The G-Immunoglobulins in Acute Leukemia in Children
Hematologic and Immunologic Relationships

By OZDEN KIRAN AND SAMUEL GROSS

CONSIDERABLE DATA are available on the effectiveness of continuous drug therapy in prolonging survival times in acute childhood leukemia and on the specific action of these drugs as cytotoxic and immunosuppressive agents. However, there is only fragmentary data on the effectiveness of the immune response in patients with acute leukemia undergoing continuous immunosuppressive therapy. A major contribution in this regard was furnished by McKelvey and Carbone in a short-term study of a group of adults and children with acute leukemia undergoing continuous therapy. In their investigation they noted that, following the introduction of therapy, the IgG levels fell within 2 to 4 weeks and returned to normal at 8 to 10 weeks, whereas the IgA levels remained constantly depressed and the IgM levels were unchanged.

In the present investigation, the effects of prolonged and intensive immunosuppressive therapy on IgG levels in acute childhood leukemia were studied with emphasis in the following areas: at the onset and during the remission-induction phase; during persistent therapy at arbitrarily designated intervals of 10 and 34 months; and during the terminal stages as an index of the effects of therapy, the response to infection, and impending death.

MATERIALS AND METHODS

Twenty-nine children, ages 3 to 12 years, with acute lymphoblastic leukemia served as subjects for the study which was divided into 4 major groupings. Group A consisted of 7 newly diagnosed patients, whose determinations were made before and following 5 weeks of induction therapy. Group B was comprised of 7 patients on continuous therapy with determinations carried out at 10 and 11 months, respectively. Group C included 13 patients on continuous therapy with the studies performed at 34 and 35 months, respectively. Group D consisted of 6 patients in the terminal stages of the disease.

Because of the difference in time and response to therapy, not all of the patients were receiving the same therapy at the time of testing. However, this difference was obviated by taking different groups at different stages and comparing their serial determinations. In general, the therapeutic regimen consisted of induction with weekly I.V. vincristine and daily oral prednisone until remission (or an average of 4 weeks), following which they received parenteral methotrexate until relapse. Thereafter, they received oral 6-

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Table 1.—Group A. Serum IgG Levels at the Onset and After 5 Weeks of Continuous Therapy

<table>
<thead>
<tr>
<th>Case &amp; Age (yr.)</th>
<th>Pretherapy WBC/mm.³ % Blasts</th>
<th>IgG (mg./ml.)</th>
<th>Therapy</th>
<th>Response</th>
<th>Post-therapy WBC/mm.³ % Blasts</th>
<th>IgG (mg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (12)</td>
<td>1500</td>
<td>100%</td>
<td>8.3 Prednisone</td>
<td>+</td>
<td>6000</td>
<td>7.3</td>
</tr>
<tr>
<td>2 (3)</td>
<td>1500</td>
<td>100%</td>
<td>11.7 Prednisone</td>
<td>++</td>
<td>4000</td>
<td>10.9</td>
</tr>
<tr>
<td>3 (4)</td>
<td>5500</td>
<td>30%</td>
<td>13.0 Prednisone</td>
<td>++</td>
<td>3600</td>
<td>5.0</td>
</tr>
<tr>
<td>4 (5)</td>
<td>7000</td>
<td>60%</td>
<td>11.0 Prednisone</td>
<td>++</td>
<td>8200</td>
<td>6.0</td>
</tr>
<tr>
<td>5 (11)</td>
<td>4450</td>
<td>35%</td>
<td>14.7 Prednisone</td>
<td>++</td>
<td>3200</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-Mercaptopurine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (11)</td>
<td>37,800</td>
<td>60%</td>
<td>12.2 Prednisone</td>
<td>++</td>
<td>1100</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-Mercaptopurine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (8)</td>
<td>18,000</td>
<td>35%</td>
<td>11.4 Prednisone</td>
<td>++</td>
<td>11,600</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methotrexate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± 1 S.D. 11.8 ± 2.2 8.5 ± 2.4 0.025 <p <0.05

* + Partial remission.
++ Complete remission.

mercaptopurine daily, followed by oral cytoxan, whereupon the cycle was repeated according to the individual responses. Two patients received prednisone and 6-mercaptopurine and one patient received prednisone and methotrexate for induction therapy. One patient was given gamma globulin. None of the other patients received exogenous gamma globulin except that which is available in the transfusion of packed red cells.

The serum immunoglobulin assays were performed according to the antibody agar plate technic of Fahey and McKelvey. Each set of determinations on 0.1 ml. of serum was run in duplicate. A known set of values was used to construct a standard line from which the unknown levels were read. The control immunoglobulin assays were done on 27 normal, age-matched children.

All of the routine hematologic data, including the bone marrow aspirations, were carried out according to standard procedures. The distinction between acute lymphoblastic and acute myeloblastic leukemia was based on the morphologic appearance with the support of the arylsulfatase histochemical technic.

The designation of remission or relapse was based on clinical findings relative to organomegaly, lymphadenopathy and the characteristics of the peripheral blood. The term, partial remission, was used in reference to diminution in clinical manifestations and/or an incomplete peripheral hematologic response, whereas complete remission referred to absence of all clinical and peripheral hematologic abnormalities. Similarly, early relapse indicated the beginning reappearance of clinical and/or peripheral hematologic abnormalities.

RESULTS

The normal mean ± 1 S.D. of IgG levels among the 27 control children was 11.3 ± 1.4 mg./ml. In group A (Table 1), the mean pretreatment IgG assay was 11.8 mg./ml. ± 2.2 mg./ml. with a range of 8.3 to 14.7 mg./ml. Five weeks later, the mean level in these patients was 8.5 ± 2.4 mg./ml. with a range of 5.0 to 10.9 mg./ml. The pretherapy levels were not significantly different from the control levels, whereas the difference between the pretherapy assays
and those performed after a time lapse of 5 weeks was significant to a level of 
\( p < 0.05 \).

In Group B (Table 2), all but one patient were in complete remission. 
Five patients were receiving parenteral methotrexate, one patient had been 
taking 6-mercaptopurine, and one patient, in early relapse, was receiving 
vincristine and prednisone. The mean IgG of this group was 12.0 \( \pm 2.9 \) mg./
ml. with a range between 8.2 and 16.9 mg./ml. One month later, repeat assays 
revealed a mean level of 10.7 \( \pm 2.9 \) mg./ml. which did not differ significantly 
either from the normal controls or the determinations carried out one month 
previously. The only major individual difference was noted in patient 8, who 
was then in complete relapse, and whose IgG level had fallen from 10.6 to 
6.1 mg./ml.

In Group C (Table 3), there were 13 patients all of whom had received 
continuous therapy for 34 months, at which time the mean IgG assay was 11.6 
\( \pm 1.2 \) mg./ml. One month later, the mean level was 10.1 \( \pm 2.0 \) mg./ml. which 
did not differ significantly either from the normal controls or the 34 month 
assay. In patient 19, following a change in therapy from cytoxan to prednisone 
and vincristine which resulted in a favorable clinical response, the IgG level 
declined from 10.5 to 9.0 mg./ml. In patient 21, a change in therapy did not 
 affect a change in the clinical state, although there occurred a decrease of 4.0 
mg./ml. in the IgG level. In patient 24, a decline of 3.6 mg./ml. followed a 
 change in therapy of cytoxan and prednisone to parenteral methotrexate. The 
mean decline of the 3 patients with therapy changes was 3.03 mg./ml. as 
compared to a mean decline of 0.92 mg./ml. in the 10 patients whose therapy was 
not altered. Sufficient numbers of patients were unavailable, however, for 
significant statistical analysis.

Group D (Table 4) represents the results obtained on 6 patients in the 
terminal stages of the disease. The mean IgG of these patients was 3.0 \( \pm 0.8 \) 
mg./ml.

In patient 8, the IgG level fell from 10.6 to 6.1 mg./ml. in 28 days, and 14 
days later it had dropped further to 2.2 mg./ml. The patient lived an additional 
20 days and died following an *E. coli* septicemia.

Patient 9 was in complete remission 135 days prior to death at a time when 
the IgG level was 13.5 mg./ml. Four months later, during the unresponsive 
stage of the disease, the IgG level was 2.0 mg./ml. at which time the patient 
developed a *phaeolomyces septicemia* and died 15 days later. The administration 
of gamma globulin one week prior to death had no beneficial effect.

Patient 15 was in relapse 42 days prior to death, at which time the IgG 
level was 10.5 mg./ml. Twenty days later the IgG level was 3.7 mg./ml. She 
then developed an *E. coli septicemia* and died within 18 days.

Patient 16, three months prior to death, had an IgG level of 9.2 mg./ml. 
and was in remission. A relapse occurred shortly thereafter. Three days prior 
to death, the patient had an IgG level of 3.6 mg./ml. and was found to have 
an *E. coli septicemia*.

Seventeen days prior to death, patient 28 was in relapse and had an IgG 
level of 7.8 mg./ml. Ten days later the IgG level was 3.5 mg./ml. At that 
time she developed an *E. coli septicemia* and expired 3 days later.
<table>
<thead>
<tr>
<th>Case &amp; Age (yrs.)</th>
<th>Duration of Current Therapy (days)</th>
<th>WBC/mm^3</th>
<th>IgG (mg/mL)</th>
<th>Status</th>
<th>WBC/mm^3</th>
<th>IgG (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>(5)</td>
<td>850</td>
<td>10.6</td>
<td>++</td>
<td>54,000</td>
<td>6.1</td>
</tr>
<tr>
<td>9</td>
<td>(10)</td>
<td>4,550</td>
<td>0%</td>
<td>++</td>
<td>6,000</td>
<td>13.5</td>
</tr>
<tr>
<td>10</td>
<td>(10)</td>
<td>6,000</td>
<td>0%</td>
<td>(30)</td>
<td>6,500</td>
<td>12.7</td>
</tr>
<tr>
<td>11</td>
<td>(8)</td>
<td>4,550</td>
<td>0%</td>
<td>(30)</td>
<td>4,550</td>
<td>0%</td>
</tr>
<tr>
<td>12</td>
<td>(5)</td>
<td>4,550</td>
<td>0%</td>
<td>(30)</td>
<td>4,550</td>
<td>0%</td>
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<tr>
<td>13</td>
<td>(10)</td>
<td>8,500</td>
<td>0%</td>
<td>(30)</td>
<td>5,000</td>
<td>0%</td>
</tr>
<tr>
<td>14</td>
<td>(7)</td>
<td>4,550</td>
<td>0%</td>
<td>(30)</td>
<td>5,000</td>
<td>0%</td>
</tr>
</tbody>
</table>

Mean±1 S.D. 12.5±2.9

* Partial Remission
** Complete Remission
Table 3.—Group C. Serum IgG after 34 and 35 Months of Continuous Therapy

<table>
<thead>
<tr>
<th>Case &amp; Age (yrs.)</th>
<th>Duration of Current Therapy (days)</th>
<th>Status* WBC/mm.³ % Blasts</th>
<th>IgG (mg./ml.)</th>
<th>Therapy</th>
<th>Status* WBC/mm.³ % Blasts</th>
<th>IgG (mg./ml.)</th>
</tr>
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<tr>
<td></td>
<td>34 Months</td>
<td></td>
<td></td>
<td>35 Months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (4) Methotrexate (150)</td>
<td>--</td>
<td>1500</td>
<td>11.5</td>
<td>Cytoxan</td>
<td>--</td>
<td>5350</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>(12)</td>
<td>16%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 (3) Methotrexate (635)</td>
<td>++</td>
<td>6550</td>
<td>10.5</td>
<td>Methotrexate</td>
<td>++</td>
<td>4950</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 (5) Methotrexate (210)</td>
<td>++</td>
<td>4400</td>
<td>10.9</td>
<td>Methotrexate</td>
<td>++</td>
<td>5100</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 (8) Cytoxan</td>
<td>(780)</td>
<td>++</td>
<td>3100</td>
<td>11.9</td>
<td>Cytoxan</td>
<td>++</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 (4) Cytoxan</td>
<td>(515)</td>
<td>--</td>
<td>900</td>
<td>8%</td>
<td>Prednisone</td>
<td>(30)</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (11) Methotrexate (128)</td>
<td>+</td>
<td>3900</td>
<td>11.8</td>
<td>Methotrexate</td>
<td>+</td>
<td>1800</td>
</tr>
<tr>
<td>21 (7) 6-Mercaptopurine (270)</td>
<td>+</td>
<td>1600</td>
<td>10.5</td>
<td>Prednisone</td>
<td>(30)</td>
<td>2450</td>
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<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 (6) Cytoxan</td>
<td>(210)</td>
<td>++</td>
<td>2750</td>
<td>10.3</td>
<td>Cytoxan</td>
<td>++</td>
</tr>
<tr>
<td>23 (7) Methotrexate (520)</td>
<td>++</td>
<td>5800</td>
<td>14.1</td>
<td>Methotrexate</td>
<td>++</td>
<td>5000</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 (6) Cytoxan</td>
<td>(420)</td>
<td>±</td>
<td>17,400</td>
<td>11.4</td>
<td>Methotrexate</td>
<td>(30)</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 (11) 6-Mercaptopurine (320)</td>
<td>++</td>
<td>4650</td>
<td>13.2</td>
<td>Methotrexate</td>
<td>++</td>
<td>6000</td>
</tr>
<tr>
<td>Prednisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 (17) Cytoxan</td>
<td>(515)</td>
<td>++</td>
<td>3850</td>
<td>10.9</td>
<td>Cytoxan</td>
<td>++</td>
</tr>
<tr>
<td>27 (7) Methotrexate (170)</td>
<td>++</td>
<td>5150</td>
<td>12.9</td>
<td>Methotrexate</td>
<td>++</td>
<td>5900</td>
</tr>
</tbody>
</table>

Mean±1 S.D.  
11.6±1.2  
10.2±2.0

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*—Complete relapse
±—Early relapse
++—Partial remission
+++—Complete remission

0.05 < p < 0.10
When patient 29 (Fig. 1) was first seen, she had a WBC of 500,000/mm$^3$ with 80 per cent blast forms and an IgG level of 11.7 mg./ml. Twenty-five days later, on prednisone and vincristine, her WBC was 800/mm$^3$ with a normal differential count, and her IgG level was 10.9 mg./ml. She relapsed after 150 days, at which time her IgG level was 8.4 mg./ml. and her WBC was 40,000/mm$^3$ with 60 per cent blast forms. Her physical examination was marked by massive hepatosplenomegaly and disfiguring lymphadenopathy. Vincristine administration resulted in a fall in WBC to 400/mm$^3$ and in the IgG level to 7.6 mg./ml. Her physical examination did not improve. Five days later she developed classic rubeola, and within 11 days she entered a complete remission with an IgG level of 11.9 mg./ml. The response lasted 2 weeks, following which she declined rapidly and had an IgG level of less than 4.0 mg./ml. She then developed a Clostridium septicum septicemia with IgG levels in the 2.5 to 2.9 mg./ml. range and expired 30 days later.

**DISCUSSION**

The data indicate that the initial IgG levels in untreated children with acute leukemia are quantitatively normal. Therapy with any of the common antileukemic drugs results in a significant drop in IgG levels within 5 weeks, following which there is a return to normal levels despite continuation of therapy. These results are substantially in agreement with the work of McKelvey and Carbone. The presence of quantitatively normal IgG globulins in the initial stage of the disease, irrespective of the total white cell count or blast cell count further indicates that the decline in IgG levels following therapy is the direct result of therapy. Implicit, also, is the knowledge that among the abnormal cells, there are sufficient numbers of cells capable of synthesizing adequate amounts of immunoglobulins.

Added evidence that IgG production is only temporarily impaired during the responsive stage is shown by the persistence of a normal range of IgG values during prolonged and continuous use of immuno suppressive agents irrespective of the clinical state. The temporary declines in IgG levels usually followed a change in therapy. Whether this phenomenon is due to the persistence of a nor-
Fig. 1.—IgG assay in patient 29. Included are the concomitant white blood counts, per cent of blasts and therapy. The peak IgG level at 6.5 months represents a response to rubeola. Prior to death the patient’s IgG level was 3.5 mg./ml. Death followed a bacterial septicemia.

During the preterminal stages the patients were no longer responsive to drug therapy, and their IgG levels were low. Yet their clinical and hematologic appearance was the same as it was at the onset of the disease. Once their IgG levels fell below 3.5 to 3.8 mg./ml., they were no longer able to respond to infection or evoke an IgG response. It appears that IgG levels during the course of the disease may well serve as a prognostic guide relative to terminal events.

The work by Noble and Fudenberg on lysozyme activity in the granulocytes in granulocytic leukemia bears some similarities to the IgG levels relative to terminal events but is more clearly related to cell maturation. Thus, in the initial and terminal stages of granulocytic leukemia, lysozymes are exceedingly low. The IgG levels, however, show no such blood count relationship; and so long as they exceed approximately 3.5 mg./ml. they appear to be capable of responding to various antigenic stimuli irrespective of the number or type of white cells.

In a recent report on survival studies in the Furth rat leukemia, some insight is offered into the relationship between prolonged survival and immunologic responsiveness. By using Freund’s complete adjuvant in combination with the separate administration of cyclophosphamide, it was possible to produce much
longer survivals than could either the adjuvant or the cyclophosphamide alone. To account for this, it was hypothesized that this combination possibly represented a heightened immunologic attack in combination with the cytotoxic action of cyclophosphamide. Although the evidence is meagre, the possibility exists that the maintenance of a heightened immunologic response may induce longer survival times in acute lymphoblastic leukemia. Additional studies along modified lines in human leukemic patients would help to establish the relationship between immunologic responsiveness and prolonged survival times.

**SUMMARY**

Serial IgG determinations were carried out on 29 children with acute leukemia. With the exception of the pretreatment assays, all determinations were performed during the course of specific antileukemic therapy. Following the initial introduction to cytotoxic agents, a significant, although temporary, reduction in IgG level occurred. The continuous use of antileukemic agents did not alter the IgG levels. Relapses, responsive to changes in therapy, were usually associated with moderate reductions in the IgG levels. Greater reductions in IgG levels occurred following the institution of changes in therapy. In both instances the declines were followed by a return in IgG to the normal range. Declines of significantly greater magnitude and rate occurred in those patients no longer capable of responding to therapy changes. In such patients, levels below 3.8 to 3.5 mg./ml. signified impending death, a lack of response to infection and inability to evoke an IgG response. An example of the interrelationships between clinical and immunologic responsiveness is shown by the patient in complete relapse with a normal IgG assay, who went into remission and had an impressive IgG response following a rubeola infection. Shortly thereafter, her disease process entered an irreversible relapse stage with an IgG level of 2.9 mg./ml., and death occurred within 30 days following a bacterial septicemia.

The relationship between hematologic and immunologic responsiveness raises the provocative question of paradoxically maintaining an immunologically responsive state as a means of prolonging the survival time in the face of persistent immunosuppressive therapy.

**SUMMARIO IN INTERLINGUA**

Determinationes serial de IgG esseva effectuate in 29 juveniles con leucc'mia acute. Con i exception dcl essayos pretractamental, omne le determinationes esseva effectuate durante le curso de un specific therapie antileucemic. Post le introduction initial de agentes cytotoxic, un significative, ben que transiente reduction in le nivello de IgG esseva constatate. Le uso continue de agentes antileucemic non alterava le nivello del IgG. Recidivas de character responsive a alterationes in le therapia esseva usualmente associate con moderate reductions in le nivello de IgG. Plus marcate reductiones del nivello de IgG occurreva post le institution de alterationes in le therapia. In ambe situationes le declinos esseva sequite de un retorno del valores pro IgG: ad intra le region normal. Declinos de un significative-mente plus grande magnitude e rapiditate occurreva in patientes qui habeava perdite le capacitate de responder a alterationes in le therapia. In tal patientes nivello de infra 3.8 a 3.5 mg per ml indicava imminentia de morte, absentia de responsa a infection, e incapacitate de evocar un responsa de IgG. Un exemplo del interrelaciones inter le responsivitate clinic e le responsivitate immunologic es monstrate per le patiente in recidiva.
complete con valores normal in le essayo de IgG le qual entrava in remission e manifestava un impressive responsa de IgG post un infeccion con rubeola. Brevemente plus tarde, le processo pathologic in iste caso entrava in un phase de recidiva irreversible con un nivello de IgG de 2,9 mg per ml, e morte superveniva intra 30 dies post le declaration de un septicemia bacterial.

Le relation inter responsivitate hematologic e responsivitate immunologic subleva le provocative question del possibilitate paradoxe de mantener un stato de responsivitate immunologic como medio de prolongar le superviventia in le presentia de un persistente terapia immunosuppressive.

REFERENCES

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