A biotin-labelled proteoglycan fraction which contained both GlcNAc and GalNAc was treated by sequential addition of chondroitinase AC/ABC and heparinase/heparitinase (Seikagaku, Tokyo, Japan) (Kato et al., 1985; Oike et al., 1980). A sharp band at the entrance of the separation gel in the untreated sample (Fig. 1; lane 1) is nearly abolished after chondroitinase treatment (Fig. 1; lane 2). A band in the molecular mass range of 150 kDa is unaffected. This band disappears after additional heparinase/heparitinase treatment and a sharp band in the range of <29 kDa appears (Fig. 1; lane 3, see arrowhead). Thus, in accordance with the amino sugar analysis, the preparation contains both chondroitin sulphate and keratan sulphate.

A fraction rich in GalNAc (Fig. 1; lane 4) was digested by chondroitinase. A smear in the range of 180–30 kDa disappeared leaving two well-defined bands of 89 kDa and <29 kDa. Further treatment by heparinase/keratanase (Seikagaku) did not change the staining pattern (not shown). Thus, this preparation contained chondroitin sulphate only.

Discussion

We describe a rapid procedure for the enzymic analysis of biotin-labelled proteoglycans by electrophoretic separation, transfer to zeta-probe membranes, and reaction with avidin-peroxidase conjugates. This method avoids the necessity of biosynthetic labelling with isotopic compounds. Biotinylation seems not to affect the enzymic degradation of the glycan chains. Therefore, the method seems to be generally applicable for the characterization of proteoglycans from tissues in which biosynthetic labelling may not be feasible. Up to 12 samples can be handled simultaneously. Electrophoresis and electrophoretic transfer are accomplished within 1 h. The sensitivity of the method as tested by the use of standard proteins detects the low nanogram range. The method may be particularly useful for the rapid screening of high numbers of proteoglycan-containing samples.

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Analysis of complement activation products in biological fluids using polyacrylamide gel electrophoresis

MICHAEL DORAN*† and DENIS J. REEN*

*Children’s Research Centre, Our Lady’s Hospital for Sick Children, Crumlin, Dublin 12 and †Department of Biochemistry, University College, Dublin, Republic of Ireland

The complement system consists of a complex series of proteins which upon activation leads to the generation of cleavage products which mediate, amplify and control inflammation. Cleavage fragments may be separated from their native components by polyacrylamide gel electrophoresis (PAGE) and identified by immunoblotting, by exploiting the cross-reactivity of antisera raised against the native protein. Heterogeneity of the native C3 molecule which appears to be unrelated to the genetically derived heterogeneity of C3 has previously been described using immunoblotting techniques. In this study these observations were confirmed and further extended using two-dimensional PAGE and immunoblotting to investigate C3 fragment heterogeneity in human biological fluids (Folkerscn et al., 1986).

Human serum was prepared from healthy blood donors and frozen at −70°C. Activated complement components were prepared by incubation of serum with inulin (15 mg/ml) for 24 h at 37°C.

Synovial fluid samples were collected in EDTA-containing tubes from patients with septic arthritis. Cerebrospinal fluid samples were obtained from patients with proven or suspected meningitis. Polyacrylamide gel electrophoresis was performed according to the method of Davis employing 5% (w/v) linear gels (Davis, 1964). SDS/PAGE was performed essentially according to Laemmli (1970) using 10% (w/v) linear gels. Molecular mass markers were included in each gel.

Two-dimensional PAGE was carried out following incubation of slices of Davis gel in equilibration buffer [20%(v/v) glycerol, 0.04 M-glycine, 0.05 M-Tris, 0.1%(w/v) SDS, pH 8.7] and then subjecting them to SDS/PAGE. Separated proteins were transferred to nitrocellulose according to Towbin

Abbreviations used: PAGE, polyacrylamide gel electrophoresis.
et al. (1979). Fragments of C3 were detected immunochemically using rabbit anti-C3c and anti-C3a polyclonal antiserum, and visualized on the blots using peroxidase-labeled anti-rabbit antiserum and 3-amino-9-ethyl carbazole substrate.

Quantification of both the native C3 molecule and its activation fragments was achieved by reflectance densitometric scanning of blots at 510 nm. Molecular heterogeneity of the native C3 was confirmed by the presence of a 190 kDa doublet on SDS/PAGE gels. The cleavage fragments C3c and C3a appeared as single bands (150 kDa and 9kDa, respectively). When the activated and non-activated samples were separated on two-dimensional PAGE and immunoblotted the heterogeneity of the native C3 190 kDa molecule was more obvious and in addition the C3c molecule separated into two distinct bands at 150 kDa (Fig. 1). This previously unrecognized heterogeneity of the native molecule is conserved in its activation fragments. Synovial and cerebrospinal fluid samples from patients with proven infection also demonstrated complement activation fragments. In several patients, marked heterogeneity of C3 activation fragments was observed. This heterogeneity, however, probably reflects further cleavage in vivo of either the native molecule or its principal activation fragments in the presence of infection.

Immunoblotting of two-dimensional PAGE gels using anti C3 antisera has demonstrated a previously unrecognized heterogeneity of the C3c molecule in human serum activated in vitro as well as the presence of multi-molecular forms of its degradation fragments in biological fluids from infected individuals.

Whether these fragments have a unique biological function is an interesting possibility.

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**Anti-Borrelia antibody in human Lyme disease**

D. G. WILLIAMS,* M. F. MUHLEMANN,† and D. J. M. WRIGHT‡

*Mathilda and Terence Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W6 7DW and †Departments of Dermatology and ‡Medical Microbiology, Charing Cross Hospital, London W6 8RF, U.K.

Lyme disease is an inflammatory disorder affecting skin, joints, nervous system and heart. The disease is a zoonosis caused by the spirochaete *Borrelia burgdorferi* which is transmitted to man by the bite of the hard tick *Ixodes ricinus* (Burgdorfer et al., 1982). The diagnosis may be apparent if there is a clear history of a tick bite followed by erythema chronicum migrans, the cutaneous hallmark of Lyme disease. In other cases, the diagnosis may often depend on serological confirmation.

The standard methods for antibody detection are indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA) (Magnarelli et al., 1984). These methods may not detect antibody in the early stages of the disease which may never reach levels of diagnostic significance when there is only skin involvement. Furthermore, cross-reactive anti-