Clin. exp. Immunol. (1980) **40**, 136–146.

Cell-mediated immune responses between HLA-identical siblings: recognition of antigenic changes associated with acute myelogenous leukaemia

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(Accepted for publication 1 October 1979)

SUMMARY

Cell-mediated immune reactions between a patient suffering from acute myelogenous leukaemia (AML) and an HLA-identical sibling were studied in order to characterize the *in vitro* reactions in MLC and CML prior to bone marrow transplantation. Our results indicated that antigenic differences were detectable between the blasts and the remission lymphocytes. While the normal sibling did not respond in MLC to her HLA-identical sister's remission lymphocytes, there was an anti-blast response. This proliferative response, however, did not lead to the development of detectable cytotoxic cells capable of destroying blast cells. Unrelated individuals, on the other hand, responded strongly both in MLC and CML to the allogeneic tumour blasts and remission lymphocytes of the patient and the lymphocytes of the healthy sibling. The kinetics and magnitude of the MLC response to blast cells was different from that to remission lymphocytes. This response indicated that the blast cells expressed antigenic differences which were recognized in MLC by both the HLA-identical sibling and unrelated individuals. Furthermore, these tumour cells were capable of sensitizing allogeneic, but not syngeneic lymphocytes to become cytotoxic, though they seemed to be more resistant to destruction in CML than normal cells.

INTRODUCTION

Current approaches for the management of leukaemia usually involve either chemotherapy or immunotherapy. While chemotherapy has proven effective against certain tumours, non-specificity and toxicity limit its application. Immunotherapy protocols attempt to activate the immune system specifically to destroy tumour cells. These approaches make the assumption, proven in animal but less well documented in human systems, that tumour cells express neoantigens after malignant transformation which make them susceptible to immune processes (Baldwin, 1977; Oettgen, 1977). In man, in fact, most if not all of the so-called tumour-specific antigens expressed on haemopoietic tumour cells were shown to represent differentiation antigens (Greaves, Brown & Rapson, 1975).

The genetics of the T cell-mediated transplantation reaction in man has been well characterized *in vitro*. The HLA-D region codes for antigens inducing strong proliferative responses of lymphocytes and measured in mixed leucocyte cultures (MLC). The HLA-A and B region codes for

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0099-9104/80/0400-0136$02.00

antigens necessary for the sensitization of killer cells, which also serve as target antigens in cell-mediated lympholysis (CML). HLA-identical siblings are bi-directionally negative in both MLC and CML (Yunis & Amos, 1971; Trinchieri *et al.*, 1973; Eijsvoogel *et al.*, 1973; Schendel, Alter & Bach, 1973; Bach & van Rood, 1976).

We had a patient, diagnosed as suffering from acute myelogenous leukaemia (AML), who was considered as a possible candidate for bone marrow transplantation. Preliminary family MLC studies indicated that one sister might be a suitable HLA-identical donor. Further studies were performed to characterize MLC and CML reactions between the two siblings as well as to determine if reactivity against tumour cells of the patient, without the interference of HLA antigens, could be expressed by cells of the normal sibling.

For these studies we utilized normal cells from the HLA-identical, healthy sister, frozen tumour cells (subsequently referred to as blasts) obtained from the patient before the initiation of chemotherapy and frozen remission lymphocytes from the patient. We addressed the following questions in MLC and CML:

(1) Does the healthy, HLA-identical sister respond differently to the blast and remission cells of the patient?

(2) Does a healthy, unrelated individual respond differently to the blasts, remission lymphocytes and healthy sister's lymphocytes?

(3) Do patient blasts and remission lymphocytes function differently as stimulators in MLC or target cells in CML?

Our results indicated that antigenic differences were detectable between the blasts and the remission lymphocytes. While the normal sibling did not respond in MLC to her HLA-identical sister's remission lymphocytes, she did respond to the blasts. This proliferative response, however, did not lead to the development of cytotoxic cells capable of destroying the blast cells. Unrelated individuals, on the other hand, responded strongly both in MLC and CML to the allogeneic blasts and remission lymphocytes of the patient and the lymphocytes of the healthy sibling. The kinetics and magnitude of the MLC response to blast cells were different from those to remission lymphocytes. These responses indicated that the blast cells expressed antigenic differences which were recognized in MLC by both the HLA-identical sibling and unrelated individuals. Furthermore, these tumour cells were capable of sensitizing allogeneic, but not syngeneic lymphocytes to become cytotoxic, though they seemed to be more resistant to destruction in CML than normal cells. We discuss the view that major histocompatibility complex (MHC) linked antigens expressed on tumour cells, rather than tumour-specific neoantigens may be responsible for these reactions.

MATERIALS AND METHODS

Patient E.A. The patient was a 23-year-old white female diagnosed as having acute myelogenous leukaemia (AML). She presented with generalized lymphadenopathy and had 80,000 WBC/mm^3 with 90% blasts in the peripheral blood and bone marrow. Demonstration of low concentrations of NaF-sensitive esterases and of receptor sites for IgG and C3 on approximately 30% of the blasts suggested a leukaemia of immature monocytic origin. This was substantiated further by the demonstration of a high density Ia expression on these blasts using a heterologous rabbit anti-human antiserum kindly provided by Dr Per Petersen, Uppsala, Sweden (Huber *et al.*, unpublished results). Following chemotherapy with adriablastin and arabinosylcytosine a remission period of 3 months was achieved. Following haematological relapse there was a central nervous system involvement. Further chemotherapy cleared the central nervous system fluid of blast cells, but did not produce a full remission. The patient died 7 months after admission before a bone marrow transplant could be attempted.

Mixed leucocyte culture (MLC). Cells for MLC were isolated from peripheral blood by density gradient separation (Böyum, 1968). An equal number (5×10^4) of responding cells from one donor were cultured with allogeneic, mitomycin C-treated (Kyowa, Japan) cells from a second donor in

medium RPMI 1640 supplemented with 25 mmol HEPES buffer (GIBCO, no. 240, Grand Island, New York), 20% male pooled serum, 2 mmol L-glutamine and antibiotics. Cultures were carried out in round-bottomed microtitre plates (Greiner, Nütringen, FRG), in a volume of 0·2 ml. The cells were incubated in a humidified environment of 5% CO_2 at 37°C. On day 5 they were labelled overnight with 2 μCi tritiated thymidine (^{3}H-TdR) (specific activity 2 Ci/mmol, Radiochemical Centre, Amersham, England) to assess the proliferative response. Cells were precipitated onto glass fibre filters by means of a cell harvester (Otto Hiller, Madison, Wisconsin) and incorporated radioactivity assessed in a liquid scintillation counter (Packard, La Grange, Illinois). The responses were evaluated in terms of per cent relative response (%RR) according to the following formula: $\%RR = (AXm - AAm)/(APm - AAm)$, where X represents the experimental stimulator, A the autologous stimulator and P a stimulator pool of six unrelated individuals.

Cell-mediated lympholysis (CML). For the generation of cytotoxic T lymphocytes (CTL) the procedure was identical to that described for MLC, except that killer cells were generated in plastic tissue culture flasks (Costar, no. 3025, Tecnomara, Zurich, Switzerland). Co-culture of 5×10^6 responder and stimulator cells each was performed at a concentration of 1×10^6 cells/ml. On day 6 they were tested for their cytotoxic ability as described below.

Sensitized cells were pooled, centrifuged and resuspended in fresh medium RPMI 1640 for counting, prepared as for MLC. Serial dilutions were made and varying numbers of effector cells in 0·1 ml medium were added to microtitre wells. Target cells usually consisted of PHA-stimulated blasts, but sometimes non-stimulated lymphocytes were used. Target cells were put into culture on day 0. They were either stimulated with 50 μl PHA-M (Difco, Detroit, Michigan) on day 3 or left alone. On the evening of day 5 the cells were labelled with 10 μCi/ml ^{51}Cr (Na_2CrO_4 in saline, 5 mCi/mM, specific activity 2–400 Ci/mmol, New England Nuclear) overnight (Claude Mawas, personal communication). The next morning they were applied directly to a density gradient (Lymphoprep, Nygaard, Oslo, Norway, 1·077 g/ml) and centrifuged at 400 ***g*** for 15 min to remove dead cells and debris. They were washed twice, counted and resuspended at a concentration of 1×10^5 cells/ml. To round-bottomed microtitre wells containing effector cells was added 0·1 ml of labelled target cells. The plates were centrifuged for 5 min at 50 ***g*** and incubated for 4 hr in a humidified atmosphere of 5% CO_2 at 37°C. To harvest the supernatant fluids the plates were centrifuged at 400 ***g*** for 10 min. One hundred microlitres of the supernatant was removed with an Eppendorf pipette, transferred to a small plastic tube and then counted in a well type γ-counter (Nuclear-Chicago, Des Plaines, Illinois). The per cent cytotoxicity (% CML) was calculated from the formula:

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100.$$

The experimental release represents c.p.m. released from ^{51}Cr-labelled target cells incubated with effector cells. The maximum release and spontaneous release represent the mean c.p.m. released from triplicate samples of target cells lysed with 2·5% Nonidet-P40 (Shell) or incubated with medium alone, respectively.

HLA typing. HLA-A and B typing was performed using routine procedures detecting 14 HLA-A and 20 HLA-B antigens (Terasaki & McClelland, 1964).

Cryopreservation. Freezing was performed according to the method of Schendel with minor modifications (Schendel *et al.*, 1979). Cells were resuspended in 2 ml freezing ampules (Nunc, Wiesbach-Biebrich, FRG) at a concentration of $10–20 \times 10^6$ cells/ml in 0·5 ml 100% human pooled serum. Immediately before freezing they were placed on ice and mixed gently with 4°C 20% DMSO in RPMI. From this time until freezing they were kept on crushed ice. Freezing was performed using a controlled rate freezing machine (Cryoson, Netherlands) at -1°C/min. The frozen vials were stored in liquid nitrogen. To thaw, ampules were vigorously shaken in a 37°C water bath until thawing began around the edge of the ampule. One mililitre of 50% serum in RPMI was gently added with a Pasteur pipette and the cells were transferred to 10 ml conical centrifuge tubes. They were centrifuged for 10 min at 300 ***g*** and resuspended in 20% serum medium for counting. Until distribution they were kept at room temperature.

RESULTS

Familial MLC and HLA typing

MLC testing was performed within family AL, which consisted of two parents, two healthy siblings and the patient, to determine whether there was an HLA-identical sibling donor for bone marrow transplantation (Table 1). The patient's blasts stimulated each responder, except C2, very strongly. Even though C2 gave 9,143 c.p.m. (%RR = 30) following stimulation with the patient's blasts we suspected that she might be HLA-identical, since the response of the other cells ranged between 74,000 and 160,874 c.p.m. (%RR = 206–460). The response, therefore, might be to antigens expressed on the tumour cells consequent to malignant transformation.

Untreated cells isolated from the patient's peripheral blood cultured with her own mitomycin C-treated tumour cells, gave 25,374 c.p.m. and her response against the other normal, mitomycin C-treated stimulator cells was significantly higher in only one case (patient X mother). The high c.p.m. in the autologous control, therefore, probably reflect the spontaneous proliferation of the blasts, rather than an immunologically specific response against allogeneic stimulator cells. Accordingly %RR was not calculated for the patient. The failure of the patient to respond in MLC is not surprising, since cell marker studies demonstrated that there were only 2·5% T lymphocytes as assessed by spontaneous rosette formation with neuraminidase-treated sheep red blood cells.

Following this suggestion that an MLC-compatible bone marrow donor might exist, HLA typing was performed. In the patient's case typing was carried out on blast cells. The results (Table 1) supported our hypothesis that the patient and C2 were HLA-A and B identical, as both had the 2,18,40 phenotype.

Kinetics of the MLC response of the HLA-identical sister and one unrelated to blast and remission cells

Complete remission in the patient was achieved after 4 weeks of induction chemotherapy. There was no evidence of blasts in the peripheral blood or bone marrow; the small lymphocytes were considered to be remission cells and cryopreserved for future studies. Using these cells as stimulators we were able to confirm that C2 was, indeed, MLC-negative with the patient (Table 2). In three experiments C2 failed to respond to the remission lymphocytes of the patient, while in three of four experiments she did respond to cryopreserved blasts. Frozen tumour cells were used and technical reasons may account for the one failure to respond to blasts. Peripheral blood blasts were used rather than bone marrow blasts, which consistently stimulated more strongly, because it was felt that it was more relevant to compare responses against blast and remission cells, both isolated from peripheral blood.

We were interested in comparing the response of C2 and an unrelated individual X to blast and remission cells to determine if the kinetics of the response would be the same to both blast and remission cells. Responding cells from C2 and X were tested with the following stimulating cells:

Table 1. MLC in family AL

HLA-Pheno.*	RC/SC	Fm	%RR†	Mm	%RR	C1m	%RR	C2m	%RR	Ptm	%RR	Poolm‡	%RR
2,12,18	F§	909	0	30,634	84	10,841	28	5,398	13	74,104	206	36,427	100
2,15,40	M§	55,267	174	527	0	12,261	37	31,949	100	145,250	460	31,959	100
n.t.	C1§	31,984	79	27,869	69	446	0	24,236	60	160,874	403	40,227	100
2,18,40	C2§	5,155	16	19,555	66	4,326	13	587	0	9,143	30	29,452	100
2,18,40	Pt§	20,317	—	35,219	—	28,022	—	16,527	—	25,374	—	25,908	—
2,3,w37	NP§	35,914	105	25,572	74	21,021	61	22,982	66	122,900	363	34,282	100

* HLA-typing: see Materials and Methods section.

† Per cent relative response $= \frac{\text{experimental c.p.m.} - \text{autologous c.p.m.}}{\text{pool c.p.m.} - \text{autologous c.p.m.}} \times 100.$

‡ Frozen cell pool composed of six randomly selected unrelated individuals.

§ F = father; M = mother; C1 = child 1; C2 = child 2; Pt = patient; NP = normal person (autologous control = 634 c.p.m.).

Table 2. MLC between C2 and remission lymphocytes or blasts of Pt

Experiment no.	Day	C2:C2m*	C2:Rm†	C2:Blm‡	Net increase c.p.m. C2:Rm − C2:C2m	Net increase c.p.m. C2:Blm − C2:C2m
1	5–6	587 ± 259		9,143 ± 3,858		8,556
2	5–6	439 ± 257	263 ± 72	1,077 ± 315	−176	638
	7–8	641 ± 52	558 ± 261	3,771 ± 1,951	−83	3,130
3	5–6	776 ± 696	341 ± 48	856 ± 99	−435	80
4	5–6	541 ± 105	376 ± 232	4,059 ± 462	−165	3,518
	7–8	1,812 ± 1,130	703 ± 164	2,973 ± 1,136	−1,109	1,161

* Autologous MLC with HLA-identical sister C2 as responder.
† MLC with C2 responding against patient's remission lymphocytes.
‡ MLC with C2 responding against patient's tumour blasts.

autologous control cells (C2 and X), remission cells (R), bone marrow blasts (BMB) and peripheral blood blasts (PBB). Three different ratios of responder to stimulator cells were cultured and the response was measured from days 3–4 to 8–9 (Fig. 1 a,b). We found that X responded far more strongly than C2 to both populations of blasts and that both C2 and X responded more strongly to bone marrow blasts than to peripheral blood blasts. The stimulation index (SI) for the peak response by X against bone marrow blasts was 207·5, against peripheral blood blasts 60·7 and remission cells 11·4. C2 gave corresponding SI of 51·5, 5·8 and 0·87, respectively. In this case the tumour cells seemed to provoke a later response than remission cells. The response of X against remission cells peaked on day 5–6, but not until day 6–7 against bone marrow blasts and had not diminished against peripheral blood blasts by day 8–9. The autologous responses were increased at this time, but not enough to account for the strong stimulation seen against bone marrow blasts (72,000 c.p.m.). The peak response of C2 against both blast populations did not occur until day 7–8.

Fig. 2 quantitates the response by C2 on day 6–7 (Fig. 2a) and 7–8 (Fig. 2b) against each stimulator population. While the peak response against normal, allogeneic cells was reached by day 6–7 and was decreasing on day 7–8, the response against the blasts was still increasing on day 7–8 and that against bone marrow blasts now surpassed the allogeneic response. Since the autologous response on day 7–8 was lower than that seen on day 6–7 the continued increase is not non-specific. Fig. 3 shows the response by X on day 6–7, the day of the peak response to the blasts. Only one day is presented, since on each day that the response was measured the qualitative order of cells in terms of their ability to stimulate was the same and only quantitative differences were seen. The response of X was very strong against the tumour cells, considerably greater than that against remission or normal allogeneic cells from the HLA-identical sibling.

Response in CML by C2 and X against blasts and remission cells

It is known that different populations of cells in both man and mouse respond primarily in MLC and CML (Bach *et al.*, 1973; Cantor & Boyse, 1975). In addition, different antigens are largely responsible for sensitizing these different populations (Yunis & Amos, 1971; Schendel *et al.*, 1973; Bach & van Rood, 1976). Since a response in MLC against blasts was seen in C2 and X, it was of interest to determine if cytotoxic lymphocytes were also produced.

Table 3 shows that the unrelated X could produce killer cells against normal cells from C2 or remission cells from the patient and that these cells could also serve as targets. Each killer population ($XC2_m$, XR_m) recognized antigens expressed on the sensitizing cells as well as on the blasts. When X was sensitized in MLC against bone marrow blasts or peripheral blood blasts much higher killing was seen. As before, bone marrow blasts were killed better than peripheral blood blasts.

When C2 was used as the responder, cytotoxic cells were produced only against the allogeneic individual X. No cytotoxic cells were ever detected against the patient's remission or blast cells. Though lack of cytotoxicity against the remission cells was expected because of the HLA identity

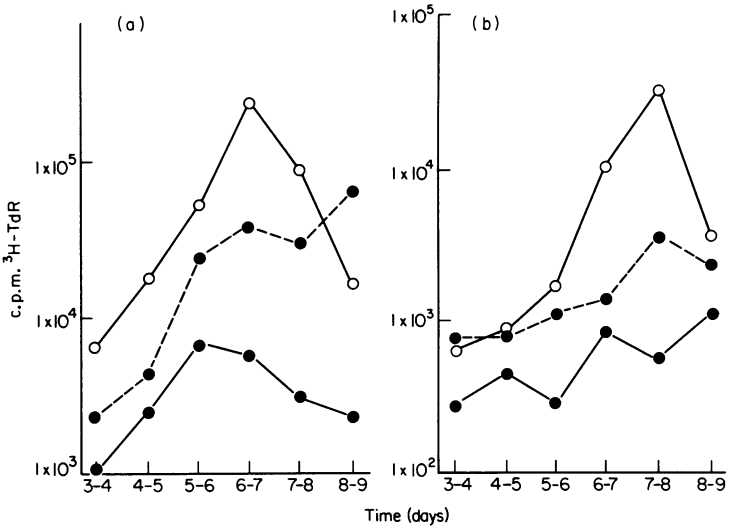

Fig. 1. Kinetics in MLC of unrelated (a) and HLA-identical sibling C2 (b) against peripheral blood blasts (●- - -●), bone marrow blasts (○——○) and remission cells (●——●). The abscissa represents time in days and the ordinate c.p.m. of ^{3}H-TdR incorporated. Autologous controls for XXm and C2C2m are respectively: day 3–4, 700, 608; day 4–5, 834, 547; day 5–6, 643, 439; day 6–7, 708, 626; day 7–8, 948, 641; day 8–9, 1,577, 1,005.

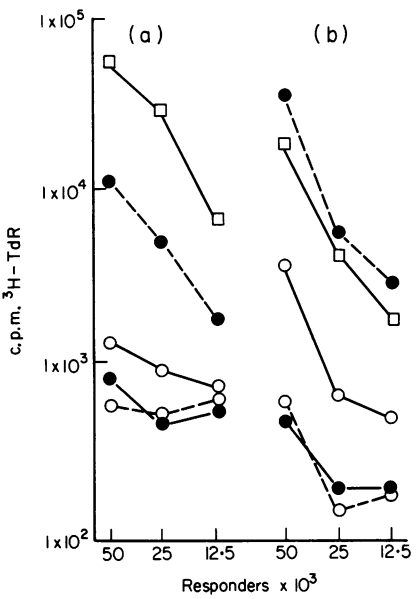

Fig. 2. Kinetics in MLC of C2 against unrelated X (□——□), bone marrow blasts (●- - -●), peripheral blood blasts (○——○), remission cells (●——●) and autologous (○- - -○) on day 6–7 (a) and day 7–8 (b). The abscissa represents number of responding cells against 5×10^4 stimulating cells and the ordinate c.p.m. of ^{3}H-TdR incorporated.

between C2 and the patient, the failure to see cytotoxicity against the blasts could not automatically be presumed because of the positive anti-blast MLC. As a further test, a three-cell experiment using stimulators from X as well as blasts showed no cytotoxicity against bone marrow blasts or peripheral blood blasts. This was not due to active suppression since cytotoxic activity against X was produced in the presence of blasts.

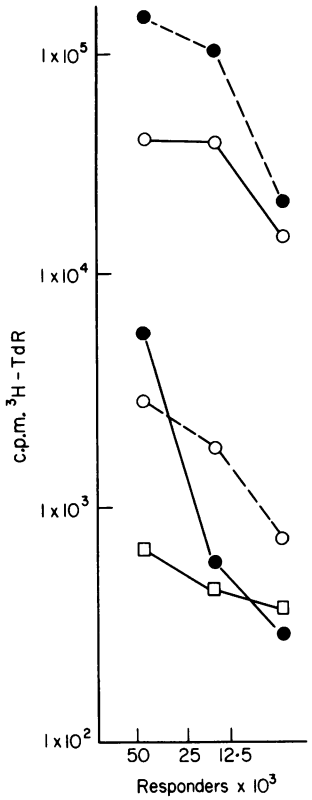

Fig. 3. Kinetics in MLC of unrelated X against bone marrow blasts (●– – –●), peripheral blood blasts (○——○), remission cells (●——●), C2 (○– – –○) and autologous (□——□) on day 6–7. The abscissa represents the number of responding cells stimulated by 5×10^4 cells and the ordinate c.p.m. of ^{3}H-TdR incorporated.

Table 3. CML by X and C2 against various target cells*

Sensitizing combination	Target cells††				
	C2†	R‡	X§	BMB¶	PBB**
XC2m	6·5	7·7	−0·2	5·2	1·9
XRm	6·9	14·7	−0·3	8·2	1·5
XXm	1·7	—	2·9	−2·0	−5·6
XBMBm	38·7	57·0	7·0	30·4	16·4
XPBBm	42·9	50·7	1·9	26·1	17·3
C2C2m	0·8	0·2	3·3	0·2	−5·8
C2Rm	−1·2	−0·3	—	−1·3	−6·0
C2Xm	1·0	1·6	33·1	−0·9	−6·6
C2BMBm	3·8	0·5	3·1	0·2	−4·3
C2PBBm	−0·5	−1·0	—	−0·4	−4·6
C2Rm+Xm	−2·1	−1·4	38·7	—	—
C2BMBm+Xm	−0·9	−0·4	34·8	−1·5	−5·8
C2PBBm+Xm	−2·3	−0·9	32·0	−1·2	−5·6

* Results expressed as per cent cytotoxicity.
† HLA-identical sister.
‡ Remission lymphocytes from patient.
§ Unrelated individual.
¶ Bone marrow blasts.
** Peripheral blood blasts.
†† Effector:target cell ratio = 40:1.

Is cross-killing of blasts and normal cells due to blast antigens?
The cytotoxicity against normal cells following sensitization to blasts may have been due to sensitization against blast antigens on the tumour, which were also expressed on PHA-stimulated cells used as targets in CML. Using non-PHA-treated target cells we could demonstrate that this was not the case (Table 4). Following sensitization to tumour blasts killing on C2 was seen even when the target cells were not PHA-stimulated and again, killing was higher on normal target cells than on tumour blasts.

Behaviour of blasts as sensitizing cells for killer cell production and as target cells
In each of these experiments highest levels of killing against blast target cells were obtained after sensitization to blast cells. Nevertheless, killing was even higher using normal lymphocytes as targets. We performed three experiments in which the behaviour of blasts as sensitizing and target cells in CML was compared. Table 5 summarizes the results. Regardless of whether normal cells or blasts were used to sensitize, lymphocytes were always killed to a higher degree than blasts. In these cases also, killing was strongest when blasts were used as stimulators.

Can CML detect the existence of non-HLA-A or B target antigens on blasts?
Although tumour blasts stimulated C2 in MLC, killer cells were not detected. Since C2 did not respond to the patient's remission cells, these MLC-stimulating antigens are either quantitatively or

Table 4. CML by unrelateds against blast and non-blast target cells

Sensitizing combination	Target cells¶ C2* **	X† **	PBB‡	Y§ **
XC2	17·4	0·6	5·3	—
XPBBm	39·4	−0·4	7·8	—
XXm	−0·3	1·1	1·4	—
YC2m	28·5	—	3·0	−0·6
YPBBm	41·5	—	14·1	3·9
YYm	−6·3	—	—	−2·2

* HLA-identical sister, non-blast target cell.
† Unrelated 1, non-blast, fresh target cell.
‡ Peripheral blood blast.
§ Unrelated 2, non-blast, fresh target cell.
¶ Effector:target cell ratio = 30:1.
** Non-PHA-treated target cell.

Table 5. Blasts as sensitizing cells and targets in CML*

Sensitizing cell/targets	Lymphocyte Pt	PBB†
Lymphocyte Pt	7·1‡	2·8
PBB	36·3	17·4
	Lymphocyte C2	PBB
Lymphocyte C2	17·8	3·8
PBB	31·3	14·7

* Values represent mean per cent cytotoxicity of three experiments.
† Peripheral blood blasts.
‡ Values represent the means of three individual experiments with three unrelated responders.

qualitatively different than cell surface antigens expressed on remission cells. Based upon typing results, however, the blasts did bear the same HLA-A and B antigens as the normal, healthy cells of C2. It has recently been reported that antigens distinct from HLA-A and B, though strongly associated with them, may serve as target antigens in CML (Schendel *et al.*, 1977; Kristensen & Grunnet, 1975). We were, accordingly, interested in whether or not we could detect the existence of additional sensitizing or target antigens on the leukaemic blasts in CML. To this end, we sensitized an unrelated individual Y, who was identical for the HLA-A and B antigens, but responded positively in MLC to C2 (representing healthy cells) and leukaemic blasts. To show that the stimulators were capable of inducing killer cell production we sensitized an unrelated individual W, who shared one defined HLA-A antigen against the same stimulator cells. If killer cells were produced by Y it would imply that they were directed against other than the HLA-A and B antigens (or those loci which seem to be in extremely high linkage disequilibrium with them and may serve as the target antigens) for which they are identical with the patient and C2. Target cells were C2, peripheral blood blasts, Y, W and two additional, unrelated individuals Z^1 and Z^2. Z^1 expressed HLA-B 18 and Z^2 expressed HLA-B 40. As expected, individual W sensitized against HLA-B 18 lysed C2, blasts, Y and Z^1. There was no autokilling. In spite of strong MLC activation, individual Y, not differing from C2 or leuakaemic blasts for any known HLA-A or B antigens, did not produce any detectable killing on any of the target cells used (Table 6).

DISCUSSION

A schematic presentation of our findings is given in Table 7. Blast cells obtained from a patient with AML induced MLC proliferation of normal peripheral blood lymphocytes obtained from an

Table 6. Is killing detectable against non HLA-A or -B antigens?

Sensitizing combination TgC*	C2†	W‡	PBB§	Y¶	Z^{1}**	Z^{2}**
HLA Diff.	—	B-18	—	—	B-18	B-40
WC2m	14·3	−3·9	6·1	11·6	25·0	
WWm	−0·9	−5·5	−2·5	−4·7	2·0	
WPBBm	19·7	−7·2	9·9	11·0	23·4	
YC2m	−1·3	−2·6	−3·4	−5·3	0·3	−3·4
YYm	−1·3	−2·9	−2·8	−5·9	−5·0	2·0
YPBBm	−0·3	−1·7	−1·6	−5·0	−7·6	−6·5

Cytotoxicity was performed on PHA-treated target cells at a ratio of 40 effector cells per target cell.

* Target cell.
† HLA-identical sister, HLA 2,18,40.
‡ Unrelated = HLA 2,15,40.
§ Peripheral blood blasts from patient.
¶ Unrelated, HLA 2,18,40.
** Z^1 and Z^2 = unrelated.

Table 7. Capacity of AML blasts to stimulate MLC and CML reactivities of HLA-identical or HLA-different lymphocytes

	Responder				
Stimulator	HLA-A	-B	-D	MLC	CML
Blasts	=	=	=	++	−
Blasts	=	=	≠	+++	−
Blasts	≠	≠	≠	++++	++

HLA-A, B and D-identical sibling, but failed to stimulate the development of cytotoxic lymphocytes. When the AML blasts were used to stimulate lymphocytes from an HLA-A, B-identical, but HLA-D different, unrelated individual the same pattern was observed. On the other hand, both strong proliferation and subsequent cytotoxicity were detected when the blasts were used to stimulate lymphocytes from unrelated individuals differing not only for HLA-D, but also for HLA-A and B. It was observed in this case, however, that the blast cells were less susceptible to lysis than normal peripheral blood lymphocytes.

Several possibilities may explain our failure to detect cytotoxic activity against the tumour blasts, despite their stimulation of strong MLC. First, our *in vitro* CML test system may not be sensitive enough to measure a weak anti-blast cytotoxic response, or the development of such cytotoxic cells may occur at a time other than that when they develop against alloantigens. Second, we observed a relative resistance of the blasts to allo-sensitized killer cells and this resistance may hinder the detection of weak cytotoxicity in the HLA-identical combination. Third, it is known in allogeneic transplantation systems that different antigens preferentially activate MLC-responding cells and cytotoxic precursors (Yunis & Amos, 1971; Trinchieri *et al.*, 1973; Eijsvoogel *et al.*, 1973; Schendel *et al.*, 1973; Bach & van Rood, 1976). Similar antigenic distinctions may also exist in some tumour systems. Although the AML blasts express antigens causing MLC proliferation, these antigens may not stimulate precursors of cytotoxic lymphocytes. In this respect, the full potential of the immune response to the tumour blasts would not be activated, at least as it can be measured *in vitro* in MLC and CML.

The nature of the antigens expressed on the AML blasts which leads them to stimulate a strong MLC response is unknown. It has been reported recently for both primary and secondary MLC that T lymphocytes from normal donors can recognize and respond by proliferation to antigens expressed on autologous B cells (Kuntz, Innes & Webster, 1976; Opelz *et al.*, 1975; Zier, Huber & Braunsteiner, 1979). Indeed, proliferative responses of autologous T cells or T cells derived from an HLA-identical sibling against unidentified antigens expressed on leukaemic blasts have been observed in this and other studies (Bach, Bach & Joo, 1969; Friedman & Kourilski, 1969; Reinsmoen, Kersey & Yunis, 1977). Preliminary studies using a heterologous antiserum directed against the constant region of the Ia-like antigens on human cells indicated that the AML blasts from this patient expressed increased amounts of Ia antigens (Huber *et al.*, unpublished results). Taken with the findings that autologous responses to such antigens can occur, the possibility remains open that the MLC proliferation by the healthy HLA-identical sister to the AML blasts was stimulated by such antigens (Kuntz *et al.*, 1976; Opelz *et al.*, 1975; Zier *et al.*, 1979). Alternatively, a leukaemic antigen or a viral antigen together with either Ia-like or tumour-expressed antigen may have been recognized.

Several other studies have examined the functional expression of HLA-associated antigens on leukaemic blasts. Zarling *et al.* have reported that cytotoxicity was generated against autologous AML blasts which did not stimulate autologous lymphocytes in MLC when a third party cell provided the MLC stimulus (Zarling *et al.*, 1976). Lee & Oliver suggested that some AML blasts are deficient in HLA-D determinants, thereby making them incapable of stimulating killer cell production, although by serological means HLA-DR determinants were detected (Lee & Oliver, 1978). These authors advanced the hypothesis that patient's blasts bore unique determinants against which killer cells could be generated. In contrast to our results, the tumour blasts examined in this study were good targets for allogeneic killer cell production, but less effective stimulators for their production as were remission cells. Sondel *et al.* could, in certain cases, detect CML responses by healthy individuals against blasts of their leukaemic sibling, though sometimes it was necessary to co-culture them in the presence of allogeneic lymphocytes (Sondel *et al.*, 1976). This, however, was not observed without exception and in some cases even in the presence of an allogeneic cell which provided a strong stimulus cytotoxicity was not detected. In accord with our results this was not the result of specific suppression by the tumour blasts, because cytotoxicity was demonstrated against allogeneic cells co-cultured in the presence of tumour cells. The partially conflicting reports concerning the stimulatory capacity of blasts in MLC and CML support the view that several mechanisms may operate in the development of and the response to AML, depending upon the stage, degree of tumour cell differentiation or immune capability of the patient. Accordingly, it is

important not to generalize from the specific, but rather to consider that individual reports may represent different disease patterns.

This work was supported by Austrian 'Fonds zur forderung der wissentschaftlichen Forschung'. We are grateful to Ms A. M. Födinger for her excellent technical help and to Dr Dolores Schendel for productive discussions and suggestions. We are especially indebted to Mrs H. L., the HLA-identical sister to the patient, for her continuous co-operation throughout the study.

REFERENCES

BACH, M., BACH, F.H. & JOO, P. (1969) Leukemia-associated antigens in mixed lymphocyte culture test. *Science*, **166,** 1520.

BACH, F.H., SEGALL, M., ZIER, K.S., SONDEL, P.M., ALTER, B.J. & BACH, M.L. (1973) Cell mediated immunity: separation of cells involved in recognitive and destructive phases. *Science*, **180,** 403.

BACH, F.H. & VAN ROOD, J.J. (1976) The major histocompatibility complex—genetics and biology. *N. Engl. J. Med.* **295,** 872.

BALDWIN, R.W. (1977) Tumor associated antigens. *Z. Krebsforsch.* **89,** 1.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Lab. clin. Invest.* **21** (Supplement 97), 77.

CANTOR, H. & BOYSE, E. (1975) Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. *J. exp. Med.* **141,** 1390.

EIJSVOOGEL, V.P., DU BOIS, M.J.G.J., MELIEF, C.J.M., DE GROOT-KOOY, M.L., KONING, C., VAN ROOD, J.J., VAN LEEUWEN, A., TOIT, E.D. & SCHELLEKENS, P.T.A. (1973) Position of a locus determining mixed lymphocyte reaction (MLR), distinct from the known HL-A loci, and its relation to cell-mediated lympholysis (CML). In *Histocompatibility Testing* 1972 (ed. by J. Dausset and J. Colombiani), p. 501. Munksgaard, Copenhagen.

FRIEDMAN, W.H. & KOURILSKI, F.M. (1969) Stimulation of lymphocytes by autologous leukemic cells in acute leukemia. *Nature*, **224,** 277.

GREAVES, M.F., BROWN, G. & RAPSON, N.T. (1975) Antisera to acute lymphatic leukemia cells. *Clin. Immunol. Immunopathol.* **4,** 67.

KRISTENSEN, T. & GRUNNET, N. (1975) Cell mediated lympholysis in man. Evidence of a separate CML locus within the major histocompatibility complex and an approach to CML typing. In *Histocompatibility Testing* 1975 (ed. by F. Kissmeyer-Nielsen), p. 835. Munksgaard, Copenhagen.

KUNTZ, M.M., INNES, J.B. & WEKSLER, M.E. (1976) Lymphocyte transformation induced by autologous cells. IV. Human T-lymphocyte proliferation induced by autologous or allogeneic non-T lymphocytes. *J. exp. Med.* **143,** 1042.

LEE, S.K. & OLIVER, R.T.D. (1978) Autologous leukemia-specific T-cell mediated lymphocytotoxicity in patients with acute myelogenous leukemia. *J. exp. Med.* **147,** 912.

OETTGEN, H.F. (1977) Immunotherapy of cancer. *N. Engl. J. Med.* **297,** 484.

OPELZ, G., KIUCHI, M., TAKASUGI, M. & TERASAKI, P.I. (1975) Autologous stimulation of human lymphocyte subpopulations. *J. exp. Med.* **142,** 1327.

REINSMOEN, N., KERSEY, J. & YUNIS, E.J. (1977) Leukemia associated antigens detected in primed lymphocyte tests. *Transplant. Proc.* **9,** 199.

SCHENDEL, D.J., ALTER, B.J. & BACH, F.H. (1973) The involvement of Ld- and Sd-region differences in MLC and CML: a three-cell experiment. *Transplant. Proc.* **5,** 1651.

SCHENDEL, D.J., WANK, R., HALL, M. & DUPONT, B. (1979) Standardization of the human *in vitro* cell mediated lympholysis technique. *Tissue Antigens*, **13,** 112.

SCHENDEL, D.J., WANK, R., HANSEN, J.A. & DUPONT, B. (1977) Cell mediated lympholysis in man: identification of a common target determinant associated with HLA-B12 and Bw35. *Transplant. Proc.* **9,** 1777.

SONDEL, P.M., O'BRIEN, C., PORTER, L., SCHLOSSMAN, S. & CHESS, L. (1976) Cell-mediated destruction of human leukemia cells by HLA identical lymphocytes: requirement for a proliferative trigger *in vitro*. *J. Immunol.* **117,** 2197.

TERASAKI, P. & MCCLELLAND, J.D. (1964) Microdroplet assay of human serum cytotoxins. *Nature*, **204,** 998.

TRINCHIERI, G., BERNOCO, D., CURTONI, S., MIGGIANO, V. & CEPPELLINI, R. (1973) Cell mediated lympholysis in man: relevance of HL-A antigens and antibodies. In *Histocompatibility Testing* 1972 (ed. by J. Dausset and J. Colombiani), p. 509, Munksgaard, Copenhagen.

YUNIS, E.J. & AMOS, D.B. (1971) Three closely linked genetic systems relevant to transplantation. *Proc. Natl. Acad. Sci. USA*, **68,** 3031.

ZARLING, J.M., RAICH, P.C., MCKEOUGH, M. & BACH, F.H. (1976) Generation of cytotoxic lymphocytes *in vitro* against autologous human leukemia cells. *Nature*, **262,** 691.

ZIER, K.S., HUBER, C. & BRAUNSTEINER, H. (1979) Restimulation in secondary MLC by autologous non-T cells. *Scand. J. Immunol.* **9,** 493.