Folic acid binding protein in acute myeloid leukaemia

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SYNOPSIS Folic acid binding protein was estimated in the serum of 94 control subjects and a normal range was established. Raised levels were found in folate deficiency and chronic myeloid leukaemia. Considerably raised levels were found in untreated acute myeloid leukaemia, most often in cases with a marked monocytic element.

A specific folic acid binding protein (FABP) exists in normal serum (Waxman and Schreiber, 1972), although only a tiny fraction of the serum folate is actually bound. This protein is found mainly in the transferrin peak on column chromatography (Markkanen and Peltola, 1971) and in the transferrin band region in polyacrylamide disc gel electrophoresis (Waxman and Schreiber, 1973); there is a peak at molecular weight 50 000 and a further one at 200 000 (Waxman and Schreiber, 1973). That it is not transferrin per se was shown by Jacob and Herbert (1974). It is present in increased amounts in folate deficiency (Waxman and Schreiber, 1973) and in both serum and cell lysates from some cases of chronic myeloid leukaemia (CML) (Rothenberg and daCosta, 1971). The physiological role of this protein is unknown.

Although 'serum folate' represents a heterogenous assortment of pteroyl compounds, the folic acid binding protein (FABP) binds pteroylmonoglutamates (PGA) more readily than other components (Waxman, 1975). Using this fact, FABP has been estimated using tritiated PGA to saturate the binders.

Methods

The method for estimation of FABP was essentially that of Waxman and Schreiber (1973), except that albumin-coated charcoal was used in place of haemoglobin-coated charcoal to avoid the necessity for decolourizing.

PROCEDURE

Aliquots of 0.4 ml of serum were incubated with 500 pg tritiated PGA (3H PGA) in buffer at 37°C for 1 hour. The incubation mixture was added to semidried albumin-coated charcoal, incubated for 10 minutes at room temperature, and centrifuged and the supernatant fluid was removed. This was treated a second time with albumin-coated charcoal to remove as much of the excess 3H PGA as possible. A control for each serum was carried out in an identical manner except that the sample was incubated with 500 ng of cold PGA initially in order to block the binding protein. Any radioactivity remaining after treatment with albumin-coated charcoal is, therefore, not bound to FABP and can be subtracted from the serum result. 0.5 ml of each supernatant fluid was mixed with 4 ml of Instagel and counted on a Phillips liquid scintillation counter, together with appropriate standard and blanks. The final results were expressed either as a percentage of the initial amount of 3H PGA bound by FABP or more conveniently in absolute terms as pg PGA bound per ml of serum.

The bone marrow aspirates from the patients with acute myeloid leukaemia (AML) were stained routinely with May Grünwald Giemsa and examined by several experienced observers: the morphological diagnoses in each case represent the consensus. In an attempt to quantify the monocyte component, the aspirates were also stained by the combined esterase method of Yam et al (1971) and scored for the percentage of reacting cells positive for alpha-naphthyl acetate esterase (NAE). Sodium fluoride rendered the score zero in each case. That this stain is highly selective for cells of the monocyte series is shown by the comparative electron microscope studies of Glick and Horn (1974).

Serum lysozyme, unbound B12 binding capacity (UBBC), transcobalamins, serum B12, folate, and...
ferritin levels were estimated at the same time (see table). Serum (and sometimes RBC) folate was estimated by standard microbiological assay using *Lactobacillus casei*.

**Results**

FABP was estimated in the serum of 94 control subjects. A range of 20–150 pg/ml was found to encompass 85% of normal sera, and the mean value was 76 pg/ml. The distribution was skew and was not rendered normal by a logarithmic presentation; for this reason standard deviations are not given. Five apparently normal female subjects had levels in excess of 300 pg/ml. No haematological abnormality could be identified in these people and no ready explanation was advanced: they were not taking oral contraceptive agents.

Eighteen patients found to be folate deficient (mean serum folate 1·2 μg/l, mean RBC folate 72) were studied and found to have levels of FABP higher than the control subjects. Their mean value was 139 pg/ml.

Eighteen consecutive new cases of AML were studied before treatment and the levels of FABP in the serum were found to be high; the mean value was 338 pg/ml. It was noticeable that some patients with particularly large monocytic components showed exceptionally high levels of FABP.

Eight patients with CML, six of whom were in the terminal blastic phase, were studied. While the mean level of FABP was twice that of the control group (being 179 pg/ml), no patient had a level over 260 pg/ml. The two patients with untreated CML and white cell counts of between 6 and $8 \times 10^{11}$ /litre had levels well within the normal range as did a patient with polycythaemia rubra vera and a high white cell count.

Levels of serum ferritin in the AML patients were consistently high, in several cases exceeding 30 times the upper limit of normal (see table).

**Discussion**

The finding of high levels of a folate binding protein (normally present in very small amounts) in the serum of patients with untreated AML is unexpected. Folate deficiency may occur in leukaemia (Rose, 1966), but this does not seem to have been the case in most of the patients studied. Not only did the levels of FABP exceed those in severe folate deficiency by a factor of 3, but the only cases in which slightly low serum folates were found were those in which the folate assay had been performed (of necessity) on serum stored without ascorbic acid. These are marked * in the table and may, of course, be falsely low.

Leukaemic cells are known to produce a variety of substances such as lysozyme (Catovsky et al, 1971), transcobalamin (Catovsky et al, 1972), and ferritin (Parry et al, 1975). In untreated AML specifically, the $B_{12}$ binding capacity is not consistently raised and in the present study there was no correlation between this and the FABP. The serum lysozyme is thought to be derived in part from the monocyte, and, in general, those of our cases with high lysozyme levels tended to have high $NAE$ scores and high levels of FABP. There was a positive correlation ($r = 0·63$) between FABP and lysozyme levels. The finding of extremely high levels of serum ferritin is in accord with that of other workers (Parry et al, 1975).

Mature granulocytes do not appear to be the major source of this binding protein for even the untreated cases of CML with extremely high white cell counts did not produce levels of FABP as high as in many cases of AML; the levels found were similar to those found in folate deficiency.

The binder in the serum of the AML patients may not be the 'normal' FABP, and no experiments were performed to characterize it. However, it is likely that it will bind folate analogues (such as
Methotrexate (MTX)) just as does 'normal' FABP. Although MTX is not used routinely in this country in early AML, this may have implications for the treatment of relapse.

After cytotoxic therapy is begun, levels of FABP fall rapidly. A study is in progress of the levels of FABP throughout treatment, remission, and relapse.

Reagents

Semidried albumin-coated charcoal is prepared by centrifuging and removing supernatant from 1 ml aliquots of an aqueous solution containing 5% activated charcoal (BDH lab. reagents) and 1% bovine albumin (Armour Pharmaceuticals Co Ltd); \( ^{3} \)H PGA stock solution containing approximately 20 Ci/mmol obtained from Radiochemical Centre, Amersham; Stock diluted to 5 ng/ml in phosphate buffer, pH 7.4; Instagel scintillator solution obtained from Packard Instrument Company.

References


