Deoxycoformycin-Induced Immunosuppression in Patients With Hairy Cell Leukemia

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Immune function in patients with hairy cell leukemia (HCL) was examined serially during treatment with alternating monthly cycles of recombinant interferon-α-2a and 2'-deoxycoformycin (dCF). At presentation, most patients had normal numbers of T lymphocytes and their cells had normal proliferative responses to mitogens (phytohemagglutinin (PHA) and concanavalin A (Con A)) and alloantigens. Patients had severe monocytopenia, decreased delayed-type hypersensitivity (DTH) reactions, and decreased peripheral blood natural killer (NK) activity. Treatment caused a profound decrease in all lymphocyte subpopulations. T cells were more affected than B cells or NK cells. Numbers of CD4+ and CD8+ lymphocytes decreased to levels <200 cells/μL in all patients during treatment. This decrease in T cell number was associated with a marked decrease in proliferative responsiveness to PHA, Con A, and alloantigens. These abnormalities persisted throughout the 14 months of treatment and have continued for up to 6 months beyond discontinuation of treatment. NK cell activity increased during treatment, but achieved a beneficial clinical response whether or not they have undergone a splenectomy. There are few complete responders (<10%), however, and IFN treatment alone does not appear to be able to cure patients of HCL. Recent data also suggest that development of antibodies to IFN-α occurs in some patients and is associated with clinical deterioration in a minority of them. Thus, many patients may require therapy other than splenectomy or IFN-α.

Recently, 2'-deoxycoformycin (dCF) also was shown to be effective in patients with HCL, both in patients with disease refractory to IFN and in previously untreated patients. Because results so far suggest that dCF alone can induce complete remissions in many patients, interest in this agent as a primary treatment for HCL has increased.

dCF is an irreversible inhibitor of adenosine deaminase (ADA). This enzyme catalyzes the deamination of adenosine to inosine and the deamination of deoxyadenosine to deoxyinosine. In the absence of ADA, adenosine and deoxyadenosine accumulate and result in myriad effects. The lymphocytolysis following ADA inhibition of dCF is believed to be mediated by intracellular accumulation of deoxyadenosine and adenosine. Another possible mechanism is the induction of DNA strand breaks. The greatest amount of this enzyme is found in cells of the lymphoid system and among these, T cells have higher levels of the enzyme than B cells, a relationship that remains true for neoplastic T and B cells. The biologic effects of ADA deficiency on the immune system are profound. Congenital ADA deficiency is responsible for one form of severe combined immunodeficiency disease. It is characterized by lymphopenia, atrophic thymus, tonsils, and adenoids, and abnormalities of T and B cell functions. A similar syndrome has been described in a patient receiving high doses of dCF for treatment of lymphoblastic leukemia. Another report described an association of dCF with severe and fatal opportunistic infections. These
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observations and a preliminary description of immune abnormalities in patients with HCL during dCF treatment led us to undertake a detailed investigation of immune function in our patients with HCL receiving dCF alone or in combination with IFN-α.

PATIENTS

Thirteen patients (12 men and one woman) were entered in a prospective study evaluating the clinical efficacy and immunologic consequences of combined therapy with alternating cycles of dCF and recombinant IFN-α2a (rIFN-α2a) in patients with hairy cell leukemia (HCL). The details regarding the criteria for diagnosis, treatment, and clinical evaluation of response to treatment have been described elsewhere. All patients received dCF 4 mg/m² by slow intravenous injection once a week for 3 consecutive weeks. The patients received no treatment for 14 days and then began therapy with rIFN-α2a (Roferon A supplied by Hoffman-LaRoche, Nutley, NJ) 3 x 10⁵ U subcutaneously (SC) daily for 4 weeks. The last day of rIFN-α2a treatment ended the 2-month cycle. Patients received dCF 4 mg/m² by slow intravenous injection once a week for 3 consecutive weeks. The patients received no treatment for 14 days and then began therapy with rIFN-α2a (Roferon A supplied by Hoffman-LaRoche, Nutley, NJ) 3 x 10⁵ U subcutaneously (SC) daily for 4 weeks. The last day of rIFN-α2a treatment ended the 2-month cycle. Patients received seven cycles (14 months) of therapy, and then all treatment was stopped. Although administration of other agents capable of inducing immune changes (corticosteroids, prostaglandin inhibitors) was generally proscribed, one patient required steroids for treatment of vasculitis. Fifteen patients were enrolled in this study; the 13 who completed all treatment are the subject of this report. Two or three peripheral blood samples were obtained at baseline, and then samples were taken at regular intervals during treatment with dCF or rIFN-α2a.

We also studied four men and one woman with HCL who were being treated with rIFN-α2a alone as part of an earlier Biological Response Modifiers Program (BRMP) trial and four men and two women receiving dCF alone after having had disease progression during treatment with IFN-α2a. None of the patients had antibodies to the human immunodeficiency virus (HIV). All treatment was administered according to protocols approved by the investigational review boards of the Frederick Cancer Research Facility and Clinical Oncology Program of the National Cancer Institute. All patients gave written informed consent.

MATERIALS AND METHODS

Cell preparation. Peripheral blood mononuclear cells (PBMCs) were obtained from whole blood after collection in preservative-free sodium heparin by Ficoll-Hypaque density-gradient centrifugation. PBMCs were washed twice in phosphate-buffered saline (PBS) containing 10% fetal calf serum (FCS) and resuspended in RPMI 1640 containing 20% FCS at a concentration of 2 x 10⁹ cells/mL. Next, an equal volume of cold (4°C) freezing medium, RPMI 1640 containing 15% dimethylsulfoxide (DMSO, Fisher, Silver Spring, MD), was added dropwise to the mononuclear cell suspension. Cell suspensions were then placed in sterile freezing ampules and cryopreserved using a Cryo-Med controlled rate freezer (Mt Clemens, MI) at 5 to 10 x 10⁶ cells/vial. After cryopreservation, the mononuclear cells were stored in liquid nitrogen freezers until needed. Just before assay, the cryopreserved lymphocytes were thawed rapidly in a 37°C water bath and washed twice in RPMI containing 20% FCS. Cells were then counted and adjusted to the desired cell density in the medium appropriate for each assay. Proliferative responses were measured in triplicate in 96-well round-bottomed microtiter plates (Costar, Cambridge, MA). Each well contained 1 x 10⁵ cells and one of the following: 2 µg/mL phytohemagglutinin (PHA, Wellcome Research Laboratories, Beckenham, England), 100 µg/mL Concanaavalin A (Con A, Calbiochem, San Diego), or 50,000 irradiated allogeneic cells. All cultures were performed in a final volume of 200 µL. The culture medium was RPMI 1640, containing 15% human serum from subjects with blood type A. Proliferative responses were measured by incorporation of [³¹⁵]thymidine over a four-hour pulse with 0.44 µCi [³¹⁵]thymidine (New England Nuclear, Boston). Proliferative responses to PHA and Con A were measured on day three and those to alloantigens were measured on day six.

Cell surface markers.

Enumeration of leukocyte subpopulations was performed by flow cytometry using a panel of monoclonal antibodies (MoAbs) directed against cell surface antigens. Cryopreserved Ficoll-Hypaque-separated mononuclear cells were used in all determinations and, where possible, all samples obtained serially from each patient were tested on a single day. The following MoAbs were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA): anti-Leu 4 (CD3), anti-Leu 3a (CD4), anti-Leu 2b (CD8), anti-Leu 11 (CD16), anti-Leu 12 (CD19), anti-Leu 10 (HLA-DQ), anti-Leu M3 (CD14), anti-Leu 19, and anti–HLA-DR. All antibodies except anti-Leu 19 were directly conjugated with FITC. The anti-Tac antibody was the gift of Dr Thomas Waldmann of the National Cancer Institute. Reactivity with anti-Leu 19 or anti-Tac was detected through a two-step staining procedure in which incubation with primary antibody was followed by incubation with the F(ab')2 fragment of goat anti-mouse IgG which was FITC labeled (Tago, Burlingame, CA). The cells were analyzed on an Ortho Cytofluorograf 30-H equipped with a model 2150 computer (Ortho Diagnostic Systems, Westwood, MA). Viable cells were selected for analysis based on propidium iodide (10 µg/mL) exclusion, as detected by red fluorescence. Cells were gated by low-angle forward- and right-angle light-scatter properties as lymphocytes or monocytes; a third analysis gate included all mononuclear cells. The percentage of cells reactive with each MoAb was determined by comparison of fluorescently labeled cells with cells that had been incubated with FITC-labeled isotype controls. The number of cells in peripheral blood that possessed a particular phenotype was determined by multiplying the percentage of MoAb reactive cells by the absolute lymphocyte (or monocyte) count determined from a simultaneously obtained WBC count with differential.

Natural cytotoxicity assay. Freshly isolated mononuclear cells were used for this assay because the recovery of natural killer (NK) activity from cryopreserved cells obtained at different times shows great variability. The assay was a standard four hour ⁵¹Cr-release cytotoxicity assay using the cell line K562 as target. The percentage of specific cytotoxicity was calculated as the percentage of ⁵¹Cr released in the experimental group minus the percentage released in the medium control. Data are expressed as lytic units, calculated from the specific cytotoxicity at each of the ratios of effector to target using an exponential-fit equation. One lytic unit (LU) is defined as the number of effector cells required to lyse 1,000 target cells (LU/10⁵ effector cells). Skin testing. Most patients had skin tests applied before and after completion of combined therapy with dCF and IFN-α. We used a Multitest CMI kit (Merieux Institute, Miami) which included the following antigens: tetanus toxoid, diphtheria toxoid, Streptococcus antigen, old tuberculin, Candida antigen, trichophyton antigen, Proteus antigen, and glycerin as a negative control. All skin tests were read 24 and 48 hours after application.

Immunoglobulin levels. Quantitative determination of immunoglobulin levels was performed by Central Laboratories of Associated Maryland Pathologists with radial immunodiffusion.

Criteria for Response

Complete remission. A complete remission was defined as resolution of splenomegaly with recovery of peripheral blood counts to a...
and ten of 13 had a nadir of <100 CD4 lymphocytes/μL. All patients had nadir CD4 lymphocyte counts <200, in number of CD4 and CD8 lymphocytes were decreases. Analysis using the paired one patient had a decrease in the number of CD8 lymphocytes, and all but counts for each patient both at presentation and at their nadir are shown in Fig 1. All I three weeks of dCF therapy. The CD4 and CD8 lymphocyte treatments below normal (150, CD8-bearing lymphocytes was 707 (range 138 to 1,299, The mean number of CD4 lymphocytes was 1010 (range 150 to 2,251). Three patients had CD4 cell numbers near normal 600 to 2,000) and the mean number of hairy cells from the peripheral blood. All patients had persistent microscopic involvement of the bone marrow as detected in some of the bilateral bone marrow aspirates and biopsies which occurs with dCF. The nadir was usually transient and associated with the maximal point of IFN-induced peripheral blood count suppression. The decrease in CD4* cell number with dCF treatment was not limited to periods of myelosuppression and persisted despite recovery of total WBC counts to normal levels in most patients. This is shown in Fig 2B. The CD4* cell number remained depressed even during times of rIFN-α administration and despite return of the patients' total WBC count to normal.

The duration of the depression in the number of CD4* cells is shown in Fig. 3. The median follow-up for these 13 patients is 246 days after completion of all therapy. Of the patients with sufficient follow-up, most continue to have depressed T cell numbers for more than a year after treatment was discontinued. CD4 cell numbers have returned to normal in only four of 13 patients. Normal CD4 cell numbers (>600/μL) were achieved at 77, 87, 162, and 237 days after discontinuation of IFN therapy. Some patients have experienced increases in CD4 lymphocyte number after completion of therapy, but total numbers remain well below normal in most patients. The median number of CD4* lymphocytes in the ten patients whose cell numbers have not returned to normal is 280/μL. There does, however, appear to be a trend toward recovery since linear regression analysis shows a significant positive linear trend (P = 0.0005).

During treatment, most patients also exhibited decreases in peripheral blood parameters and remission, disappearance of hairy cells from the peripheral blood and a decrease in the hairy cell infiltrate in the bone marrow by >50%. Minor remission. A minor remission was defined as improvement in peripheral blood parameters and >50% decrease in the number of circulating hairy cells with a decrease in the hairy cell infiltrate in the bone marrow by <50%.

Statistical methods. Group-to-group mean comparisons were analyzed with Student's t test. Changes from baseline for each immune parameter were assessed using a paired sample t test to incorporate the pair-to-pair variability of the patients' data. Patients served as the pairing factor; differences were computed by subtracting average baseline measurements and posttreatment values. Linear trends were detected using regression analysis on parameters regressed over time on study. Significance was determined on all statistical tests at the 0.05 level. The t test, paired sample t test and regression analyses were performed using the TTEST, MEANS, and GLM procedures in the Statistical Analysis System (SAS) available on the IBM 370 at the National Institutes of Health (NIH), Division of Computer Research and Technology.

RESULTS

Although all patients experienced decreases in the percentage of hairy cells in the bone marrow after combined treatment with dCF and IFN-α, there were no complete responders. All patients had improvements in their peripheral blood counts, and those with circulating hairy cells at diagnosis had rapid and complete disappearance of those cells from the peripheral blood. All patients had persistent microscopic involvement of the bone marrow as detected in some of the bilateral bone marrow aspirates and biopsies performed multiple times before and after the completion of the seventh and last cycle of therapy.

Effect of combined dCF and rIFN-α on PBMC Number. Despite frequent peripheral blood involvement with hairy cells, the number of CD4- and CD8-bearing lymphocytes at presentation was normal in most patients (Fig 1). The mean number of CD4* lymphocytes was 1010 (range 150 to 2,251, normal 600 to 2,000) and the mean number of CD8-bearing lymphocytes was 707 (range 138 to 1,299, normal 150 to 600). Three patients had CD4 cell numbers below normal (150, 266, 340) at presentation. The initial treatments with dCF caused a marked decrease in lymphocyte levels. This was particularly marked in the T-cell population and was usually evident by completion of the first three weeks of dCF therapy. The CD4 and CD8 lymphocyte counts for each patient both at presentation and at their nadir are shown in Fig 1. All 13 patients tested had a decrease in the number of CD4* lymphocytes, and all but one patient had a decrease in the number of CD8* lymphocytes. Analysis using the paired t test showed that the decreases in number of CD4 and CD8 lymphocytes were statistically significant (P < 0.0001 and P < 0.0002, respectively). All patients had nadir CD4 lymphocyte counts <200, and ten of 13 had a nadir of <100 CD4* lymphocytes/μL.
in numbers of B cells (CD19+) and NK cells (Leu 19+). The CD19+ lymphocytes appeared to be as susceptible to reduction in number by dCF as the T cell populations but appeared to recover more quickly. Figure 4A shows data from a representative patient. An oscillatory pattern is evident with nadirs of CD19+ cells after dCF treatment followed by recovery to normal cell numbers during the month of treat-

ment with rIFN-α2a. A similar pattern is evident for Leu 19+ NK lymphocytes (Fig 4A); however, in contrast to T and B lymphocytes, the NK cells appeared to be more resistant to the effects of dCF since they rarely reached the nadirs of B and T cells, although their absolute numbers did decrease below baseline.

As described previously,24 all our patients suffered from
severe monocytopenia at presentation. We used the differential count from the peripheral blood smear and antibodies to the CD14 antigen (Leu M3) to enumerate monocytes before and during combination therapy. No monocytes (CD14+ cells) were detectable at presentation in any of the 13 patients tested. Monocytes generally returned on completion of the first cycle of treatment (after three doses of dCF and 28 days of IFN-α) and persisted throughout the remainder of treatment. There were, however, marked oscillations in the number of CD14+ monocytes which varied according to the agent being administered (Fig 4B). In direct contrast to the behavior of lymphocytes, once monocytes appeared, their number increased during treatment with dCF and decreased dramatically during IFN-α therapy. In Fig 4C, B cell number and monocyte number for a single patient are superimposed on the same graph. The periodicity of the curves for CD14 and CD19 cell numbers was completely out of phase. These data in combination with the observed suppressive effects on T-cell number suggest differential effects of dCF and IFN on lymphocyte and monocyte populations.

Functional Studies. The decrease in T-cell number was accompanied by a marked change in patients' immune function. Sufficient numbers of cells were available to perform functional studies on samples collected before, during, and after completion of therapy in seven of 13 patients. Before treatment, cells from all seven patients responded normally to the mitogen PHA and six of seven responded normally to Con A and alloantigens. During treatment, the ability of patients' cells to respond to PHA and Con A decreased markedly in six of seven and five of seven patients tested, respectively. The proliferative response of cells to alloantigens became severely impaired in all seven patients tested. The proliferative responses of cells from two typical patients are shown in Fig 5. The loss of responsiveness to these stimuli correlated with the decreases observed in T-cell number. Function was maximally depressed when CD4 cell number decreased to <200. In general, the response to PHA was least affected by treatment and did not decrease dramatically until CD4 cell numbers were <150/μL. The responses to Con A and alloantigens appeared to be much more sensitive to reduction in T-cell number and were depressed at levels at which PHA responsiveness was maintained. This deterioration in in vitro measures of T-cell function occurred despite a remarkable hematologic improvement experienced by most patients in the study.

In vivo T-cell function was assessed by examining the patients' ability to manifest a delayed-type hypersensitivity reaction to a panel of recall antigens. Ten patients were tested for their responsiveness to eight recall antigens before and after treatment with dCF and rIFN-α. Before therapy, six patients did not react to any antigens and one patient each reacted to two, three, four, and five antigens. No patient reacted to all antigens tested. On completion of therapy, three patients remained totally anergic; four lost reactivity to two, two, three, or four antigens (three becoming anergic), and three patients recovered reactivity to one, one, or two antigens. No patient reacted to all antigens tested after completion of therapy.

We also examined peripheral blood NK activity before and during treatment. Despite normal numbers of Leu 19+ lymphocytes in most patients, NK activity was well below normal in most patients prior to initiation of therapy (Fig 6). Before treatment, peripheral blood NK activity in our patients with HCL was significantly less than that observed in a population of normal donors examined in our laboratory (88 ± 8 LU/10⁶). Patients without hairy cells in the peripheral blood had significantly lower NK activity (20 ± 6 LU/10⁶, P < .001), as did patients with hairy cells in the peripheral blood (8 ± 1 LU/10⁶, P < .01). All patients experienced an increase in NK activity sometime during treatment when compared with multiple pretreatment samples. When the means of NK activity and Leu 19+ lymphocyte number for all points after initiation of therapy were compared with pretreatment means, there was a significant increase in NK activity (P < .001) without a significant change in Leu 19+ cell number (P < .41). This was true whether or not hairy cells had been present in the peripheral blood prior to treatment. Similar to the observed effects on B cell and NK cell number, a variable pattern was found in NK activity during treatment. Increases in both Leu 19+ cell number and NK activity generally accompanied IFN-α therapy, and decreases in
both cell number and NK activity were associated with dCF treatment.

B-cell function was assessed indirectly by serial measurement of serum immunoglobulin levels during therapy. All patients had normal levels of IgM, IgG, IgD, and IgA at presentation, and levels of these immunoglobulins did not change significantly during treatment. IgE levels were above normal at initiation of therapy in five of 12 patients (three with very high levels and two with borderline elevations). Eleven of 12 patients in whom serial measurements were available for study showed significant increases in IgE levels during treatment (Fig 7). No individual developed manifestations of atopic disease in association with their elevated IgE levels.

**Effect of dCF or IFN on Cell Number and Function.** Since our treatment protocol included alternating therapy with both dCF and IFN-α, documentation of the agent responsible for the severe depression observed in T-cell number and function is important. Because of the previously described effects of dCF, our first assumption was that dCF was responsible for the immunosuppression. To verify this, we examined cells from patients with hairy cell leukemia who had been treated with rIFN-α alone or with dCF following treatment failure with IFN-α. Treatment with rIFN-α alone did not result in major decreases in T-cell number (Fig 2A) or function, whereas dCF treatment alone caused decreases in both T-cell number and function as measured by the same tests described for combined alternating therapy (data not shown). Figure 8 shows the results of sequential therapy with IFN-α and dCF in a single patient. During IFN-α treatment, CD4 number and Con A responsiveness remained normal, but the patient did not respond to IFN-α therapy, and the monocyte count remained low. In patients who respond to IFN-α treatment, monocyte number increases. On initiation of treatment with dCF, CD4 cell number and Con A responsiveness decreased while CD14+ monocytes appeared in the peripheral blood. These findings coincided with the patient’s favorable clinical response to dCF treatment, suggesting that the decrease in T cell number and associated loss of function are caused by dCF treatment.

**Clinical Infections.** Four of 15 patients treated with alternating cycles of dCF and IFN-α developed localized Herpes zoster infections. (Table 1). One patient developed infection during therapy, and the other three developed infection 6, 6, and 9 months after completing all therapy. We also observed two additional cases of localized Herpes zoster among seven patients with HCL treated with dCF alone after failing IFN-α therapy. Among the six cases, four patients received acyclovir therapy and two patients received no antiviral therapy. There were no cases of cutaneous or visceral dissemination, and one of six patients has developed postherpetic neuralgia in the involved dermatome.
DISCUSSION

The clinical course of HCL is variable, but infection, often caused by uncommon agents, represents the major cause of morbidity and mortality in these patients. Increased susceptibility to infection has been attributed to granulocytopenia, mononcytopenia, qualitative defects in the few cells present, and/or deficiencies in the patients' immune status. The immune deficiencies which have been described include decreased NK activity, decreased delayed-type hypersensitivity reactions, absolute deficiencies of T cells, and decreased lymphocyte proliferative responsiveness to mitogens and to allogecnic or autologous cells. Response to splenectomy or IFN therapy is generally associated with a decreased incidence of infection. The recent increase in the use of the potentially immunosuppressive agent dCF in patients with HCL led us to perform a detailed analysis of its effects on immune function.

Severe and apparently long-lasting immune suppression was observed in patients with HCL during and after therapy with alternating monthly cycles of dCF and rIFN-α. Based on data obtained from a few patients treated with IFN-α or dCF alone or sequentially, we believe that this severe immune suppression is related to dCF treatment. The lympholytic properties of dCF have been well described, as has its potential for inducing immune suppression in patients with HCL. Most of our patients had normal numbers of T cells and normal in vitro proliferative responses to mitogens and alloantigens at the initiation of therapy. Our patients did exhibit certain abnormalities of immune function, including severe monocyctopenia, decreased DTH responses, and decreased NK activity. In general, those parameters not dependent on T-cell activity (monocyctopenia and NK activity) returned toward normal when patients began to respond to therapy.

After initiation of therapy, patients tended to have rapid disappearance of hairy cells from the peripheral blood, followed by a short duration of granulocytopenia, anemia, and thrombocytopenia. This was followed by a general improvement in total WBC, RBC, and platelet counts. Despite the recovery of granulocyte counts, there was a persistent lymphocytopenia, particularly involving T lymphocytes. CD4$^+$ cells decreased rapidly to low levels, usually $<100/\mu L$ and persisted at these low levels during most of the treatment period. Absolute numbers of CD4$^+$ cells remained at low levels up to 1 year after discontinuation of therapy, and only four of 13 patients who have completed therapy have had CD4 cell numbers return to normal levels. CD8$^+$ cells were similarly affected.

Table 1. Localized Herpes Zoster Infection in dCF-Treated Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Treatment</th>
<th>Location</th>
<th>Timing</th>
<th>Antiviral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dCF/IFN-α</td>
<td>L5-S1</td>
<td>11 mo into treatment</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>dCF/IFN-α</td>
<td>Trigeminal</td>
<td>6 mo after treatment</td>
<td>Acyclovir IV</td>
</tr>
<tr>
<td>3</td>
<td>dCF/IFN-α</td>
<td>C4-C5</td>
<td>6 mo after treatment</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>dCF/IFN-α</td>
<td>T6-T8</td>
<td>9 mo after treatment</td>
<td>Acyclovir IV/PO</td>
</tr>
<tr>
<td>5</td>
<td>dCF</td>
<td>Trigeminal</td>
<td>6 mo into treatment</td>
<td>Acyclovir PO</td>
</tr>
<tr>
<td>6</td>
<td>dCF</td>
<td>S2</td>
<td>11 mo after treatment</td>
<td>Acyclovir PO</td>
</tr>
</tbody>
</table>

IV, intravenously; PO, orally.

Accompanying the decrease in number of T cells was a loss of immune function. Peripheral blood cells from most patients had a marked decrease in their ability to respond to PHA, Con A, and alloantigens. This decrease paralleled the loss of lymphocytes and correlated with the number of CD4- and CD8-bearing lymphocytes. There appeared to be a hierarchy of responsiveness, with the response to PHA appearing to be the least dependent and the response to alloantigens being the most dependent on the number of T cells. The prolonged duration of low number of T cells suggests that there may be some effect on the stem cell committed to lymphocyte development. The number of T cells should return to normal within a few months if the treatment has not affected the immature stem cell. Because all our patients are adults, the thymus would have involuted and become dysfunctional, impairing the ability of our patients to replete their peripheral T-cell compartments. However, the residual postthymic T cells would be expected to be able to expand to normal levels shortly after removal of the suppressive agent. This has not yet happened in our patients. Continued observation over time will be required to appreciate the extent and duration of our patients’ immunodeficiency fully.

Our observations of decreased NK activity in patients at presentation despite apparently normal numbers of cells of NK phenotype confirm the observations of other investigators. Leu 19-bearing NK cells did decrease in number during treatment, but not to the extent of the CD4 and CD8 cells. The magnitude and duration of the effect on Leu 19 cells was even less since actual numbers did not decrease to the levels observed with T and B cells, and the decrease observed during dCF treatment was followed by partial recovery during therapy with IFN-α. This recovery of cell number did not occur in the T cell populations and suggests a more permanent or long-lasting effect on T cells v large granular lymphocytes. Despite an overall decrease in the number of Leu 19 cells over time, patients actually exhibited an increase in NK activity (Fig 6). The NK activity also appeared to vary cyclically, being highest during or just after IFN-α treatment and lowest following dCF administration. An overall increase in NK activity in patients with hairy cell leukemia who respond to treatment with IFN-α has been described previously.

Our observations suggest that B cells are also affected by this treatment. There was a clear drop in number of B cells following treatment with dCF. This effect on B cells was more transient than the suppression of the number of T cells since there was a recovery of the number of B cells toward
normal during the 28 days of IFN-α treatment. As a crude assessment of B-cell function we measured serum immunoglobulin levels. We observed no significant changes in the levels of IgM, IgG, IgA, or IgD but did see an increase in IgE levels in most patients. The increase in IgE production observed during treatment is probably an indirect result of dCF-induced immunosuppression. Although we have no data reflecting IgE levels in patients treated with IFN-α alone, increased IgE levels have been reported in other immunosuppressed states characterized by partial or complete T-cell deficiency (ataxia-telangiectasia, and Wiskott-Aldrich and Di George syndromes). IgE production is closely regulated by a balance of T-helper/inducer and suppressor function. dCF may cause an imbalance that results in loss of suppression and a concomitant increase in IgE levels. Another possibility is that patients' hairy cells may secrete IgE-binding factors that have been shown to potentiate IgE production by human B cells. These effects could be related to an as yet unknown effect of dCF or may be mediated indirectly by interleukin-4 (IL-4), which might be produced by the hairy cells or dying dCF-treated T cells.

The severe and persistent T-cell dysfunction observed in our patients treated with IFN-α and dCF has important implications for management of HCL. The recent description of pathologic complete responses to dCF in patients with HCL raises the prospect that dCF may represent curative therapy for this disease. Our results and the results of other studies of patients with HCL examined after longer follow-up suggest that CRs occur less frequently than initially reported. Earlier claims of high CR rates used more relaxed definitions of CR. Bilateral bone marrow aspirates and biopsies were not performed, and other investigators did not require confirmation of a CR by repeat marrow examination 2 months later. The various studies using dCF for treatment of HCL used different dose schedules and drug administration. Our lack of complete responses may be related to decreased dose intensity of dCF administration, not just to a more careful bone marrow examination.

The physician treating a patient with HCL has at least three options: splenectomy, IFN-α, or dCF. Since none of these treatments appears capable of completely eradicating the disease, the choice of treatment is likely to be based on other considerations, such as duration of response, convenience, cost, and toxicity. The morbidity of splenectomy is well known, as is the toxicity of IFN. The acute and chronic toxicities associated with dCF are just now being recognized. Before dCF is widely used, the prospects for increased complications secondary to chronic T-cell dysfunction must be considered. Although T-cell levels in many of our patients are as low as those found in patients with AIDS (<200 CD4 cells/μL) is used as a threshold for AZT administration), none of our patients experienced an opportunistic infection during or following treatment with dCF and IFN-α. Four patients have experienced localized dermatomal H zoster, one patient during therapy and three patients after completion of combined dCF and IFN therapy. Two other patients have suffered the same complication after treatment with dCF alone following treatment failure with IFN. All four patients receiving combined treatment with dCF and rIFN-α had subnormal numbers of CD4 cells at the time of their infection. The occurrence of H zoster in dCF-treated patients with cancer has been noted previously. None of the patients in that study had HCL, and all of the described cases had undergone dissemination. Infection remained localized in all six of our patients. One patient at a different institution has developed Pneumocystis carinii pneumonia (PCP) following treatment with dCF for HCL (N.A. Siddiqi, personal communication, December 1987). At the time of this opportunistic infection, the patient’s total lymphocyte count was 1,484/μL with a CD4 lymphocyte count of 30/μL and CD8 lymphocyte count of 237/μL. Her serum was negative for antibodies to HIV, and she had an uneventful course with a complete recovery following treatment with trimethoprim/sulfamethaxoxole.

Although PCP has been described in patients with HCL who have not received dCF, a contribution of the underlying dCF-induced immune suppression must be considered, particularly in patients with normal peripheral blood granulocyte counts and minimal residual disease after therapy. Similarly the contribution of dCF to the development of localized H zoster must be considered. Shearer et al described the selective loss of major histocompatibility complex self-restricted T-cell immune responses during development of AIDS. This population of T cells is responsible for the recognition and elimination of virally infected cells in vivo. dCF may be inducing an AIDS-like state and may be responsible for the increased frequency of H zoster infection. These cases should serve as a warning to consider treatment with dCF very carefully. Some patients in our study have not been at risk for an opportunistic infection for a long time, and the time for recovery to normal T cell numbers and function after discontinuation of therapy has yet to be determined. Pending the results of long-term follow-up of dCF–treated patients, dCF should be considered experimental therapy reserved for use only in patients who have failed splenectomy and IFN therapy.

dCF-induced immune suppression may have potential benefits for certain patient populations. The partially selective effect on T cells with relative sparing of B cells, large granular lymphocytes, polymorphonuclear lymphocytes, and monocytes suggest that dCF may be useful in organ transplantation in which T cell specific effects are desired. It may also prove useful in other clinical situations where immune suppression is a desired effect, eg, the treatment of autoimmune diseases.

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Deoxycoformycin-induced immunosuppression in patients with hairy cell leukemia

WJ Urba, MW Baseler, WC Kopp, RG Steis, JW Clark, JW 2d Smith, DL Coggin and DL Longo

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