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Letter to the Editor

Size-exclusion high-performance liquid chromatography of immune complexes isolated from patients with acute leukaemia

Preliminary observations

Sir,

Size-exclusion high-performance liquid chromatography (SE-HPLC) is an important tool for the rapid and effective separation of protein mixtures. There have been many SE-HPLC studies on immunoglobulins (Ig), their subunits and their complexes with other molecules either obtained in vitro or isolated from patients' sera [1–4]. We have made an attempt to analyse immune complexes from leukaemia patients. The relationship between the concentration of immune complexes and disease evolution is well known [5,6]. We have analysed by SE-HPLC the IgG1–2–4 immune complexes obtained from two patients with acute myeloid leukaemia (AML), isolated by polyethylene glycol (PEG) precipitation and purified by protein A–Sepharose affinity chromatography.

EXPERIMENTAL

Patients

We studied two patients with acute myeloid leukaemia, a 43-year-old man (FAB-M5) with 41% blast cells (patient A) and a 58-year-old woman (FAB-M2) with 72% blast cells (patient B). The patients were at the onset of their disease and had not yet received therapy. In order to obtain a control of the elution profile, a third patient, a 42-year-old man with active chronic hepatitis type B, was studied.

Isolation of immune complexes

Immune complexes from 1 to 4 ml of serum were isolated by PEG precipitation according to Chia et al. [7]. The final precipitate was suspended in 0.1 *M* phosphate buffer (pH 7.4) and frozen at −70°C until taken for analysis.

Purification of immune complexes

Protein A–Sepharose affinity chromatography of precipitated immune complexes was performed according to the method of Chenais et al. [8]. The material

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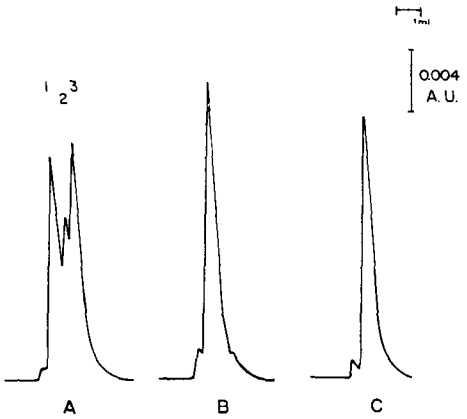

Fig. 1. SE-HPLC of protein A–Sepharose bound peaks obtained from a hepatitis patient (A) and two AML patients (B and C). Chromatographic conditions as described under Experimental.

eluted with 1 *M* acetic acid was dialysed overnight at 4°C in 0.1 *M* phosphate buffer (pH 7.4) and subsequently concentrated to 100 μl (Minicon B15; Amicon, Danvers, MA, U.S.A.).

Size-exclusion high-performance liquid chromatography

The HPLC system consisted of a Clar pump (Violet, Rome, Italy), a Synchropac GPC 300 column (250×4.6 mm I.D.) and a 30×4.6 mm I.D. guard column (Synchrom, Linden, IN, U.S.A.). Isocratic elutions were performed with 0.1 *M* phosphate buffer (pH 5.8) at a flow-rate of 0.5 ml/min; 1–20 μl samples were injected; the absorbance was monitored at 280 nm with an LC 75 spectrophotometric detector (Perkin-Elmer, Norwalk, CT, U.S.A.). A calibration graph was prepared with HMW and LMW gel filtration calibration kits from Pharmacia (Uppsala, Sweden) and human IgG (Sigma, St. Louis, MO, U.S.A.).

Quantification of antigens and antibodies

IgG class immunoglobulins in the chromatographic fractions from the hepatitis patient were measured by radial immunodiffusion (LC-Partigen plates; Behring Institute, Marburg, F.R.G.). An ELISA kit from Abbott (Abbott Tark, North Chicago, IL, U.S.A.) was used for the determination of hepatitis markers.

RESULTS AND DISCUSSION

With the hepatitis patient, the SE-HPLC elution profile revealed three major peaks representative of three different molecular masses: 160 000 (peak 1, Fig. 1A), 20 000 (peak 2, Fig. 1A) and 8000 (peak 3, Fig. 1A). Peak 1 is the IgG component of the mixture (concentration in the injected sample 0.15 mg/ml), while peaks 2 and 3, devoid of immunoglobulins, might be attributable to hepatitis antigenic components. However, no hepatitis antibodies (peak 1) or antigens (peaks 2 and 3) were detected.

The two AML patients' elution profiles were significantly different from that

of the hepatitis patient: the elution profile of patient A (Fig. 1B) showed one major peak of molecular mass 160 000, preceded and followed by two minor peaks of 600 000 and 8000, respectively. The elution profile of patient B (Fig. 1C) also showed a major peak of 160 000 preceded by a minor peak of 600 000. Our preliminary results show a striking similarity between the elution profiles of the two AML patients, and also confirm some previous observations concerning the composition of the immune complexes in AML, which demonstrated the prevailing presence of immunoglobulins [9].

In conclusion, our preliminary results show that the composition of immune complexes in leukaemia patients might differ, probably in some substantial way, from that observed in some other diseases (e.g., hepatitis). However, additional data are needed from such pathological groups in order to confirm our preliminary observations.

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