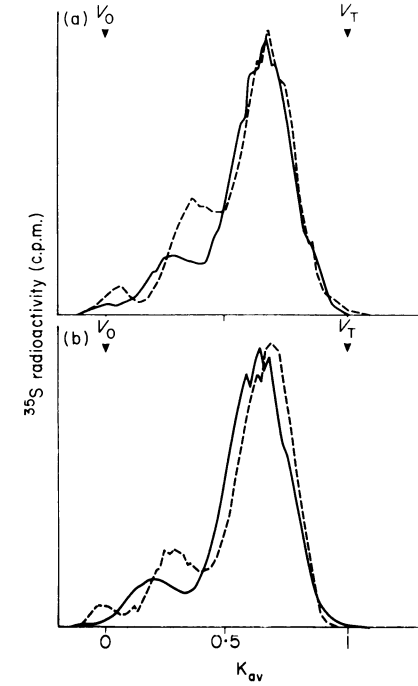

Figure 3. Profiles from CL-6B gel-filtration chromatography of ^{35}S radiolabelled macromolecules derived from (a) cell pellets and (b) media of T cells (——) and NK cells (- - -) cultured in the presence of 1 mM para-nitrophenyl-β-D-xylopyranoside.

Further but smaller increases occurred with higher concentrations in NK cells, but in T cells increments of xyloside concentrations above 0·5 mM were associated with a progressive fall in label incorporation (Table 1).

The nature of the molecules produced in the presence of 1 mM xyloside was examined by gel-filtration chromatography on Sepharose CL-4B and CL-6B. Molecules with two distinct hydrodynamic sizes were seen in the cell-associated and medium compartments of NK and T cells (Fig. 3). The larger molecules were smaller, as assessed by their elution positions on Sepharose

Table 2. Effect of xyloside on growth of NK cells and T cells. Results are expressed as a percentage of cell numbers in the absence of xyloside, mean ± SD of nine experiments using seven different donors

Xyloside (mM)	0·25	0·5	1·0	2·0
NK cells	122·5 ± 51·8	115·8 ± 45·8	94·3 ± 57·6	55·3 ± 35·7
T cells	79·6 ± 17·0	77·4 ± 34·8	68·3 ± 40·5	49·7 ± 31·4

CL-6B (K_{AV} for NK cells 0·3, for T cells 0·25), than the proteoglycans produced by cells cultured in the absence of xylosides. Their proteoglycan nature was demonstrated by their shift of K_{AV} after alkali treatment. The smaller molecules, comprising approximately 90% of the radiolabelled pool in both medium and cell pellets, were not affected by alkali and were thus glycosaminoglycan chains. Glycosaminoglycans produced in the presence of xylosides were smaller (K_{AV} on CL6B, 0·7 for NK cells, 0·66 for T cells), than those produced by untreated cells. The culture of NK and T cells in the presence of xyloside results, therefore, in an increase of radiolabel incorporation and this is accounted for by the biosynthesis of free glycosaminoglycans. The synthesis of proteoglycans in the presence of xylosides is reduced by approximately 50% in T cells and NK cells. The differential alteration of radiolabel incorporation in cell-associated and media fractions was predominantly in the latter compartment (approximately five-fold increase), with only modest increases being seen in the former.

Effect of xyloside on the growth of NK-cell and T-cell populations

Cultures of highly purified NK-cell and T-cell populations were transferred into fresh feeder mixure in the presence of increasing concentrations of xyloside. Equal numbers of cells were plated at each xyloside concentration and after a further 10–14 days growth cell counts were performed. In all cases T-cell growth was inhibited by increasing concentrations of xyloside, as shown in Table 2. In some experiments NK cell growth showed a similar pattern of inhibition but three of the seven donors tested showed stimulation of growth at low xyloside concentrations. Representative experiments using three different donors illustrate the heterogeneity of the response (Fig. 4).

Effect of culture with xyloside on cytolytic activity

Purified T-cell and NK-cell populations grown in IL-2 for 10–14 days in the presence of increasing concentrations of xyloside were tested for cytotoxic activity against the NK-cell target K562. In some experiments the assays were carried out in the presence of the same concentration of xyloside in which the cells had been grown; preliminary experiments had shown that xyloside concentrations of up to 2 mM had no effect on the cytotoxic activity of fresh NK cells (data not shown). Growth in xyloside concentrations of up to 2 mM had no significant effect on the cytolytic activity of cultured NK cells or on the lower levels of activity induced in cultured T-cell populations, as shown in Table 3.

Effect of culture with xyloside on self-killing

Purified T-cell and NK-cell populations were grown in the presence or absence of 2 mM xyloside for 7–10 days. The effector

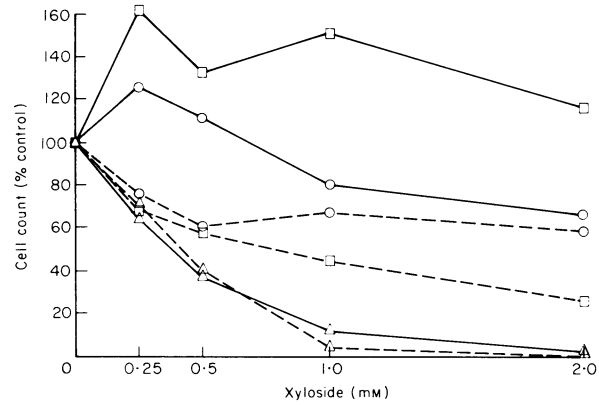

Figure 4. Effects of xyloside on growth of T cells and NK cells. Donor A (□); Donor B (○); Donor C (△); continuous line, NK cells; dashed line, T cells.

Table 3. Effect of xyloside concentration on cytolytic activity against the NK-cell target, K562. Results were expressed as a percentage of the specific cytotoxicity for untreated cells. E:T ratio 2·5:1 for NK cells and 10:1 for T cells. Mean ± SD of nine experiments. Absolute cytotoxic activities were 48·4 ± 22·0% for NK cells and 27·0 ± 12·2% for T cells

Xyloside (mM)	0·25	0·5	1·0	2·0
NK cells	87·4 ± 20·5	89·6 ± 17·0	91·9 ± 20·0	87·3 ± 47·8
T cells	85·1 ± 26·7	92·1 ± 36·8	78·1 + 29·0	89·9 ± 39·0

cells were labelled with ^{51}Cr and tested for susceptibility to autolysis by incubating for 4 hr in the presence or absence of unlabelled K562 target cells in the same concentration of xyloside in which the effector cells had been grown. In all three experiments NK and T cells grown in xyloside did not show higher levels of isotope release than those grown in the absence of xyloside, either in the presence or absence of unlabelled target cells, as shown in Table 4.

DISCUSSION

We have shown that primary cultures of both T and NK cells are able to incorporate ^{35}S-sulphate solely into chondroitin-4-sulphate proteoglycans which are both cell associated and secreted, confirming other similar findings (Levitt & Lee-Ho, 1983; Hart, 1982; Dvorak *et al.*, 1983; Parmley *et al.*, 1985). It has been reported that these cells also synthesize heparan sulphate (Hart, 1982; Levitt & Lee-Ho, 1983; Parmley *et al.*, 1985) and chondroitin-6-sulphate (Hart, 1982) but neither of these species were identified in the human NK and T cells used in our experiments. We have demonstrated that during normal culture conditions free glycosaminoglycan chains are present in addition to proteoglycans in cell-associated material, but that only the latter species is secreted into the medium. Such a finding has not been reported previously, but examination of gel-filtration profiles from long-term NK-cell clones published by MacDermott *et al.* (1985) also suggests the presence of free

Table 4. Effect of xyloside on autolytic activity of cultured NK cells and T cells from three donors. Results are expressed as absolute percentage isotope release from labelled effector cells

	NK cells		T cells	
	Spontaneous	+K562*	Spontaneous	+K562†
Exp. 1				
Untreated	9·2±0·4	8·5±0·3	13·0±1·7	9·5±1·0
2 mM xyloside	8·1±1·4	4·2±2·6	14·8±0·8	13·4±1·3
Exp. 2				
Untreated	17·7±1·4	10·8±0·5	13·1±0·8	12·5±0·2
2mM xyloside	13·2±0·4	12·9±0·2	15·0±0·3	14·9±0·4
Exp. 3				
Untreated	21·2±1·0	17·0±0·3	7·2±0·3	7·3±0·1
2 mM xyloside	17·6±0·4	16·6±1·1	11·1±0·3	12·9±3·3

* E:T ratio = 2·5:1.
† E:T ratio = 10:1.

glycosaminoglycan chains. The resistance to Pronase degradation of both T-cell and NK-cell derived proteoglycans may indicate the presence of clustered serine/glycine-repeat sequences in the core protein, which were predicted for heparin attachment regions by Robinson *et al.* (1978) and confirmed by Bourdon *et al.* (1985) using DNA sequence analysis in rat yolk sac tumour chondroitin sulphate proteoglycan. Pronase resistance appears to be a characteristic of haemopoietic cell proteoglycans and has been demonstrated for NK and mast cell chondroitin sulphate proteoglycans (MacDermott *et al.*, 1985; Stevens *et al.*, 1986) but not for T-cell derived proteoglycan, and suggests a possible role for the NK-derived molecules as carriers of proteolytic enzymes during target-cell lysis.

Radiolabelling of both cell types in the presence of varying concentrations of xyloside showed a concentration-dependent increase of secreted free glycosaminoglycans and 50% reduction in proteoglycan production. This increase was maximal at 0·5 mM with little increment above this level in NK cells (and a fall in T cells). This is similar to findings in the bone marrow system (Spooncer *et al.*, 1983) and may indicate that at this concentration the glycosyl transferase system is saturated or that when xyloside concentrations are above 0·5 mM, a higher proportion of the glycosaminoglycans produced are degraded. Further experiments using agents which prevent lysosomal degradation (e.g. chloroquine) would help to clarify this.

Although chondroitin sulphate proteoglycans are secreted during target-cell lysis by cultured NK cells (Schmidt *et al.*, 1985), our results using cells grown in the presence of xyloside have suggested that optimal production of the intact molecule may not be essential for lytic activity. NK cells cultured in xyloside concentrations of up to 2 mM showed reduced synthesis and secretion of proteoglycan, while still incorporating high levels of ^{35}S sulphate into material which co-migrated with free glycosaminoglycan chains. It is conceivable that in this situation the free glycosaminoglycan, together with the protein core, may be able to substitute for the intact proteoglycan in any function in target-cell lysis. However, the effect of xyloside on the synthesis and secretion of the protein core of the proteoglycan is not known. Alternatively, the intact proteoglycan may play a physiological role in target-cell lysis, but this function may not be essential in the artificial situation of an *in vitro* cytotoxicity assay in which effectors and targets are placed at high density in close apposition.

The results of experiments using ^{51}Cr-labelled effector cells strongly suggest that normal production of the intact proteoglycan molecule is not essential for the protection of effector cells from autolysis, at least in the 4-hr cytotoxicity assays employed here. NK cells grown in 2 mM xyloside were no more susceptible to autolysis following target-cell contact than those grown in the absence of xyloside. Again, it is possible that the glycosaminoglycans secreted in the presence of xyloside may be capable of replacing the intact proteoglycan in any role in effector-cell protection *in vitro*.

The growth of NK cells from some donors was stimulated at low xyloside concentrations, and similar findings have been reported for long-term bone marrow cultures (Spooncer *et al.*, 1983). Evidently, xylosides may exert pleiotropic effects by inhibiting the synthesis of a variety of proteoglycans and this may affect different cell types in different ways. In all our experiments T-cell growth was inhibited at all xyloside concentrations tested. Whether T-cell and NK-cell proteoglycans differ functionally is not clear. However, the primary role of T-cell proteoglycan may not be concerned with cytotoxic function as non-cytolytic $CD4^+$ T-cell clones still produce significant amounts of these macromolecules (Steward *et al.*, 1987). In this context it has been reported that a factor co-purifying with T-cell chondroitin sulphate proteoglycan can stimulate murine B-cell proliferation and differentiation (Levitt & Olmstead, 1985). Whether the proteoglycan functions as a carrier molecule for this B-cell stimulatory activity or whether the factor alone is active is uncertain. It is conceivable that the same or a similar proteoglycan molecule may function as a carrier for different secreted products by different cell types. In the case of NK cells this may be a molecule involved in the lytic process while in the case of helper T cells it may be a component concerned with T–B co-operation for antibody production.

ACKNOWLEDGMENTS

Thanks to Ms D. Burt for technical assistance and Ms E. Mercer for typing the manuscript. This work was assisted by the Cancer Research Campaign.

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