Biochemical studies on a human leukaemia-associated antigen

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The establishment of several continuously proliferating cell lines has greatly facilitated research into cellular differentiation and has increased our understanding of leukocyte proliferative disorders, in particular leukaemia. One such cell line is HL-60 [1]. This human promyelocytic cell line has been used not only in studies of myeloid differentiation, but also as an immunogen for the production of monoclonal antibodies, which have had many applications in leukaemia research and have done much to increase our knowledge of the leukaemic condition.

In a previous publication, we described the production and classification of a monoclonal antibody, NC-2, to an unusual surface protein expressed on the HL-60 cell line, but present on no other cell line examined [2]. This antibody was subsequently shown to react with leukocytes from 5% of normal individuals, with a much higher percentage (35%) of leukaemia patients reacting. On the basis of these data there appeared to be a link between the expression of this particular antigen and the incidence of leukaemia [3]. Links between the expression of specific cell surface antigens and a variety of diseases have already been established. In the present study, we have focused on the biochemical characterization and cellular distribution of the alloantigen reacting with NC-2 and have shown it to be a proteinase-sensitive glycoprotein which is present, not only in the plasma membrane, but also in the cytoplasm of reacting cells.

SDS/PAGE analysis of 125I-labelled HL-60 cell surface proteins, which had been immunoprecipitated with NC-2, showed two major bands, at 50 kDa and 15 kDa in a 12.5% gel (Fig. 1a; lane A). In addition, there was some material of much higher molecular mass which failed to enter this gel or gels made with a lower percentage (7%, w/v) polyacrylamide. The 15 kDa protein is unlikely to be a catabolic fragment of the 50 kDa protein, since a large range of pro-

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Fig. 1. Immunoprecipitation and digestion of NC-2 reactive material
(a) NC-2 antigen immunoprecipitated from 125I-labelled HL-60 cells and separated on a 12.5% (w/v) SDS/polyacrylamide gel (lane A). Precipitated antigen was digested for 4 h at 37°C with 1 mg/ml trypsin before electrophoretic separation (lane B). (b) Labelled immunoprecipitated NC-2 antigen was digested with 2.0 m-units of glycopeptidase F for 18 h at 37°C (lane B). Lane A shows an undigested control sample.

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Absorption and analysis of clofazimine and its derivatives

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Introduction

Clofazimine (B663), a red iminophenazine dye [1], is very effective in the treatment of leprosy. Its mode of action is thought to be due to its inhibition of the template function of DNA. Resistance to the anti-leprosy agents rifampicin and dapsone has been widely reported and in 1982 a case of clofazimine-resistant leprosy was described [2]. Hence, a number of analogues of clofazimine were synthesized in order to develop agents that might overcome such resistance [3-5]. Studies on clofazimine and analogues were performed to determine which compounds had the required absorption characteristics in mice. The use of h.p.l.c. for the analysis of clofazimine and analogues was also investigated.

Materials and methods

Clofazimine and derivatives were synthesized as previously described [5]. The drugs were made up and thoroughly homogenized in 0.5% (w/v) carboxymethyl-cellulose and 0.5% (v/v) Tween 20. Animals (8-10-week-old Schofield mice) received a daily dosage of 20 mg day⁻¹ kg⁻¹ by gavage (0.4 ml) over a period of 3 weeks using a modified 5 cm human spinal needle. After 21 days the mice were killed. The lungs, liver, spleen and pelvic fat were removed and weighed. They were then placed in pre-weighed 5 ml Bijou bottles containing 0.5 ml of 25% (v/v) acetic acid. Organs were pooled, if appropriate. Serum samples were also taken. All samples were stored at -20°C. For drug extractions tissues were finely chopped, homogenized in 25% (v/v) acetic acid in a Elvehjem homogenizer and then carefully extracted (×4) with chloroform or other appropriate solvent, followed by 50% (v/v) phosphoric acid or hydrochloric acid [6]. Groups of three to six mice were used for each drug studied. The amount of drug extracted was then determined spectro-