Immunologic Rebound After Cessation of Long-term Chemotherapy in Acute Leukemia

By Luis Borella, Alexander A. Green, and Robert G. Webster

The aim of this study was to determine the kinetics of repopulation of blood and bone marrow lymphocytes, immunoglobulin synthesis, and antibody production after stopping long-term combination chemotherapy in children with acute lymphocytic leukemia (ALL) in continuous remission for 2½–3 yr. The phase of recovery after immunosuppressive drugs were discontinued was characterized by a rise of lymphocytes and increased immunoglobulin and antibody production, and it was dependent on the age of the patients at the beginning of treatment. Thus, after the drugs were stopped, blood and bone marrow lymphocytes and serum IgG and IgM increased significantly in the group of patients younger than 5 yr but not in the older group. Lymphocytosis was more pronounced and earlier in bone marrow, suggesting an initial expansion of this cell compartment. After the drugs were discontinued one-fourth of these patients had a rise in antibody to the Hong Kong influenza virus without evidence of reexposure to the same antigen. Although the mechanisms of this age-dependent immunologic rebound are uncertain, we postulate that the number of long-lived, drug-resistant, memory lymphocytes increases in peripheral blood as a function of age and antigenic stimulation. Practical implications derived from this study are: an increase of bone marrow lymphocytes above 40% in children with ALL in whom chemotherapy has been stopped does not indicate relapse but may be a manifestation of immunologic recovery; and a rise in antibody titers after cessation of immunosuppressive drugs may reflect immunologic rebound to an “old” antigen and not necessarily be secondary to an active infectious process.

A MARKED IMPROVEMENT in the prognosis of childhood acute lymphocytic leukemia (ALL) has occurred in the recent past. At St. Jude Children’s Research Hospital, the 3-yr “leukemia-free” survival of children with ALL admitted to a combination chemotherapy program for ALL, “Total Treatment V Protocol,” is 63%. The present policy at this institution is to discontinue all antileukemic-immunosuppressive therapy when patients with ALL have remained in continuous complete remission for 2½–3 yr. Thus, more
than 50% of the children admitted to Total V Protocol are now in the “off therapy” phase of treatment.

Although the immunosuppressive effects of long-term combination chemotherapy in patients with ALL have been recently described,1 no information is available on the kinetics of immunologic recovery when chemotherapy is discontinued. Studies on the repopulation patterns of lymphoid cells and on their functional recovery following long-term immunosuppressive therapy may not only provide information on the immunocompetence of patients with ALL, but may also increase our understanding of the recovery phase after prolonged immunosuppression in other clinical situations.

The aim of the present study was to investigate the kinetics of repopulation of blood and bone marrow lymphocytes, immunoglobulin synthesis, and antibody production following cessation of all antileukemia drugs in children with ALL who had been in continuous complete remission and receiving combination chemotherapy for 28–36 mo. We had previously reported the immunosuppressive effects of long-term chemotherapy in the same group of patients before the treatment was stopped.1 This report is a follow-up of these children during the “off chemotherapy” or recovery phase. The data to be presented indicate that there is a temporal relationship between immunoglobulin production and the kinetics of bone marrow and blood lymphocyte recovery following cessation of therapy. The results also demonstrate that after stopping immunosuppressive treatment, enhancement of specific antibody synthesis against an antigen may occur without extrinsic restimulation.

MATERIALS AND METHODS

Eighteen children with ALL who had been treated according to the St. Jude Total Treatment V Protocol1 were included in this study. They had received a combination of five drugs—prednisone, vincristine, 6-mercaptopurine, methotrexate, and cyclophosphamide—and cranial irradiation according to schedules, dosages, and routes of administration previously reported.1 After 28 mo in continuous complete remission, each patient was randomized to have therapy stopped or continued for another 8 mo. The first 18 patients whose therapy was discontinued are included in this study. All but one have been in continuous complete remission for 36–43 mo and off chemotherapy for 2–15 mo. One patient had leukemia relapse 40 mo after diagnosis and 12 mo following cessation of chemotherapy. There were 12 females and six males, and at the time of diagnosis their ages ranged from 2-4/12 to 15-10/12 yr, with a median of 4-10/12 yr. Total white blood cell and differential counts and bone marrow examinations were performed at frequent intervals during chemotherapy, on the day that the drugs were stopped, and at monthly intervals during the following 6 mo. Differential counts on 200 nucleated cells were performed in each bone marrow specimen by a single laboratory technician who was unaware of this study. The slides were then coded, and the bone marrow smears reexamined by one of us (AG); there was agreement in the results obtained by both examiners.

Serum samples for immunoglobulin determination were obtained at the time when chemotherapy was stopped in all but two patients. In these two children, the serum immunoglobulins were determined for the first time 2 and 4 wk after stopping therapy. Following cessation of treatment, serum immunoglobulins were assayed at monthly intervals by a modification3 of the single radial diffusion method of Mancini et al.4 Quantitative immunodiffusion plates and standard sera were obtained from Pfizer Diagnostics, New York, N. Y. Standard curves and normal ranges for age used in this laboratory have been reported.8
The children in this study had received the monovalent type A2/Aichi/2/68 (Hong Kong variant) influenza vaccine (Zonome, Eli Lilly & Co., Indianapolis, Ind.) before the 1969 epidemic of Hong Kong influenza. Their initial response to immunization has been reported. Of the 18 children, only 12 were included in the evaluation of antibody response to influenza virus without antigenic restimulation because of the following reasons: these patients had not been reimmunized with influenza vaccine within 2 mo before and 5 mo after therapy was stopped, influenza was not represent in the community at that time, and these children did not have clinical evidence of influenzalike illness prior to or after therapy was discontinued. Six patients that had received influenza vaccine within 2 mo before cessation of therapy were excluded from this part of the study. Samples of the sera used for immunoglobulin assays were stored at 20°C until viral antibody titers were determined. Before titration, the sera were treated with receptor-destroying enzyme to remove nonspecific serum inhibitors.5 Hemagglutination-inhibition assays were performed in plastic trays (WHO type) with the use of four hemagglutinating doses of virus as previously described.2 Analysis of significance was performed using the Student’s t test. Since the immunoglobulin concentration in general populations does not conform to the normal Gaussian distribution, serum immunoglobulin levels were converted to logarithms and compared with values obtained from a control group of 40 normal children. The “normal bounds” for each age group were obtained by taking the antilogs of the mean logs ± twice the pooled standard deviation of the logs as described by Buckley et al.6

RESULTS

Following long-term immunosuppressive therapy, there was a significant lymphopenia in the peripheral blood and bone marrow of children with ALL in remission. On the day when the drugs were stopped, the average number of lymphocytes was 920/cu mm in blood and 7.8% in bone marrow. In normal children of the same age, the average number of blood lymphocytes ranges from 2500 to 4500/cu mm and the average per cent of lymphocytes in bone marrow is 13.7 After the immunosuppressive treatment was discontinued there was a rise in blood and bone marrow lymphocytes. This increase was independent of sex, race, or duration of treatment. However, an inverse relationship was found between lymphocyte rise and age. The median age of these patients at time when treatment was begun was 5 yr; thus, we divided them into two groups: younger and older than 5 yr. There were nine children in each group. As shown in Fig. 1, in the younger children the median number of blood lymphocytes increased from 350 on the day that treatment was stopped to 1750 8–10 wk later and to 2350 on week 20. Statistical analysis of
the data demonstrates a significant difference ($p < 0.01$) between the values of 4–6 and 8–10 wk, indicating that the number of peripheral blood lymphocytes continues to rise beyond the first 6 wk after cessation of therapy. In contrast, on day zero in the older children the median number of lymphocytes was 1200. This value did not increase after 8 wk, off chemotherapy and was 1900 at the 20th wk. These data indicate that both the reduction in the number of peripheral blood lymphocytes during long-term combination chemotherapy and the rise that follows cessation of chemotherapy are more pronounced in younger than in older children.

After cessation of immunosuppressive therapy, bone marrow lymphocytes also increased. In contrast to peripheral blood, these values are expressed as per cent of total number of bone marrow cells. Quantitative techniques to estimate in absolute terms the rise of bone marrow cells are not available. However, since the cellularity of the bone marrow samples appeared to increase during the “off therapy” period, it is likely that the lymphoid population in the bone marrow increased more than is apparent by the percentage. As shown in Fig. 2, the rise in bone marrow lymphocytes occurred earlier than in the peripheral blood, particularly in the younger age group where within 4–6 wk following cessation of chemotherapy there was a 4.5-fold increase. Within the same period the mean rise in the older children was 2.6-fold. Thus, while following cessation of therapy, bone marrow lymphocytes increased in both groups; the difference was greater in the younger than in the older group. Since bone marrow lymphocytosis preceded peripheral blood lymphocytosis, it is likely that bone marrow repopulation by lymphocytes was intrinsic and not of blood origin.

Review of the medical records in these patients indicated that they had been free of infections during the “off therapy” phase. Therefore, it is unlikely that the expansion in the lymphoid compartment can be explained by primary stimulation with viral or bacterial antigens, unless these were asymptomatic infections. Another possibility considered was a lack of correlation between the increase in lymphocytes and functional recovery. A simple way to look at this problem was to determine whether the changes in lymphoid population were accompanied by a rise of their end product, the immunoglobulins, and
whether changes in immunoglobulin levels were also age dependent. As shown in Fig. 3, following cessation of chemotherapy there was an increase in the levels of serum IgC and IgM in the younger children. The most significant change was an almost threefold rise in IgG, occurring about 2 mo after therapy was stopped. IgM increased from a median value of 0.6 mg/ml to 1.2 mg/ml. Conversely, in the older group during the first 6 mo off chemotherapy, IgG and IgM levels did not vary significantly. During the same period, median values for serum IgA did not change in either group. We conclude that there is a temporal relationship between the rise in lymphocytes and the rise in serum IgG and IgM and that both phenomena are age dependent.

In normal children, serum immunoglobulin levels fall within a wide range that change with age. Normal boundaries have been established from the antilog of the mean normal log values ± twice the standard deviation of the logs. The question arose of how the levels of immunoglobulins obtained before and after cessation of therapy compare with values obtained in matched normal controls. It was found that most serum immunoglobulin levels in this group of children with ALL during and after therapy were within the normal boundaries for age. Values below and above the normal range were found only in children in the group younger than 5 yr. In this group, before cessation of treatment IgG and IgM were below normal in three children. Two months after the drugs were stopped, IgG and IgM were above normal in two patients. However, there was no statistically significant difference between IgG, IgM, and IgA values in normal children and immunoglobulin levels in the group of
children with ALL, either before or after cessation of therapy. This is an apparent paradox, since we have demonstrated that in the younger group there was a statistically significant rise in serum IgG and IgM when the drugs were discontinued. This discrepancy demonstrates that a biological phenomena such as the rise in immunoglobulins following cessation of chemotherapy would be missed if, instead of a longitudinal comparison of the same individuals as a function of time, one would attempt to compare values obtained at a single time interval with values from a group of matched controls.

The data presented above suggest that cessation of therapy was followed by a sudden burst of proliferation and differentiation of immunocompetent cells. To further explore the nature of this activity, the following question was asked. Without extrinsic antigenic stimulation will cessation of chemotherapy induce the synthesis of antibodies against an antigen to which the patient had been primed early during the suppressive phase? Four of 12 patients had a significant rise of antibodies against the hemagglutinin of the A2/Hong Kong influenza virus without evidence of reimmunization or reexposure. The age of these children was 3, 4, 5, and 5½ yr at beginning of treatment. The objection may be raised that the antibody response observed in these four patients was secondary to an inapparent influenza infection. This possibility is extremely unlikely because: chemotherapy was stopped in each of these children at different times over a wide period between May 1970, and June 1971; and at no time during this period was influenza A or B reported in the USA. (Personal communication from Dr. Walter R. Dowdle, Chief, Respiratory Virology Unit, Center for Disease Control, Atlanta, Ga.) The kinetics of rise in antibody titers in three of these patients, which are shown in Fig. 4 (upper row, LM, JB, LC), demonstrate temporal correlation between rising antibody titers and serum IgG levels. Another patient (LW) had a slight increase of both IgG and antibody titers. In two other children, antibodies to Hong Kong virus were undetectable before and after cessation of chemotherapy. Patient MT in Fig. 3 (lower row) belongs to this group. In this child the level of serum IgG did not increase during the off chemotherapy phase. In the remaining five children, antibody titers did not change after therapy was stopped. Patient FG (Fig. 3, lower right quadrant) illustrates this group. FG was 15 yr old when chemo-

Fig. 4. Relationship between serum IgG and A2/Hong Kong antibody titers in children with ALL following cessation of chemotherapy.
therapy was discontinued. At this time IgG was 24 mg/ml, and the Hong Kong influenza antibody titer 1:280. Five months later IgG was 15 mg/ml, and there was no change in antibody titer. The small number of patients in each group precludes any meaningful analysis of antibody response as a function of age. However, from this data we can conclude that extrinsic antigenic stimulation may not be necessary to reinduce antibody synthesis during the recovery phase that follows cessation of immunosuppressive therapy.

DISCUSSION

This study demonstrates the kinetics of repopulation of blood and bone marrow lymphoid compartments and a rebound of immunoglobulin and antibody synthesis following cessation of therapy in children that had received immunosuppressive drugs continuously for 2½–3 yr.

Because these children have acute lymphocytic leukemia, caution should be exercised in the interpretation of the data, particularly regarding extrapolation to patients without leukemia. Nevertheless, since all these children were in complete remission during the whole period of study, it is likely that the phenomenon described here may also be true for patients without lymphoproliferative diseases receiving immunosuppressive drugs.

Between 4 and 12 wk after cessation of long-term immunosuppressive therapy, there was a significant expansion of the lymphocyte compartment in blood and bone marrow and a rise of serum IgG and IgM. Previously, Ragab et al. had reported a rise in serum IgG and bone marrow lymphocytes in a group of children with ALL in whom chemotherapy had been discontinued. However, this was a limited observation since those patients had received various treatments, the drugs were stopped for only 4 wk, and there was no temporal analysis on repopulation kinetics or antibody production.

The relative homogeneity of diagnosis, treatment, and clinical course of the patients included in this study had permitted us to analyze the effects of immunosuppressive therapy on lymphoid cells and immunoglobulins as a function of age. Thus, we found that in children younger than 5 yr there was a significant lymphopenia and depression of IgG and IgM immunoglobulins; however, following cessation of chemotherapy, peripheral blood lymphocytes, bone marrow lymphocytes, and serum IgG and IgM increased at or above normal values. The lymphocyte rise was more pronounced and earlier in the bone marrow than in peripheral blood, suggesting a primary expansion of the intrinsic bone marrow lymphoid cell population. In the group of older children, however, we did not observe significant increase in blood lymphocytes or serum immunoglobulins. In this study we also demonstrated a rebound of antibody production against an antigen to which these patients were exposed during the immunosuppressive phase without evidence of reexposure to the same antigenic determinant.

A comment should be made on the immediate practical implications of this study. It has been stated that more than 40% lymphocytes in the bone marrow of a patient with acute lymphocytic leukemia indicates relapse. However, this study demonstrates that this is not true during the early phase of immu-
nologic recovery that follows cessation of long-term immunosuppressive therapy. In addition, caution should be exercised in the interpretation of antibody titers as a diagnostic aid in patients with ALL in remission in whom, because of infectious complications, immunosuppressive therapy is stopped for more than a week. A rise in antibodies may reflect immunologic rebound to an “old” antigen and not necessarily be secondary to the infectious process for which chemotherapy was stopped.

Although the mechanisms of the age-dependent immunologic rebound are uncertain, we will attempt to reconcile this observation with recent knowledge on the functional characteristics of lymphoid cells. Bone marrow is the major source of potentially immunocompetent cells in the adult mouse. Our observation on the early rise in bone marrow lymphocytes during the recovery phase suggests that this is also true in humans. Further support for a bone marrow origin of immunocompetent cells in man was provided by the successful immunologic reconstitution of an infant with lymphopenic hypogammaglobulinemia following bone marrow transplantation. The morphologic features of the precursor cell in bone marrow has not been characterized, but there are data indicating that it may resemble a small lymphocyte. Some of these bone marrow-derived cells circulate throughout and are processed by the thymus, reentering the peripheral blood and lymphoid compartments with new characteristics. Davies et al. have found that the majority of blood lymphocytes in mice are thymus-processed cells. Most of them are long-lived lymphocytes that also carry immunologic memory and/or the several functions involved in cell-mediated immunity. In contrast, it appears that the main function of bone marrow lymphocytes is to provide self-renewal, pluripotent stem cells, and the precursor of antibody-forming cells. The sensitivity of bone marrow-derived and thymus-processed cells to metabolic inhibitors has not been analyzed critically. However, it is known that the anamnestic responses in which long-lived lymphocytes participate are less affected by chemotherapy than primary responses.

Based on the above information, we postulate that in younger children the compartment of long-lived, or memory-carrying lymphocytes is relatively small compared to the compartment of “virgin” or short-lived lymphocytes. Under the effect of antigenic stimulation, the number of long-lived lymphocytes would increase as a function of age and become the largest fraction of circulating lymphocytes in older individuals. To explain the findings that in this age group peripheral blood lymphocytes do not significantly decrease during, or increase after, therapy, we also postulate that these long-lived lymphocytes are less sensitive to chemotherapy than the bone marrow-derived non-thymus-processed cells. Further evidence to support this hypothesis is that, as previously reported, the in vitro response to phytohemagglutinin by peripheral blood lymphocytes of these patients was not depressed by long-term immunosuppressive therapy. In addition, in the same children the anamnestic response to the neuraminidase of the Hong Kong influenza virus was less affected by chemotherapy than the primary response to the hemagglutinin determinant of the same virus.
All the drugs that these patients had received during $2\frac{1}{2}$–3 yr have been reported to produce immunosuppression both in animals and man.2,15–17 Furthermore, inoculation of an antigen during therapy may induce specific immunologic tolerance that can be demonstrated after the drugs are stopped.15,18 In this study, we describe a different phenomenon: following cessation of chemotherapy there was an increase of serum IgG with a concomitant rise of antibody titers to an antigen to which the patients had not been recently reexposed. This phenomenon bears some similarity with the enhancement effect of some immunosuppressive drugs, such as x-ray, colchicine, and 5-fluoro-2'-deoxyuridine on antibody production.17,19–20

Enhanced production of antibodies to bovine gamma globulin in rabbits treated with 6-mercaptopurine also has been reported. This effect was seen as late as 20 days after the drug was discontinued.21 However, the main difference is that in this study the patients were inoculated with the Hong Kong influenza virus about 1–2 yr before cessation of therapy. Several alternatives that are not mutually exclusive can be offered to explain this phenomenon. First, during the suppressive phase the antigen remained in the reticuloendothelial system and became accessible to an expanded lymphoid population that developed when therapy was discontinued. Accordingly, the response would be secondary to "intrinsic antigenic stimulation." Second, a small population of long-lived lymphocytes carried immunologic memory for the hemagglutinin determinant of the virus proliferate when immunosuppressive drugs were stopped. Third, the memory cells or thymus-processed lymphocytes do not proliferate during the recovery phase; however, they may now interact with the progenitors of antibody-producing cells that are released from the bone marrow lymphoid cell compartment. The consequence of this interaction would be an enhancement of IgG and antibody synthesis. That serum IgA did not significantly change in younger or older children during the off therapy phase suggests that cell interactions for the synthesis of IgA may be entirely different, or if they are the same, they occur at a later time or in a different cell compartment. These hypotheses are susceptible to experimental testing and will be explored in patients in whom chemotherapy will be stopped in the near future. The findings of an age-related immunologic rebound after cessation of long-term therapy should provide the basis for future studies on the relationship between differentiation of immunocompetent cells as a function of age and the functional individuality of bone marrow-derived and thymus-processed cells.

ACKNOWLEDGMENT

The technical assistance of Mrs. Edna Duck and Mr. L. Kerr is gratefully acknowledged.

REFERENCES


Immunologic Rebound After Cessation of Long-term Chemotherapy in Acute Leukemia

Luis Borella, Alexander A. Green and Robert G. Webster