Inhibition of the Primary Immune Response in Man by Anti-Metabolites

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SUMMARY

The effect of anti-tumor agent therapy on the primary immune response was studied in 45 patients with malignant and inflammatory diseases. The % complete inhibition of the primary response to tularemia and to Vi and pneumococcal polysaccharide antigens by 6 therapeutic programs were: intermittent i.v. methotrexate, 50%; intensive 5-day methotrexate, 71%; daily i.v. methyl-GAG, 46%; intensive 5-day 6-mercaptopurine, 75%; 6-mercaptopurine, methotrexate, vincristine, and prednisone in combination, 60%; vincristine and prednisone in combination, 60%.

At least 25% of antigenic stimulations in each treatment program resulted in low but significant antibody titers in spite of chemotherapy.

There was an inverse relationship between antigenic strength and the degree of suppression of the immune response to the given antigen.

The degree of immunosuppression did not correlate with the toxicity of the treatment programs.

Chemotherapy did not affect isoantibody titers or established delayed hypersensitivity.

Anti-tumor agents can inhibit many host defense mechanisms (8), including primary and secondary antibody responses (4, 8), the development of delayed hypersensitivity (9), the exudative inflammatory response (7, 21), and allograft rejection (30, 32). These observations have led to the clinical use of anti-metabolites in the therapy of “autoimmune” and inflammatory diseases and also in the therapy of graft rejection in renal homotransplantation (19, 20, 22, 24). The observations mentioned above have also suggested mechanisms for the increased susceptibility to infection noted during anti-metabolite therapy (10).

There have been only a few studies of immune suppression by anti-metabolites in man (16, 22, 23). The present study reports observations in man on primary immune suppression by other drugs or dosage schedules, or both. This would allow a better understanding of altered host defense during cancer chemotherapy and perhaps suggest improved chemotherapeutic approaches to the treatment of “autoimmune” diseases.

MATERIALS AND METHODS

Forty-seven patients were admitted to the study on the basis of their inclusion in a chemotherapy program for the treatment of their disease. Thirty-five patients had acute leukemia, 4 had other nonleukemic neoplastic disorders, and 8 had inflammatory ocular diseases. They ranged in age from 3 to 70 years with a median of 17.

Six therapeutic programs were studied (Table 1). Eight patients received intermittent methotrexate (MTX) i.v. at a dose of 25 mg/sq m of body surface area every 4th day (intermittent MTX). These patients all had inflammatory ocular syndromes (35). Seven patients received 9-15 mg/sq m of MTX i.v. daily for 5 days (intensive MTX). Eight patients were given 100-150 mg/sq m of methylglyoxal bis-guanylhydrazone i.v. daily (methyl-GAG). Eleven patients received 300-2000 mg/sq m of 6-mercaptopurine (6-MP) i.v. daily for 5 days (intensive 6-MP). Eight patients were given 5- to 10-day courses of combination chemotherapy with 4 drugs (2) (intensive combination). Finally 5 patients received prednisone (40 mg/sq m daily) and vincristine (2 mg/sq m i.v. weekly) in combination (combination Pred-VCR).

As a control group, 22 patients with a variety of malignant diseases for which they were not receiving therapy were studied. These patients were all ambulatory, hematologically normal, and in a normal nutritional state in spite of their malignant disorders.

Patients receiving continuous anti-metabolite therapy (methyl-GAG, combination Pred-VCR) received antigenic stimulation 1 week after the start of therapy. Patients receiving intermittent therapy (intermittent MTX) were stimulated 24 hr after the 3rd dose. Patients receiving the intensive 5- to 10-day therapies (intensive 6-MP,
MTX, and combination) were stimulated 24 hr after the 1st dose of the course. A total of 57 primary antigenic stimulations were given to these 47 patients.

The antigens used were: Vi antigen (Vi) (33); tularemia vaccine (Tu) (12); and pneumococcus Type III polysaccharide (Pn) (5). The dose of each antigen was 0.1 mg dissolved in 1 ml of saline given s.c. in the deltoid area. Antibody titers were determined prior to immunization and at 2 and 4 weeks after stimulation. Antibody titers to Vi were determined by the hemagglutination method (13). Titers to Tu and Pn were measured by a modified bentonite flocculation method (34).

As an index of the effect of therapy on established immunity, delayed hypersensitivity to Dermatophytin and Dermatophytin "O" was evaluated before and during therapy. Patients receiving intensive therapy were tested 24 hr after the end of the course. Patients receiving continuous or intermittent therapy were tested after 2 weeks of treatment. The test consisted of intradermal injections of 0.1 ml of 1:30 through 1:10,000 dilutions and measurement of the resultant induration at 24 and 48 hr. Levels of circulating isoantibodies were also measured (11).

Appropriate clinical parameters were followed closely.

RESULTS

The effect of chemotherapy on the primary antibody response in these patients is shown in Chart 1. Intensive MTX and intensive 6-MP therapy were most effective in blocking the primary response. Of antigenic challenges given during therapy, 71—75% failed to elicit an antibody response. Methyl-GAG (i.v.) therapy was least effective in suppressing the primary antibody response. Antibody production was completely suppressed only 46% of the time. Intermittent MTX was also relatively ineffective in immune suppression, and complete inhibition of antibody production occurred only 50% of the time. For the Pred-VCR and intensive combination therapy, antibody production was completely blocked in 60% of the instances. In general the response to both antigens was suppressed in patients who received more than 1 antigenic stimulation. The responses were the same in simultaneous stimulations with Vi and Pn and with Vi and Tu.

The antibody titers in patients who responded to the primary antigens during chemotherapy were low. Examined separately (column 8, Chart 1) they showed a median titer of 3.0, compared to a control value of 7.0. This difference was not quite significant at the 5% level, however (0.10 > P > 0.05).

The antigen used was important in the evaluation of the effect of therapy. The antigen giving the highest control titers was the least inhibited by therapy and vice versa. Thus, as seen in Table 2, antibody production was completely suppressed only in 50% of Tu stimulations, but in 56% of Vi stimulations and 78% of Pn stimulations. This was true for each type of therapy. It can

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**TABLE 1**

<table>
<thead>
<tr>
<th>Drug program</th>
<th>Dose (mg/sq m)</th>
<th>Route</th>
<th>Schedule</th>
<th>No. of patients</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent MTX</td>
<td>25</td>
<td>i.v.</td>
<td>Every 4th day throughout study</td>
<td>8</td>
<td>Uveitis</td>
</tr>
<tr>
<td>Intensive MTX</td>
<td>9—15</td>
<td>i.v.</td>
<td>Daily for 5 days</td>
<td>7</td>
<td>Acute leukemia</td>
</tr>
<tr>
<td>Methyl-GAG</td>
<td>100—150</td>
<td>i.v.</td>
<td>Daily throughout study</td>
<td>8</td>
<td>Acute leukemia (6 patients)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other malignancies (2 patients)</td>
</tr>
<tr>
<td>Intensive 6-MP</td>
<td>300—2000</td>
<td>i.v.</td>
<td>Daily for 5 days</td>
<td>11</td>
<td>Acute leukemia (9 patients)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other malignancies (2 patients)</td>
</tr>
<tr>
<td>Intensive combination</td>
<td>6-MP 60</td>
<td>p.o.</td>
<td>Daily</td>
<td>8</td>
<td>Acute leukemia</td>
</tr>
<tr>
<td></td>
<td>MTX 20</td>
<td>i.v.</td>
<td>Every 4th day</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pred 40</td>
<td>p.o.</td>
<td>Daily</td>
<td>8</td>
<td>Acute leukemia</td>
</tr>
<tr>
<td></td>
<td>VCR 2</td>
<td>i.v.</td>
<td>Weekly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Pred 40</td>
<td>p.o.</td>
<td>Daily</td>
<td>8</td>
<td>Acute leukemia</td>
</tr>
<tr>
<td></td>
<td>VCR 2</td>
<td></td>
<td>Given together in 5—10-day courses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The following abbreviations are used: MTX, methotrexate; methyl-GAG, methylglyoxal bisguanylhydrazone; 6-MP, 6-mercaptopurine; Pred, prednisone; VCR, vincristine.
also be seen that the therapeutically suppressed antibody titers were higher to Tu than to Vi or Pn. None of the control patients failed to respond to these antigens.

Ten patients, 2 each in the first 5 treatment programs, were stimulated with Dermatophytin antigens. There was no inhibition of the delayed hypersensitivity response during chemotherapy. These were patients who did show primary immune suppression.

In 20 patients, serum isoantibodies were measured prior to therapy and 4 weeks after antigenic stimulation. No significant changes in antibody titers were noted. Median anti-A and anti-B titers prior to therapy were both 256 and during therapy were 256 and 384, respectively.

Table 3 gives the toxicity data for the 6 therapeutic programs. Intermittent MTX was without toxic manifestations during the study period. The other therapies were all associated with leukopenia, oral or gastrointestinal toxicity, or both, and either fever or distinct infection in some of the patients. Methyl-GAG and intensive combination therapy were, over-all, the most toxic. No patients died during the study.

Only administration of Tu was associated with side effects. Moderate local tenderness developed 24–48 hr after injection. Two patients (8%) had a 24-hr episode of fever following injection of the vaccine.
DISCUSSION

In this study 5 anti-tumor agents used in 6 therapeutic programs were shown to be potent inhibitors of the primary immune response in man. Intensive 6-MP and intensive MTX therapy were most effective, and daily i.v. methyl-GAG and intermittent i.v. MTX were least effective. Therapy completely blocked the response in the majority of cases, and the titers of patients who did respond were lower than the controls. These results suggest that, where maximal rapid immunosuppression is desired, a combination of intensive i.v. 6-MP and MTX therapy would probably give the best results. After 5 days of such therapy maintenance might be achieved with conventional, less toxic doses of these agents.

Conversely, at least 25% of the antigenic stimulations resulted in some degree of primary antibody response in each of the therapeutic programs. This was in spite of the significant degrees of leukopenia and other toxic manifestations produced by most of these programs. Lack of complete primary immune suppression during chemotherapy in man has been observed by several authors (16, 22). However, Santos et al. (23), using Tu and Vi antigens, demonstrated complete suppression during intensive 6-MP, MTX, 5-fluoro-2'-deoxyuridine, and cyclophosphamide therapy. Administration of the antigen at 4 rather than 24 hr after the 1st dose of therapy or the greater dose and duration of drug in Santos' study may explain this difference. Failure of complete suppression during a type of therapy which is both toxic and associated with infection suggests there may be severe limitations on the use of anti-tumor agents for immunosuppression.

Untreated patients with acute leukemia have normal primary antibody responses (15, 26). The control series of the current investigation shows that patients with metastatic cancer can respond to Vi and Tu antigens, demonstrated complete suppression during intensive 6-MP, MTX, 5-fluoro-2'-deoxyuridine, and cyclophosphamide therapy. Administration of the antigen at 4 rather than 24 hr after the 1st dose of therapy or the greater dose and duration of drug in Santos' study may explain this difference. Failure of complete suppression during a type of therapy which is both toxic and associated with infection suggests there may be severe limitations on the use of anti-tumor agents for immunosuppression.

There is a dose-response relationship for 6-MP in immune suppression in man. A dose of 1.5 mg/kg daily does not inhibit the primary response (22). At 2.5 mg/kg daily there was 58% complete inhibition (16), while at 3.5-7.0 mg/kg (23) and at the dose used in the current study, 75-100% inhibition occurred. In general as drug toxicity increased the effectiveness of immune suppression increased.

Since this toxicity relationship exists for 6-MP it seemed likely that intensive combination or methyl-GAG therapy would be more effective than single drug treatment. However, the 4-drug combination therapy was only as effective as single drug treatment and less effective than intensive 5-day therapy. Methyl-GAG was the least effective therapy. The reasons for this unexpected observation are not clear since each agent of the 4-drug combination has immunosuppressive properties and leukopenia was produced. Methyl-GAG may have qualitative differences about its immunosuppressive properties.

This study has also shown that the type of antigen used is most important in evaluating the effects of immunosuppressive therapy. Thus, weak degrees of suppression could be uncovered by an antigen such as Pn. Stronger antigens, such as Tu, would be better for the evaluation of varying intensities of suppression since it is more difficult to inhibit the response to this antigen.

Anti-tumor agents can inhibit a variety of immune responses in animals (8). This effect is associated with a complete arrest of the hemocytoblastic transformation and subsequent proliferation of lymphocytes and plasma cells (1, 31). Andres and co-workers (1) have also demonstrated that cellular proliferation eventually escapes the suppressive effects of drug treatment.

In man the agents which can inhibit the primary antibody response now include 6-MP, MTX, methyl-GAG, vincristine, cyclophosphamide, 6-thioguanine, and 5-fluoro-2'-deoxyuridine (16, 22, 23). Clinically, azathioprine, actinomycin C, and adrenal steroids have been used extensively in the maintenance of renal homografts (20). Inhibition of the secondary response in man by anti-metabolites has not been demonstrated. This is not surprising in that 6-MP does not block the cellular changes in lymph nodes after secondary stimulation (1).

Established antibody levels (such as isoantibodies) in man do not change during chemotherapy (25). Levels of specific immune globulins also have not been found to change (18).

These studies have several clinical implications. Anti-tumor agents are used successfully in the therapy of several inflammatory diseases (22, 24, 35) and to maintain homografts (20). On the other hand, the profound alterations of host defense mechanisms produced complicate their use. Several authors have suggested that the increasing incidence of opportunistic infections in cancer patients was due to chemotherapy (10, 27). In a recent study of the causes of death among patients with acute leukemia, disseminated fungus infections had increased from 8% to 23% of all disseminated infection during the most recent 4 years of the 10-year study period (6).

ACKNOWLEDGMENTS

The technical assistance of Mrs. Mary Strott is gratefully acknowledged.

REFERENCES


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