# AUTOLOGOUS LEUKEMIA-SPECIFIC T-CELL-MEDIATED LYMPHOCYTOTOXICITY IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

By S. K. LEE* and R. T. D. OLIVER‡

*(From the Imperial Cancer Research Fund, Department of Medical Oncology, St. Bartholomew's Hospital, London, E.C.1., England)*

It is more than 80 years since the first attempts were made to immunize human cancer patients against their own tumors (for review see reference 1). As yet, there are no immunological manipulations of proven value in terms of ensuring long-term cure, though there are randomized control studies demonstrating short-term prolongation of survival in patients with acute myelogenous leukemia receiving bacille Calmette-Guérin (BCG)[1] with or without allogeneic leukemic blasts (for review see reference 2). In experimental animals, particularly inbred mice, it has been considerably easier to demonstrate tumor-specific antigens, both by serological and cell-mediated techniques and to prolong survival by immunizing against these antigens (3). Despite a welter of publications, there are few studies in human tumor systems where the concept of tumor-specific antigens has been clearly established and universally confirmed. In particular, though many authors have demonstrated lymphocyte-mediated cytotoxicity against tumor lines in both normal individuals and patients with tumors (4, 5), evidence for T-lymphocyte-mediated autologous tumor cytotoxicity has been very difficult to establish.

In vitro assays of transplantation rejection using the mixed lymphocyte culture and cell-mediated lympholysis tests, have provided clear insight into the nature of antigenic determinants necessary for generation of cytotoxic T cells. The pioneering studies of Eijsvoogel et al. (6) on HLA recombinant families demonstrated the need for two types of antigens, the first type coded for by genes closely associated with those of the HLA D locus cause lymphocyte activation, whereas the second class of determinants, closely associated with the serologically defined HLA A, B, and C locus antigens, acts as the target for the cytotoxic T lymphocytes. Eijsvoogel et al. (7) also showed that production of cytotoxic T lymphocytes was possible even if the lymphocyte-activating determinant and serologically defined antigens were present on different cells. Zarling et al. (8) applied this principal to study leukemia patients and then reported one patient where stimulation of remission lymphocytes by mitomycin C inactivated autologous myeloblasts and a third party inactivated allogeneic lymphocytes in short-term culture led to the production of cytotoxic cells against autologous blasts but not the remission lymphocytes.

We report and confirm this observation in a series of 14 patients with acute myelogenous leukemia and investigate the nature of the effector cell, the helper

* Present address: Department of Immunology, National Institute of Medical Research, London N7, England.

‡ Present address: Department of Oncology, Institute of Urology, London WC2, England.

[1] *Abbreviations used in this paper:* AML, acute myelogenous leukemia; BCG, bacille Calmette-Guérin; CML, cell-mediated lympholysis; cpm, counts per minute; PBS, phosphate-buffered saline; PHA, phytohemagglutinin.

antigen on the third party cell, and the target antigen on the leukemia blast, and consider the relevance of these observations for development of rational immunotherapy for this disease.

## Materials and Methods

*Patients.* Criteria for the diagnosis of acute myelogenous leukemia and therapeutic protocols used have been reported in detail elsewhere (9, 10). All the patients studied were in complete remission and received either chemoimmunotherapy with BCG plus irradiated allogeneic leukemic myeloblasts according to the protocol reported by Powles et al. (11, 12) or the same chemotherapy plus BCG alone (2). The leukemic myeloblasts were removed from patients at the time of diagnosis, using the IBM Blood Cell Separator (IBM Corp., White Plains, N.Y.) (13) and then frozen in liquid nitrogen using techniques reported elsewhere (14). Before use they were thawed and treated with 10,000 rads in a linear accelerator.

*Cytotoxicity Assay.* Remission lymphocytes were prepared from defibrinated peripheral blood using the Ficoll-Hypaque technique (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N.J.) and adjusted to a concentration of $1 \times 10^6$/ml. They were set up in mixed cell culture with equal volumes of stimulators adjusted to the same concentration after inactivation with mitomycin C (25 $\mu$g/5 $\times 10^6$ cells in 1 ml for 30 min at 37°C and then washed twice). After 6–7 days, culture in 5% $CO_2$ and air at 37°C using RPMI 1640 medium supplemented with 4 mM glutamine and 10% AB serum, the cells were harvested and washed. To measure cytotoxicity $5 \times 10^5$ primed cells were incubated with $1 \times 10^4$ $^{51}$Cr-labeled target cells (200 $\mu$Ci/$10^7$ cells incubated for 1 h at 37°C and washed twice) in a total volume of 0.2 ml with RPMI-1640 and 10% AB serum in V-shaped Cooke microtiter plates (M220). After 4 h the plates were spun gently and the 0.1-ml supernate was assayed for counts per minute (cpm) $^{51}$Cr release using a gamma counter.

*Preparation of T- and B-Rich Effector Cell Population.* The technique used was that of Greaves et al. (15) in 1974. Briefly, 200 $\mu$l of packed sheep erythrocytes (washed twice in acetate buffer, pH 5.5) was incubated with 15 U of neuraminidase (Behring Diagnostics, American Hoechst Corp., Somerville, N.J.) for 1/2 h at 37°C. Then it was washed once in phosphate-buffered saline (PBS) and resuspended in 1 ml TC199 to give a 20% cell suspension. An equal volume of leukocytes separated by the Ficoll-Hypaque technique ($4 \times 10^7$ cells/ml) was mixed with equal quantities of erythrocyte suspension together with 3 ml of TC199 and 3 ml of heat-inactivated fetal calf serum. The mixture was incubated for 10 min at 37°C, then spun at 1,500 rpm (300 *g*) for 5 min, reincubated at 4°C for 20 min, and then the cells were gently resuspended, respun, and left at 4°C for 1 h. The rosette-forming cells were separated from nonrosetting cells after a second centrifugation on Ficoll-Hypaque gradient. The sheep erythrocytes were removed from rosette-forming cells by Tris ammonium chloride lysis.

Horse anti-human thoracic duct lymphocyte serum was absorbed with chronic lymphatic leukemia cells (sequential absorption for 30 min at room temperature with cells from three different patients) according to the technique of Brochier et al. (16), and then spun at 100,000 *g* for 1 h at 40°C. Chimpanzee anti-human chronic lymphatic leukemia serum after absorption with pooled platelets was provided by Shroeder and Van Rood. Before testing, effector cells from a 6-day culture were treated with an equal volume of absorbed serum and incubated at 37°C for 30 min, spun, and then resuspended in an equal volume of rabbit complement diluted 1:2 in TC199. Incubation was continued for 1 h at room temperature and then the cells were washed twice in TC199 and tested for cytotoxicity as described above.

## Results

Table I summarizes details of cpm data on results from 14 patients and shows how the spontaneous release and specific release are calculated. When remission lymphocytes from the acute myelogenous leukemia (AML) patients were cultured with autologous blast cells and allogeneic lymphocytes, it became clear that the occurrence of chromium release was greater here than in the control cultures (even for the cells where the spontaneous release is quite high).

TABLE I
*Summary cpm Data from Patients with AML*

| Patient | $^{51}$Cr release from autologous blast cells | | |
|---|---|---|---|
| | Spontaneous | Test* | Total‡ |
| | % | % | |
| M. B. | 395 (14)§ | 1,475 (43)‖ | 2,890 |
| P. W. | 507 (31) | 1,178 (60) | 1,627 |
| J. R. | 335 (45) | 379 (11) | 740 |
| G. P. | 171 (9) | 390 (12) | 1,944 |
| J. K. | 672 (33) | 926 (19) | 2,017 |
| M. S. | 1,739 (36) | 3,317 (51) | 4,812 |
| I. C. | 1,224 (35) | 1,856 (28) | 3,486 |
| A. T. | 588 (21) | 818 (10) | 2,822 |
| T. W. | 304 (25) | 866 (56) | 1,200 |
| M. F. | 397 (27) | 585 (17) | 1,491 |
| E. B. | 393 (20) | 396 (0.2) | 1,940 |
| F. H. | 257 (39) | 283 (6) | 600 |
| B. R. | 670 (23) | 684 (1) | 2,915 |
| E. H. | 180 (30) | 180 (0) | 601 |

* Remission lymphocytes primed by autologous leukemic blasts plus allogeneic lymphocytes.
‡ Cell frozen and thawed × 3.
§ Spontaneous cpm × 100/total cpm.
‖ (Test cpm − spontaneous cpm) × 100/total cpm − spontaneous cpm.

That this release is not a nonspecific effect of short-term culture is shown in Table II, which demonstrates that in those cultures where there is significant cytotoxicity against autologous blasts, there is not a concomitant cytotoxicity against the autologous remission lymphocytes. Culture of remission lymphocytes alone for 6–7 days (column 1) produced weak cytotoxicity against the autologous blast cell in 3 of the 14 patients (3–6% release). Culture of remission lymphocytes with inactivated autologous blast cells alone (column 2) produced weak killing (1–8% release) in nine of the patients (median 5%). Culture with inactivated allogeneic lymphocytes (column 3) produced slightly greater killing (6–30% release) in 5 of the patients (median 8%); whereas culture with a mixture of mitomycin C-treated autologous blast cells and allogeneic lymphocytes (column 4) produced killing in 11 of 14 experiments (6–60% release, with a median of 19%). This activity was greater in those who had been in remission longer than 6 mo (nine out of nine positive), compared with those in remission for less than 6 mo (two out of five positive). As a measure of reproducibility, results from two patients tested on several occasions are shown in Table III. Patient M. B. had been in remission for 36 mo and had stopped receiving maintenance chemotherapy 2 yr previously. Patient T. W. received his last course of maintenance chemotherapy after the second assay and when tested 2 days after completing this course of treatment, had lost all reactivity. This gradually returned, and when tested 2 mo after chemotherapy, was greater than before chemotherapy.

*Fractionation Studies of Effector Cells.* Two approaches have been used to investigate the nature of the cell involved (Tables IV, V). In the first experi-

TABLE II
*Specificity Controls Anti-Leukemia Cytotoxicity Assay*

| Patient | Time in CR* | $^{51}$Cr release from autologous leukemia blasts | | | | $^{51}$Cr release from allogeneic lymphocytes‡ | | | | $^{51}$Cr release from autologous lymphocytes‡ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| | *mo* | % | | | | % | | | | % | | | |
| M. B. | 36 | ND | 6 | 8 | 43 | ND | 0 | 23 | 29 | ND | ND | ND | ND |
| P. W. | 8 | 0 | 0 | 0 | 60 | 0 | 9 | 44 | 35 | 0 | 0 | 0 | 0 |
| J. R. | 10 | 0 | 2 | 0 | 10 | 0 | 0 | 34 | 17 | 0 | 0 | 0 | 0 |
| G. P. | 12 | 6 | 8 | 13 | 12 | 3 | 11 | 60 | 11 | 0 | 0 | 0 | 0 |
| J. K. | 15 | 0 | 0 | 0 | 19 | 2 | 0 | 50 | 47 | 0 | 0 | 2 | 6 |
| M. S. | 10 | ND | 6 | ND | 52 | ND | 0 | ND | 6 | 0 | 0 | 3 | 0 |
| I. C. | 20 | 3 | 5 | 30 | 28 | 5 | 3 | 10 | 8 | 0 | 0 | 0 | 0 |
| A. T. | 14 | 0 | 0 | 6 | 10 | 4 | 0 | 10 | 12 | 0 | 0 | 3 | 0 |
| T. W. | 10 | 0 | 2 | 0 | 9 | 2 | 1 | 48 | 74 | 0 | 0 | 0 | 0 |
| M. F. | 5 | 0 | 1 | 6 | 19 | 0 | 0 | 5 | 6 | 0 | 0 | 0 | 0 |
| E. B. | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 |
| F. M. | 5 | 0 | 5 | 0 | 6 | 0 | 0 | 9 | 3 | 0 | 0 | 0 | 0 |
| B. R. | 4 | 3 | 1 | 0 | 1 | 0 | 0 | 19 | 10 | 0 | 0 | 0 | 0 |
| E. M. | 3 | 0 | 0 | 0 | 0 | 3 | 5 | 14 | 26 | 0 | 0 | 0 | 0 |

* CR, Complete remission; ND, not done.

‡ Cultured with PHA for 3 days before labeling.

*1*, Culture of remission lymphocytes for 7 days, *2*, culture of remission lymphocytes for 7 days with autologous leukemic blasts, *3*, Culture of remission lymphocytes for 7 days with allogeneic lymphocytes, *4*, Culture of remission lymphocytes for 7 days with autologous blasts and allogeneic lymphocytes.

ments, after a 7-day culture with autologous blasts and allogeneic lymphocytes, the remission lymphocytes were treated with either a horse anti-human thoracic duct lymphocyte serum absorbed with chronic lymphatic leukemia cells, shown to be specific for T cells, or chimpanzee anti-human chronic lymphatic leukemia serum absorbed with pooled platelets and shown to be specific for B cells. In all three experiments, the reactivity was lost after treatment with the anti-T serum, but after treatment with anti-B reactivity was unchanged in two of the experiments and only slightly reduced in the third (Table IV).

In the second type of experiment, effector cells recovered from cultures of remission lymphocytes with Mitomycin-C, inactivated autologous blast cells and allogeneic lymphocytes were separated into T- and B-rich fractions using the neuraminidase-treated sheep erythrocyte technique described in the Materials and Methods. The two populations were tested against autologous blasts and cytotoxicity could only be demonstrated in the E rosette-positive population (Table V).

*Nature of Determinant on Third Party Allogeneic Cell.* To compare the effectiveness of lymphocyte-activating determinants of the HLA D region and the serologically defined antigens of the HLA A, B, and C region, experiments were undertaken using mitomycin C-inactivated lymphoblastoid cells instead of allogeneic lymphocytes as third party cells in the mixed cultures. Cytotoxic cells reactive with autologous blast cells were produced when Daudi cells were

TABLE III

*Reproducibility of Autologous Leukemia Blast Cell Cytotoxicity Assay*

| Primary mixture | Autologous blasts as targets | | | | Autologous PHA transformed lymphocytes as targets | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| T. W. | 0 | 0 | 6 | 13 | 7 | 13 | 2 | 4 |
| | 8 | 0 | 0 | 25 | 0 | 0 | 0 | 0 |
| | ND | 0 | 0 | 0 | ND | 3 | 2 | 3 |
| | 0 | 0 | 0 | 7 | 2 | 2 | 2 | 3 |
| | ND | 0 | ND | 6 | ND | 0 | ND | 0 |
| | ND | ND | ND | 8 | ND | ND | ND | 2 |
| | ND | 3 | ND | 10 | ND | 0 | ND | 0 |
| | ND | 11 | ND | 34 | ND | 0 | ND | 0 |
| M. B. | 12 | 3 | 7 | 118 | 0 | 0 | 0 | 0 |
| | 0 | 5 | 18 | 14 | 0 | 0 | 0 | 0 |
| | ND | 6 | 8 | 43 | ND | ND | ND | ND |
| | ND | 10 | ND | 45 | ND | ND | ND | ND |
| | 0 | 15 | 0 | 62 | 2 | 4 | 5 | 0 |

Explanation of columns is the same as Table II.

TABLE IV

*Effect of Anti-T- and B-Cell Serum on Cell-Mediated Autologous Leukemia Cell Lysis*

| Treatment of effector cell | Viable effector cell recovered after antisera treatment | Cytotoxicity* against autologous leukemia blasts | | |
|---|---|---|---|---|
| | Expt. 2 | Expt. 1 | Expt 2. | Expt. 3 |
| | % | | % | |
| Untreated | 100 | 45 | 51 | 14 |
| Complement alone | 88 | 43 | 46 | 14 |
| Anti-CLL‡ (absorbed with platelets and thymocytes) + complement | 61 | 44 | 28 | 12 |
| Anti-T cell (absorbed with CLL) + complement | 18 | 0 | 0 | 0 |

* Viable effector cell to target cell ratio 50:1.

‡ CLL, chronic lymphocytic leukemia.

TABLE V

*Effect of T- and B-Cell Separation on Cell-Mediated Autologous Leukemia Cell Lysis*

| Treatment of effector cells | E Rosette-positive cells in effector population | | Cytotoxicity against autologous leukemia blasts | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| | % | | % | |
| Unfractionated | 72 | 79 | 28 | 4 |
| T cells* | ND | 91 | 27 | 10 |
| B cells* | ND | 4 | 0 | 0 |

* See Materials and Methods for technique of separation.

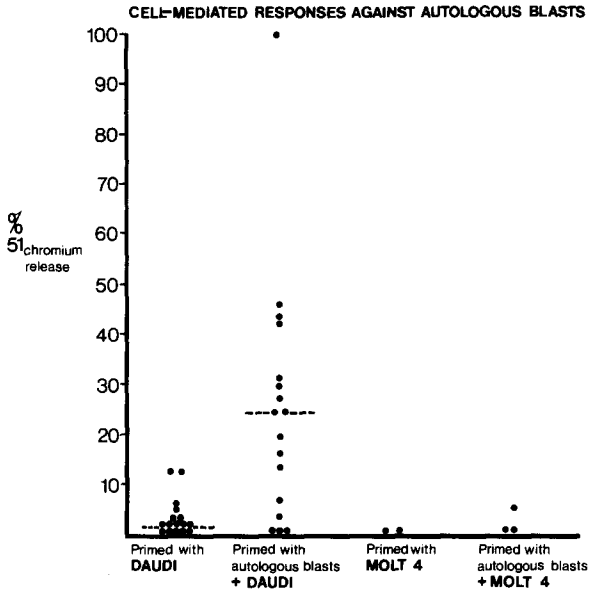


FIG. 1. Cytotoxicity against autologous blast cell after priming with Daudi or Molt 4.

TABLE VI
*Comparison of Allogeneic Blasts and Remission Lymphocytes as Stimulators and Targets in CML Assay*

| | | Target | |
|---|---|---|---|
| | | Allogeneic lymphocyte | Allogeneic blast |
| | | % | % |
| Stimulator | Allogeneic lymphocyte | 13* | 11.6* |
| | Allogeneic blast | 4.1* | 4.7* |

* Percentage $^{51}Cr$ release is mean of four experiments.

mixed with autologous blast cells and used to stimulate remission lymphocytes but not when Molt 4 cells were mixed with autologous blast and used to stimulate remission lymphocytes (Fig. 1). Further evidence that myeloid leukemia blasts in general have a poorer capacity for lymphocyte activation comes from comparative study of remission lymphocytes and acute myelogenous leukemic blast cells used as allogeneic stimulators and targets in cell-mediated lympholysis assays. Table VI shows, that as stimulators, myeloid leukemia blast cells are considerably less effective than remission lymphocytes, whereas there is minimal difference in terms of the effectiveness of myeloid blast cells as targets for mixed lymphocyte culture-generated cytotoxic lymphocytes.

*Nature of Target Antigen on Autologous Blast Cell.* Two approaches have been used to study this problem. Firstly, mixing equal quantities of unlabeled target cells with $^{51}Cr$-labeled blast cells showed that it was only possible to inhibit cytotoxicity using unlabeled autologous leukemic myeloblasts, but not unlabeled phytohemagglutinin (PHA)-transformed remission lymphocytes or

TABLE VII
*Cold Target Experiments*

| Exp. | Targets | | | |
|---|---|---|---|---|
| | Autologous blast ($^{51}Cr$) ($1\times10^4$) | Autologous blasts ($^{51}Cr$) ($1\times10^4$) + autologous blasts ($1\times10^4$) | Autologous blasts ($^{51}Cr$) ($1\times10^4$) + autologous remission lymphocytes ($1\times10^4$) | Autologous blasts ($^{51}Cr$) ($1\times10^4$) + allogeneic blasts ($1\times10^4$) |
| | % | % | % | % |
| 1 | 34 | 5 | 36 | 31, 34, 37 |
| 2 | 21 | 1 | 18 | (23*), 19 |
| 3 | 12 | 0 | 6 | 12, 18 |
| 4 | 43 | 30§ | 41§ | 35‡·§ |
| | | 6‖ | 39‖ | 26‡·‖ |
| | | 0¶ | 40¶ | 33‡·¶ |

* Cold targets = Daudi.
‡ Cold targets = allogeneic PHA blasts.
§ Cold targets $1\times10^3$.
‖ Cold targets $1\times10^4$.
¶ Cold targets $4\times10^4$.

TABLE VIII
*Autologous Blasts as Targets after Priming*

| Patient | Autologous blast | Allogeneic lymphocytes | Allogeneic lymphocytes + autologous blasts | Allogeneic blast | Allogeneic blast + allogeneic lymphocytes |
|---|---|---|---|---|---|
| | % | % | % | % | % |
| T. W. | 0 | 0 | 9 | 0 | 0 |
| L. F. | 0 | 0 | 16 | 0 | 0 |
| M. B. | ND | 0 | 23 | 7 | 5 |
| T. W. | ND | 1 | 51 | 0 | 0 |

allogeneic leukemic myeloblasts, Daudi cells, or allogeneic lymphocytes (Table VII).

Secondly, taking pairs of AML patients, stimulating remission lymphocytes from one patient with allogeneic blast cells from the other patient even in the presence of allogeneic lymphocytes from that second patient would not produce cytotoxic cells against autologous blasts, even though the allogeneic lymphocytes from that second patient could cooperate with the autologous blasts to cause generation of cytotoxic cells (Table VIII).

Indirect evidence to support the suggestion from these experiments that the target antigen on the autologous cell is unique for each patient, comes from studies using allogeneic blast cells to prime remission lymphocytes. In a series of 23 experiments, using AML remission lymphocytes primed with allogeneic leukemic myeloblasts, mean specific release from autologous blast was 5%, compared to 6% mean release when allogeneic lymphocytes were used, 4% mean release when the specific autologous blast cells were used and 19% mean release when a mixture of autologous blasts and allogeneic lymphocytes were used for priming.

To exclude the involvement of the patient's own D locus determinants known to be present on B cell and myeloid leukemic blast cells, experiments were performed priming remission lymphocytes with purified autologous B cells plus Daudi cells. No cytotoxicity was generated against the autologous blast cell. In addition after priming remission lymphocytes with autologous blast cells and allogeneic lymphocyte or Daudi cells, effector cells with cytotoxicity against the specific leukemic blast cell showed no cytotoxicity against purified autologous T or B cells. In a single experiment, effector cells generated by priming with autologous blast plus Daudi cells showed no cytotoxicity against autologous remission bone marrow.

## Discussion

The idea of immunological stimulation by one molecule augmenting response to an otherwise nonimmunogenic molecule is not new. In experimental animals, the use of carrier molecules to stimulate antibody response to haptens is a standard procedure and the interdependence of T and B cells in these responses is well documented (for review, see reference 17). In man, Eijsvoogel et al. (6) were the first to demonstrate the interaction between the two molecules that was necessary for the generation of cytotoxic T cells against transplantation antigens. He also showed that it was not necessary for the HLA D lymphocyte-activating determinant to be on the same cell as the HLA A, B, or C target antigen (7). After this, Schendell and Bach (18) were able to demonstrate the same principal in mice and, more recently, Zarling et al. (8) took this principal and applied it to study response of leukemic patients to their own blast cells. They showed that remission lymphocytes cultured with autologous blasts and allogeneic lymphocytes generated cytotoxic cells reacting with autologous leukemic blasts in one out of six patients studied. Sondell et al. (19) showed that lymphocytes from siblings HLA identical with patients with acute leukemia could also respond to patient's blasts and allogeneic lymphocytes and generate cytotoxic cells in three out of four patients tested.

Our data confirms these observations in a larger series of patients but, in addition, has investigated in greater depth the underlying mechanism. The studies using Daudi, a lymphoblastoid cell line which has lost all evidence of HLA A, B, and C locus antigens but retains HLA D locus antigens (20) and Molt 4, a leukemia cell line which has no detectable HLA D antigens but readily demonstrable HLA A and B locus antigens (21) would suggest that, as in the response against transplantation antigens in allogeneic cell-mediated lympholysis (CML), the HLA D region stimulus is what is lacking from cultures stimulated by autologous blast cells alone.

The evidence from Table V that even as stimulators of allogeneic CML, blast cells are deficient of HLA D-associated lymphocyte-activating determinants compared to normal peripheral blood cells is in contrast to the relative ease with which HLA D-associated serologically defined antigens (formerly known as 'Ia' like, now designated as DR i.e. D related) have been demonstrated on AML blasts (22). Further investigation of this observation may provide important evidence to help resolve the controversy of whether the two techniques are defining the same antigenic determinant. The lack of cytotoxicity of primed remission lymphocytes against autologous B cells excludes the possibility that

the target antigen on the leukemic blast is a normal B-cell antigen which Opelz et al. (23) have suggested may be the cause of autologous stimulation of T cells by B cells.

Though there are many studies in the literature on cytotoxic cells against human tumors, with the exception the study of Jondal et al. (24) on a single patient with Burkitt's lymphoma, none of these systems have been clearly demonstrated to be mediated by T cells. The observations reported here are very important because of the significant role T cells have been shown to have in tumor rejection in experimental animals (for review see reference 25).

Three approaches reported in this paper (cold target inhibition experiments, failure to generate cytotoxicity with allogeneic blast cells, and the crossover experiments with pairs of patients) suggest that the target antigenic determinant on the patient's blast cell is unique for that patient's blast. Obviously, only by testing an extremely large panel of allogeneic lymphocytes and blast cells would it be possible to exclude that there is any HLA restriction influencing the target antigen as reported for mouse cytotoxicity reactions (26). Likewise, only extensive cross-testing will enable one to exclude that the target antigen is an altered normal transplantation antigen as has been found for some tumor antigens in the mouse (27).

In spite of the need for more information on these points, the observation is important from two points of view. First, from the theoretical point of view, most experimental animals' tumors with a viral etiology have common tumor-specific antigens, whereas unique determinants are more usually found in non-virally induced tumors (28, 29). Although this is not proof of the etiology of leukemia as the target could possibly be HLA restricted, the failure to isolate infectious viruses from human leukemias using the techniques which easily detect viruses in animal tumors is increasingly raising doubts about the role of viruses in the pathogenesis of human leukemia. This viewpoint has been supported by the failure of Ihle et al. (30, 31) to find evidence of viral expression in the majority of radiation-induced lymphomas in C57B1/6 mice.

Secondly, from the practical viewpoint of clinical studies of immunotherapy, the demonstration of a unique antigenic determinant on the patient's blast cell raises doubts as to the value of using allogeneic blast cells for immunotherapy in this disease. So far, in a study comparing BCG with BCG and allogeneic blast cells in patients with AML, we have found no difference in remission lengths or survival of our patients (2). Clearly it will be important to see if the principals which have been developed from our in vitro studies can be applied in vivo using an animal model before using this approach in clinical studies. In the meantime it is important that the failure of current studies of immunotherapy in man to lead to an increase in the long-term cure of patients should not lead to the conclusion that active specific immunotherapy is of no benefit. The studies reported here would suggest that it has not so far been adequately tested.

## Summary

Short-term culture of acute myelogenous leukemia patient's remission lymphocytes with inactivated autologous leukemic blast cells plus allogeneic

lymphocytes, generated effector T lymphocytes which were cytotoxic for the specific autologous blast cell in 11 of 14 patients studied. Experiments using Daudi and Molt 4 lymphoblastoid cell lines as a third-party helper cell suggest that an HLA D locus incompatability is necessary to provide effective help in this system. Cold target inhibition experiments, crossover studies between pairs of patients, and experiments with allogeneic leukemic blast cells as priming stimulus suggest that the target antigen is only present on the specific autologous blast cell.

We gratefully acknowledge the helpful advice of Professor N. A. Mitchison during the course of this work, Dr. M. Steel for providing us with stock of Molt 4 and Daudi lymphoblastoid cell lines, and Anthea Minchom for her secretarial assistance.

*Received for publication 15 November 1977.*

## References

1. Currey, G. A. 1972. 80 years of immunotherapy. *Br. J. Cancer.* 26:141.
2. Oliver, R. T. D. 1977. Active specific and non-specific immunotherapy for patients with AML. Proceedings of the 3rd International Congress on Immunology. D. Nelson, editor. In press.
3. Baldwin, R. W. 1977. Immune responses to tumour specific and embryonic antigens on chemically induced tumours. Proceedings of the 3rd International Congress on Immunology. D. Nelson, editor. In press.
4. Hellstrom, I., H. Sjogren, and G. A. Warner. 1971. Demonstration of cell mediated immunity to human neoplasms of various histological types. *Int. J. Cancer.* 7:1.
5. Takasugi, M., M. R. Mickey, and P. I. Terasaki. 1974. Studies on specificity of cell mediated immunity to human tumours. *J. Natl. Cancer Inst.* 53:1527.
6. Eijsvoogel, V. P., M. J. G. S. du Bois, C. J. M. Melief, W. P. Zeylemaker, L. Raat-Koning, and L. de Groot-Kooy. 1973. CML in relation to MLC and HLA. *Transplant. Proc.* 5:1301.
7. Eijsvoogel, V. P., M. J. G. J. de Bois, A. Meinesz, A. Bierhorsh-Eijlander, W. P. Zeylermaker, and T. Th. A. Schelleken. 1973. The specificity and activation mechanism of CML in man. *Transplant. Proc.* 5:1675.
8. Zarling, J. M., P. C. Raich, M. McKeough, and F. H. Bach. 1976. Generation of cytotoxic lymphocytes in vitro against autologous human leukaemia cells. *Nature (Lond.).* 262:691.
9. Crowther, D., R. L. Powles, and C. J. T. Bateman. 1973. Management of adult acute myelogenous leukaemia cells. *Br. Med. J.* 1:131.
10. Crowther, D., M. E. J. Beard, and C. J. T. Bateman. 1975. Factors influencing prognosis in adults with acute myelogenous leukaemia. *Br. J. Cancer.* 32:456.
11. Powles, R. L., D. Crowther, C. J. T. Bateman, M. E. J. Beard, T. J. McElwain, J. Russell, T. A. Lister, J. M. A. Whitehouse, P. F. M. Wrigley, M. Pike, P. Alexander, and G. Hamilton Fairley. 1973. Immunotherapy for AML. *Br. J. Cancer.* 28:365.
12. Powles, R. L., J. Russell, T. A. Lister, R. T. D. Oliver, J. M. A. Whitehouse, R. Peto, B. Chapuis, D. Crowther, and P. Alexander. 1977. Immunotherapy for AML. *Br. J. Cancer.* 35:265.
13. Powles, R. L., T. A. Lister, and R. T. D. Oliver. 1974. A safe method for collecting leukaemia cells for use as immunotherapy. *Br. Med. J.* 4:375.
14. Oliver, R. T. D. 1976. Cryopreservation of leukaemia cells and their use for active specific immunotherapy. *INSERM (Inst. Natl. Sante Rech. Med.) Colloq.* 62:335.
15. Greaves, M. F., G. Janossy, and M. Doenoff. 1974. Selective triggering of human T

and B lymphocytes in vitro by polyclonal mitogens. *J. Exp. Med.* 140:1.
16. Brochier, J., Y. A. Abon-Hamed, J. P. Gueko, and J. P. Revillard. 1976. Study of human T and B lymphocytes with heterologous antisera. *Immunology*. 31:749.
17. Hogg, N., U. Koozinowski, and N. A. Mitchison. 1977. Principals governing the response to cell surface antigens. Proceedings of the 3rd International Congress on Immunology. D. Nelson, editor. In press.
18. Schendell, D. J., and F. H. Bach. 1974. Genetic control of cell mediated lympholysis in mouse. *J. Exp. Med.* 140:1534.
19. Sondell, P. M., C. O'Brien, L. Porter, S. F. Schlossman, and L. Chess. 1976. Cell mediated destruction of human leukaemic cells by MHC identical lymphocytes. *J. Immunol.* 117:2197.
20. Fellows, M., F. Mortchelewiey, M. Kamoun, and J. Dausset. 1975. Use of a lymphoblastoid cell line to define B lymphocyte specificities probably controlled by the MHC region. Histocompatibility Testing. F. Kissmeyer, editor. Munksgaard, A/S, Copenhagen. 708.
21. Royston, I., R. B. Pitts, R. W. Smith, and P. R. Graze. 1975. In vitro immunisation against cultured human B and T lymphoblastoid cell lines. *Transplant. Proc.* 7:531.
22. Schlossman, S. F., L. Chess, R. E. Humphreys, and J. L. Strominger. 1976. Distribution of Ia like molecules on the surface of normal and leukemic human cells. *Proc. Natl. Acad. Sci. U. S. A.* 73:1288.
23. Opelz, G., M. Kiuchi, M. Takasugi, and P. I. Terasaki. 1975. Autologous stimulation of human lymphocyte subpopulations. *J. Exp. Med.* 142:1327.
24. Jondal, M., E. Svedmyr, E. Klein, and S. Singh. 1975. Killer T cells in Burkitt's lymphoma biopsy. *Nature (Lond.)*. 255:405.
25. Allison, A. C. 1977. Immunological surveillance of tumours. *Cancer Immunol. Immunotherapy*. 2:151.
26. Zinkernagel, R. M., and P. C. Doherty. 1975. H-2 compatibility requirement for T cell mediated lysis of targets infected with lymphocyte choriomeningitis. *J. Exp. Med.* 141:1426.
27. Garrido, F., H. Festensein, and V. Shirmacher. 1976. Further evidence for derepression of H2 and Ia like specificities of foreign haplotypes in mouse tumour cell lines. *Nature (Lond.)*. 261:705.
28. Old, L., and E. A. Boyse. 1964. Immunology of experimental tumours. *Annu. Rev. Med.* 15:167.
29. Klein, G. 1969. Experimental studies in tumor immunology. *Fed. Proc.* 28:1739.
30. Ihle, J. N., R. McEwan, and K. Bengali. 1976. Radiation leukaemia in C57BL/6 mice. I. Lack of serological evidence for role of endogenous ecotropic viruses in pathogenesis. *J. Exp. Med.* 144:1391.
31. Ihle, J. N., D. R. Joseph, and N. M. Pazmino. 1976. Radiation leukaemia in C57BL/6 mice. II. Lack of ectropic virus expression in the majority of lymphomas. *J. Exp. Med.* 144:1406.