Alteration of Antibody Synthesis in the Rat by Cytosine Arabinoside\textsuperscript{1,2}

Malcolm S. Mitchell\textsuperscript{3,4}, Stephen R. Kaplan, and Paul Calabresi

Departments of Medicine and Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

SUMMARY

Cytosine arabinoside (ara-C) was effective in altering hemolysin antibody synthesis in the rat. The kinase that activates the drug was present in adequate amounts in lymphoid tissue. γG synthesis was completely suppressed in all experimental animals treated with 2,000 mg/kg/day for 5 days (Day 0 to Day 4 after antigen), but γG hemolysin also was absent in several animals treated with a nontoxic course of 200 mg/kg/day for 5 days and was decreased even in rats that had received 20 mg/kg/day. It was possible, with appropriate dosage schedules, selectively to inhibit γM synthesis, abbreviate its duration, or augment its synthesis and duration, as reflected in serum levels of hemolysin. No concomitant enhancement of γM and γG synthesis could be demonstrated.

Challenge of animals previously treated with ara-C resulted in secondary hemolysin responses similar to those obtained in controls. ara-C was capable of increasing as well as inhibiting humoral antibody production, the effect obtained depending upon the magnitude and scheduling of the dosage. Toxicity did not seem a sufficient explanation of the effects observed in these experiments.

INTRODUCTION

Many antineoplastic agents have demonstrated the ability to alter the immune response, although considerable variation has been noted among the drugs used and the species of animal tested. In general, alkylating agents and purine antagonists have been considerably more effective than pyrimidine analogs (16). However, cytosine arabinoside (\(\text{1,3-D-arabinofuranosylcytosine, cytarabine, ara-C}\)), a new pyrimidine antagonist characterized by an alteration of the sugar moiety (Chart 1), has proved to be a potent immunosuppressive agent in the mouse, inhibiting the primary response to sheep erythrocytes (12, 14, 15), bovine gamma-globulin (3), and allogeneic tumor cells (4). Complete suppression of the secondary response to tetanus toxoid also was achieved in the rabbit (18). The primary and secondary responses in patients with neoplasia were inhibited completely, but often temporarily, by injections or infusions of ara-C (21). A high dose of ara-C, given 1 hour before injection of antigen, has been reported to produce antibody titers exceeding those of control animals (3), but scarcity of ara-C limited the extent of these observations.

The rat is highly resistant to the toxic effects of ara-C. The single-dose \(LD_{50}\) has not yet been determined precisely, but it is certainly more than 1,000 mg/kg, and the 5-day \(LD_{50}\) is greater than 1,000 mg/kg/day (3, 26). Our purpose was to investigate systematically the effects of ara-C upon humoral antibody production in this species, on the premise that, because of a favorable therapeutic index, effects other than complete suppression of immunity might be achieved. Changes in the two types of antibody sequentially produced, during the primary immune response, polymeric rapidly sedimenting \(\gamma M\) (\(1gM, 19 S \gamma r\)) and monomeric \(\gamma G\) (\(IgG, 7 S \gamma 2\)), were studied after administration of drug. Results indicate that with appropriate dosage schedules of ara-C it is possible to obtain selective inhibition of synthesis of \(\gamma G\) or \(\gamma M\) antibody, or acceleration and prolongation of \(\gamma M\) synthesis.

MATERIALS AND METHODS

Animals

Sprague-Dawley female rats 10 to 12 weeks of age, weighing about 200 grams, were used throughout these experiments.

Antigen

Sheep erythrocytes in Alsever’s solution (Baltimore Biological Laboratories, Baltimore, Maryland) were washed three times in 0.85% saline and were standardized spectrophotometrically to a concentration of 1%, or about 1.7 \(\times 10^8\) cells per ml by enumeration in a Coulter Model A counter. One ml of saline suspension was injected intravenously into all animals as antigen, both for primary and challenge stimulation. For titrations (v.i) a fourth washing was performed in \(pH\) 7.5 Veronal-buffered saline (VBS) (20) before a 1% suspension in VBS was prepared.

Drug

ara-C was obtained through the courtesy of Dr. Carter D. Brooks of the Upjohn Company as a dry powder or a 50...
ara-C on Antibody Synthesis

Chart 1. Structure and probable sites of action of cytosine arabinoside. ara-C differs from cytidine at the 2' and 3' positions of the sugar moiety where the hydrogen atoms are in the trans instead of the cis configuration. The principal block exerted by ara-C was thought to occur at the conversion of cytidine diphosphate to deoxycytidine diphosphate [a] (6), but inhibition of DNA polymerase [b] may be at least as important (13). Incorporation of ara-C into DNA and RNA has been demonstrated, [c], [d], (6—8) and may account for some rapid, irreversible effects of the drug. ara-C, cytosine arabinoside; ara-C-DP and ara-C-TP, di- and triphosphate derivatives of ara-C; CDP, cytidine diphosphate; dCDP and dCTP, di- and triphosphate forms of deoxycytidine.

mg/ml solution. Concentrations of 1, 50, or 200 mg/ml in saline were used as indicated in each experiment. The drug was injected intraperitoneally into all experimental animals. When repeated doses were given, the first dose was injected within 10 minutes after the antigen was administered.

Titration of Antibody

Tittrations for hemolysins against sheep erythrocytes were performed in disposable plastic microtrays, with 25-μl dispensing droppers and diluters (Linbro Chemical Company, New Haven, Conn. and Cooke Engineering Co., Alexandria, Virginia). Serial 2-fold dilutions of antisera were made in pH 7.5 VBS. Twenty-five μl of 1% erythrocytes were added to each well, and the trays were shaken to mix. After a 15-minute incubation at 37°C, 25 μl of a 1:10 dilution of guinea pig complement (reconstituted lyophilized guinea pig serum) (Microbiological Associates, Bethesda, Maryland) were added, and the trays were shaken again. Hemolysis was completed after an additional 30-minute incubation at 37°C, with a final agitation at 15 minutes. Titrations were read after the trays had been at room temperature for 15 minutes to permit setting of any unlysed erythrocytes. Hemolysis was evaluated with a magnifying mirror; the last well in which 100% hemolysis was present was considered the endpoint. The titer was expressed as the log₂ of the reciprocal of the dilution of serum present at the endpoint.

Twenty-five μl of a 1:2 dilution of each serum were incubated with an equal volume of 0.2 M 2-mercaptoethanol (ME) in VBS at 37°C for 1 hour (23), and the mercaptoethanol-resistant hemolysin titer was determined. Calculation of the titer of ME-sensitive antibody was performed as follows: Mean total titer was converted to an arithmetic value, and the mean ME-resistant antibody titer, similarly converted, was subtracted. The result was then reconverted to a logarithm (base 2) for graphic presentation. Note that this is not the same as simply subtracting logarithms, which is equivalent to dividing the total by ME-resistant antibody. For the same reason the sum of ME-resistant and -sensitive antibody titers is not equal to total hemolysins when all are expressed as log₂.5

Sucrose gradient ultracentrifugation was performed upon 12 representative specimens from immunized control and experimental animals using an SW-50L rotor in a Spinco L2-65A centrifuge. Three-tenths ml of a 1:3 saline dilution of each serum was layered onto 4.6 ml of a linear 10 to 40% sucrose gradient. After centrifugation of 35,000 rpm (approx. 100,000 X g) for 16 hours at 8°C, 0.4-ml fractions were collected and analyzed individually for protein concentration (optical density at 280 m) and for antibody titer. Sensitivity to mercaptoethanol also was determined on each fraction.

ME-resistant antibody was found exclusively in the 7 S region, but ME-sensitive antibody was not simply 19 S; 10 to 11 S ME-sensitive hemolysins also were present early in the response of controls, and this species of antibody persisted along with heavier antibody in experimental animals whose ME-sensitive titers were prolonged (Chart 2). The nature of this intermediately sedimenting globulin was not further elucidated, but similar antibody has been described in the horse’s response to certain haptens (22) and in the human isoagglutinin system (27). With these qualifications in mind, ME-resistant antibody will be referred to as “γG” and ME-sensitive antibody as “γM” for the sake of simplicity. γA globulin also may be partially resistant to mercaptoethanol, but since this globulin does not appreciably fix complement (17), its contribution to the hemolysin titer probably was negligible.

A standard antisera against sheep erythrocytes (Difco Laboratories, Detroit, Michigan) was included with each set of titrations to insure uniformity of reagents from experiment to experiment. All sera from an experiment were titrated at the same time.

Experimental Procedure

Animals were injected with ara-C before or after antigen, as a single injection or daily for 5 days as summarized in Table 1.

5For example, if the total hemolysin titer were 2₅ and ME-resistant antibody were 2₄, ME-sensitive antibody = 32₈ = 2₆; log₂ = 4.5. This calculation assumes that titers of total hemolysins, ME-resistant and -sensitive antibodies have a simple relationship to each other, that the titer of each can be related to the absolute amount of antibody by a similar constant. Since γM is a more potent hemolysin than γG on a mol for mol basis (28) even this method tends to underestimate γM. All titers of ME-sensitive antibodies (“γM”) should therefore be viewed as relative to each other but not necessarily directly comparable with titers of ME-resistant antibodies (“γG”).
Controls received 0.5 to 1.0 ml of saline instead of drug. Blood for determination of antibody was obtained from the tail of each animal on Days 0, 2, 4, 6, 8, 10, 14, and 20 after administration of antigen. Serial total leukocyte counts and weights were determined during and after the course of treatment to gauge toxicity. Leukopenia was defined as a white blood count less than 8,000 in the etherized rat. A weight loss of more than 5 grams was considered significant. Many groups of rats were reinjected with antigen, 6 to 10 weeks after the initial injection. Blood was obtained just before reinjection and on Days 2, 4, 6, 8, and 10 afterwards. After separation from the clot, each serum was heated at 56°C for 30 minutes to inactivate complement and was stored at −18°C for 1 to 3 weeks before titration.

Charts 2. Results of sucrose gradient ultracentrifugation. Note the progress from a mixture of 19 S and 7 S (and intermediate) hemolysins on Day 8 (A) to purely 7 S (in this particular control animal) on Day 20 (B). In the experimental animal (C) ME-sensitive antibody persisted indefinitely. A, control (saline-treated) rat; Day 8 after antigen. B, control (saline-treated) rat; Day 20 after antigen. C experimental rat treated with ara-C, 2000 mg/kg/day × 5, (day 0 to 4 after antigen); Day 20 after antigen. ME, 2-mercaptoethanol.

**Table 1**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Timing relative to administration of antigen</th>
<th>Total hemolysins</th>
<th>γG hemolysins</th>
<th>γM hemolysins</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Single doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+ 10 min</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>+ 10 min</td>
<td>0</td>
<td>0</td>
<td>Acceleration; prolongation&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>+ 10 min</td>
<td>0</td>
<td>0</td>
<td>Prolongation</td>
</tr>
<tr>
<td>2000</td>
<td>+ 10 min</td>
<td>Partial suppression</td>
<td>0</td>
<td>Partial suppression</td>
</tr>
<tr>
<td>200</td>
<td>Day −2</td>
<td>Abbreviation</td>
<td>0</td>
<td>Abbreviation</td>
</tr>
<tr>
<td>200</td>
<td>Day −2</td>
<td>Abbreviation</td>
<td>0</td>
<td>Abbreviation</td>
</tr>
<tr>
<td>200</td>
<td>Day +2</td>
<td>Partial suppression</td>
<td>Partial suppression</td>
<td>Prolongation</td>
</tr>
<tr>
<td>2000</td>
<td>Day +2</td>
<td>Partial suppression</td>
<td>Partial suppression</td>
<td>Prolongation</td>
</tr>
<tr>
<td>200</td>
<td>−1 hr</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>−1 hr</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B. Course of 5 daily doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Days 0 to 4</td>
<td>Acceleration; prolongation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>Acceleration; prolongation&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>Days 0 to 4</td>
<td>Partial suppression</td>
<td>Partial suppression</td>
<td>Prolongation</td>
</tr>
<tr>
<td>200</td>
<td>Days 0 to 4</td>
<td>Partial suppression</td>
<td>Partial suppression (complete in 2/11)</td>
<td>Prolongation&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>Days 0 to 4</td>
<td>Nearly complete</td>
<td>Complete suppression</td>
<td>Partial suppression&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>Days −8 to −4</td>
<td>0</td>
<td>Increase of peak titer; prolongation</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Days −6 to −2</td>
<td>Partial suppression</td>
<td>Partial suppression</td>
<td>Prolongation</td>
</tr>
<tr>
<td>20</td>
<td>Days 2 to 6</td>
<td>Partial suppression</td>
<td>Abbreviation (no γM after Day 8)</td>
<td></td>
</tr>
</tbody>
</table>

Effects of various dosage schedules of cytosine arabinoside upon antibody synthesis (primary immune response). Compared with saline-injected controls in the same experiment. 0 = No effect.

<sup>a</sup>Weight loss, leukopenia, diarrhea; death in 4 of 6.

<sup>b</sup>Weight loss, within 10 min after antigen; −1 hr, 1 hour before antigen; Day −2, 2 days before antigen; Day +2, 2 days after antigen; etc., etc.

<sup>c</sup>Indicates that serum antibody titers appeared earlier and persisted longer than in controls.

<sup>d</sup>Weight loss in 4 of 12, without leukopenia.
ent in the serum until after a 2-day latent period. After a peak at 6 to 8 days, the titer decreased, reaching a plateau of 2 to 3 weeks after antigenic stimulation. γM synthesis began on about the 3rd day, reached a peak by the 6th to 8th day, and declined rapidly after the 10th day. A “double-humped” appearance was often seen, with a peak on Day 4 and a lesser secondary peak on Day 8, with the total hemolysins and γM antibody. γG synthesis began on approximately the 5th day, rose only slightly by the 8th day, and declined very little thereafter. After the 14th day, virtually all antibody in the serum was of the γG type. A dose of ara-C insufficient to cause even minimal toxicity, 20 mg/kg/day for 5 days, administered from Day 0 to Day 4 after antigen, had a distinct effect on the synthesis of antibody. More profound suppression was obtained with 2,000 mg/kg/day after 5 days. It is apparent from Chart 3 that synthesis of both γM and γG hemolysins was inhibited by these repeated daily doses given after the antigen. γG was in fact completely suppressed in all animals by 2,000 mg/kg/day. In all experiments, γM when present persisted for at least several weeks in the serum of animals in which γG synthesis had been suppressed.

Four of 6 animals given the highest dose of ara-C died with severe diarrhea within 5 days after the course of therapy had ended; the remaining 2 rats were leukopenic (WBC, 3000 to 7000/cu mm) for 5 days after the drug was discontinued. However, both survivors, and a third rat just before its death, produced γM antibody which, though reduced in titer, persisted longer than γM of controls.

The variation in response of individual rats to 200 mg/kg/day for 5 days, given from Day 0 to Day 4 after antigen, is shown in Chart 4. Two animals failed to produce γG hemolysins without manifesting weight loss or a significant decrease in their total leukocyte counts. Both produced a reduced amount (titer, 4) of γM. Other rats achieved normal γG titers with a delay in the onset of synthesis (8 to 10 days rather than

RESULTS

Results obtained with each dosage schedule are summarized in Table 1. Specific immunologic responses will be considered in more detail by individual category. Statistical analysis of difference was performed by the ranking method of Wilcoxon (31) as modified by White (30). Significance was accepted at the 5% level.

Suppression of Antibody Synthesis

Evidence that ara-C is capable of suppressing the primary immune response in the rat is illustrated in Chart 3. In the primary response of control animals, no hemolysins are present in the serum until after a 2-day latent period. After a peak at 6 to 8 days, the titer decreased, reaching a plateau of 2 to 3 weeks after antigenic stimulation. γM synthesis began on about the 3rd day, reached a peak by the 6th to 8th day, and declined rapidly after the 10th day. A “double-humped” appearance was often seen, with a peak on Day 4 and a lesser secondary peak on Day 8, with the total hemolysins and γM antibody. γG synthesis began on approximately the 5th day, rose only slightly by the 8th day, and declined very little thereafter. After the 14th day, virtually all antibody in the serum was of the γG type. A dose of ara-C insufficient to cause even minimal toxicity, 20 mg/kg/day for 5 days, administered from Day 0 to Day 4 after antigen, had a distinct effect on the synthesis of antibody. More profound suppression was obtained with 2,000 mg/kg/day after 5 days. It is apparent from Chart 3 that synthesis of both γM and γG hemolysins was inhibited by these repeated daily doses given after the antigen. γG was in fact completely suppressed in all animals by 2,000 mg/kg/day. In all experiments, γM when present persisted for at least several weeks in the serum of animals in which γG synthesis had been suppressed.

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The duration of γM synthesis could be abbreviated by a few dosage schedules, one of which is shown in Chart 6. Pretreatment with a single dose of 200 mg/kg, 2 days before antigen, led to a decline of γM levels after the fourth day, as contrasted with controls whose decline began 4 days later. Levels of γG were unaffected, indicating a primary effect of the drug upon γM. Increasing the dosage to 2000 mg/kg, 2 days before the antigen, failed to abbreviate γM synthesis more than 200 mg/kg, but the general effect was similar.

The synthesis of γM antibody thus seemed to be most vulnerable to attack when ara-C was given shortly before or virtually concomitantly with antigen. Complete inhibition of γM synthesis, however, could not be achieved in any of these experiments.

"Augmentation" of Antibody Synthesis

It was also possible to demonstrate accelerated appearance of γM antibody in the serum of drug-treated rats as illustrated...
Chart 7. "Augmentation" (acceleration and prolongation) of γM synthesis. Note significantly higher titers in experimental animals on the 4th day after antigen and subsequent to the 14th day. γG was unaffected by this schedule. ara-C, cytosine arabinoside.

in Chart 7. When 2 mg/kg/day of ara-C were given from Day 0 to Day 4 after antigen, titers of γM hemolysins significantly greater than in controls were detected on Day 4, and the presence of γM in the serum was prolonged. Peak total and ME-sensitive hemolysin titers were not significantly increased, however. Synthesis of γG hemolysins was unaffected. It was not established whether this represented augmented synthesis of antibody, although that was our presumption, or whether more rapid release of a long-lived antibody was elicited.

A pretreatment schedule of 200 mg/kg/day for 5 days, ending 4 days before the antigen was administered, caused a persistence of higher titers of γG in the serum (mean γG titer = 7, versus 4 in controls), but total hemolysins were similar to control values. A single dose of ara-C given 1 hour before the antigen failed to augment synthesis of either γM or γG when a dose of 200 to 1000 mg/kg was tried.

Response to Challenge of Animals Previously Treated with ara-C

After a second injection of antigen, 46 days after the primary stimulus, 2 surviving animals previously treated with 2000 mg/kg/day for 5 days (Day 0 to Day 4 after antigen) achieved hemolysin titers equal to those of controls (Chart 8). These animals, with previously complete suppression of γG...
Presence of Activating Kinase in Rat Lymphoid Tissue

Previous studies by others [Kreis, cited by Smith (26)] had suggested that ara-C might not be "activated" to phosphorylated derivatives in the rat. Since our results indicated that the drug was highly effective, analysis for the presence of deoxycytidine kinase, the activating enzyme, was performed upon lymphoid tissue obtained from these rats. Approximately 0.04 mmoles of phosphorylated derivatives per hour per 0.1 ml of cells was produced from tritium-labeled ara-C. This represents a sixth of the specific activity of the kinase present in human leukocytes obtained from patients with granulocytic leukemia (10). It has also been possible to demonstrate significant decreases in the rate of thymidine-3H incorporation into DNA when normal rat bone marrow or spleen cells, or spleen cells from immunized animals, were incubated with ara-C at concentrations varying from $10^{-6}$ M to $10^{-4}$ M. These preliminary data indicate that these cells may be a tenth as sensitive as human blood cells or mouse tumor cells, which is consistent with, though not necessarily completely explained by, data on phosphorylation noted above.

DISCUSSION

It has become increasingly evident that many compounds once considered "immunosuppressive" are capable of altering immune responses in a variety of ways, depending upon the magnitude and scheduling of the dosage. Chamougan and Schwartz (5) demonstrated that a course of 6-mercaptopurine given to rabbits, with a 2- to 5-day respite before injection of antigen, caused an augmented antibody response to bovine gamma-globulin. The same course given after the antigen, previously had been shown to be immunosuppressive (25). Buskirk et al. (3) found that a number of agents, including ara-C in mice, were capable of increasing peak titers above control values when given 1 hour before the antigen.

We have not yet been able to augment both $\gamma M$ and $\gamma G$ concomitantly by any schedule, perhaps because the dose of antigen used has been too high (5) or because of species differences. With an antigen similar to ours, however, Gray et al. (14) also could not reproduce Buskirk's results in the mouse. At least accelerated release, and very possibly accelerated production of $\gamma M$ hemolysins, has been achieved in this study by low-dose treatment after antigen, with elevated titers of antibody persisting considerably longer than controls. An observation of possible relevance was made by Adler (1) who noted a biphasic production of $\gamma M$ hemolysins in the mouse, with a cessation on the fourth or fifth day after intraperitoneally injected antigen and a secondary upsurge after the eighth day. A similar biphasic synthesis was observed in many rats in this study. Adler adduced as an explanation a production of antibody in extrasplenic sites, such as lymph nodes, after the eighth day that nearly counterbalanced the rate of catabolism of serum antibody. It is, therefore, a possibility that ara-C in low doses continued the production of $19 S$ rat hemolysins by extrasplenic lymphoid organs after initial rapid synthesis by the spleen. Paradoxical augmentation of antibody synthesis by low doses of a chemotherapeutic agent was noted in vitro by Ambrose and Mets (2). They found that low levels of actinomycin D in the medium during the productive phase of antibody formation enhanced the secondary response of rabbit lymph node fragments, whereas high levels of the drug were inhibitory. These investigators postulated an inhibition of an unidentified repressor of antibody synthesis, perhaps non-antibody-coded messenger RNA's (2). Chamougan and Schwartz (5) suggested an adjuvant action of nucleic acids released by drug-treated cells upon neighboring cells as the explanation of augmented synthesis seen with 6-mercaptopurine. In the final analysis, however, the precise mechanism(s) of augmentation of antibody synthesis and release are unclear.

Adler (1) could not be certain whether the second phase of $19 S$ hemolysin synthesis in the mouse required the persistence of antigen, but Uhr (29) has shown that the synthesis of ME-sensitive antibody to bacteriophage $\phi X-174$ most probably is antigen dependent. The effects of ara-C upon the synthesis of $\gamma M$ hemolysins, whatever the exact lymphoid organs involved, could be mediated by an effect upon the continued interaction of antigen and lymphoid cells, the processing (uptake, degradation) of antigen, or the rate of catabolism of $\gamma M$. We have insufficient data to determine which of these effects are operative. Harris and Hersh (15) have found that the duration of suppression of mouse agglutinins was greatest when antigen and ara-C were given simultaneously and by the same route. Their evidence, though consistent with an effect of ara-C upon uptake and processing of antigen by macrophages, is less than conclusive proof at present. In any case, it is of considerable interest that ara-C under certain conditions exhibited some selectivity for $\gamma M$ synthesis, since it is the synthesis of $\gamma G$ globulins that is usually most sensitive to antimetabolites (23, 24).

ara-C has been effective in suppressing antibody synthesis, often completely, during the primary or secondary response to a variety of antigens in several animal species including man (3, 4, 12, 15, 18, 21). This suggests that our inability to abolish antibody synthesis in this study may have been at least partially a function of the antigen-antibody system involved. Sheep erythrocytes constitute a strong primary antigenic stimulus in rodents, and $\gamma M$ hemolysins are potent antibodies on moles-per-titer basis (28). A small amount of $\gamma M$ hemolysin released into the serum would thus lead to a relatively high titer in the sensitive microassay employed. It is of interest that we have recently been able to suppress hemagglutinins to human 0 erythrocytes completely in the Sprague-Dawley rat by treatment with a 5-day course of 1000 mg/kg/day of ara-C. Many of these animals have in fact been made "tolerant," as defined
by failure to develop serum agglutinin titers over a 60- to 70-day period, by administration of appropriate doses of drug and of antigen. (R. O. Gordon, M. E. Wade, and M. S. Mitchell, unpublished data.)

In this study several animals treated with nontoxic doses of ara-C (e.g., 200 mg/kg/day for 5 days) showed decreased production of antibody. The lack of obvious toxicity, as measured by changes in weight and in the elements of the peripheral blood, in many subjects in whom definite suppression has been achieved (cf. also Ref. 21), has emphasized that immunosuppression can be a somewhat selective process. Whether antimitabolites act in a manner more specific for immunity than their known inhibition of nucleic acid synthesis is at best doubtful, but general host debility clearly is not a requisite. Conversely, rats made severely toxic by near-lethal doses of ara-C failed to show complete suppression of synthesis. Schwartz (24) noted that a regimen of 6-mercaptopurine capable of producing severe toxicity (leukopenia, death) in rabbits failed to cause suppression of the secondary response, whereas a shorter, higher dosage schedule resulting in no greater toxicity achieved complete suppression (19, 25). It seems a fair assertion, therefore, that toxicity is neither necessary nor sufficient to explain immunosuppression mediated by antimitabolites.

Cytosine arabinoside originally seemed to act principally by inhibiting the conversion of cytidine to deoxycytidine, thus inhibiting DNA synthesis (6) (Chart 1), but recent data suggest that effects upon DNA polymerase (13) and effects via incorporation into DNA and RNA (6–8) may well be of equal importance. The drug must first be phosphorylated to its active derivatives ara-C diphosphate and ara-C triphosphate by deoxycytidine kinase; inactivation occurs by deamination to uracil arabinoside (9). Since Kreis (quoted in Ref. 26) found that 70% of the drug was excreted unchanged in the urine of the rat, and since the antitumor activity in the rat was reported to be limited (32), it appeared likely that the drug was not activated in that species (26). There are now indications from our work and that of others (11) that a disproportionate distribution of the activating kinase is present in this species and that, perhaps, accounts for the effectiveness of ara-C upon the synthesis of antibody at nontoxic doses. In our experiments there was little apparent effect upon leukopoiesis, frequency of bowel movements, or body weight except at very high doses while effects upon immunity were seen at cumulative doses in the range of 10 to 20 mg/kg. Durham and Ives (11) found that intestine, liver, and other nonlymphoid tissues in the rat contained very low levels of deoxycytidine kinase while lymph nodes, spleen, and thymus had much higher levels, but the proportion of lymphocytes in the marrow was not stated. Thus, in the rat an activating system for ara-C exists in the tissues where immunosuppression is achieved and is lacking in most others. (Development of resistance to ara-C by bone marrow cells more rapidly than by lymphoid cells during therapy is another theoretical explanation for differential effects upon immunity and leukopoiesis, apart from static considerations of levels of enzyme. This possibility is currently under investigation.) While such a fortunate situation as encountered with ara-C may not obtain for all drugs or species of animal, a more rational selection of agents causing alteration of immune responses may possibly be achieved through a knowledge of the specific distribution of enzymes affecting metabolism of the drug in host tissues.

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Malcolm S. Mitchell, Stephen R. Kaplan and Paul Calabresi


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