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'ANTIGENIC' DISPARITY BETWEEN LEUKAEMIC LYMPHOBLASTS AND NORMAL LYMPHOCYTES IN IDENTICAL TWINS

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SUMMARY

Circulating lymphocytes of a normal boy were unequivocally stimulated by irradiated leukaemic lymphoblasts from his identical twin with acute lymphoblastic leukaemia. This reaction indicates that there is an 'antigenic' disparity between these two types of cells. It is possible that leukaemic cells may acquire new surface antigens or that existing surface antigens of lymphoid cells may be modified during the process of leukaemogenesis.

INTRODUCTION

In vitro stimulation of lymphocytes by autologous leukaemic cells has been reported in the past few years, indicating the histoincompatibility between these two types of cells (Viza *et al.*, 1969; Friedman & Kourilsky, 1969; Powles *et al.*, 1971; Leventhal *et al.*, 1971). We recently studied mixed cell interactions between lymphocytes from a normal boy and lymphocytes or leukaemic lymphoblasts from his identical twin with acute lymphoblastic leukaemia, at times of remission and relapse of the leukaemia, and observed evidence that leukaemic lymphoblasts possess new or altered antigens.

MATERIALS AND METHODS

Circulating lymphocytes and leukaemic lymphoblasts were obtained according to a method previously described (Han & Sokal, 1970). Partially purified lymphocytes or leukaemic cells were suspended in RPMI 1640 culture medium at a concentration of 10^6 cells per ml. When used as target cells, these were irradiated to create a one-way mixed cell reaction. Each experiment included (a) mixed cell cultures containing equal numbers of lymphocytes (reactive cells) and syngeneic lymphocytes or leukaemic lymphoblasts (target cells), in 2 ml of culture medium and 0·2 ml of autologous (reactive cell donor) plasma, (b) cultures containing 10^6 lymphocytes and 0·1 ml of reconstituted phytohaemagglutinin (PHA-M,

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Difco Laboratories, Detroit, Michigan) and (c) controls: 10^6 lymphocytes alone and irradiated target cells alone, in the same volumes of culture medium and autologous plasma. Duplicate cultures were incubated at 37°C for 7 days. Lymphocyte response was determined by the ^{3}H-thymidine incorporation method (Han *et al.*, 1972) following the addition of one μCi ^{3}H-thymidine (specific activity 2·0 Ci/mM, Nuclear Chicago) 4 hr before harvesting the cells. The results were expressed as counts per minute (cpm).

CASE HISTORIES

Twin A (T.L.): A 26-month-old white male, the first-born of identical twins, was admitted to the hospital in October 1969 because of pallor and easy bruising. Four weeks before admission he had developed anorexia and lethargy. One week prior to admission he was noted to bruise easily. On examination, he was pale but in no acute distress. The liver was palpable 4 cm below the right costal margin and the spleen tip, just below the left costal margin. The white blood cell count was 17,500 per cu mm with 52% lymphoblasts. The haemoglobin was 6·1 gm/100 ml and the platelets were 45,500/cu mm. Bone marrow aspiration confirmed the diagnosis of acute lymphoblastic leukaemia.

Therapy with prednisone, 60 mg daily and vincristine, 1 mg weekly was given. Four weeks after admission, both peripheral blood count and bone marrow became normal. Maintenance therapy included methotrexate, 15 mg twice a week and monthly pulses of prednisone and vincristine. He remained in complete remission for 12 months. However, in November 1970, bone marrow examination showed complete replacement by lymphoblasts. Therapy with prednisone and mercaptopurine was begun, but only partial response was achieved. In April 1971, his bone marrow was again completely replaced by lymphoblasts. Subsequent therapy with cytoxan, L-asparaginase, adriamycin and guanozole (3,5-Diamino-1,2,4-Triazole), failed to control his leukaemia. On 1 October, 1971, his white blood cell count was 29,000/cu mm, with 93% lymphoblasts. He died 2 weeks later, with pseudomonas septicaemia.

At autopsy, leukaemic infiltrates of the liver, spleen, kidney, lymph nodes, bone marrow and meninges were seen. Pseudomonas septicaemia with severe haemorrhagic bronchopneumonia was considered the immediate cause of death.

Twin B (S.L.): This 26-month-old white boy was seen in October 1969, during the original admission of his twin brother. He was studied to establish precise zygosity in view of high incidence of concurrent leukaemia in identical twins. Physical examination revealed no abnormalities. Haemogram and bone marrow examination revealed no evidence of leukaemia. He was last seen in October 1971, when physical examination and haemogram revealed no evidence of abnormality.

Past history. The twins were the first children of a 24-year-old mother. Their delivery occurred prematurely on 24 August, 1967. At 5 months of age, the patient had a bilateral inguinal herniorrhaphy. He had been in good health otherwise until onset of the leukaemia.

Evidence for identity of the twins

In their physical features, these two children resembled each other; hair, eye colour and ear configuration were similar. The placenta and the amniotic and chorionic membranes at birth appeared characteristic of a uniovular pregnancy.

Study of their fingerprints revealed similarity of patterns on the fingers, palms and feet, suggesting that they were monozygotic.

Studies of blood grouping before transfusion showed both twins to be O, cDE/ce(R_2r), MsNs, P_1-, Lu(a^-b^+), kk, Le(a^-b^+), Fy(a^-b^+), Jk(a^+b^-), Mi(a^-), Vw^-, Vel^+, Wr(a^-).

Leucocyte (HL-A) typing (Amos *et al.*, 1969) indicated that both twins were HL-A, 2, 3, 6^b, 7, 8.

RESULTS

Mixed cell reactions of the identical twins and one unrelated normal individual are shown in the Table. Experiment 1 was performed on 19 June, 1970, when the patient was in complete remission and receiving maintenance methotrexate therapy. In this experiment, the

TABLE 1. Mixed cell reaction in identical twins

Experiment and dates	Status of leukaemic twin	Composition of culture	^{3}H-thymidine incorporation (cpm)
Experiment 1			
19.6.70	In remission	S.L.	106
		S.L.+PHA	36,435
		T.L.*	14
		S.L.+T.L.*	48
		T.L.	48
		T.L.+PHA	56,624
		S.L.*	7
		T.L.+S.L.*	25
Experiment 2			
1.10.71	In relapse	S.L.	410
		S.L.+PHA	39,600
		T.L.*	6
(WBC 29,000/cu mm, lymphoblasts, 93%)		S.L.+T.L.*	2,907
		B.D. †	178
		B.D.+PHA	39,914
		B.D.+T.L.*	43,035

* Irradiated 6000 r.
† Unrelated healthy individual.

lymphocytes from the normal twin (S.L.) and from the leukaemic twin (T.L.) did not stimulate each other, indicating complete histocompatibility; ^{3}H-thymidine incorporation in the control cultures was negligible. The PHA response of each twin was within the normal range. In contrast, lymphocytes of the normal twin were unequivocally stimulated by his brother's circulating cells, obtained when the patient was in relapse and receiving high dose methotrexate therapy (Experiment 2). Thymidine uptake in the mixed cell culture (S.L.+T.L.*) was approximately seven times greater than that of the control culture. A much greater blastogenic effect of these leukaemic cells, for lymphocytes of an unrelated individual (B.D.), was observed, suggesting that they were highly antigenic.

DISCUSSION

The stimulation of lymphocytes obtained from three of five patients with leukaemia during remission by autologous leukaemic cells collected previously and stored alive in liquid nitrogen was first reported by Viza *et al.* (1969). Similar findings in six patients with acute leukaemia (five with lymphoblastic leukaemia and one with acute myeloblastic leukaemia) were described by Friedman & Kourilsky (1969). Powles *et al.* (1971) have recently reported that the remission lymphocytes of nine patients with acute leukaemia were stimulated by autologous leukaemic cells. The stimulation of lymphocytes from the normal twin by leukaemic cells from the other identical twin with acute leukaemia has been described (Bach *et al.*, 1969). This *in vitro* lymphocyte stimulation by autologous or syngeneic leukaemic cells clearly indicates that there is an 'antigenic-disparity' between these two types of cells and suggests that leukaemic cells acquire new or altered antigens during the process of leukaemogenesis.

It is generally agreed that there is a good correlation between the intensity of mixed cell reactions and differences among HL-A antigens (Ivanyi *et al.*, 1967). The intense stimulating effect of leukaemic cells from our patient on allogeneic normal lymphocytes (see Table 1) is most likely due to already existing HL-A incompatibility between these two types of cells, although 'leukaemic' antigens may have contributed.

The possible loss or masking of surface antigens of leukaemic cells has been suggested by Viza *et al.* (1969); these authors found that the leukaemic cells from one patient with leukaemia not only failed to stimulate autologous normal lymphocytes, but also failed to activate the normal allogeneic lymphocytes. Lymphocytes obtained from this patient during remission, on the other hand, stimulated normal allogeneic lymphocytes. Although it is possible that antigens of leukaemic cells may be lost or masked, a more likely cause of their findings is that the viability of these leukaemic cells may have been very poor, following storage at $-180°C$ and thus, the stimulating activity of these cells may have been lost. Storage of cells in liquid nitrogen usually does not destroy viability or impair antigenicity; however, poor viability of such cells is not infrequently observed after thawing. It is known that the stimulatory effect of circulating lymphocytes or cultured lymphoid cells in mixed cell culture, correlates with their viability (Han *et al.*, 1972).

The mechanism of the mixed cell reaction between leukaemic cells and autologous or syngeneic lymphocytes is not fully understood. It has been reported that there are no differences between the HL-A antigens of leukaemic cells and normal lymphocytes from the same patients (Kourilsky *et al.*, 1968). Therefore, it is possible that another antigenic system would be involved in this reaction or that new 'leukaemic' antigens are not recognized by the standard HL-A typing method. It is known that currently available HL-A typing sera do not identify all transplantation antigens, and that mixed lymphocyte culture reveals histoincompatibilities in pairs of unrelated individuals with 'identical' HL-A antigens (VanRood & Eijsvoogel, 1970; Kissmeyer-Nielsen & Svejgaard, 1970). We have previously demonstrated the stimulation of lymphocytes by autologous cultured lymphoid cells with similar HL-A antigens (Han *et al.*, 1972).

Cell-mediated immunity against tumour-specific antigens in patients with a variety of solid tumours has recently been demonstrated by Hellstrom *et al.* (1971). Their findings suggest that the lymphocytes from these patients are sensitized by tumour-specific antigens. There has been some evidence that lymphocytes from patients with leukaemia may also

be sensitized against autologous leukaemia-associated antigens (Viza *et al.*, 1969; Powles *et al.*, 1971; Halterman & Leventhal, 1971). Viza *et al.* (1969) observed an increase in the intensity of the reaction against autologous leukaemic cells in one patient, after immunization with irradiated autologous leukaemic cells, collected before chemotherapy was instituted. Similarly, Powles *et al.* (1971) observed an increased lymphocyte reactivity against autologous leukaemic cells in each of nine patients following autoimmunization. Halterman and Leventhal reported that the leukaemic cells of one patient stimulated autologous lymphocytes although these cells did not stimulate lymphocytes of a normal identical twin.

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