The Treatment of Autoimmune Hemolytic Anemia with 6-Mercaptopurine and Thioguanine

By Robert Schwartz and William Dameshek

Prior to 1949, the treatment of autoimmune hemolytic anemia (AIHA) was frequently disappointing. Despite splenectomy and the use of blood transfusions, many patients had an unremitting and progressively deteriorating course which ended fatally. With the introduction of ACTH and cortisone, the treatment of this disorder was greatly facilitated and beneficial results became far more consistent. Today the corticosteroids, and especially the newer synthetic agents, such as prednisone, may be said to constitute the treatment of choice in AIHA, splenectomy being reserved for refractory cases.

During the past three years we have become interested in another series of compounds, the antimetabolite purine analogues, as a mode of treatment for this condition. Following the demonstration that six-mercaptopurine (6-MP) could suppress immune responses in experimental animals, we explored the possible value of this material in human autoimmune disease, notably in AIHA and systemic lupus, and reported the results in a preliminary paper. It is the purpose of this paper to present our experiences in the treatment of 14 cases of autoimmune hemolytic anemia with 6-MP and a related analogue, thioguanine.

Materials and Methods

Fourteen patients with autoimmune hemolytic anemia, eight women and six men, aged 14 to 72 years, were studied (tables 1, 2). The disease was characterized by a chronic or subacute hemolytic anemia, in association with a positive direct Coombs' test. In three cases, the indirect Coombs' test was also positive. Jaundice and splenomegaly were variable findings. Red cell survival time, using the Cr²¹ technic, was greatly reduced in the ten cases in which this parameter was studied. In three patients, the hemolytic anemia was associated with another disease: chronic lymphocytic leukemia (one patient) and systemic lupus erythematosus (two patients). In addition, one patient was a "lupus suspect." Hematologic evaluation, including determinations of hemoglobin, red blood cell count, hematocrit, white blood cell count, platelet count, Coombs' tests, Cr²¹ red cell survival and LE cell preparations, were done by standard technics. Since AIHA is usually a disorder of considerable gravity and may be lethal, control (untreated) patients were not included in this study.

Six-mercaptopurine (Purinethol, B. W.), 50 mg. tablets, and thioguanine (Thioguan, B. W.), 40 mg. tablets, were used in a dosage of approximately 2.5 mg/Kg., in divided doses, usually taken with meals. Three patients were treated with 6-MP; the remaining 11 received thioguanine.

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*The tablets for these studies were generously supplied by the Burroughs Wellcome Co., Tuckahoe, N. Y.
Sustained, long-term (13 months) remission after a 1 month's course of thioguanine.

Clinical Summary
First diagnosed in 1958; remission achieved with prednisone, but long-term maintenance required. After 2 years developed osteoporosis and diabetes necessitating discontinuance of prednisone. Relapse promptly occurred and when hemoglobin was 8 Gm.%, thioguanine was started. Hemoglobin promptly rose, but leukopenia and thrombocytopenia developed and thioguanine was discontinued.

R. M.  41 F
Gradual onset of weakness and jaundice. Past and family histories of migratory polyarthritis. L.E. preparations and latex fixation tests negative. No corticosteroid therapy given.

R. T.  57 F
Gradual onset of weakness. Past history of myxedema (controlled by thyroid extract since 1957). Brother had lymphosarcoma with autoimmune hemolytic anemia.

E. S.  54 F
Rapid onset of fatigue and jaundice. Entered hospital semicomatous with 3 Gm.% hemoglobin.

D. E.  29 F
Gradual onset of severe anemia and hepatitis. Required 8 L. of blood during first month of illness. L.E. test positive.

C. C.  37 M
Da'co'd lupus developed in 1951. Autoimmune hemolytic anemia diagnosed in 1955. Widespread skin eruption, false positive test for syphilis and positive L.E. tests found. Required long-term corticosteroid therapy for hemolytic anemia, but rash unaffected. Remission on prednisone never satisfactory.

N. K.  72 F
Chronic lymphocytic leukemia with autoimmune hemolytic anemia. Gangrene of left leg due to vascular compression by enlarged lymph nodes necessitated amputation. Unresponsive to corticosteroids in massive doses. Eventually died of sepsis.

J. M.  58 M

A. P.  39 F
Gradually increasing fatigue and pallor. Epilepsy 14 years and under treatment with Dilantin. Extremely ill on ad-

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Table 1.—Clinical Data in 14 Patients with Autoimmune Hemolytic Anemia Treated with Antimetabolites

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Summary</th>
<th>Result with Antimetabolite Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. N.</td>
<td>46</td>
<td>F</td>
<td>First diagnosed in 1958; remission achieved with prednisone, but long-term maintenance required. After 2 years developed osteoporosis and diabetes necessitating discontinuance of prednisone. Relapse promptly occurred and when hemoglobin was 8 Gm.%, thioguanine was started. Hemoglobin promptly rose, but leukopenia and thrombocytopenia developed and thioguanine was discontinued.</td>
<td>Sustained, long-term (13 months) remission after a 1 month's course of thioguanine.</td>
</tr>
<tr>
<td>R. M.</td>
<td>41</td>
<td>F</td>
<td>Gradual onset of weakness and jaundice. Past and family histories of migratory polyarthritis. L.E. preparations and latex fixation tests negative. No corticosteroid therapy given.</td>
<td>Prompt, long-term (12 months) remission after a 3 months' course of thioguanine. No therapy required at present.</td>
</tr>
<tr>
<td>R. T.</td>
<td>57</td>
<td>F</td>
<td>Gradual onset of weakness. Past history of myxedema (controlled by thyroid extract since 1957). Brother had lymphosarcoma with autoimmune hemolytic anemia.</td>
<td>Slow (2 months) development of remission. Relapse occurred when thioguanine was stopped. A second remission (incomplete) achieved with re-institution of thioguanine. Prednisone then started. Requires 7.5 mg. prednisone daily to maintain remission.</td>
</tr>
<tr>
<td>E. S.</td>
<td>54</td>
<td>F</td>
<td>Rapid onset of fatigue and jaundice. Entered hospital semicomatous with 3 Gm.% hemoglobin.</td>
<td>Remission ensued within 2 months. Initial dose of 2.5 mg./Kg. of 6-MP reduced to 1.2 mg./Kg. because of leukopenia. No relapse 2 months after discontinuance of 6-MP. No therapy required at present.</td>
</tr>
<tr>
<td>C. C.</td>
<td>37</td>
<td>M</td>
<td>D'aco'd lupus developed in 1951. Autoimmune hemolytic anemia diagnosed in 1955. Widespread skin eruption, false positive test for syphilis and positive L.E. tests found. Required long-term corticosteroid therapy for hemolytic anemia, but rash unaffected. Remission on prednisone never satisfactory.</td>
<td>Incomplete remission of hemolytic anemia maintained after prednisone discontinued. Rash improved and hyperglobulinemia disappeared. Severe relapse after discontinuing 6-MP; subsequently responded to prednisone, this time with a complete remission.</td>
</tr>
<tr>
<td>N. K.</td>
<td>72</td>
<td>F</td>
<td>Chronic lymphocytic leukemia with autoimmune hemolytic anemia. Gangrene of left leg due to vascular compression by enlarged lymph nodes necessitated amputation. Unresponsive to corticosteroids in massive doses. Eventually died of sepsis.</td>
<td>No response.</td>
</tr>
<tr>
<td>A. P.</td>
<td>39</td>
<td>F</td>
<td>Gradually increasing fatigue and pallor. Epilepsy 14 years and under treatment with Dilantin. Extremely ill on ad-</td>
<td>After failure to respond to as much as 150 mg./day of prednisone, complete remission.</td>
</tr>
</tbody>
</table>
Results

Nine patients attained satisfactory responses to antimetabolite therapy and five patients failed to improve significantly. Of the first group, three subsequently required either combined antimetabolite-corticosteroid maintenance or were eventually maintained on small daily doses of corticosteroids. The other six patients achieved and maintained excellent remissions without additional corticosteroid therapy. One of the patients in the “failure” group had a temporary benefit from thioguanine, but gastrointestinal and bone marrow toxicity forced its discontinuance. Of the five therapeutic failures, four were men.

This series of patients may be divided into the following categories: (1) those in whom an antimetabolite was the only agent used; (2) those who failed to respond satisfactorily to corticosteroids and in whom an antimetabolite was eventually used; (3) those in whom an initial course of antimetabolite therapy was followed either by combined antimetabolite-corticosteroid treatment, or by corticosteroid treatment alone.

Two patients, R. M. and E. S., were in category (1). Both these women with moderately severe hemolytic anemia responded very well, R. M. to thioguanine and E. S. to 6-mercaptopurine. Neither of these patients require maintenance therapy.
**Table 2.—Laboratory Studies**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hemoglobin (Gm. %) before</th>
<th>Reticulocytes (%) before</th>
<th>WBC (×1000) before</th>
<th>Platelets (×100,000) before</th>
<th>Bilirubin (mg. %) before</th>
<th>Direct Coombs before</th>
<th>Indirect Coombs before</th>
<th>T½ Cr* RBC (days) before</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. N.</td>
<td>8.6</td>
<td>14.0</td>
<td>—</td>
<td>6.3</td>
<td>3.8</td>
<td>2+</td>
<td>2+</td>
<td>—</td>
</tr>
<tr>
<td>R. M.</td>
<td>7.5</td>
<td>14.6</td>
<td>23.8</td>
<td>0.9</td>
<td>18.3</td>
<td>7.5</td>
<td>4.8</td>
<td>10.2</td>
</tr>
<tr>
<td>R. T.</td>
<td>6.6</td>
<td>11.5</td>
<td>17.4</td>
<td>1.6</td>
<td>5.4</td>
<td>6.8</td>
<td>2.4</td>
<td>1.0</td>
</tr>
<tr>
<td>E. S.</td>
<td>3.3</td>
<td>11.6</td>
<td>26.0</td>
<td>0.9</td>
<td>8.5</td>
<td>7.7</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>D. E.</td>
<td>5.4</td>
<td>10.8</td>
<td>19.3</td>
<td>2.9</td>
<td>8.4</td>
<td>4.2</td>
<td>5.5</td>
<td>2.4</td>
</tr>
<tr>
<td>C. C.</td>
<td>10.5</td>
<td>11.7</td>
<td>18.6</td>
<td>5.8</td>
<td>12.9</td>
<td>5.6</td>
<td>5.6</td>
<td>3.1</td>
</tr>
<tr>
<td>N. K.</td>
<td>5.7</td>
<td>4.9</td>
<td>12.0</td>
<td>9.2</td>
<td>401</td>
<td>211</td>
<td>10.1</td>
<td>3.4</td>
</tr>
<tr>
<td>J. M.</td>
<td>8.0</td>
<td>7.1</td>
<td>9.5</td>
<td>15.7</td>
<td>6.3</td>
<td>8.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A. P.</td>
<td>3.9</td>
<td>13.1</td>
<td>27.4</td>
<td>4.0</td>
<td>20.8</td>
<td>5.8</td>
<td>4.0</td>
<td>8.2</td>
</tr>
<tr>
<td>D. S.</td>
<td>8.0</td>
<td>11.3</td>
<td>17.0</td>
<td>6.3</td>
<td>5.0</td>
<td>3.2</td>
<td>—</td>
<td>4+</td>
</tr>
<tr>
<td>J. D.*</td>
<td>5.4</td>
<td>—</td>
<td>27.8</td>
<td>—</td>
<td>15.6</td>
<td>4.9</td>
<td>10.2</td>
<td>—</td>
</tr>
<tr>
<td>L. B.</td>
<td>8.8</td>
<td>12.7</td>
<td>33%</td>
<td>17.0</td>
<td>13.4</td>
<td>15.1</td>
<td>4.1</td>
<td>3.9</td>
</tr>
<tr>
<td>C. B.</td>
<td>16.0</td>
<td>14.2</td>
<td>3.4</td>
<td>4.0</td>
<td>13.8</td>
<td>14.6</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td>R. P.</td>
<td>9.6</td>
<td>11.8</td>
<td>1.1</td>
<td>7.2</td>
<td>5.6</td>
<td>6.3</td>
<td>3.0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

*Treated for one week only.
AIHA TREATMENT WITH 6-MERCAPTOPURINE AND THIOGUANINE

Table 3.—Corticosteroid "Failures" Treated with Antimetabolites

<table>
<thead>
<tr>
<th>Patient</th>
<th>Reason for Steroid &quot;Failure&quot;</th>
<th>Response to Antimetabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. N.</td>
<td>Osteoporosis and diabetes.</td>
<td>good</td>
</tr>
<tr>
<td>C. C.</td>
<td>Required 175 mg. prednisone/week; L.E. skin eruption unresponsive.</td>
<td>fair</td>
</tr>
<tr>
<td>N. K.</td>
<td>Failed to respond to 1 mg./Kg./day of prednisone.</td>
<td>poor (good on L.E. rash)</td>
</tr>
<tr>
<td>J. M.</td>
<td>Failed to respond to 0.5 mg./Kg./day of prednisone.</td>
<td>poor</td>
</tr>
<tr>
<td>A. P.</td>
<td>Failed to respond to 1 mg./Kg./day of prednisone.</td>
<td>good</td>
</tr>
<tr>
<td>D. S.</td>
<td>Required 210 mg. Medrol/week.</td>
<td>poor</td>
</tr>
<tr>
<td>C. B.</td>
<td>Required 350 mg. prednisone/week.</td>
<td>good</td>
</tr>
<tr>
<td>R. P.</td>
<td>Required 140 mg. prednisone/week.</td>
<td>good</td>
</tr>
<tr>
<td>J. D.</td>
<td>Required 300 mg. prednisone/week.</td>
<td>died after 1 week</td>
</tr>
</tbody>
</table>

Category (2), nine patients in whom corticosteroids failed to achieve satisfactory control of the disease, was characterized by: (a) failure to respond to large doses (0.5-1 mg./Kg./day of prednisone or equivalent doses of other synthetic corticosteroids given for at least 10-14 days; (b) requirement of more than 100 mg. prednisone/week (or equivalent doses of other synthetic corticosteroids) as maintenance therapy; (c) development of serious or potentially serious side effects (osteoporosis, diabetes, infection, vasculitis). The patients in this category are listed in table 3. Of these nine corticosteroid "failures," four had a good response to antimetabolites, one had a partial response and four had poor responses. Three of these four patients died. Two patients in this group also failed to benefit from splenectomy.

Category (3) consists of three patients (D. E., R. T. and L. B.) in whom a first course of antimetabolite was followed by subsequent use of corticosteroids. The clinical and hematologic signs and symptoms of D. E. and R. T. were "titrated" by the alternate or combined use of antimetabolite and corticosteroid. Although R. T. responded well to both agents, a more rapid effect was achieved with the use of prednisone. D. E., a woman with autoimmune hemolytic anemia as a feature of systemic lupus erythematosus, was, in addition, a moderately severe manic-depressive and prednisone was administered to her only with considerable caution for a short period of time under psychiatric supervision. L. B. failed to respond to thioguanine, but later had an excellent response to prednisone.

Toxicity

Three patients developed signs of myelotoxicity with the appearance of either leukopenia or thrombocytopenia (table 4). In two of these the drug was discontinued, while in the third patient (E. S.), it was continued at a lower dosage and the leukocyte count gradually increased to normal values. An occasional finding was the development of megaloblastoid cells in the bone marrow during antipurine therapy, perhaps indicating interference with the nucleic acid metabolism of developing erythrocytes. Two patients de-
TABLE 4.—TOXIC REACTIONS OF ANTIMETABOLITE THERAPY

<table>
<thead>
<tr>
<th>Patient</th>
<th>Toxicity</th>
<th>Effect of Toxicity on Therapy</th>
<th>Result of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. N.</td>
<td>leukopenia, thrombocytopenia</td>
<td>6-mercaptopurine discontinued</td>
<td>good</td>
</tr>
<tr>
<td>R. M.</td>
<td>none</td>
<td>—</td>
<td>good</td>
</tr>
<tr>
<td>R. T.</td>
<td>none</td>
<td>—</td>
<td>good</td>
</tr>
<tr>
<td>E. S.</td>
<td>leukopenia</td>
<td>—</td>
<td>good</td>
</tr>
</tbody>
</table>

| D. E. | G.I. (mild, controllable) | none | good |
| C. C. | none                  | —    | fair  |
| N. K. | none                  | —    | poor  |
| J. M. | none                  | —    | poor  |
| A. P. | none                  | —    | good  |
| D. S. | leukopenia, G.I.     | 6-mercaptopurine discontinued | poor |
| L. B. | G.I.                  | 6-mercaptopurine discontinued | poor |
| C. B. | G.I. (mild)           | none | good  |
| R. P. | G.I. (mild, controllable) | none | good  |
| J. D. | marked megaloblastic changes | died after 1 week of therapy | poor |

Developed severe gastrointestinal side effects (nausea, bloating, anorexia) which forced discontinuance of the drug, thereby accounting for two of the treatment “failures.” Three patients had mild gastrointestinal symptoms which were relieved by alkali or encouragement. None of the fourteen patients developed irreversible myelotoxicity; this observation may reflect the hyperplastic bone marrow uniformly present in this disease. There was no correlation between the development of myelotoxicity and the eventual outcome of the treatment (table 3). No differences in toxicity between thioguanine and 6-mercaptopurine were noted. None of these patients developed infection other than trivial viral upper respiratory disease, and in none of these did the viral infection seem more severe than might otherwise be expected.

Effect of Treatment on the Hematologic Abnormalities

In those patients who responded to antipurine treatment, the first sign of recovery was invariably a sharp drop in the reticulocyte count; this usually occurred two-three weeks after the initiation of therapy and was followed within several days by a gradual increase in the hemoglobin value toward normal. This effect on the reticulocytosis has also been observed during therapy with corticosteroids, although in some patients so treated, the reticulocyte value may rise prior to the onset of remission. Hyperbilirubinemia, if present, cleared during this time. In some patients, a reduction in transfusion requirement ensued before any effect on the blood could be observed (D. E., A. P.). During remission the survival of Cr tagged red blood cells was either notably improved or became normal. Three patients of the series had positive indirect and direct Coombs' tests. In two, the indirect test became negative, while it continued positive in the third (R. P.). Subsequent serologic studies in this woman demonstrated that the indirect Coombs' test was due to

*Recent experience indicates that the imidazole derivative of 6-mercaptopurine (BW57-322) seems to induce fewer gastrointestinal reactions than the other antipurine analogs.*
a transfusion induced isoantibody (anti-Kell). In one patient the direct Coombs' test became negative, while in the remaining 13 patients it remained persistently positive, although, in some, weaker than prior to therapy.

**Discussion**

Shortly after two groups of French physicians, Widal, Abrami and Brulé,\(^5\) and Chauffard and Troisier,\(^6\) recognized and described the syndrome of acquired hemolytic anemia, its successful treatment by splenectomy was reported by Micheli in 1911.\(^7\) Although this procedure soon became widely popular in the treatment of a variety of hematologic disorders, it was later realized that splenectomy was of possible benefit in only about one-half of the cases of autoimmune hemolytic anemia.\(^5\) Even among those patients who obtained initial benefit from surgery, the relapse rate was high. In spite of Lederer's enthusiastic reports of 1925\(^9\) and 1930,\(^10\) it was soon clear that blood transfusion alone rarely induced a remission in documented cases of autoimmune hemolytic anemia. It was evident that its role was no more than palliative and the beneficial effects transient. Since splenectomy and blood transfusions do not attack the fundamental basis of the disease, which is the production of autoantibodies that attack the patients' red blood cells, the disappointing results obtained with these measures should not be surprising.

Since it seemed quite apparent that the chief necessity in the treatment of the self-perpetuating disorder of autoimmune hemolytic anemia was the control of antibody production, various agents were used to “blockade” or reduce the activity of the reticuloendothelial system. Thus Congo red\(^11\) was first used—unsuccessfully—following which nitrogen mustard was tried.\(^12\) This was dramatically successful in the first case in which it was used, but in four other cases the results were either poor or inconstant. Other alkylating agents were also used with occasional success. While these early clinical trials with cytotoxic agents were proceeding, Dougherty and White reported their discovery that ACTH caused a profound atrophy of the lymphatic tissue of mice;\(^13\) a simultaneous reduction in the titer of heteroimmune antibody was demonstrated.\(^14\) It had been shown previously that one of a series of steroid compounds isolated from the adrenal cortex by Kendall and his associates, and named by them Compound E (later to be known as cortisone), had a similar effect in rats.\(^15\) When, in 1949, sufficient ACTH had been produced to permit its use in man, it was therefore tried in cases of autoimmune hemolytic anemia. Since the first reports of their use in this disease, showing the striking improvement that occurred, numerous papers have amply documented the effectiveness of ACTH and the corticosteroids. Today, corticosteroids represent the standard therapeutic agents in autoimmune hemolytic anemia. Although the precise mechanism of their action in ameliorating the hemolytic process is not clear, a number of studies have indicated that the suppression of autoantibody formation may be one of their major effects.\(^16\)

With discovery of the inhibitory effects of 6-MP on antibody formation in rabbits, it seemed desirable to assess the effects of this agent and an analogue, thioguanine, in autoimmune diseases of man. Although numerous studies have indicated a variety of possible mechanisms of action of 6-MP, recent
experiments have demonstrated that this purine analogue is converted in vivo to its active form, 6-mercaptopurine ribonucleotide, which inhibits the conversion of inosine monophosphate to other purine ribonucleotides (adenosine monophosphate and guanosine monophosphate). This disruption of purine biosynthesis has, in turn, profound effects on nucleic acid metabolism.

The suggestion that 6-mercaptopurine might interfere with antibody synthesis was first made by Sterzl and Houb; however, their initial experiments failed to demonstrate an effect in adult rabbits immunized with heat-killed *Salmonella paratyphi* B organisms. Later, studies in our laboratory showed that 6-mercaptopurine suppressed antibody formation in rabbits injected with purified proteins. A state resembling acquired immunologic tolerance could be induced in adult rabbits with this agent, and the suppression of transplantation immunity to skin grafts was demonstrated. The inhibition of immune reactions has been confirmed in several species challenged with a variety of antigenic stimuli, including bacteria in mice, kidney homografts in dogs, neural tissue in rabbits and skin homografts in chickens. Some species, such as the guinea pig, appear to resist the anti-immune effects of this drug, but are susceptible to another antimetabolite, amethopterin. In these studies on laboratory animals, the choice of species, the nature and dose of the antigen and the dose and method of preparation of the drug were found to be extremely important variables.

The mode of action of 6-mercaptopurine in suppressing the immune reaction is not completely known. Earlier studies indicated that the drug did not affect the synthesis of normal proteins. Sterzl has shown that, like x-radiation, the maximum effect of the drug occurs during the inductive phase of antibody formation; Condie, however, demonstrated that in rabbits treated with large doses of both antigen and 6-mercaptopurine, the secondary immune response could be inhibited. Our own studies have shown that the proliferation of immunologically competent cells which arise in the lymphoid systems of homograft-bearing animals is prevented by 6-mercaptopurine. As long as the growth of these cells is blocked by 6-mercaptopurine, the homograft is retained. Although 6-mercaptopurine causes toxic side effects in rabbits, such as weight loss, anorexia, diarrhea and leukopenia, these are insufficient to explain its immunologic effects, since, in a recent study of a large group of antimetabolites, many were found which caused identical or even more severe side effects, but which were without effect on antibody production.

The demonstration that 6-mercaptopurine acts mainly on the primary immune response may appear at variance with its effects in patients with autoimmune disease, where autoantibody production is well under way by the time treatment is started. Although no convincing data are yet available to answer this problem, we presume that these patients are undergoing a chronic immune response analogous to the primary response, but self-perpetuating, because of the constant stimulus to proliferation of antibody-forming cells by the normal cell antigens.

The reason why certain individuals produce antibodies reactive against their own tissues is no clearer than the reason why the majority of us do not undergo autodestructive immune reactions. As the result of our clinical studies
of patients with autoimmune hemolytic anemia as a feature of chronic lymphocytic leukemia,\textsuperscript{33} and of our experimental studies of runt disease in the F\textsubscript{1} hybrid mouse,\textsuperscript{34} we have proposed that one mechanism of autoimmunization in man is by the acquisition of abnormal immunologically competent cells.\textsuperscript{35} These aberrant cells react to normal body antigens, as can be found on the erythrocyte, with the production of an antibody which induces a disease. The proliferation of abnormal antibody forming cells is thus a central and obligatory feature of this process. A natural corollary to this hypothesis is the treatment of autoimmune diseases with cytotoxic agents, particularly with those whose main effect is on lymphoid cells.

Thus the agents shown to exert an effect on autoimmune hemolytic anemia—nitrogen mustard,\textsuperscript{12,36} TEM, urethane, radioactive gold,\textsuperscript{37} ACTH, corticosteroids, 6-MP and thioguanine—share the common attribute of cytotoxicity, and particularly of toxicity to lymphoid cells. Presumably their mode of action at the clinical level is due to the destruction of antibody-forming cells, and the observed differences among them are due to their relative degree of efficiency at the cellular level.

Three patients in this series (R. M., Y. N. and E. S.) are of particular interest. Each of these middle-aged women had excellent responses to relatively short courses (one to three months) of either 6-MP or thioguanine. On discontinuance of antimetabolite therapy, each patient maintained her remission for a very lengthy period without additional treatment. The length of remission in these patients was from four to 14 months. One woman of this group (Y. N.) had previously relapsed promptly when maintenance prednisone therapy was discontinued because of the development of osteoporosis and diabetes. A one-month course of thioguanine was followed by a 14-month remission; she then developed a relapse and, at the time of this writing, she is undergoing a second antimetabolite induced remission. In our experience, long-term remissions without the use of maintenance corticosteroids are unusual in autoimmune hemolytic anemia, and most patients promptly relapse when their small daily dose of prednisone is discontinued. Thus in a previous study of 43 cases of autoimmune hemolytic anemia, 70 per cent of the patients in whom steroid therapy was discontinued relapsed.\textsuperscript{38} The results in R. M. and E. S. might be considered as spontaneous remissions occurring during the course of treatment, but this occurrence in three out of 14 patients would seem highly unlikely. These patients might be construed as analogous to the experimental animals which developed immunologic tolerance to heterologous antigens after a short course of 6-MP treatment. Perhaps the antimetabolite therapy they received induced a state of tolerance to their own tissues.

The eventual role of 6-MP and its analogues in the treatment of AIHA is difficult to assess at this time. They may be preferred in patients with complications which prohibit the use of corticosteroids, such as severe diabetes or psychoses. They may be useful as adjuncts to corticosteroid therapy, thereby permitting lower doses of both drugs to be used. They might be useful in patients failing to respond either to corticosteroids or to corticosteroids and splenectomy.

It is important to bear in mind that antimetabolites are potentially dan-
dangerous drugs. Suppression of the bone marrow by them may be severe. Although no patients in whom permanent damage to the bone marrow were encountered in this study, strict supervision of the patient must be observed. Gastrointestinal reactions, while they were always relieved by cessation of treatment, could be troublesome, particularly in light of reports of hepatitis induced by these agents. In view of these “side effects,” 6-MP and thioguanine can by no means be recommended for the routine treatment of this disease at present. That these “myelotoxic” agents often have striking effects in raising the red cell count in cases of AIHA is in itself of considerable theoretical interest.

The results of the present study are at least encouraging and indicate that further clinical trials of these drugs are in order. As broader experience with them is gained, their place in the treatment of AIHA will be easier to assess.

Summary

Fourteen patients with autoimmune hemolytic anemia were treated with either 6-mercaptopurine or thioguanine. Nine patients responded and five failed to improve. Eight patients developed side effects, either hematologic or gastrointestinal, of varying degrees of severity; in three the antimetabolite had to be discontinued, while in others adjustment of the dosage or the administration of antacids was sufficient to control side effects. Included in this series are nine patients who failed to respond adequately to corticosteroid therapy; four of these had a good effect from antimetabolite therapy.

Although these results indicate that antimetabolites may reverse the course of autoimmune hemolytic anemia, the eventual role of these agents in the treatment of this disorder requires further study.

Summario in Interlingua

Dece-quatro patientes con hemolytic anemia autoimmun esseva tractat con (1) 6-mercaptopurina e (2) thioguanina. Novem del patientes respondeva; cinque non se meliorava. Octo patientes disveloppava adverse effectos secundari de character hematologic o gastrointestinal, de varie grados de severitate. In tres casos il esseva necessari discontinuar le administration del antimetabolito durante que in alteres un ajustamento del dosage o le administration de antiacidos esseva sufficiente pro supprimer le adverse effectos secundari. Iste serie include novem patientes qui non habeva respondite adequatamente a therapia a corticosterone; quatro de illes respondeva favorabilemente al therapia antimetabolitic.

Ben que iste resultatos indica que antimetabolitos pote reverter le curso de hemolytic anemia autoimman, le uso futur de iste agentes in le tractamento de iste morbo require studios additional.

APPENDIX

Selected Case Reports

R. M. (NECH No. 131–411). This 41 year old white woman developed increasing fatigue in January 1959. In August 1959, her family physician noted that she was jaundiced. No specific therapy was given and the degree of jaundice and weakness gradually increased.
In January 1960, she was referred to the Hematology Service for evaluation. The past history revealed that she had intermittent swelling and pain of the knees, ankles and elbows since 1953. On physical examination she was a moderately ill-appearing woman with pallor and jaundice of the skin and sclerae. The liver was felt 4 cm. below the right costal margin. There was no splenomegaly. The remainder of the physical examination was normal. The laboratory examinations on admission showed: hemoglobin 8.6 Gm. per cent, red cells 2.3 million/mm.³, hematocrit 34 per cent, reticulocytes 10.4 per cent, platelets 483,510/mm.³, white blood cells 18,300 with a normal differential count. The blood smear showed marked spherocytosis, polychromatophilia and normoblastenia. The direct Coombs’ test was positive.

A Cr⁵¹ labeled red cell survival gave a T½ of 13.1 days. The total serum bilirubin was 3.7 mg. per cent, with 3.1 mg. per cent indirect reacting bilirubin. The erythrocyte sedimentation rate was 77 mm./hour. The remainder of the laboratory tests were normal. Two L.E. cell preparations and a latex fixation test were negative. Six-mercaptopurine in a dosage of 150 mg./day was begun after collection of baseline studies. However, after three days of therapy the patient developed severe nausea. Because of this, the 6-MP was discontinued and treatment with thioguanine, 150 mg./day was begun. She tolerated this medication well and developed no side effects. Within one week the hemoglobin value began to rise and the reticulocyte count began to decrease. This rise in hemoglobin was sustained and reached normal levels within one month. The dosage of thioguanine was gradually reduced and, after 10 weeks of therapy, was discontinued (fig. 1). The patient has remained in complete remission during the ensuing two years. A repeat Cr⁵¹ survival done while she was in remission showed a T½ of 27 days. In August, 1961, the Coombs’ test was found to be negative.

Y. N. (NECH No. 117-010). This 47 year old housewife was first admitted to the New England Center Hospital in January 1958, because of jaundice. Three months prior to admission she noted recurrent bouts of fever, vomiting and weakness. Because of these symptoms she was admitted to another hospital where a cholecystectomy was performed. At the time of surgery the spleen was found to be enlarged. Because of persistent anemia—in spite of the transfusion of 14 units of blood—and jaundice, she was transferred to the New England Center Hospital for evaluation. Further investigations disclosed, in addition to the pallor, jaundice, splenomegaly and anemia, a marked reticulocytosis, spherocytosis, erythroid hyperplasia of the bone marrow and a positive direct Coombs’ test. A diagnosis of autoimmune hemolytic anemia was made and treatment with triamcinolone (Aristocort), 64 mg./day was begun. Because of the appearance of glycosuria and hypokalemia the Aristocort was discontinued in February 1958. In April 1958, she developed marked weakness and malaise and was again found to be anemic (hemoglobin 7.4 Gm. per cent). Prednisone in a dosage of 50 mg. daily was administered. The hemoglobin gradually rose to normal values and the dose of prednisone was gradually reduced to 5 mg./day. However, in February 1960, she again developed symptoms of diabetes and, in addition, was found to have osteoporosis of the lumbar spine. Because of these findings the prednisone was discontinued. Two months later, the hemoglobin had dropped from 14 Gm. per cent to 8 Gm. per cent. The direct Coombs’ test was again found to be positive. At that time, treatment with thioguanine in a dose of 150 mg./day was begun. Within two weeks, the hemoglobin was 15 Gm. per cent. However, because of the development of thrombocytopenia and leukopenia, the thioguanine was discontinued after one month. The patient remained completely well for the next 14 months. She required no therapy and the hematologic values remained within normal limits. However, in June 1961, she again developed fever, malaise and vomiting and was found once again to be severely anemic (hemoglobin 5.0 Gm. per cent). She was readmitted to the hospital, where pallor, jaundice and splenomegaly were found on physical examination, and anemia, reticulocytosis, spherocytosis, normoblastemia and a positive direct Coombs’ test were found on laboratory examination. She was given two units of blood, which raised the hemoglobin value from 5.0 Gm. per cent to 7.5 Gm. per cent, and treatment with 6-imidazole-6-mercaptopurine (BW 57-322) 300 mg./day was begun. The hemoglobin rose to 11.0 Gm. per cent within four weeks and clinical and laboratory signs of abnormal hemolysis began to subside. In November 1961, she was found to
Fig. 1—Satisfactory response to a short course of 6-MP in a patient with severe autoimmune hemolytic anemia. Because of the development of leukopenia, the dosage was reduced to 50 mg/day; on this dosage the hemoglobin continued to rise.
AIHA TREATMENT WITH 6-MERCAPTOPURINE AND THIOGUANINE

Fig. 2.—Good response to thioguanine. A relapse followed discontinuance of the drug, but remission followed combined thioguanine-prednisone therapy. Patient is presently maintained on small doses of thioguanine. (Note: 500 cc. of blood were administered on March 14.)
Fig. 3.—Slow, but steady improvement in thiosemicarbazide relapse follow its discontinuance, and a second remission was achieved with combined thiosemicarbazide therapy. Note lack of hemotoxicity.
AIHA TREATMENT WITH 6-MERCAPTOPURINE AND THIOGUANINE

Fig. 4.—Failure to respond to large doses of prednisone (up to 150 mg./day) with subsequent excellent remission on thioguanine.

have the following hemogram: hemoglobin 13.2 Gm. per cent, hematocrit 41 vols. per cent, red cell count 3.86 million/mm.³, platelet count 400,000/mm.³, reticulocytes 4 per cent, white cell count 7,300 with a normal differential. The direct Coombs’ test was positive. At the present time the patient is maintained on 200 mg./day of BW 57-322. She is asymptomatic and her hematologic values remain normal.

D. E. (NECH No. 132-912). This 29 year old housewife developed severe fatigue and headaches in January 1960. In February 1960, she noted jaundice and dark urine and had several episodes of nausea and vomiting. She was admitted to another hospital where she was found to have jaundice and hepatosplenomegaly. There was marked anemia and reticulocytosis and the liver function tests indicated hepatitis. She was transfused repeatedly, 16 units of blood being given at approximately daily intervals. Because no response to this therapy took place, the patient was transferred to the New England Center Hospital in March 1960. On physical examination she was a moderately ill, depressed woman with pallor and jaundice of the skin and mucous membranes. The liver was felt 3 cm. below the right costal margin and the spleen 3 cm. below the left costal margin. The remainder of the physical examination was normal. The initial laboratory data were: hemoglobin 6.3 Gm. per cent, hematocrit 20 vols. per cent, red blood cell count 1.6 million/mm.³, platelets 553,000/mm.³, reticulocytes 16 per cent, white blood cell count 5,400/mm.³ with 59 per cent polys, 4 per cent bands, 24 per cent lymphocytes, 8 per cent monocytes, 2 per cent eosinophils, 1 per cent myelocytes and 2 per cent metamyelocytes. The blood sedimentation rate was 42 mm./hour. Bone marrow aspiration showed marked erythroid hyperplasia. The direct Coombs’ test was positive. The serum albumin was 3.5 Gm. per cent and serum globulin was 3.9 Gm. per cent; total serum bilirubin was 3.4 mg. per cent (1.0 mg. per cent direct fraction). The SGOT was 190 units, the SGPT was 220 units and the LDH was 290 units. An L.E. cell preparation was positive. The fecal urobilinogen was 1030 mg./24 hours and the Cr²⁺ red cell survival showed a T½ of 3.2 days. A liver biopsy showed moderately active hepatitis.

A diagnosis of SLE with autoimmune hemolytic anemia and lupoid hepatitis was made and treatment with thioguanine, 150 mg./day was instituted. During the next four weeks there was return of the abnormal liver function tests to norm: 

Fig. 5—Sustained remission of autoimmune hemolytic anemia following a 3 months' course of thioguanine.
hemoglobin value. The requirement for transfusions stopped and the reticulocyte count fell toward normal. In May 1960, she developed increasing nervousness and insomnia. She was therefore readmitted to the NECH for further evaluation. On physical examination she was found to be markedly agitated. There was neither pallor nor jaundice. The liver was felt 1 cm. below the right costal margin and the spleen was not felt. The hemoglobin was 10.3 Gm. per cent, hematocrit 30 per cent, red blood cell count 3.6 million/mm.³, platelets 538,000, reticulocytes 7.6 per cent, white blood cell count 5,300 mm.³ with a normal differential. The L.E. and Coombs tests were again positive. A psychiatric consultant diagnosed agitated depression and she was begun on therapy with tranquilizers and prednisone, 50 mg./day. The latter was gradually reduced to 20 mg./day. The thioguanine was continued as before. During the ensuing months her hematologic condition remained stable, with hemoglobin values ranging between 10-11 Gm. per cent. In August 1961, the maintenance of prednisone was discontinued and the dosage of thioguanine was reduced to 40 mg./day. In September 1961, she underwent a series of electroshock treatments which resulted in marked improvement of her mood and mentation. At the present time she is maintained on 40 mg. of thioguanine daily and her condition remains stable.

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The Treatment of Autoimmune Hemolytic Anemia with 6-Mercaptopurine and Thioguanine

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