The Immunosuppressive Effects of Long-Term Combination Chemotherapy in Children with Acute Leukemia in Remission

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SUMMARY

The immunosuppressive effects of maintenance combination chemotherapy given for periods ranging from 8 to 28 months to 20 children with acute lymphocytic leukemia in remission were investigated. There was depression of both the primary antibody production to the hemagglutinin antigen of the Hong Kong influenza virus and the anamnestic response to the neuraminidase of the same virus. The primary response (hemagglutination inhibition) was affected to a greater extent than the secondary (neuraminidase inhibition) response. A preferential depression of 2-mercaptoethanol-resistant hemagglutination inhibition antibodies (IgG) was also observed. One-fourth of all acute lymphocytic leukemia patients had low serum IgG levels. In vitro transformation of lymphocytes was a poor index of immunocompetence. After 2 years of continuous chemotherapy, children with low IgG and suppressed antibody production had normal response to phytohemagglutinin. This initial study on the immunocompetence of patients with leukemia undergoing long-term combination chemotherapy should provide a baseline to establish correlations between immunological parameters and infectious complications.

INTRODUCTION

It has been stated that combination chemotherapy is mandatory in the treatment of acute leukemia (10). Combination chemotherapy in children with acute lymphocytic leukemia at St. Jude Children's Research Hospital has resulted in prolonged remissions and a 17% 5-year cure rate (9, 19). Continuous administration of immunosuppressive therapy has, however, increased the risk and severity of infections (15). Several recent reviews discuss the effects of antineoplastic drugs upon different stages of the immune response in both animals and man (8, 13, 24). Studies in humans have been aimed at determining the degree of immunosuppression in patients receiving short-term courses of single or combined antimetabolites (11–14, 22). However, the effects of prolonged maintained combination chemotherapy upon immunocompetence have not been investigated. Knowledge of the immunosuppressive effects of long-term combination chemotherapy is now urgently needed because of the increasing number of prolonged remissions and potential 5-year cures among children with acute lymphocytic leukemia receiving this form of treatment (19).

This study was aimed at determining the immunosuppressive effects of long-term combination chemotherapy in children with acute lymphocytic leukemia. Several unique features of this investigation were as follows. (a) All patients were in remission and received the same combination chemotherapy continuously for periods ranging from 8 to 28 months. (b) Patients and controls were immunized with the Hong Kong influenza virus vaccine prior to the Hong Kong influenza virus epidemic. This permitted the assay of the primary antibody response to one of the antigenic determinants of the virus, the hemagglutinin, and the secondary antibody response to the other determinant, the neuraminidase (4, 23). (c) The effect of long-term chemotherapy upon 2 other parameters of immunocompetence, lymphocyte response to PHA and serum immunoglobulin levels, was also determined and compared.

MATERIALS AND METHODS

Selection of Patients. Immunological studies were performed in 20 children with acute lymphocytic leukemia and 22 nonleukemic children. All children with leukemia were in complete remission during the entire period of immunological evaluation. They were in the maintenance phase of the treatment, which consisted of daily 6MP, weekly MTX, and Cyclo, and a 2-week course of VCR and prednisone every 10 weeks (Table 1). They had this therapy for periods ranging from 8 to 28 months. Another group of 22 children with acute lymphocytic leukemia that were not inoculated with the Hong Kong influenza vaccine also received the same maintenance treatment for periods ranging from 4 to 26 months. They were included in this study solely in an attempt to determine clinically whether during an epidemic of influenza there was any difference in the incidence or severity of influenza-like illness between vaccinated and nonvaccinated children with acute lymphocytic leukemia.

All patients with acute lymphocytic leukemia had complete physical examinations, including white blood cell, differential, and platelet counts, weekly. The doses of antineoplastic drugs were adjusted for each individual patient to maintain the white
Table 1
Maintenance combination chemotherapy of children with acute lymphocytic leukemia in remission

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/sq m)</th>
<th>Route</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MP</td>
<td>50</td>
<td>p.o.</td>
<td>Daily</td>
</tr>
<tr>
<td>MTX</td>
<td>20</td>
<td>p.o. or i.v.</td>
<td>Weekly</td>
</tr>
<tr>
<td>Cyclo</td>
<td>200</td>
<td>p.o. or i.v.</td>
<td>Weekly</td>
</tr>
<tr>
<td>Prednisone</td>
<td>40</td>
<td>p.o.</td>
<td>Daily for 15 days every 10 weeks</td>
</tr>
<tr>
<td>VCR</td>
<td>1.5</td>
<td>i.v.</td>
<td>Weekly for 3 weeks every 10 weeks</td>
</tr>
</tbody>
</table>

Blood cell count between 2000 and 3000/cu mm. The control group consisted of 22 children without neoplastic diseases seen at the Nutrition and Metabolic Clinic for evaluation of growth, development, and nutritional status. The age of leukemic and control children ranged from 2 to 15 years and the median was 5 years; 38 were female and 26 were male.

**Vaccine and Vaccination.** Monovalent type A2/Aichi/2/68 (Hong Kong variant) influenza virus vaccine prepared by zonal centrifugation (Zonomune) containing 800 chick cell agglutinin units/ml was obtained from Eli Lilly and Company, Indianapolis, Ind. The vaccine was administered s.c. at the following dosage: for children over 10 years of age, 0.5 ml; for children 6 to 10 years old, 0.25 ml; and for children 3 months to 6 years old, 2 doses of 0.1 ml each at an interval of 2 weeks.

**Viruses.** The A2/Aichi/2/68 (Hong Kong) and the A3/Tokyo/3/67 (Tokyo) strains of influenza virus were grown in the allantois of 11-day-old chick embryos. The viruses were concentrated by differential centrifugation and stored at 4°C with 0.1% sodium azide as preservative.

**Titration of Antibodies to the Hemagglutinin and Neuraminidase Antigens of Influenza Virus.** Blood samples were obtained before vaccination and at weekly intervals thereafter for 4 weeks. To reduce the immediate suppressive effects of chemotherapy, all blood samples were obtained 7 days after the last and immediately before the next weekly injection of MTX and Cyclo and at least 4 weeks after the last dose of VCR and prednisone. The sera were stored at −20°C and treated with receptor-destroying enzyme to remove nonspecific serum inhibitors (3) before serological assays were done. Hemagglutination inhibition tests were done in plastic trays (WHO type) with the use of 4 hemagglutinating doses of virus as described previously (7).

Neuraminidase assay was done with fetuin as substrate by the method of Warren (25) except that the color was extracted into 1-butanol containing 5% (v/v) concentrated hydrochloric acid (1). Neuraminidase inhibition tests were performed as previously described (26).

**Mercaptoethanol Treatment of Sera.** Antisera were treated with a final concentration of 0.1 M 2-mercaptopethanol as described by Reisner and Franklin (20). The sera for hemagglutination inhibition tests were first treated with receptor-destroying enzyme as described above, and the antisera used in the neuraminidase inhibition test were extensively dialyzed against phosphate-buffered solution after treatment with 2-mercaptoethanol.

**Criteria for Clinical Diagnosis of Influenza.** The children in this study were vaccinated against Hong Kong influenza virus during the first 2 weeks of December 1968. Written parental consent was obtained in all cases. The Hong Kong influenza epidemic was first detected in the local community (Memphis, Tenn.) on December 20, 1968, reached a peak on January 10, 1969, and was over by January 31, 1969. The following list of symptoms was considered for clinical diagnosis of influenza virus infection during the epidemic period: fatigue, headache, myalgia, temperature over 38°C, lacrimation, coryza, sore throat, cough, hoarseness, nausea, and vomiting. Since identification of the virus was not attempted, we have used the term influenza-like illness rather than influenza to describe the infections. Siblings of leukemic children served as normal controls.

**In Vitro Stimulation of Peripheral Blood Lymphocytes with PHA.** For avoidance of any interference from previous immunization upon the response of peripheral blood lymphocytes to PHA stimulation, this test was performed at least 6 months after influenza vaccination. The 13 patients evaluated were still in continuous complete remission and receiving the same uninterrupted combination chemotherapy. Fifteen ml of venous blood were removed 7 days after the last dose of MTX and Cyclo, mixed with 250 units of heparin, and allowed to sediment for 2 hr at 37°C in a 20-ml plastic syringe in a vertical position. The WBC-rich plasma was transferred to a sterile tube, and the cells were then cultured in glass tubes according to the method described by Hersh and Oppenheim (14). Each culture contained $2 \times 10^6$ WBC; 40 to 60% of these were lymphocytes. Half of the cultures were inoculated with 0.025 ml of PHA (Difco Laboratories, Detroit, Mich.). For each patient and each determination, sets of 4 stimulated and 4 nonstimulated cultures were established. Three days after initiating the culture, 1.5 μCi of thymidine-3H (Schwarz BioResearch, Inc., Orangeburg, N. Y.) with a specific activity of 3.0 Ci/mmol were added to each tube and the cells were incubated for an additional 18 hr. The cells were washed twice with 0.9% NaCl solution, and the nucleoproteins were precipitated with 3 ml of 5% tricholoroacetic acid; 1 ml of bovine serum albumin (500 μg) was added as carrier protein. The precipitates were taken up in 1.0 ml of NCS (Amersham-Searle, Chicago, Ill.), mixed with 10 ml of toluene-based scintillant, and counted in a liquid scintillation spectrometer. All these procedures were performed at 4°C. The results were expressed as the relationship between PHA stimulation in cultures from leukemic children over the PHA stimulation in control cultures (Fig. 3). PHA stimulation was defined as the ratio of cpm in cultures with PHA over the cpm in cultures without PHA. In several cultures, the percentage of blasts per $10^6$ WBC was also calculated; there was a good agreement between PHA stimulation as determined by the incorporation of thymidine-3H and blastic transformation determined morphologically.

**Serum Immunoglobulin Levels.** Serum immunoglobulin concentrations were determined by a modification (6) of the single radial diffusion method of Mancini et al. (17). Quantitative immunodiffusion plates and standard sera were
obtained from Melpar, Inc., Falls Church, Va. All values reported were from preimmunization sera. The results were compared with the curve of immunoglobulin concentrations of sera obtained from 50 normal children used as standard reference in this hospital. There was agreement between immunoglobulin levels in our control group and those reported by Buckley et al. (2). Immunoglobulins were considered low when the values were below the normal bounds for age as described by Buckley et al. (2).

RESULTS

Hemagglutinin and Neuraminidase Antibody Levels after Vaccination of Normal and Leukemic Children with Hong Kong Influenza Virus. Both groups of children in this study received Hong Kong influenza vaccine before the onset of the Hong Kong influenza virus epidemic in 1968. Therefore, this was their first exposure to the Hong Kong influenza virus hemagglutinin. On the other hand, the neuraminidase on the Hong Kong influenza virus was not different from the neuraminidase on the preceding influenza viruses (4), and many of these children would have had experience with this antigen before vaccination.

Children with acute lymphocytic leukemia on long-term maintenance chemotherapy had a depressed immune response to the Hong Kong influenza virus vaccine. However, several differences were noticed between the antibody response to the hemagglutinin and neuraminidase antigens (Chart 1, Table 2). (a) In the control children and children with acute lymphocytic leukemia, the mean preimmunization hemagglutination inhibition antibody titers were below the lowest dilution tested; in contrast, the mean log₂ initial titers to the neuraminidase were 5.9 in the normal children and 4.9 in the acute lymphocytic leukemia group. (b) All control children responded to both antigenic determinants of the virus, but in the acute lymphocytic leukemia group, 5 and 2 patients failed to produce antibodies to the hemagglutinin and the neuraminidase, respectively. (c) At the peak of the antibody response, the mean hemagglutination inhibition titer of patients receiving immunosuppressive drugs was at least 10-fold below that of the control group. The mean neuraminidase inhibition antibody titer was depressed less than the hemagglutination inhibition response, and most of these children were producing neuraminidase antibodies in excess of 1:128 and as high as 1:2048.

These results indicate that long-term combination chemotherapy depresses both the primary and secondary immune response. Antibody production to the new hemagglutinin antigen of Hong Kong influenza was inhibited to a greater extent than the response to the antigenic determinant present in influenza viruses from preceding years: the neuraminidase.

Table 2

The immune response of normal and leukemic children to influenza virus (A₂/Hong Kong) Mean antibody titers of 20 children with acute lymphocytic leukemia and 22 nonleukemic children 4 weeks after immunization with the A₂/Hong Kong influenza virus vaccine.

<table>
<thead>
<tr>
<th>Test</th>
<th>Leukemic children in remission</th>
<th>Normal children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prebleed</td>
<td>Post vaccination</td>
</tr>
<tr>
<td>Hemagglutination inhibition</td>
<td>2.0ᵇ</td>
<td>4.4</td>
</tr>
<tr>
<td>Neuraminidase inhibition</td>
<td>4.9</td>
<td>7.0</td>
</tr>
</tbody>
</table>

ᵇ Antibody titers are expressed as mean log₂/0.25 ml. Postvaccination sera were obtained 4 weeks after immunization.

Less than mean

Chart 1. Hemagglutination and neuraminidase antibody titers after vaccination of normal and leukemic children with the Hong Kong influenza virus vaccine. The probability that the differences between the groups with acute lymphocytic leukemia (ALL) and normal groups may occur by chance alone is <0.001 for hemagglutination inhibition and 0.001 for neuraminidase inhibition.
measure of the IgM response, the appearance of by treatment with 2-mercaptoethanol. All other 4-week sera had a less chemotherapy also induced qualitative changes, the sera were above studies indicated a quantitative difference in antibody Neuraminidase antibodies against the neuraminidase antigen had 2-mercaptoethanol-resistant hemagglutination inhibition antibodies 4 weeks after acute lymphocytic leukemia and normal children. The antibodies directed against the neuraminidase antigen of Hong Kong virus (23). Children with acute lymphocytic leukemia also had preimmunization titers to the Tokyo 67 strain; however, these were below the initial titers of the controls. A slight rise was also noticed around the 3rd week, but at 4 weeks they had returned to the preimmunization level.

These data show that most children had previous exposure to the Tokyo influenza virus and immunization with the Hong Kong virus failed to induce a true secondary response to the hemagglutinin antigen of the Tokyo virus.

Incidence of Influenza-like Illness in Vaccinated and Nonvaccinated Groups of Normal and Leukemic Children. The incidence of influenza-like illness in normal and leukemic groups of children receiving Hong Kong influenza vaccine is

Sensitivity of Antibodies to 2-Mercaptoethanol. The above studies indicated a quantitative difference in antibody response. To determine whether or not prolonged chemotherapy also induced qualitative changes, the sera were titrated before and after treatment with 2-mercaptoethanol. Although 2-mercaptoethanol sensitivity is not an absolute measure of the IgM response, the appearance of 2-mercaptoethanol-resistant antibodies usually parallels the IgG response.

As shown in Table 3, only one-third of the children with acute lymphocytic leukemia that responded to the hemagglutinin antigen had 2-mercaptoethanol-resistant hemagglutination inhibition antibodies 4 weeks after immunization. Conversely, in the control group, all children had 2-mercaptoethanol-resistant hemagglutination inhibition antibody, and in all determinations the reduction in antibody titers following 2-mercaptoethanol treatment was less than 50% of the total. On the other hand, in all 4-week sera from the children with acute lymphocytic leukemia and control children, the antibodies against the neuraminidase antigen were mainly 2-mercaptoethanol resistant. There was no difference in the amount of 2-mercaptoethanol-sensitive neuraminidase inhibition antibodies between the children with acute lymphocytic leukemia and normal children.

These results indicate that prolonged chemotherapy inhibited the primary IgG antibody response to a greater extent than the primary IgM response. However, there was no detectable difference in the quality of the secondary response to the neuraminidase antigen. As previously mentioned, the neuraminidase is not different from the enzyme on preceding influenza viruses.

Kinetics of Antibody Response to the Hemagglutinin of the Tokyo and Hong Kong Influenza Viruses. In the interpretation of the above results, we assumed that most of these children had previous contact with influenza viruses from preceding years. Thus, it was important to determine whether they had preexisting antibodies to the hemagglutinin of the influenza virus from the year before (Tokyo/67) and to compare the kinetics of response to these 2 viruses following immunization with the Hong Kong influenza virus vaccine. The results shown in Chart 2 demonstrate that the sera of both children with acute lymphocytic leukemia and control children had higher initial titers to the Tokyo/67 virus than to the Hong Kong strain. In the control groups, the anti-Hong Kong hemagglutination inhibition log₂ titer rose in the first 2 weeks from less than 2 to 8.5. In contrast, in the same group there was only 1 log₂ difference between the initial and the 2-week antibody titer to the Tokyo virus. This slight increase can be explained in terms of steric inhibition of hemagglutination by antibodies directed against the neuraminidase antigen of Hong Kong virus (23). Children with acute lymphocytic leukemia also had preimmunization titers to the Tokyo 67 strain; however, these were below the initial titers of the controls. A slight rise was also noticed around the 3rd week, but at 4 weeks they had returned to the preimmunization level.

These data show that most children had previous exposure to the Tokyo influenza virus and immunization with the Hong Kong virus failed to induce a true secondary response to the hemagglutinin antigen of the Tokyo virus.

Table 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Children with acute lymphocytic leukemia in remission</th>
<th>Normal children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemagglutination inhibition</td>
<td>9/15</td>
<td>0/18</td>
</tr>
<tr>
<td>Neuraminidase inhibition</td>
<td>0/17</td>
<td>0/18</td>
</tr>
</tbody>
</table>

* The number of 4-week sera that had all antibody activity removed by treatment with 2-mercaptoethanol. All other 4-week sera had a less than 50% reduction in titer after 2-mercaptoethanol treatment.

* The size of the samples did not permit the measurement of neuraminidase inhibition and mercaptoethanol sensitivity in 4 of the control sera.

Incidence of Influenza-like Illness in Vaccinated and Nonvaccinated Groups of Normal and Leukemic Children. The clinical diagnosis of influenza-like illness was made during the period of Hong Kong influenza epidemic in the local community according to the symptoms described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>No. with illness (total)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemic children</td>
<td>Vaccinated</td>
<td>2/20</td>
<td>10.0</td>
</tr>
<tr>
<td>Leukemic children in remission</td>
<td>Not vaccinated</td>
<td>10/22</td>
<td>45.5</td>
</tr>
<tr>
<td>Siblings of leukemic children</td>
<td>Not vaccinated</td>
<td>13/51</td>
<td>25.5</td>
</tr>
</tbody>
</table>

* Hong Kong influenza vaccine.
The relationship between serum immunoglobulins and antibody response

Table 5
The relationship between serum immunoglobulins and antibody response

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>IgG (mg/100 ml)</th>
<th>IgA (mg/100 ml)</th>
<th>IgM (mg/100 ml)</th>
<th>HI</th>
<th>NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. J.</td>
<td>3</td>
<td>375</td>
<td>30</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. C.</td>
<td>4</td>
<td>390</td>
<td>30</td>
<td>52</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D. M.</td>
<td>4</td>
<td>375</td>
<td>&lt;15</td>
<td>45</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T. S.</td>
<td>3</td>
<td>350</td>
<td>30</td>
<td>98</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. J.</td>
<td>12</td>
<td>370</td>
<td>74</td>
<td>46</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Group 1: Immunized children with acute lymphocytic leukemia with low IgG

15 patients 2–12

849 (480–1200) 90 (50–180) 60 (27–105)

2/15<sup>a</sup> 1/15<sup>b</sup>

Group 2: Immunized children with acute lymphocytic leukemia

22 patients 2–12

1037 (670–1450) 124 (60–200) 64 (30–130)

0/22 0/18

<sup>a</sup> Absence of hemagglutination antibodies/total tested.
<sup>b</sup> Absence of neuraminidase antibodies/total tested.

shown in Table 4. The incidence of illness in nonvaccinated leukemia children was 45.5% and in vaccinated leukemic children it was 10%, a figure below the incidence of illness in normal children (25.5%). The incidence of influenza-like illness in the vaccinated leukemic group of children was significantly lower than in the nonvaccinated group of leukemic children: p = 0.01 by the X<sup>2</sup> test. However, since evaluation was solely on clinical grounds and no virus isolation was attempted, these results suggest only that the Hong Kong influenza vaccine reduced the incidence of influenza in children with acute lymphocytic leukemia in remission.

Relationship between Serum Immunoglobulins and Antibody Response. The levels of serum IgG, IgM, and IgA were determined to investigate whether or not there was a correlation between the effects of long-term chemotherapy upon serum immunoglobulins and antibody response. As shown in Table 5, one-fourth of the children receiving immunosuppressive drugs had low serum IgG. Four of these 5 patients also had low IgA. Three of these 5 children had no hemagglutination inhibition antibodies to the Hong Kong virus vaccine. On the other hand, only 2 of the 15 children with acute lymphocytic leukemia who had normal levels of immunoglobulins failed to produce hemagglutination inhibition antibodies. These results indicate that long-term combination chemotherapy may depress serum IgG and IgA. The lack of apparent effects upon IgM may be related to the previously described prolongation of IgM antibody response to the hemagglutinin-antigenic determinant of the influenza virus.

Response of Peripheral Blood Lymphocytes to PHA Stimulation. The response of peripheral blood lymphocytes to PHA is nonspecific. On the other hand, it has been correlated with other in vitro specific immune responses and has been shown to be markedly depressed by immunosuppressive drugs (14). We investigated whether combination chemotherapy given without interruption for 17 to 28 months had any effect upon this in vitro response and whether there was any correlation between PHA stimulation and duration of treatment. The method used to determine PHA response in cultures from patients and controls is described in the text. ○, children with no hemagglutination inhibition antibody response. Three of them had low IgG.
transformation or thymidine-$^3$H incorporation and either the length of treatment or hemagglutination or neuraminidase inhibition antibody response. Thus, the in vitro response of peripheral blood lymphocytes to PHA was not affected by and cannot be used as a sensitive parameter of the immunosuppressive effects of long-term chemotherapy.

**DISCUSSION**

The major aim of this study was to determine the effects of long-term combination chemotherapy upon immunocompetence in children with acute lymphocytic leukemia in remission. At the time of this study all patients were in continuous complete remission and receiving maintenance chemotherapy for periods ranging from 8 to 28 months.

Long-term chemotherapy with a combination of 6MP, MTX, Cyclo, VCR, and prednisone depressed the antibody response to the hemagglutinin and neuraminidase antigens of the Hong Kong influenza virus. Antibodies to the hemagglutinin determinant were inhibited to a greater extent than antibodies to the neuraminidase.

Results from this and other studies (4, 23) indicate that the hemagglutinin antigen of Hong Kong influenza virus was a new antigen in 1968 and distinct from the hemagglutinin antigen on the influenza virus in the preceding years. However, the neuraminidase on Hong Kong influenza virus was identical with the neuraminidase antigen on the influenza viruses that were current in the preceding years.

We can conclude that this maintenance combination chemotherapy inhibited both the primary and the secondary antibody response. However, the primary response was more affected than the anamnestic response. These effects may vary with other antigens and different routes of inoculation.

All the drugs used in this maintenance phase have been shown to depress the primary immune response in animals and man (8, 12, 22). Previous studies in man had been designed, however, to determine the immunosuppressive effects of single (11, 12, 22) or combined antimetabolites (12) given in short-term courses. Hersh et al. (12) and Santos (22) had reported that 6MP given in a weekly total dose of 1.5 to 10 g/sq m depressed the primary response to tularemia, Vi, and pneumococcal antigens. Similar inhibition of antibody production was obtained with Cyclo given i.v. at a dose of 7 mg/kg for a period of 7 consecutive days. These weekly doses were approximately 7 to 10 times higher than the maintenance dosage of this study. Thus, significant differences in dosage, schedule, and duration of treatment prevents any fruitful comparison between the results from this and previous studies.

In animals treated with 6MP, the suppression of Igg antibodies led to a prolongation of the IgM response (21). The important concept of the feedback control of IgM production by IgG antibodies is based on these experimental data. Santos reported that in 5 patients treated with 6MP the anti-Vi antibody activity was present only in the 19 S fraction of their sera (22). In our study, children receiving combination chemotherapy also showed a preferential suppression of IgG antibody response, but only to the primary stimulation with the hemagglutinin antigen. The clinical information obtained suggested that, during an epidemic of influenza, children with leukemia in remission may be protected by prompt immunization. However, a definitive answer should wait for prospective studies with systematic virus isolation.

Serum IgG was depressed in one-fourth of the children with leukemia in remission. This is in agreement with our own unpublished observation of low levels of serum IgG in a larger group of patients treated with the same chemotherapy protocol. The apparent discrepancy with other reports of normal IgG levels in patients with leukemia in remission can be explained by the shorter duration of the maintenance phase or by differences in drug schedule in those studies (16, 18). In the report of McKelvey and Carbone (18), the immunoglobulin levels were determined only in the first 7 months of the maintenance phase. The patients studied by Kian and Gross (16) received various treatments, but only 1 or 2 drugs were used simultaneously. In our study, 3 of the 5 children with low IgG failed to produce hemagglutination inhibition antibodies. These 2 parameters of severe immunosuppression in the same individuals may indicate genetic hypersusceptibility to chemotherapy or minor individual differences in drug dosage.

In vitro lymphocyte transformation has been used as a sensitive and reproducible way of evaluating the immunocompetence of circulating lymphocytes. Intensive combination chemotherapy with 6MP and MTX completely abolished transformation after 3 days of treatment (14). However, our study conclusively shows that combination therapy given continuously for more than 2 years does not affect the response of peripheral blood lymphocytes to PHA. Since we were more intrested in the long-term than in immediate effects of this treatment, the samples were obtained 7 days after the last weekly doses of Cyclo and MTX. Therefore, the immediate suppressive effects of these drugs were not measured since a substantial recovery of the lymphocytes may occur as soon as 3 days after stopping a short-term course of combination chemotherapy (14).

The use of combination chemotherapy has increased the duration of continuous complete remission and the 5-year cure rate (9, 19). However, serious infections threatened the life of these patients during the prolonged maintenance phase of treatment. At this institution, children with acute leukemia in remission have died mainly of nonbacterial infections not preceded by granulocytopenia (15). This study provides an initial profile on the effects of this long-term chemotherapy upon the immunocompetence of these children. Currently, the levels of secretory immunoglobulins and IgA antibodies and the response of peripheral blood lymphocytes to hemocyanin (5) are also under investigation. These data are now being used in the clinical follow-up of these children with acute lymphocytic leukemia in remission with the hope to establish definitive correlations between these parameters of immunocompetence and incidence and severity of infections.

**REFERENCES**


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