cutaneous vascular tachyphylaxis (Greaves and Shuster, 1967) as well as attempts to recover prostaglandins in a wider range of inflammatory reactions.

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Clinical Significance of 7s IgM in Monoclonal IgM Diseases

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Summary

The sera from 117 patients with diseases associated with a high production of monoclonal IgM were analysed for the presence of low molecular weight (7s) IgM by using a simple thin-layer Sephadex technique. 7s IgM was found in the sera of patients with myelomata (66%), lymphomata (45%), and Waldenström's macroglobulinaemia (20%), but was absent from the sera of patients with benign monoclonal macroglobulinaemia.

This technique provides a cheap and practical test which may be valuable in selecting patients with lymphomata from those with benign lesions.

Introduction

A high production of monoclonal 19s IgM is a major feature of some lymphomata, Waldenström's macroglobulinaemia, IgM myelomatosis, and primary cold agglutinin disease and may also be associated with chronic lymphatic leukaemia, rheumatoid arthritis, and malignant diseases. The 19s IgM molecule is a polymer of five 7s IgM units, each 7s unit comprising two μ -heavy chains and two light chains. It has recently been shown that the production of low molecular weight (7s) IgM may also be seen in some of these diseases.

Solomon and Kunkel (1967) found 7s IgM in addition to 19s IgM in the serum of a patient with a neoplastic lesion of the plasma-cell/lymphatic system, and Bush, Swedlund, and Gleich (1969) and Hansson and Laurell (1969) also showed 7s IgM in the sera of patients with macroglobulin diseases. Separation of the 7s subunits from the 19s IgM was achieved by Hansson and Laurell using crossed-immunoelectrophoresis and by the other two groups using sucrose density-gradient ultracentrifugation.

Though the presence of 7s IgM is a normal finding in the sera of some lower vertebrates (Marchalonis and Edelman, 1965; Clem and Small, 1967) and even in human neonates (Perchalski, Clem, and Small 1968), the significance of its presence in the serum of human adults is unknown.

In this paper the clinical importance of finding 7s subunits in adult sera in the presence of macroglobulin disease is investigated, and a simpler method for their detection than has hitherto been described is presented.

Patients and Methods

Sera were analysed in a blind trial of 117 patients with diseases associated with excess monoclonal IgM production. They were patients in whom a definitive diagnosis had been possible through the kindness of our clinical colleagues, many in the Medical Research Council's Working Party on Leukaemia in Adults (see Hobbs, 1969), and many pathologists who had kindly sent material to Professor C. V. Harrison. The diagnostic criteria used were lymphomata, invasive destruction of lymph node architecture; Waldenström's macroglobulinaemia, infiltration of preserved architecture; myelomatosis, typical bone lesions; and cold haemagglutinin disease, typical serology (thanks to Drs. S. Worledge and A. Cooper). Nine patients diagnosed as benign monoclonal macroglobulinaemia had all been followed up for at least six years during which time no increase in the IgM, nor any form of neoplasia, had been detected. The 117 patients were classified as in the Table.

Incidence of 7s IgM and Bence Jones protein (B.J.)

Underlying Disease	No. of Patients					% of Patients with IgM Subunits in Serum
	Total	B.J. in Urine	Type of IgM			
			19s	19+7s	7s	
Myeloma	3	3	1	2	—	66
Lymphoma	33	33	18	11	4	45
Waldenström's macroglobulinaemia	25	24	20	5	—	20
Primary cold agglutinin Chronic lymphatic leukaemia	45	4	41	4	—	9
Rheumatoid arthritis	1	0	1	—	—	0
Benign	1	0	1	—	—	0
	9	0	9	—	—	0

Spots of serum (0.25 μ l) were run on horizontal microscope slides spread with 0.75 mm (0.03 in) of superfine Sephadex G200 along which 0.9% saline flowed from a reservoir via 3 MM chromatography paper wicks. The completion of the run was shown by the position reached by 2% blue dextran

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(molecular weight 2000,000; > 19s) applied as a marker. The marker also contained 2% haemoglobin (molecular weight 65,000; 4s). The positions of the marker spots were indicated on the underside of the slide for subsequent comparison with precipitin lines. The presence or absence of 7s IgM subunits was shown by immunodiffusion and precipitation against sheep antihuman μ which was placed in troughs cut in the Sephadex (see Fig. 1). Precipitin lines opposite blue dextran do not distinguish between 19s IgM and complexes of larger size (23s, 29s, etc.). For simplicity, where 19s IgM is used in this article it implies 19s or greater. The above methodology with the use of microscope slides (as yet unpublished) was largely developed by J. Kershaw.

It must be stressed that sample overloading results in trailing through the Sephadex, possibly leaving 19s IgM to subsequently form precipitin lines in the low-molecular-weight

FIG. 1—Thin-layer chromatography in superfine Sephadex G200 on microscope slides, followed by diffusion against antiscum to identify μ antigen in molecules of different sizes.

area. To avoid this IgM concentrations in the samples applied were adjusted to not exceed 2 g/100 ml. All samples which apparently showed precipitin lines in the 7s area were run again at 1 g/100 ml. Sera were only recorded as containing 7s IgM when this was found also in the second run. The limit of detection is of the order of 10-20% of the total IgM as 7s IgM.

Sera containing cryomacroglobulins were also found to trail at room temperature. This was overcome by performing runs with such sera in an incubator at 37°C.

The urines from these patients were also analysed for Bence Jones protein by electrophoresis and immunoelectrophoresis, concentrating samples up to 300-fold where necessary (Hobbs, 1967).

Results

7s IgM subunits were found in the sera of 22 patients out of the 61 who had myelomata, lymphomata, and Waldenström's macroglobulinaemia (60 of whom also had Bence Jones proteinuria) and in only four patients with primary cold agglutinin disease who had proved associated lymphomata. Four patients with lymphomata had 7s IgM alone with no detectable 19s IgM. In no other patients were 7s subunits or Bence Jones proteinuria detected.

Discussion

The method presented for the detection of 7s IgM subunits in the serum is a cheap and practical one, results being obtainable within 24 hours without the use of special equipment or trained personnel. It is also shown by blind trials to be efficacious: selection of all four patients with associated lymphomata from 45 cases of primary cold agglutinin disease was achieved by the detection of 7s IgM in those four sera alone. Examination of sera from nine patients with benign monoclonal macroglobulinaemia showed the presence of 19s IgM only.

The method also detects other low-molecular-weight IgM units such as μ -chains. None were found in the present series, though they have been described in lymphomata (Franklin, 1970).

The sera used in this study had been stored in a deep freeze, but 7s IgM subunits found were not artefacts produced in vitro by temperature-induced damage to the 19s IgM since freezing and thawing causes no ill effects on these immunoglobulin pentamers (Bush *et al.*, 1969).

There are two points of special interest in the results. Firstly, apart from one patient, all cases with myelomata, lymphomata and Waldenström's macroglobulinaemia were found to have Bence Jones proteinuria, suggesting impaired immunoglobulin synthesis. Secondly, the incidence of 7s subunits in the serum in addition to 19s IgM rises with increase in the degree of cellular dedifferentiation and invasion in the underlying lesions: benign lesions 0%, Waldenström's macroglobulinaemia 20%, lymphomata 45%, and myelomata 66%. Of the patients with lymphomata 12% had 7s IgM subunits alone.

This correlation suggests that the property of combining 7s subunits to form 19s IgM is normally found in the mature adult cell and that this property may be impaired in cells which are either immature, 7s IgM being a normal finding in some lower vertebrates and human neonates, or which have become dedifferentiated. These findings add further support to the hypothesis (Hobbs, 1967) that biochemical dedifferentiation parallels neoplastic dedifferentiation.

The presence of 7s subunits in the sera of patients with lesions producing monoclonal IgM may therefore carry a prognostic significance which follow-up studies on patients in whom these subunits have been found will help to clarify.

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Requests for reprints should be sent to Professor J. R. Hobbs, Westminster Medical School, London S.W.1

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