PROTEOGLYCANS FROM SHEEP, PIG, RAT AND HUMAN SPLEENS HAVING CHEMICAL AND BIOLOGICAL RESEMBLANCES TO THAT IN KURLOFF CELLS

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1. Introduction

Kurloff cells appear to be lymphocytes which contain characteristic inclusions [1, 2] that have been shown to consist mainly of a chondroitin sulphate protein [3, 4]. The infrared spectrum of this proteoglycan shows prominent unidentified bands absorbing at 805 cm^{-1} and 1260 cm^{-1} [4, 5] in addition to those bands at 720 cm^{-1}, 850 cm^{-1} and 928^{-1} characteristic of chondroitin 4-sulphate [6], while the material also shows strong ultraviolet absorption with maxima at 257 nm in acid medium and at 265 nm in alkaline medium. Samples of the proteoglycan and of the corresponding glycosaminoglycan derived from it by proteolysis have been shown to be specifically toxic to macrophages *in vitro* at high dilution [7].

The spleens of pregnant or oestrogen treated guineapigs are a rich source of Kurloff cells and have been used as a starting material for the preparation of proteoglycans [3, 4, 8]. Although Kurloff cells, identifiable histologically by their characteristic inclusion bodies, have not been described in species other than the guinea-pig, this report describes the purification and characterization of proteoglycans from sheep, pig, rat and human spleens obtained during pregnancy.

2. Experimental

Spleens were obtained from pregnant sheep, pigs, rats and at autopsy from a young woman who died in the immediate post-partum period. In all cases, spleens were homogenised in a Griffith's tube in 0.9% (w/v) saline (2 g of tissue and 10 ml of saline) and then heated at 55° for 75 min to lyse the cells [7].

The suspension was centrifuged at 1200 *g* and the supernatant set aside. The residue was suspended in saline and heated at 55° for a further 60 min and recentrifuged, after which both supernatant solutions were combined and polyanions precipitated with 9-aminoacridine hydrochloride [9]. Proteoglycans were then purified and freed from nucleic acids as described previously [4].

The ultraviolet absorption spectra of samples dissolved in 0.2 M sodium acetate, pH 6.8, at appropiate concentrations, were plotted manually using a Beckman DB-G spectrophotometer. Infrared spectra were obtained by dissolving 1 mg of proteoglycan in 1 ml of 0.1% KCl (w/v). Samples were then freeze-dried and the resulting powder pressed into a disc. Spectra were recorded using a Unicam SP200 spectrophotometer. Gel chromatography of proteoglycans was carried out using a column (0.9 cm × 73 cm) packed with Sephadex G-200

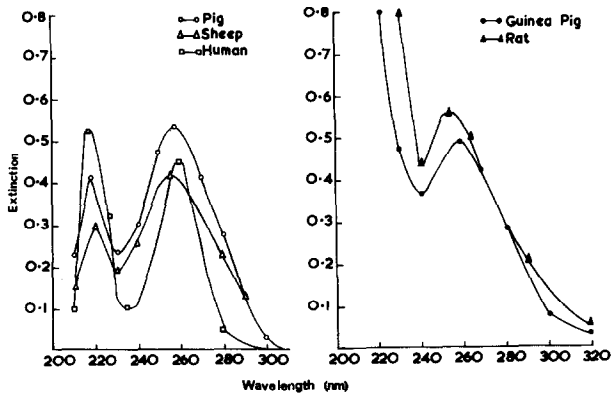

Fig. 1. Ultraviolet absorption spectra in aqueous solution, pH 6.8, of the sodium salts of proteoglycans from guinea-pig, sheep, pig, rat and human spleens. Concentrations (μg/ml) were as follows: guinea-pig 20, pig 8, rat 24, sheep 12, man 7.

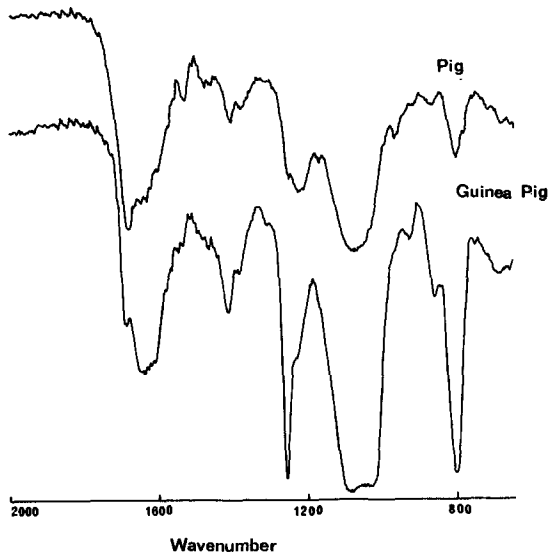

Fig. 2. Infrared absorption spectra of proteoglycans from guinea-pig and pig spleens. 1 mg and 250 μg respectively with 100 mg of KCl were dissolved in water, freeze-dried and then pressed into a disc. A Unicam SP 200 recording spectrophotometer was used.

suspended in 0.2 M sodium acetate pH 6.8 which was also used to elute the column. Fractions of 0.85 ml were collected and their uronic acid contents determined [10].

Purified proteoglycans from each species were tested for their toxicity to macrophages in vitro at a concentration of 1 μg/ml using the cells of peritoneal exudates of guinea-pigs as described [7].

3. Results and discussion

Material containing uronic acid was isolated from the spleens of all species examined and after purification and removal of nucleic acids [4] samples of each were eluted from Sephadex G-200 with the void volume, suggesting that in each case uronic acid was present in the form of proteoglycan, as was the Kurloff cell material previously isolated from guinea-pig spleens [3]. Each sample of proteoglycan including that from Kurloff cells had a characteristic ultraviolet absorption spectrum with a maximum at 257 nm, apart from human material where the maximum and minimum were shifted by 3 nm to longer wavelengths (fig. 1). In addition infrared bands absorbing at 805 cm^{-1} and 1260 cm^{-1} were observed in each case as well as those bands at 720 cm^{-1}, 850 cm^{-1} and 928 cm^{-1} typical of chondroitin 4-sulphate and at 1240 cm^{-1} typical of the sulphate group. The infrared spectra of proteoglycans purified from pig and from guinea-pig spleens are shown in fig. 2.

When the proteoglycan from each species was added to cultures of guinea-pig peritoneal exudate cells *in vitro*, at a concentration of 1 μg/ml it caused swelling of macrophages followed in some cases by complete disruption of the cells. This phenomenon was similar to that first observed with the purified proteoglycan from guinea-pig spleens [7]. The majority of swollen cells were unable to exclude eosin when this was added to cultures as a test of cell viability, except for cells showing only the early signs of damage.

Although cells similar to Kurloff cells have not been found in species other than the guinea-pig, it is of interest that proteoglycans chemically similar to, and with the same spectral characteristics as the proteoglycan from Kurloff cells can be obtained from human, rat, pig and sheep spleens. The significance of the biological activity of the Kurloff proteoglycan and of the proteoglycans isolated from the other species examined is yet uncertain, although

it has been suggested that the proteoglycans of Kurloff cells may interfere with cell mediated-immunity [7], and perhaps prevent rejection of the foetus during pregnancy. The fact that proteoglycans resembling that in Kurloff cells may be extracted from the spleens of pregnant animals of several species as divergent as rat and human, may mean that there is a specialized line of lymphoid cells in other species, which although not histologically distinguishable as is the Kurloff cell, may contain similar proteoglycans having the same biological function.

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References

[1] J.C.G. Ledingham, J. Pathol. Bacteriol. 50 (1940) 201.
[2] A.H.E. Marshall and K.V. Swettenham, J. Anatomy 93 (1959) 348.
[3] M.F. Dean and H. Muir, FEBS Letters 4 (1969) 4.
[4] M.F. Dean and H. Muir, Biochem. J. 118 (1970) 783.
[5] M.F. Dean and H. Muir, unpublished results.
[6] M.B. Mathews, Nature 181 (1958) 421.
[7] A.H.E. Marshall, K.V. Swettenham, B. Vernon-Roberts and P.A. Revell, Intern. Arch. Allergy 40 (1971) 137.
[8] H. Muir and A.H.E. Marshall, Nature 191 (1961) 706.
[9] H. Muir and S. Jacobs, Biochem. J. 103 (1967) 367.
[10] T. Bitter and H. Muir, Anal. Biochem. 4 (1962) 330.