Deficiency of Antibody-dependent Cellular Cytotoxicity and Mitogen-induced Cellular Cytotoxicity Effector Cell Function in Patients with Acute Myelogenous Leukemia in Remission

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ABSTRACT

Patients with acute myelogenous leukemia in remission have pronounced deficiency in antibody-dependent cellular cytotoxicity (ADCC) and mitogen-induced cellular cytotoxicity. The deficiency in ADCC was partly explained by reduction in the number of circulating effector cells (Fc receptor-bearing cells) demonstrable at a time when white blood cell and platelet counts were normal. These cytotoxic functions, as well as the circulating numbers of T-cells and Fc receptor-bearing cells were further decreased by the administration of monthly cycles of combination chemotherapy with 1-β-D-arabinofuranosycytosine and 6-thioguanine. Following each cycle of chemotherapy, progressive recovery of these functions occurs during the third and fourth weeks with occasional increases above base line in patients in whom chemotherapy is withheld for longer than five weeks. In selected patients, recovery of one cytotoxic function preceded the other, indicating that these functions are mediated by different effector cells. Administration of a single dose of daunomycin i.v. had no effect in either of these cytotoxic functions or in the circulating numbers of lymphocytes. The decrease in ADCC effector cell function induced by phase cycle-specific agents correlated with the level of reactivity exhibited by patients after achieving bone marrow and clinical remission. Patients showing low levels of reactivity postremission experienced highest degree of depression. In two patients, complete abrogation of ADCC effector function was demonstrated with minimal recovery even six weeks after stopping chemotherapy.

These findings indicate that effector cells in ADCC and mitogen-induced cellular cytotoxicity are highly susceptible to phase cycle-specific agents, and their recovery takes longer than that of other lymphoid and nonlymphoid populations.

INTRODUCTION

Cytotoxic drugs used for the treatment of neoplastic disorders can induce significant alterations of the functions of T- and B-lymphocytes (1, 14, 17). Little is known, however, of the effects of drugs on lymphocyte-mediated cytotoxicity. The present investigations evaluated the effects of chemotherapy given at monthly intervals on ADCC and MICC, respectively.

This study was designed to answer the following questions: (a) are cytotoxic effector cell function, i.e., ADCC and MICC, depressed in patients with AML in remission who have normal numbers of circulating granulocytes and platelets; (b) do monthly courses of maintenance chemotherapy with ara-C and 6-TG (given to patients in remission) affect lymphocyte effector cell functions; and (c) what is the kinetics of recovery for lymphocyte subpopulation after each cycle of chemotherapy.

The results obtained clearly demonstrate that effector cell function in ADCC and MICC is significantly reduced in patients achieving bone marrow remission. Further reduction of this cytotoxic effector function occurs as a result of monthly courses of chemotherapy. The decrease in effector function in ADCC was partly explained by decreased number of circulating Fc-R, which contain the effector cells participating in ADCC. The effects of chemotherapy on these functions were maximal 2 to 3 weeks postinitiation of therapy and persisted past the time granulocytic and megakaryocytic recovery occurred.

MATERIALS AND METHODS

Patient Population. Fifteen adult patients (26 to 54 years) in bone marrow remission after receiving combination chemotherapy with ara-C, 6-TG, and daunomycin were evaluated prior to receiving their first course of maintenance chemotherapy and at weekly or monthly intervals thereafter. During remission induction, ara-C was given as a bolus every 12 hr for 7 days. The control population was composed of individuals between the ages of 21 and 70 healthy enough to work and evaluated in the same laboratory over the period of July 1976 to July 1978. Maintenance chemotherapy consisted of monthly cycles of ara-C (100 mg/sq m) i.v. every 12 hr for 10 dosages and 6-TG (100 mg/sq m) p.o. every 12 hr for 10 dosages repeated every 28 days. Every third cycle, the patients received instead a single dosage of daunomycin (60 mg/sq m i.v.). In selected cases, 6-TG was withheld secondary to liver toxicity. These patients received ara-C alone at the dosage indicated above.

Chromium Release Assay. Release of 51Cr from target cells is an effective method for studying cytolysis. Once released, the isotope is not reutilized by either lymphoid or target cells (2). The isotope is presented to target cells in its chromate form but released in a hexavalent or trivalent form, probably bound to small peptides. The later observation may explain why the isotope is not reutilized after release.

ADCC. The target cell used was a human B-lymphoblastoid cell line (LA-237) coated with either heterologous (rabbit) or homologous (anti-HLA) antisera. The heterologous antiserum was raised by repeated immunization of rabbits with 50 × 10^7 LA-237 cells given i.v. biweekly over a 4-month period. Homologous, monospecific anti-HLA-B5 antiserum was obtained

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from multiparous women. Maximal activity was obtained with a 1:5000 dilution of heterologous and a 1:1000 dilution of the homologous antisera, and these dilutions were used in all experiments. To assay effector cell function in ADCC, varying numbers of mononuclear cells in 0.1 ml of Eagle’s minimal essential medium containing 20% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) were mixed with 0.1 ml \( 51^{Cr} \)-labeled targets (10\(^3\)/tube) and 0.1 ml of either antisera. The mixture was centrifuged at 400 \( \times \) \( g \) for 2 min and incubated at 37\( ^{\circ} \)C in a rocker platform for 2 hr in 5% CO. To terminate the reaction, 1 ml of cold phosphate-buffered saline (pH 7.4) (NaCl, 8 g/liter) (Grand Island Biological Co.) was added to each tube, followed by centrifugation at 400 \( \times \) \( g \) for 10 min. The supernatant was decanted into fresh culture vessels, and the amount of \( 51^{Cr} \) released was measured using a \( \gamma \) counter (Nuclear Chicago Corp.).

Percentage of specific lysis was calculated using the following formula:

\[
\text{% specific lysis} = \frac{\text{Counts released in presence of antisera} - \text{Counts released in control cultures}}{\text{Counts released in detergent (Triton)} - \text{Counts released in control cultures}} \times 100
\]

Control cultures contained effector cells and targets in the absence of antisera. Counts released by 0.1\% Triton averaged approximately 80% of total counts incorporated by targets.

**Mononuclear Cell Preparation.** Mononuclear cells were obtained from heparinized (20 units/ml) peripheral blood by flotation in a Ficoll-Hypaque gradient according to the method described by Boyum (4). Harvested cells were washed 3 times with Hanks’ balanced salt solution and resuspended in Eagle’s minimal essential medium supplemented with glutamine, penicillin (100 units/ml) and streptomycin (100 mg/ml) (Grand Island Biological Co.). Phagocytic cells were routinely removed with the carbonyl iron-magnet technique (12). After purification, the samples contained 99\% lymphocytes which were over 95\% viable.

**Lymphocyte Surface Receptor Assays.** T-lymphocytes and Fc-R were measured by their ability to bind spontaneously to SRBC or to antibody IgG-coated SRBC, respectively, using standard methodological procedures (11).

**Mitogen-induced Cellular Cytotoxicity.** Effector cells and \( 51^{Cr} \)-labeled LA-237 were incubated at a 50:1 ratio for 2 hr in the presence of 10 \( \mu \)g reagent grade PHA (Burroughs Wellcome and Co., Research Triangle Park, N. C.). Control cultures consisted of effector cells and targets in the absence of PHA. At completion of the incubation time, the supernatant was assayed for chromium release with a \( \gamma \) counter, and percentage of specific lysis was calculated according to the formula described for ADCC (see above).

**Statistical Analysis.** Two-sample Student’s \( t \) test and paired \( t \) test were used to analyze these data.

**RESULTS**

Fifteen adult patients with AML in remission were evaluated for effector cell function in ADCC at a time when their WBC and granulocyte counts were normal (Table 1). As demonstrated, effector cell function in ADCC was markedly reduced when either heterologous (rabbit) or homologous (anti-HLA) serum was used to coat \( 51^{Cr} \)-labeled targets (52 and 38\% of control values, respectively). Comparison of ADCC effector function in individual patients to the day control revealed a reduction in ADCC of the order of 2.5 to 10 times (Table 2). In addition, the number of circulating Fc-R and B-cells was markedly decreased, whereas percentage of T-cells was unaffected (Table 3). When individual patients were examined, no correlation could be established between percentage of Fc-R and ADCC. Furthermore, presence of very low numbers of Fc-R (1 to 2\%) did not predict the degree of ADCC cytotoxic capacity the patient might exhibit. As demonstrated in Table 4, Patients D. S. and E. F. had ADCC activity in the normal range, whereas Patients N. H. and I. F. had markedly depressed ADCC activity, while all showed a profound decrease in percentage of Fc-R. This clearly demonstrates that the effects of therapy and disease on Fc-R participating in ADCC varies from patient to patient.

MICC cytotoxic activity was also markedly reduced in this population, suggesting a deficiency or functional derangement of this effector cell population as well (Table 1).

**Effects of Maintenance Chemotherapy on ADCC Effector Cell Function.** The patients evaluated received monthly courses of combination chemotherapy with ara-C and 6-TG according to the protocol described in “Materials and Methods.” Chemotherapy was only given when WBC was \( \geq 4000/\) cu mm, and platelet count was \( \geq 100,000/\) cu mm. As shown in Table 5, administration of chemotherapy resulted in progressive reduction in ADCC effector function which coincided with a parallel decrease in the percentage of Fc-R. This was already apparent on the first week following chemotherapy at a time when WBC and platelet counts were still normal. Decreased

Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>ADCC (%) specific lysis</th>
<th>No. of cells ( \times 10^5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML (remission)</td>
<td>15</td>
<td>7.1 ± 2.4</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>7.4 ± 2.8</td>
</tr>
</tbody>
</table>

\* Target cells coated with rabbit antiserum.
\* Target cells coated with human (anti-HLA) serum.
\* Mean ± S.D.
\* \( p < 0.001 \)
\* \( p < 0.01 \)

\* Caucasian population composed of individuals healthy enough to work and between the ages of 21 and 70.
Deficiency of ADCC and MICC Effectors

Progressive recovery in ADCC function was noted. The levels of activity demonstrated were always below 1 S.D. of those encountered in the normal population. On the contrary, individuals with high effector function showed less reduction in this cytotoxic activity following chemotherapy.

The effects of a single dose of daunomycin (60 mg/sq mm) in ADCC effector functions and on Fc-R was evaluated in 2 patients. As shown in Table 7, ADCC effector function increased progressively during this period of time and usually reached levels above those encountered before its administration. Clearly, daunomycin in a single dose had no effects on ADCC or MICC effector cell function.

By contrast, administration of ara-C as a single agent induced the same effects as the combination of the latter with 6-TG (Table 6).

Correlation between Fc-R and ADCC Effector Function. The chemotherapy used in this study markedly reduced the relative and absolute number of Fc-R. In multiple opportunities, percentage of Fc-R was less than 5% and not infrequently was 1%. This low percentage of Fc-R persisted beyond the time other cell lines were fully recovered, indicating that the reconstitution of Fc-R is delayed compared with granulocytes, platelets, or T-cells with surface receptors for SRBC. Patients with low percentage of Fc-R still were capable of showing adequate levels of ADCC effector cell function (Table 4). The degree of cytolysis, however, varied markedly between patients. Two (N. H. and L. L.) had low or no reactivity. Patient E. F. showed intermediate levels, whereas Patient D. S. reactivity was in the normal range.

Effects of Chemotherapy on MICC Function. The ability of patients’ lymphocytes to affect the destruction of 51Cr-labeled targets in the presence of PHA was markedly decreased. In some patients, decreased MCC correlated with decreased ADCC activity. In some cases, however, a clear disparity between these functions could be demonstrated. As shown in Table 8, Patients N. H., F. H., and D. E. exhibited normal ADCC effector function while their MCC activity was minimal. Opposite findings are demonstrated for Patients F. D., D. S., and J. McF.

MCC was further reduced by cyclic administration of ara-C and 6-TG (Table 5). Marked decrease was already observed at 1 week, with persistence for 3 weeks, and usually recovery after 4 weeks. In patients for whom chemotherapy was withheld ≥5 weeks, MCC levels rebounded to values above those encountered prior to start of that particular chemotherapy cycle. The kinetics of recovery for the overall population was similar to that encountered for ADCC. When individual patients were evaluated, kinetics of recovery for MCC and ADCC differed at times, indicating that these functions or the responsible effectors are differentially affected by the drugs administered.
Decreased numbers of single-cell conjugates with antibody-coated sheep erythrocytes. Percentages of Fc-R cells were quantitated by measuring binding to antibody-coated sheep erythrocytes. Most of these patients form heterogeneous populations participating in MICC (8, 30). Deficiency in Fc-R is readily detected. The latter is a heterogeneous population of cells that contains effector cells participating in ADCC (8, 22) and possibly one of the cell populations participating in MICC (8, 30). Deficiency in Fc-R after remission induction is in part selective since T-cell numbers were only moderately decreased, and granulocyte and platelet counts were within normal limits.

DISCUSSION

Adult patients with AML, in remission after intensive chemotherapy, show profound decrease in ADCC and MICC activity. It is possible that the decrease in ADCC activity is partly due to decreased number of circulating effector cells. Preliminary results in our laboratory indicate that these patients form smaller numbers of single-cell conjugates with antibody-coated targets (13) indicating a decrease in effector frequency.4 In addition, a significant decrease in the relative and absolute numbers of circulating Fc-R is readily detected. The latter is a heterogeneous population of cells that contains effector cells participating in ADCC (8, 22) and possibly one of the cell populations participating in MICC (8, 30). Deficiency in Fc-R after remission induction is in part selective since T-cell numbers were only moderately decreased, and granulocyte and platelet counts were within normal limits.

These observations indicate that recovery of Fc-R following aggressive chemotherapy is slower than for other lymphoid and nonlymphoid cell populations. In support of this is the observation that delay in institution of maintenance chemotherapy allows for further recovery in the numbers and function of Fc-R. Two patients on maintenance chemotherapy in whom treatment was discontinued for over 8 weeks (due to liver toxicity) showed complete recovery of their percentage of Fc-R and cytolytic effector function. From the data obtained, it is not possible to determine whether the decrease in cytolytic effector function is due to effects of the disease and/or the therapy. Judging from the effects of maintenance chemotherapy, it seems clear that at least 2 of the drugs used for remission induction (ara-C and 6-TG) destroy or inactivate Fc-R, thus decreasing ADCC and MICC effector function. It is reasonable to assume therefore that therapy must have a determining role in the induction of the functional derangement described.

After one course of combination chemotherapy with ara-C and 6-TG, a profound decrease in ADCC, MICC, and percentage of Fc-R occurs. These changes are most pronounced during the second and third weeks postchemotherapy, coinciding with the nadir of the drug effects on granulocyte and platelet counts. In most patients, percentage of Fc-R are markedly reduced (≤5% of circulating lymphocytes), and in some patients, they may not even be detectable at the peak of the chemotherapy effect. Evaluation of circulating Fc-R and ADCC effector function after a single dose of daunomycin (60 mg/sq mm) revealed no changes. By contrast, single agent ara-C given every 12 hr for 5 days had similar effects to those seen when ara-C is given in combination with 6-TG. Clearly, Fc-R and ADCC effectors are highly susceptible to phase cycle-specific agents, suggesting that they belong to the proliferating pool of circulating lymphocytes (short-lived lymphocytes) (9, 10). In addition to blood, ADCC effector cells have been detected in normal spleen (26). Physical stress, i.e., running, produces marked increases in ADCC effector cell function and in the circulating number of Fc-R. These cells seem to come

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**Table 5**

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>% of Fc-R</th>
<th>ADCC (heterologous)</th>
<th>ADCC (homologous)</th>
<th>MICC</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.6 ± 5.7a</td>
<td>22.6 ± 14.6</td>
<td>13.6 ± 7.1</td>
<td>13.8 ± 4.3</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>5.7 ± 3.2</td>
<td>14.3 ± 5.1b</td>
<td>10.3 ± 4.3c</td>
<td>6.7 ± 2.7</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>3.7 ± 1.8</td>
<td>17.1 ± 6.1b</td>
<td>8.0 ± 3.5b</td>
<td>5.0 ± 3.0b</td>
<td>15</td>
</tr>
<tr>
<td>28</td>
<td>9.6 ± 4.9</td>
<td>33.7 ± 8.1b</td>
<td>21.5 ± 9.3</td>
<td>17.8 ± 5.8</td>
<td>12</td>
</tr>
<tr>
<td>≥35</td>
<td>12.2 ± 5.3</td>
<td>42.3 ± 16.0b</td>
<td>25.3 ± 8.8b</td>
<td>30.6 ± 12.8</td>
<td>7</td>
</tr>
</tbody>
</table>

*a Mean ± S.D.

*b p < 0.001.

*c p < 0.01.

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**Table 6**

<table>
<thead>
<tr>
<th>Day of chemotherapy</th>
<th>% of Fc-R</th>
<th>ADCC 1a (% of specific lysis)</th>
<th>ADCC 2b (% of specific lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>3</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

*a Targets coated with rabbit antiserum (effector cell:target cell, 50:1).

*b Targets coated with human anti-HLA serum (effector cell:target cell, 50:1).

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4 J. Zighelboim, manuscript in preparation.
from the spleen, since splenectomized subjects have markedly decreased responses. Preliminary data indicate that patients receiving ara-C and 6-TG have mild or no increase in ADCC effector cell function after physical stress, suggesting the depletion of these cells from the spleen as well (data not shown).

The literature contains conflicting reports as to the nature of the effector cells participating in ADCC and MICC. It is quite clear from reviewing these reports that the type of effectors participating in these cytolytic reactions is determined by the nature of the targets (22) as well as the origin of the antisera used (30). When using nucleated targets, ADCC and MICC seem to be mediated by several types of effector cells in humans (22) and rodents (21). In humans, ADCC is mediated by a subpopulation of Fc receptor-bearing lymphocytes (8, 19, 22), whereas MICC is mediated by T-lymphocytes with and/or without Fc receptors (3, 8, 21). Our data demonstrate that ADCC and MICC effector function can be found independently of specific lysis (heterologous) and (homologous) targets coated with heterologous (rabbit) antiserum.

Table 8

Comparison between ADCC and MICC function in patients with AML in remission who are receiving monthly course of maintenance chemotherapy

<table>
<thead>
<tr>
<th>Subject</th>
<th>ADCC (heterologous)</th>
<th>ADCC (homologous)</th>
<th>MICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. H.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F. H.</td>
<td>35</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>D. E.</td>
<td>23</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>F. D.</td>
<td>6</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>D. S.</td>
<td>2</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>J. McF.</td>
<td>3</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

The degree of deficiency in ADCC activity induced by maintenance chemotherapy correlated with level of reactivity existing after remission induction. Clearly, patients with low levels of ADCC showed more pronounced decrease in response to chemotherapy. Two patients with low levels of reactivity showed complete abrogation of ADCC and MICC function with no recovery even 6 weeks postchemotherapy. It is possible that these individuals have a smaller pool of effector cells and therefore were more susceptible to the effects of these drugs. ara-C has been shown to have a variety of immunosuppressive effects (14). Its short half-life, however, has limited its usefulness as an immunosuppressive agent. ara-C can inhibit antibody responses in mice (20) and humans while having no effects on established delayed hypersensitivity, allograft survival in rodents or dogs, and development of graft-versus-host reaction (15). The mechanism(s) by which ara-C or the latter in combination with 6-TG reduce ADCC and MICC reactivity has not been fully elucidated. ara-C is a phase cycle-specific agent that destroys dividing cells, and part of its effects are most certainly due to the destruction of ADCC and MICC effectors. In vitro, ara-C does not inactivate ADCC or MICC effector cells, indicating a lack of direct cytotoxic or inactivating effect on these cells. Whether daunomycin is immunosuppressive in humans is still unknown. In mice, this agent can affect antibody responses and cellular responses to a tumor allograft (25). In this study, a single dose of daunomycin (60 mg/sq m) had no effect on ADCC and MICC effectors. Furthermore, it actually permitted the recovery of their function.

It is unclear whether individuals with low levels of effector cell function are biologically underprivileged. One way of determining this would be by comparing remission duration and survival in patients with AML according to their levels of cytotoxic effector function. This study is currently under way.

It seems clear that following remission induction leukemic patients are immunologically compromised and that this is further aggravated by monthly courses of chemotherapy. These observations emphasize the need for good rationale in scheduling immunotherapy program when used in conjunction with intensive chemotherapy regimens.

The role of ADCC in vivo is still uncertain. Some investigators have suggested that this cytolytic reaction is important in graft (24) and tumor rejection (23), in certain autoimmune reactions (6), and in hemolytic disease in the newborn (29). The biological significance of the in vivo function(s) of ADCC effector cells in normal and diseased individuals is awaiting definition. It

Table 7

Effects of a single dosage of daunomycin on ADCC effector functions

<table>
<thead>
<tr>
<th>Patient T. H.</th>
<th>Patient C. H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADCC (heterologous)</td>
<td>ADCC (homologous)</td>
</tr>
<tr>
<td>Day of maintenance chemotherapy</td>
<td>% of Fc-R</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

Other investigations have attempted to define the origin and phenotypic characteristics of ADCC effectors active against antibody-coated nucleated targets. While there is agreement that these cells are not B-cells (27, 31), there is controversy as to whether these cells belong to the T-cell population (18) or whether they are neither T- nor B-cells (5, 28). Kay et al. (18) and West et al. (26) have shown that ADCC effector cells bear low-affinity receptors for sheep erythrocytes which would suggest these cells belong to the T-cell population.
seems clear that these cells are actively engaged in DNA synthesis and readily move into the circulation in response to stress. These observations suggest that these cells might be an important component of host defenses against invading microorganisms and related disease processes.

ACKNOWLEDGMENTS

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