A SERUM DNA-BINDING PROTEIN ABSENT IN MALIGNANT DISEASE

J. G. LEWIS and C. M. ANDRÉ
Clinical Biochemistry Department, Christchurch Hospital, Christchurch, New Zealand

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1. Introduction

DNA-binding proteins are known to be present in human serum and some have been described [1,2]. Their biological significance is unknown and the relationship between serum and intra-nuclear DNA-binding proteins is obscure although the latter may be related to the control of gene expression. The appearance of carcinofetal antigens such as CEA [3], alpha-fetoprotein [4] and the DNA-binding protein C3DP [2] in malignant disease may indeed be the result of derepression of the original foetal genome. A unique 36 000 dalton DNA-binding protein in foetal cord and malignant disease serum has been reported [5]. This prompted us to investigate the profile of DNA-binding proteins in normal subjects, foetal cord sera as well as sera from malignant and non-malignant diseases.

This report describes the presence of a 45 000 dalton DNA-binding protein which is present in normal adult serum but absent from foetal cord and the majority of malignant disease sera examined.

2. Materials and methods

Serum was obtained from patients having a variety of known malignant and non-malignant diseases. Normal and foetal cord serum was obtained from clinically well subjects.

DNA-cellulose was prepared as in [6]. Mono-specific antiserum to human serum proteins were obtained from Behringwerke A. G. and Dakopatts. Protein determinations were performed by the Lowry method [7]. Dodecyl sulphate disc-gel electrophoresis was carried out in 7% polyacrylamide gels [8]. Molecular weight standards were IgG (155 000), bovine serum albumin (68 000), aldolase (40 000), pepsin (35 000), carbonic anhydrase (29 000), trypsin (22 500) and haemoglobin (16 000).

Briefly, serum (0.5 ml) was chromatographed on mini-columns (0.5 × 6 cm) with 10 mM phosphate buffer, pH 6.6. After partial immunoglobulin removal the bound material was eluted using the same buffer containing 0.5 M NaCl. This eluate was extensively dialysed against 10 mM phosphate buffer, pH 6.6, containing 1 mM EDTA prior to DNA-cellulose chromatography, (1 g protein/100 mg DNA). All unbound material was removed and the DNA-bound fraction totally eluted with 10 mM phosphate buffer containing 0.4 M NaCl. Following dialysis against distilled water and protein determination, 60 μg lyophilised material was subjected to SDS–polyacrylamide gel electrophoresis after reduction with mercaptoethanol.

3. Results

The serum DNA-binding protein profiles from foetal cord and normal adult are shown in fig.1. The most interesting difference was the presence of a 45 000 dalton DNA-binding protein in normal adult serum but apparently absent in the foetal cord fractions. A similar molecular weight DNA-binding protein is seemingly absent in many cancer patients' serum but present in the majority of non-malignant disease sera examined, fig.2 and fig.3 respectively. The complete results are shown in table 1. Overall, 60% of the 44 cases of malignant disease sera studied displayed the foetal cord characteristic with the absence of a 45 000 dalton DNA-binding protein. On the other

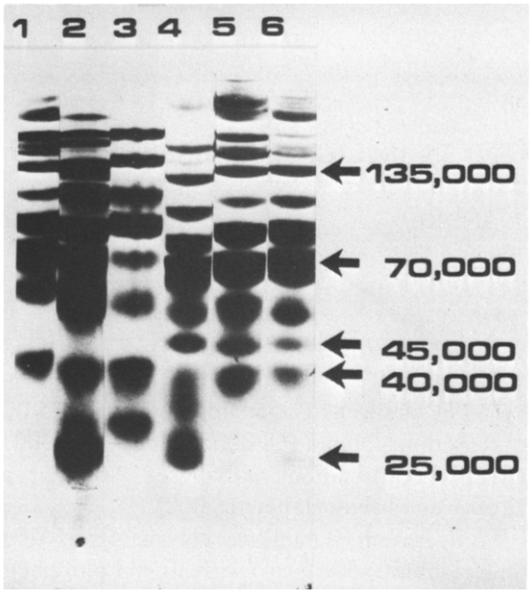

Fig.1. SDS–polyacrylamide gels of DNA-binding proteins from foetal cord (1–3) and normal adult serum (4–6). The molecular weights of various proteins are shown.

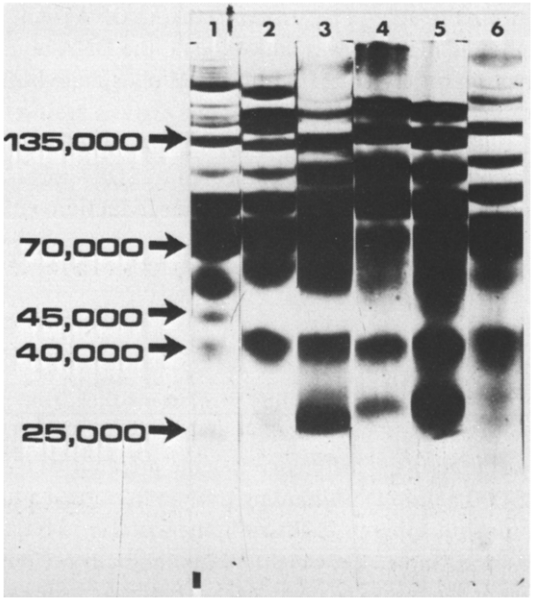

Fig.2. SDS–polyacrylamide gels of serum DNA-binding proteins from normal adult (1), foetal cord (2) and malignant disease. Serum from carcinomas of prostate, pancreas, colon and lung denoted by 3, 4, 5 and 6, respectively. The colonic carcinoma patient (5) shows an indistinct 45 000 dalton band.

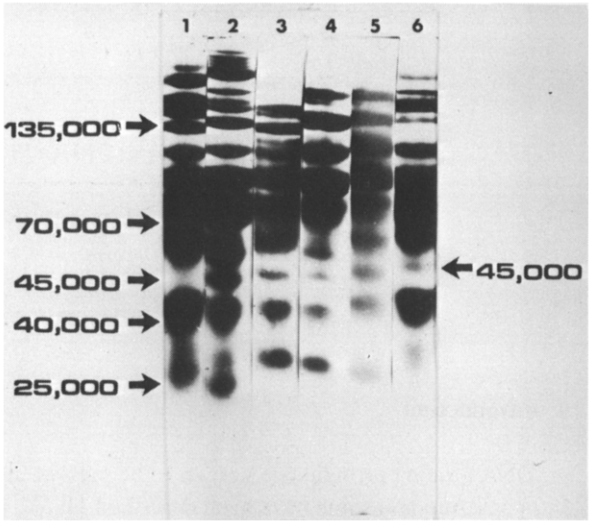

Fig.3. SDS–polyacrylamide gels of serum DNA-binding proteins from foetal cord (1), normal adult (2) and non-malignant disease. Samples 3, 4, 5 and 6 denote serum from psoriasis, dermatitis, bronchitis and rheumatoid arthritis patients, respectively.

hand only 20% of the 30 cases of non-malignant disease sera displayed the absence of this protein. Immunodiffusion against both pooled normal and pooled foetal cord serum DNA-binding protein preparations (1 mg/ml) revealed immunoreactivity towards albumin, α-1-antichymotrypsin, α-2-macroglobulin, C3 complement and IgG. Additional immunoreactivity towards immunoglobulins A and M were present in the normal adult DNA-binding fraction.

4. Discussion

This paper reports for the first time the observation of a serum DNA-binding protein of mol. wt 45 000 present in normal adults but apparently absent in both foetal cord and the majority of malignant disease sera examined. Parsons et al. [9] have described a lower molecular weight malignancy associated DNA-binding protein having elevated levels in malignant disease sera. Their 40 000 dalton protein, derived from C3 complement, also appears on our gels in seemingly elevated amounts in malignant disease sera (fig.2).

Preliminary attempts to identify the 45 000 dalton

Table 1
Absence of a 45 000 dalton DNA-binding protein in serum

Group	No. patients studied	No. patients displaying absence of 45 000 dalton protein
Normal adult	29	–
Foetal cord	20	20
Malignant disease:		
Carcinoma, primary site		
Breast	4	1
Colon	9	4
Lung	3	3
Prostate	4	2
Others	13	9
Sarcoma	3	2
Lymphoma	5	3
Multiple Myeloma	3	2
Total	44	26
Non-malignant disease:		
Cirrhosis	4	3
Psoriasis	7	1
Systemic Lupus Erythematosus	4	–
Asthma	3	1
Emphysema	1	1
Others	11	–
Total	30	6

serum DNA-binding suggest a glycoprotein of α-2 electrophoretic mobility antigenically similar to α-2-HS-glycoprotein.

It is known that α-2-HS-glycoprotein possesses opsonic properties and the ability to alter tumour growth [10]. The relationship of this protein to the immunological status of the patient requires further investigation. It is tempting to speculate that its ability to bind DNA may facilitate the clearance of free DNA from the serum which is elevated in malignant disease [11].

Acknowledgements

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