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THE INHIBITORY EFFECT OF
LEUKAEMIA ASSOCIATED ANTIGEN AND
LEUKAEMIC SERUM ON THE
LEUKOCYTE MIGRATION OF CHILDREN WITH
ACUTE LEUKAEMIA IN REMISSION

By

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The immunopathology of leukaemia is one of the widely investigated fields of oncology. It seems to be well established that "new" membrane antigens appear on the surface of leukaemic cells (19). These antigens can be solubilized, extracted and antiserum can be raised against them in xenogenous systems (18). The existence of these leukaemia-associated antigens (LAA) has been demonstrated by various techniques, partly through the detection of antibodies directed against these new antigens. The techniques include immunofluorescent methods, immunoprecipitation, gel-diffusion, cytotoxicity reactions, complement fixation, passive cutaneous anaphylaxis reaction, etc. (3, 4, 5). Another approach to the detection of LAA-s is the use of cell-mediated immune reactions, such as mixed lymphocyte cultures (12, 17), *in vitro* cytotoxicity and delayed type skin reaction to leukaemic cell membrane extracts (10).

Leukocyte migration inhibition as described by Søborg & Bendixen (16) can be used for the testing of delayed type

hypersensitivity to various substances. Using this method we investigated the following problems:

1. Is it possible to produce a leukaemic cell membrane preparation that acts as LAA?
2. What is the effect, if any, of this extract on the leukocyte migration of children with acute lymphoblastic leukaemia (ALL) in remission?
3. Will the use of leukaemic serum influence the migration of leukocytes, and alter the effect of the leukaemic cell extracts?

MATERIALS AND METHODS

Patients

Leukocyte migration tests were carried out using the cells of 20 children who were in full remission of ALL. The patients were either maintained on 6-mercaptopurine (75 mg/m²/day) and Methotrexate (15 mg/m²/week), or were undergoing prophylactic irradiation of the skull together with half-dose 6-mercaptopurine maintenance. Cells from 17 children hospitalized for minor surgical intervention were used as controls.

Leukaemic cell preparations were made from blasts obtained from two children suffering from ALL. Blood for both the cell preparation and serum was drawn prior to all therapy or transfusions.

Methods

A. Leukaemia blast preparations: 30–40 ml blood was drawn into a heparinized tube before any treatment was started. After allowing the blood to sediment for an hour at 37°C, the plasma layer rich in leukaemic blasts was aspirated and mixed with an equal volume of TC-199 medium (Difco). The blasts were separated by Ficoll-Uromiro gradient centrifugation. The cell preparation obtained in this way was washed three times in TC-199, and sonicated using an LKB sonicator. The resulting suspension was centrifuged at 100,000 × g for 30 minutes, at +4°C. The aspirated supernatant was passed through a Millipore filter (pore size: 0.22 μ) and stored at –20°C. Protein concentration of this preparation was adjusted to 1 mg/ml before use with TC-199 medium. Serum was obtained at the same time as the blasts and was also stored at –20°C.

To exclude a cytotoxic effect, allogeneic lymphocytes were cultured in a serial dilution of both the blast preparations and sera. Trypan-blue exclusion revealed 0–6 per cent damaged cells after 24 hours.

B. Leukocyte migration inhibition studies were carried out according to the method described by Søborg & Bendixen (16) with minor modifications. Briefly,

cells were obtained after spontaneous sedimentation. Red blood cells were removed through haemolysis with a 9:1 mixture of 0.83 per cent ammonium chloride and 0.02 per cent EDTA, and white blood cells washed three times in TC-199 medium. The cell suspension contained neutrophils and lymphocytes in the same ratio as on blood films. Cell concentration was adjusted to $5 \times 10^6 - 1 \times 10^8$ neutrophil leukocyte pro ml. Culture media were supplemented with 10 per cent heat-inactivated human AB serum or 10 per cent heat-inactivated leukaemic serum. The cell suspensions were drawn into glass capillary tubes with an inner diameter of 1.0 mm. After sealing, the capillary tubes were spun at 600 g for 10 minutes. The tubes, cut below the cell-fluid interphase, were fixed in special plastic chambers using hot paraffin. Two tubes were placed in one chamber (capacity: 1.0 ml), cells migrating in opposite directions. After filling the chambers with AB serum or leukaemic serum supplemented culture medium, the chambers were closed with a cover-slip. Injecting 0.1 ml of the leukaemic blast cell preparation into the appropriate chambers made the final protein concentration 0.1 mg/ml. The chambers were placed in a 37° C thermostat and incubated for 24 hours. The migration areas were projected onto white paper, using a photographic blow-up machine. The areas were drawn, cut out and weighed on an analytical scale. Results are expressed as migration index

$$(MI), \text{ which is: } MI = \frac{\text{migration area (mg) with antigen}}{\text{migration area (mg) without antigen}}$$

Each value represents the mean of triplicate cultures.

C. *Statistical analysis* of the results was carried out using the Student's *t*-test.

RESULTS

1. The effect of 0.1 mg/ml leukaemic blast preparation on the migration of cells from 14 controls; MI in cultures supplemented with AB-serum: 0.9 ± 0.23 . MI in cultures with leukaemic serum: 1.10 ± 0.48 . MI's of 11 leukaemic children in remission were 0.49 ± 0.25 in AB serum and 0.48 ± 0.26 in leukaemic serum. The MI's of the leukaemic children are significantly lower than those of controls ($P < 0.05$) in both AB and leukaemic serum supplemented cultures (Fig. 1). No difference was observed between the effects of the two blast cell preparations.

2. The migration areas of cultures without antigen were also compared. Comparing the results in leukaemic serum to those of the AB serum cultures, the cells of 17 control children

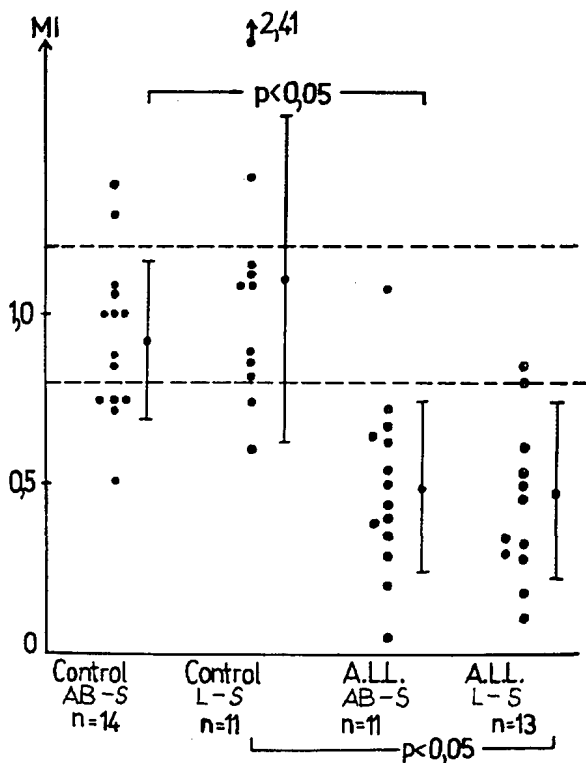


Fig. 1.

The effect of ALL associated antigen on the peripheral leukocyte migration of children in remission of ALL and controls.

ALL = group of children with ALL in remission.

AB-S = in presence of AB serum.

L-S = in presence of leukaemic serum.

had a mean MI of 0.61 ± 0.36 , while those of 20 leukaemic children in remission had an MI of 0.41 ± 0.18 . The difference is significant ($P < 0.05$, Fig. 2). It is interesting to note that the two leukaemic sera differed in their inhibitory effect. With serum 1, the MI of control children was

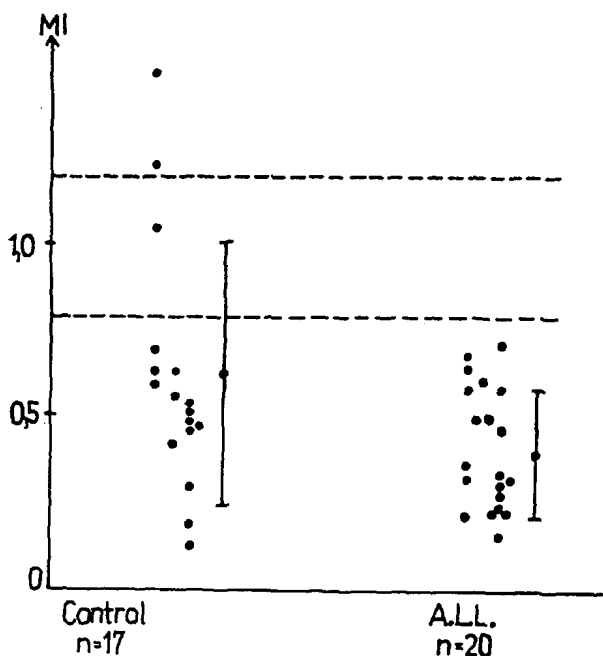


Fig. 2.

The effect of leukaemic serum on the peripheral leukocyte migration of children in remission of ALL and controls.

Abbreviations as in Fig. 1.

0.96 ± 0.36 , of leukaemic children 0.47 ± 0.19 ($P < 0.005$). With serum 2, MI of control children was 0.38 ± 0.15 , of leukaemic patients 0.35 ± 0.17 .

DISCUSSION

The leukocyte migration inhibition method has been widely used for the detection of antigens associated with malignant tumours (1, 2, 11, 20, 21). LAA has been demonstrated before with the stimulatory effect of leukaemic blast cells

(12, 17), or soluble leukaemic cell-membrane extracts (6) on autologous or allogeneic lymphocytes. The leukocyte migration inhibition technique is suitable for the demonstration of the antigenicity of both leukaemic blasts and soluble membrane extracts. If the antigen is "recognized" by the lymphocytes, the migration will be inhibited (20).

In our investigations, the leukaemic blast cell preparation, which contained all the water soluble cell constituents, behaved as specific antigen. This antigen significantly inhibited the migration of cells from other leukaemic children in remission, while it was ineffective on the cells of controls. We suggest, therefore, that our blast cell preparation contains the leukaemia associated antigen. The presence of leukaemic serum in the culture medium did not influence the inhibitory effect of the LAA. This is in contrast to our previous observations, where we were able to demonstrate blocking factors in the sera of so far untreated leukaemic children (13).

The blocking effect of serum from patients with malignant disease on cell-mediated immune responses is well documented (7, 8, 15), but perhaps it can only be demonstrated in certain models, while it remains unnoticed in others. Present investigations in our laboratory on the spontaneous rosette forming capacity of leukaemic lymphocytes favour this view too, since leukaemic serum does not significantly influence the number of rosetting cells (14). Testing more than one aspect of cell-mediated immune responses in clinical immunological investigations is therefore mandatory.

When the results obtained in the absence of antigen were compared, a significant inhibition was observed in the chambers where leukaemic serum was used instead of AB serum. The degree of inhibition was significantly greater on the migration of cells from leukaemic, than from control children. A difference was observed between the effects of the two leukaemic sera. Serum 1 only inhibited the migration of cells obtained from leukaemic children in remission, whereas serum 2 inhibited the migration of cells from both leukaemic and control children.

Although the leukaemic sera were not found to be cytotoxic when tested on allogeneic lymphocytes, it is possible that they contained non-specific toxic substances, which could interfere with lymphocyte functions. As large numbers of malignant cells are destroyed even before treatment, membrane bound leukaemia-specific antigens may also enter the circulation, and cause an immunologically specific inhibition.

Sjögren (15) demonstrated in the serum of patients with malignant diseases the presence of antigen-antibody complexes which can alter immunological functions. Furthermore, Carvalho (3) found a "leukaemic specific" inhibitory "antibody"-substance in the sera of 92 per cent of normal donors. Finally, the leuko-agglutinating effect of leukaemic sera should also be taken into consideration (9). In case of such an activity, an agglutinating barrier is formed at the front of the migratory fan, thus preventing further migration.

SUMMARY

The effect of leukaemia associated antigen (LAA) prepared from leukaemic lymphoblasts and that of pretreatment leukaemic sera was tested on the migration of peripheral leukocytes obtained from children with acute lymphoblastic leukaemia in remission, and from controls.

LAA specifically inhibited the migration of cells drawn from leukaemic children but it had no effect on the migration of cells from controls. Leukaemic serum did not influence the degree of inhibition induced by LAA, but it did exert an inhibitory effect on its own, when compared to results obtained in the presence of AB serum.

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