

# HYPERSENSITIVE REACTIONS AND ANTIBODY FORMATION DURING L-ASPARAGINASE TREATMENT OF CHILDREN AND ADULTS WITH ACUTE LEUKEMIA

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One hundred and fourteen patients with acute leukemia, 57 children (10 AML and 47 ALL) and 57 adults (37 AML and 20 ALL) were treated with L-asparaginase (Asnase) 200 or 1000 IU/kg daily for 30 days unless withdrawn on account of side effects. Combinations with other cytotoxic drugs were used in all but eight patients. Hypersensitive reactions, decrease in Asnase activity in plasma, and bivalent antibodies to Asnase appeared more frequently in adults (28%, 46%, and 79%, respectively) than in children (16%, 17%, and 25%, respectively). There was a clear association between these three parameters. Thus hypersensitive reactions generally developed at the time of or after the decrease in plasma Asnase activity. Antibodies were detected only where Asnase activity had disappeared from the plasma. This time sequence, and in vitro experiments, suggest the formation of antigen-antibody complexes which might be responsible for inactivation of Asnase and for the development of hypersensitive reactions. However in many cases antibodies were found without concomitant enzyme inactivation or hypersensitive reactions. Antibodies to Asnase of IgE type (reagins) were found in only 10 children and 6 adults. There was no correlation between hypersensitive reactions, decrease in Asnase activity, and IgE antibodies. The frequency of remission among patients developing bivalent antibodies to Asnase was 68% (13/19) in contrast to 27% (3/11) among patients whose sera contained no detectable antibodies to Asnase, but the difference was not statistically significant.

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THE ENZYME L-ASPARAGINASE (ASNASE) IS effective in inducing remission in many patients with acute leukemia, particularly the lymphoblastic type in children. The most commonly used type of Asnase is derived from *Escherichia coli* and is thus a foreign protein

for the patients. In practice, hypersensitive reactions have been observed frequently and they are, in fact, the most undesirable side effects in Asnase therapy. To study this problem further, we have analyzed formation of antibodies to Asnase in 114 enzyme-treated pa-

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tients with acute leukemia, and studied their importance for hypersensitive reactions, maintenance of enzyme activity in serum, and for the therapeutic result. Preliminary results have been published elsewhere.<sup>2,5,10</sup>

#### MATERIALS AND METHODS

One hundred and fourteen patients with acute myeloblastic and lymphoblastic leukemia, 57 children (10 AML and 47 ALL) and 57 adults (37 AML and 20 ALL) were treated with Asnase (Crasnitine,<sup>®</sup> Bayer, Molecular weight about 130,000<sup>14</sup>), 1000 IU/kg body weight, intravenously with daily injections. A few patients at the very beginning of the study were given 200 IU/kg intravenously or intramuscularly. The treatment period was scheduled to be 30 days but had to be interrupted in a great many cases, particularly with adults, because of side effects (see below). Nearly all patients received the Asnase treatment in combination with other drugs; only two adults and six children received single drug therapy. Most adult patients were newly discovered, previously untreated, whereas the majority of children were in late relapse. The adults received the Asnase treatment as part of the CAPA-program (cyclophosphamide, 50 mg/m<sup>2</sup> i.v. twice daily + cytosine arabinoside, 50 mg/m<sup>2</sup> i.v. twice daily + prednisolone, 40 mg/m<sup>2</sup> i.v. twice daily for four consecutive days, followed by a 30-day course of Asnase, 1000 IU/kg i.v.<sup>6</sup> Six adult patients and 15 children received only prednisolone in addition to Asnase. Thirty-six children received Asnase in combination with various other chemotherapeutic drugs, i.e., cyclophosphamide, methotrexate, 6-mercaptopurine or vincristine.

Seventeen patients had multiple courses of Asnase treatment although only the results after the first course are included in the figures and the tables. Six adults had two courses and 11 children had two to four courses. The mean length of the second course was 11.7 days for adults (six patients) and 24.2 days for children (five patients).

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During the course of Asnase treatment, plasma samples were collected from the patient 24 hours after injection (i.e., immediately before the next injection) and stored at -20°C until analyzed. Asnase activity and antibodies to Asnase in plasma were determined by techniques described previously.<sup>2</sup> Thus, the enzyme activity was determined by measuring the liberation of ammonia by the Berthelot reaction;<sup>4</sup> significant Asnase activity is 0.3 IU/ml or higher. Antibodies to Asnase were determined using radioimmunosorbent techniques.<sup>16,18</sup> CNBr-activated Sephadex (Pharmacia, Uppsala) or cellulose particles (Merck, Darmstadt)<sup>15,17</sup> were coated with Asnase and incubated with plasma samples. One part of the particle suspension was exposed to <sup>125</sup>I-labeled Asnase<sup>16</sup> and the other to <sup>125</sup>I-labeled antibodies to IgE.<sup>18</sup> Antibodies reacting with labeled Asnase possessed two or more binding sites and are referred to as bivalent antibodies in the present work. IgE antibodies (reagents) behaved as functionally monovalent antibodies in this system.<sup>2</sup> The "bivalent" antibodies are expressed in per mille of a standard serum collected from one patient who had a high concentration of antibodies. The Asnase specific IgE (reagents) are expressed in sorbent units. For both types of antibodies, a value of 2 or higher represents a significant antibody concentration.

The statistical evaluation of differences in frequencies between groups was made with the chi-square test. In the text "significant" implies that  $p < 0.05$ .

#### RESULTS

##### Hypersensitive Reactions

Twenty-two percent of all leukemia patients had hypersensitive reactions in connection with Asnase treatment (Table 1). The reactions were urticaria, chills, fever, flush, blood pressure fall, dyspnea, or Quincke edema—the

TABLE 1. Incidence of Hypersensitive Reactions, Development of Bivalent Antibodies to Asnase, and Decrease in Asnase Activity

|                   | Hyper-sensitive reactions | Asnase antibodies | Decrease Asnase activity |
|-------------------|---------------------------|-------------------|--------------------------|
| Children          | 9/57 (16%)                | 9/36 (25%)        | 8/46 (17%)               |
| Adults            | 16/57 (28%)               | 26/34 (79%)       | 16/35 (46%)              |
| Children + adults | 25/114 (22%)              | 35/70 (50%)       | 24/81 (30%)              |

symptoms appearing in direct connection with administration of the antigen (Asnase). These reactions occurred at highly variable times (6–33 days) after Asnase treatment had been initiated (Fig. 1). In general, adults had their hypersensitive reactions earlier than children.

The hypersensitive reactions occurred in 28% of the adults and 16% of the children; the difference is not statistically significant. In neither age group was there any significant difference between the two types of acute leukemia with respect to incidence of hypersensitive reactions. Thus, among children, 2 of 10 (=20%) AML patients and 7 of 47 (=15%) ALL patients developed hypersensitive reactions, and among adults the corresponding figures were 11 of 37 (=30%) and 5 of 20 (=25%).

#### Development of Antibodies to Asnase

Antibodies to Asnase of IgE class (reagins) were found only occasionally (see below). Determination of bivalent antibodies to Asnase was undertaken in 102 of the 114 patients. However, it turned out that no such antibodies could be detected as long as enzyme activity was present in the plasma (see below). For this reason 32 cases had to be excluded, having Asnase activity in all plasma samples, leaving 70 patients for analysis of bivalent antibodies after the disappearance of Asnase activity. The adult patients developed antibodies much more frequently (79%) than did children (25%) ( $p < 0.001$ ) (see Table I) and in general the antibody concentrations were higher in adults (Fig. 2). Out of 11 children who had

two to four courses of Asnase treatment, none developed antibodies or hypersensitive reactions. In contrast, two of six adults receiving a second course of treatment developed antibodies after the first series of Asnase injections; one patient had a Quincke edema on day 12 after start of the second course and the other patient died on the third day. The four patients lacking antibodies after the first course of treatment had no side reactions during their second course.

Asnase antibodies were detected 8–39 days after Asnase treatment had been started (Abscissa, Fig. 5). They persisted in the peripheral blood for highly variable periods, from a few days to more than 1200 days (Fig. 3).

No difference was found between AML and ALL patients with respect to incidence of antibody formation in either age group. Thus, among children, 2 of 6 (=33%) AML patients and 7 of 30 (=23%) ALL patients developed bivalent antibodies to Asnase. The corresponding figures for adults were 19 of 23 (=83%) and 7 of 11 (=73%).

The frequency of remission among antibody producers was 68% (13/19) and among non-producers 27% (3/11) on the same treatment program, but this difference is not statistically significant. Neither was any significant difference found in length of remission time or survival time between immunized and nonimmunized patients.

#### Decrease in Asnase Activity

As previously described<sup>1,2,10,12</sup> a fall in Asnase activity was frequently seen in connec-

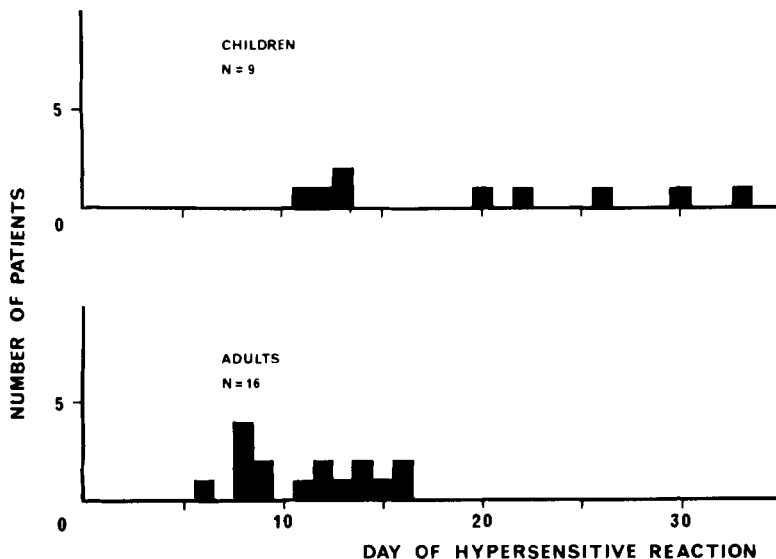
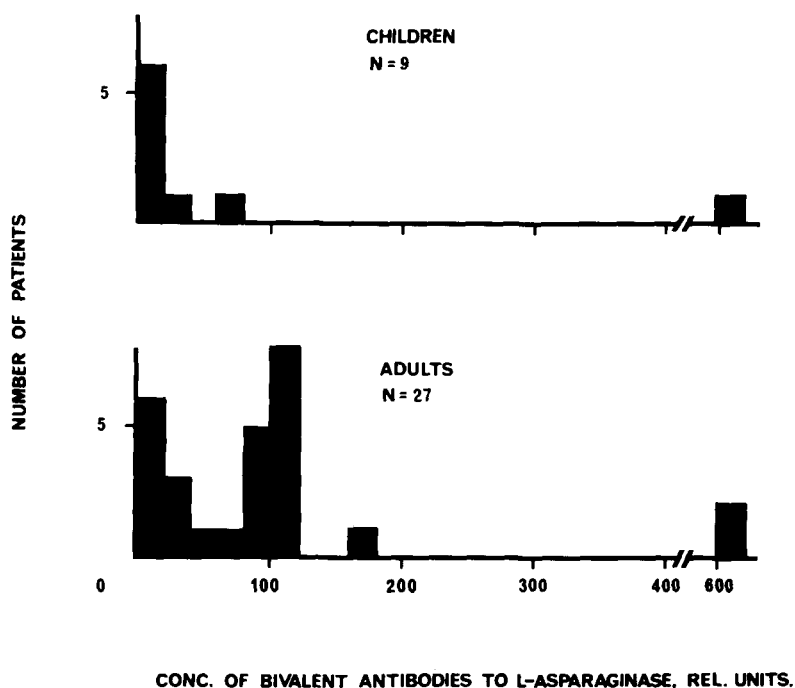


FIG. 1. Hypersensitive reactions during Asnase treatment of acute leukemia. Duration to reaction from start of treatment.

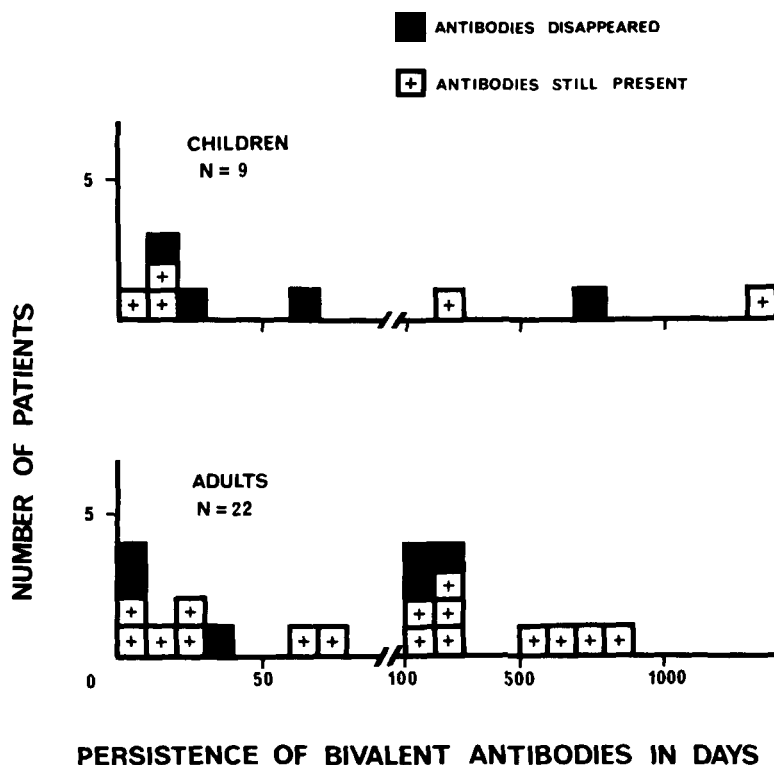
FIG. 2. Concentration of bivalent antibodies to Asnase in 36 patients with acute leukemia who developed antibodies during enzyme treatment. Multiple antibody analyses were made in most patients. The highest value within 30 days after the end of Asnase treatment is noted in the figure.



tion with the formation of antibodies to the enzyme. This is exemplified in Fig. 4, demonstrating typical courses of Asnase treatment: one without any complications (top) and another course which had to be interrupted be-

cause of a hypersensitive reaction at day 19 (bottom). The first patient had his daily Asnase injection for 30 days. Apart from some decrease in enzyme activity between day 9 and 16, the activity level was normal. It dropped

FIG. 3. Asnase-treated patients with acute leukemia who developed bivalent antibodies to the enzyme. Persistence of antibodies in the peripheral blood.



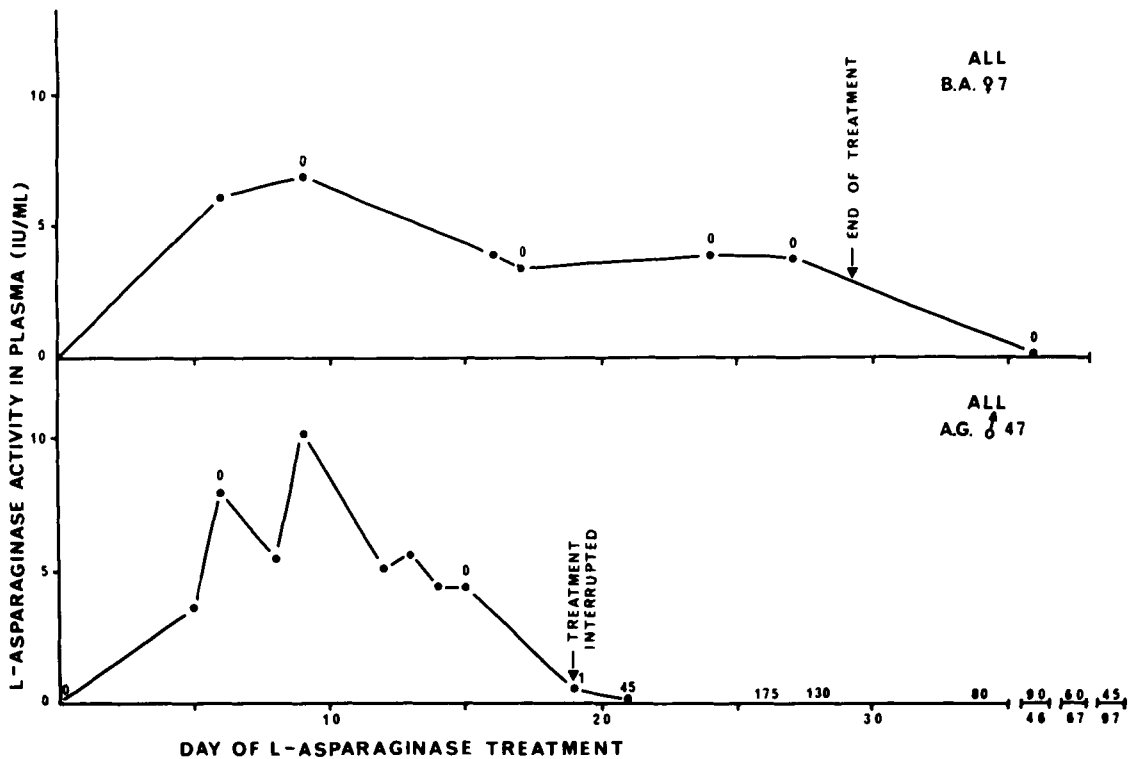


FIG. 4. Asnase activity during enzyme treatment of two patients with acute lymphoblastic leukemia; one without any complications (top) where the Asnase treatment was given for the scheduled time (30 days) and one (bottom) where the enzyme treatment had to be interrupted because of a hypersensitive reaction. The concentrations of bivalent antibodies to Asnase are indicated above the enzyme activity curves.

to zero within 7 days after the last Asnase injection. No antibodies were found at any time. In the second case (Fig. 4, bottom), however, enzyme activity fell continuously starting at day 12, i.e., 7 days before the hypersensitive reaction, which did not occur until the enzyme activity was close to zero. Neither were any antibodies to Asnase detected until the plasma was free from enzyme activity and after the hypersensitive reaction. The antibodies then decreased in concentration but persisted in the circulation for at least 60 days (see also Fig. 3).

Repeated enzyme activity tests were made during the Asnase treatment of 81 patients. A fall in Asnase activity was observed more frequently among adults (46%) than among children (17%) (Table 1).

#### Relationship between Hypersensitive Reactions, Antibodies to Asnase, and Enzyme Activities

The parameters in Table 1 were compared in order to obtain information about possible

relationships (Table 2). Hypersensitive reactions and presence of bivalent antibodies were clearly related since 21/22 patients with clinical reactions developed antibodies to Asnase (Table 2A). However, in quite a few cases, particularly among adults, bivalent antibodies were present without any clinical signs of hypersensitive reactions.

Decreased Asnase activity was recognized in as many as five of eight children and seven of nine adults with hypersensitive reactions (Table 2B) but it also occurred without such reactions, particularly among adults.

Table 2C shows that there was a stronger correlation between decreased enzyme activity and the development of bivalent antibodies to Asnase, the latter being found as frequently as in six of seven children and 15 of 16 adults with decreased Asnase activity. The antibodies appeared at the same time as the decrease in Asnase activity was observed (as illustrated by the filled circles on the 45° dashed line in Fig. 5), or later (circles below the line). Bivalent antibodies never appeared before a decrease

TABLE 2. Relationship between Hypersensitive Reactions, Antibodies to Asnase, and Enzyme Activities

| A. Antibodies-Hypersensitive reactions (70* patients)      |                   |                          |             |                       |
|------------------------------------------------------------|-------------------|--------------------------|-------------|-----------------------|
|                                                            | Asnase antibodies | Hypersensitive reactions |             | Significance          |
|                                                            |                   | Present                  | Not present |                       |
| Children<br>(36 patients)                                  | Present           | 7                        | 2           | $p \leq 0.001$        |
|                                                            | Not present       | 1                        | 26          |                       |
| Adults<br>(34 patients)                                    | Present           | 14                       | 13          | $0.01 < p \leq 0.05$  |
|                                                            | Not present       | 0                        | 7           |                       |
| B. Asnase activity-Hypersensitive reactions (81* patients) |                   |                          |             |                       |
|                                                            | Asnase activity   | Hypersensitive reactions |             | Significance          |
|                                                            |                   | Present                  | Not present |                       |
| Children<br>(46 patients)                                  | Decrease          | 5                        | 3           | $0.001 < p \leq 0.01$ |
|                                                            | No decrease       | 3                        | 35          |                       |
| Adults<br>(35 patients)                                    | Decrease          | 7                        | 9           | $p > 0.05$            |
|                                                            | No decrease       | 2                        | 17          |                       |
| C. Antibodies-Asnase activity (59* patients)               |                   |                          |             |                       |
|                                                            | Asnase antibodies | Asnase activity          |             | Significance          |
|                                                            |                   | Decrease                 | No decrease |                       |
| Children<br>(33 patients)                                  | Present           | 6                        | 1           | $p \leq 0.001$        |
|                                                            | Not present       | 1                        | 25          |                       |
| Adults<br>(26 patients)                                    | Present           | 15                       | 4           | $0.01 < p \leq 0.05$  |
|                                                            | Not present       | 1                        | 6           |                       |

\* Number of patients in whom both parameters could be investigated.

in enzyme activity. If antibodies were absent, there was as a rule, particularly among children, no decrease in Asnase activity (Table 2C).

The importance of Asnase antibodies for the enzyme activity was studied further in *in vitro* experiments. These demonstrated an inactivation of Asnase when incubated with plasmas containing antibodies (Fig. 6), the extent of the reduction of enzyme activity being correlated to the concentration of antibodies.

As mentioned above, antibodies to Asnase of IgE type (reagins) were found occasionally (16/100 tested patients). All 16 patients are listed in Table 3. Half of them had bivalent antibodies as well. Six of seven patients who had hypersensitive reactions and four of five cases where a decrease in Asnase activity was found, belonged to this "combined" antibody group. Only one of the eight "pure" reagin cases developed a hypersensitive reaction; decreased enzyme activity was observed in one other.

#### DISCUSSION

Previous studies on antibody formation after Asnase treatment have demonstrated the ap-

pearance of different types of antibodies, such as precipitating antibodies,<sup>9,11,12,13</sup> complement fixing antibodies,<sup>12</sup> and passive hemagglutinating antibodies to Asnase. Analyses of the immunoglobulin class of Asnase-antibody complexes have revealed mostly IgG<sup>9,12</sup> and IgM. Specific IgE antibodies (reagins) have also been detected.<sup>2,12</sup> Clearly, Asnase antibodies are related in some way to hypersensitive reactions and to neutralization of Asnase.<sup>2,12,13</sup> For example, in the present work antibodies were found in most patients (21 out of 22) with hypersensitive reactions and in 21 out of 23 patients with decreased Asnase activity.

However, no absolute correlations have been found. Thus, the incidence of antibody formation is higher than that of hypersensitive reactions, e.g., 70% and 30% in a previous work<sup>11,13</sup> and 50% and 22% in the present one. In our study, the formation of antibodies was also more common (50%) than a decrease in Asnase activity during treatment (30%).

Evidently, many patients developed bivalent antibodies to Asnase which neither inactivated Asnase enzyme activity nor resulted in hypersensitive reactions. We have not analyzed these antibodies further to show differences from inactivating antibodies and thus we can not

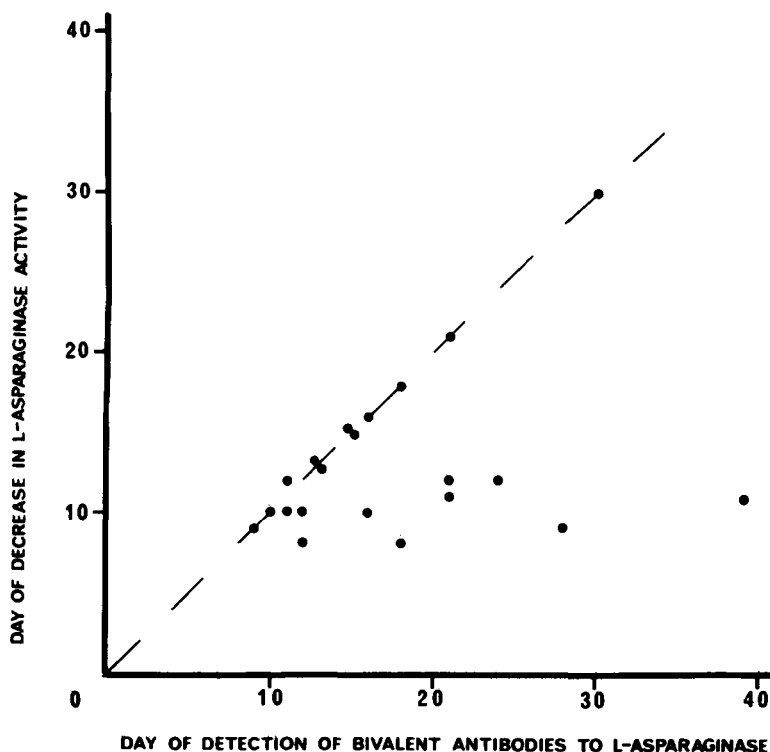


FIG. 5. The relationship between the time from start of treatment to decrease in Asnase activity and to detection of bivalent antibodies to Asnase in patient with acute leukemia. Each circle represents one patient. The day of decrease in Asnase activity is defined as the first sampling day with decreased enzyme activity, which is followed by a series of continuously decreasing activity values, e.g., day 12 in Fig. 4, bottom.

tell if they were due to impurities in the enzyme preparation. Pinsky et al.<sup>13</sup> found that the precipitation pattern differed between antibodies neutralizing Asnase activity and those which did not, and Peterson et al.<sup>12</sup>

found that passive hemagglutinating antibodies were better correlated with allergic reactions than precipitating or complement-fixing antibodies.

The clinical value of antibody and enzyme activity analyses is limited in predicting hypersensitive reactions. In general, antibodies are not detected before the reactions. However, our studies on patients receiving multiple courses of Asnase treatment indicate that knowledge about the presence or absence of antibodies is valuable if additional courses of Asnase treatment are being considered. A decrease in enzyme activity was found in many patients (12/17) prior to hypersensitive reactions, but this was also observed in 12 out of 64 patients without reactions. This lack of precision makes the clinical value of enzyme analyses doubtful as well. Similar conclusions were drawn by Dunncliff et al.<sup>3</sup> while more optimistic results were obtained in the study by Peterson et al.<sup>12</sup>

The mechanism underlying decreased enzyme activity during Asnase treatment is not fully understood. Neutralizing effects of antibodies to Asnase are well documented<sup>2,9,11,12,13</sup> but there is no complete inactivation of the enzyme in vitro. This has been shown in studies in enzyme activity of Asnase-antibody com-

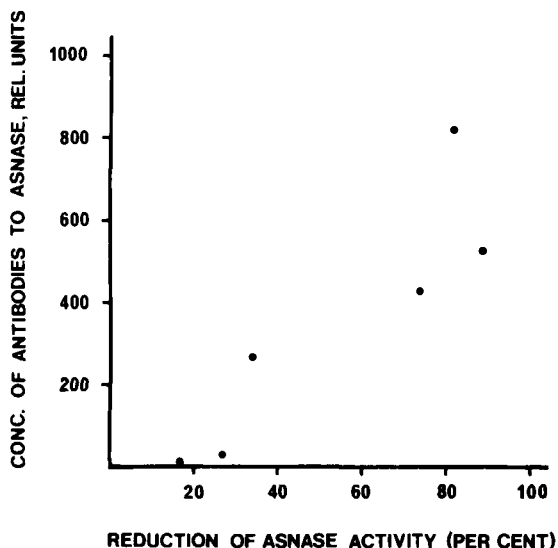


FIG. 6. In vitro reduction of Asnase activity by bivalent antibodies. Plasma samples with different concentrations of antibodies were incubated with known amounts of Asnase for 1 hour at +4°C.

TABLE 3. Patients Developing IgE Antibodies (Reagins) to Asnase

|                           | Case no. | Diagnosis | Reagins | Bivalent antibodies | Decrease in Asnase activity | Hypersensitive reactions |
|---------------------------|----------|-----------|---------|---------------------|-----------------------------|--------------------------|
| Children<br>(10 patients) | 2        | ALL       | +       | —                   | —                           | —                        |
|                           | 20       | ALL       | +       | —                   | —                           | —                        |
|                           | 49       | ALL       | +       | —                   | —                           | —                        |
|                           | 52       | ALL       | +       | —                   | —                           | —                        |
|                           | 4        | ALL       | +       | —                   | —                           | —                        |
|                           | 3        | ALL       | +       | +                   | —                           | +                        |
|                           | 54       | ALL       | +       | +                   | —                           | +                        |
|                           | 5        | ALL       | +       | +                   | +                           | +                        |
|                           | 9        | AML       | +       | +                   | +                           | +                        |
|                           | 55       | ALL       | +       | +                   | +                           | +                        |
| Adults<br>(6 patients)    | 122      | AML       | +       | —                   | —                           | —                        |
|                           | 109      | ALL       | +       | —                   | +                           | —                        |
|                           | 152      | AML       | +       | —                   | —                           | +                        |
|                           | 157      | AML       | +       | +                   | +                           | —                        |
|                           | 106      | AML       | +       | +                   | —                           | +                        |
|                           | 150      | AML       | +       | +                   | —                           | +                        |

plexes.<sup>9,12,19</sup> Therefore, some other phenomenon must explain the complete clearance of enzyme activity in the circulation of some patients, e.g., increased elimination by deposition of antigen-antibody complexes in the reticuloendothelial system<sup>12</sup> or decreased stability of the enzyme in an antibody complex.

Adult patients displayed stronger immunologic reactions to Asnase treatment than children, as demonstrated by higher incidence of hypersensitive reactions, formation of bivalent antibodies, high concentrations of antibodies, and decrease in enzyme activity during asparaginase treatment. Thus, children tolerated Asnase treatment better than adults and were able to receive repeated courses without complications. This difference between children and adults is difficult to explain. It is not due to the well-known prevalence of acute lymphoblastic leukemia in children and acute myeloblastic leukemia in adults, since comparison within each age group disclosed the same frequency of immunologic reactions in these two types of leukemia. Different types and schedules of treatment in children and adults could help to explain the difference in immunologic reactions. There were two principle differences in this respect. Firstly, adults were in general treated with Asnase early in the disease (during induction of the first remission) whereas in children the enzyme was used only for the induction of the second or later remission. It is conceivable that the immune response to Asnase at later stages of the disease is inferior to the response at earlier stages, though no such differences appears to have been reported.

Secondly, different drug combinations besides Asnase were used in adults and children. Adults generally received intense chemotherapy (CAPA)<sup>6</sup> which was probably more immunosuppressive than the less intensive concomitant chemotherapy generally applied in children. It is thus not likely that differences in drug combinations would result in more frequent immunologic reactions in adults.

Finally, the observed difference in immune response to Asnase between adults and children may be due to more intense exposure of adults to *E. coli* during previous infections. In such cases treatment with *E. coli* Asnase would induce a stronger immune response to the enzyme.

The Asnase antibodies which were important for the hypersensitive reactions were in both adults and children of the bivalent type, —probable IgG antibodies. IgE antibodies to Asnase were analyzed in parallel but were detected in only a minority of patients. There appeared to be no relationship between IgE antibodies and hypersensitive reactions, or inactivation of Asnase activity.

Although bivalent antibodies to Asnase seemed to be harmful in many cases, being related to hypersensitivity reactions, patients who developed antibodies responded to treatment as well as those who did not. In fact, among the adult patients who were randomized to the CAPA-program,<sup>6</sup> those who developed antibodies had a higher incidence of remission (68%) than those who did not (27%). However, the difference is not statistically significant. The immunocompetence of patients

with malignancies may be important for prognosis. Thus Hersh et al.<sup>7</sup> have shown that there may be a better response to treatment in leukemia patients in whom cell-mediated

immunity is preserved. Similarly, the antibody response to Asnase may reflect the immunocompetence of the patients which may play a role in their response to treatment.

#### REFERENCES

1. Capizzi, R. L., Bertino, J. R., and Handschumacher, R. E.: L-asparaginase. *Ann. Rev. Med.* 21:433, 1970.
2. Dohlwitz, A., Franzén, S., Holmgren, A., Killander, A., Killander, D., Wide, L., and Åhström, L.: Studies on antibody formation in patients treated with L-asparaginase. *Recent Results Cancer Res.* 33:198, 1970.
3. Dunncliff, M. A., Eigner, E. A., Jaffe, N., Traggis, D., and Rosenoer, V. M.: Plasma asparaginase concentration and the prediction of anaphylactic reactions. *Proc. Am. Assoc. Cancer Res.* 11:22, 1970.
4. Fawcett, J. K., and Scott, J. E.: A rapid and precise method for the determination of urea. *J. Clin. Pathol.* 13:156, 1960.
5. Gahrton, G., Dohlwitz, A., Engstedt, L., et al.: Hypersensitivity reactions and antibody formation to L-asparaginase during induction therapy in acute leukemia. 1st International Meeting on Therapy of Acute Leukemia, Rome, December 6-8, 1973 (abstract) p. 117.
6. Gahrton, G., Engstedt, L., Franzén, S., et al.: Induction of remission with L-asparaginase, cyclophosphamide, cytosine arabinoside and prednisolone in adult patients with acute leukemia. *Cancer* 34:472, 1974.
7. Hersh, E. M., Whitecar, J. P., McCredie, K., Bodey, G. P., and Freireich, E. J.: Chemotherapy, immunocompetence, immunosuppression and prognosis in acute leukemia. *N. Engl. J. Med.* 22:1211, 1971.
8. Hill, J. M., Loeb, E. A., MacLellan, A., et al.: Response to highly purified L-asparaginase during therapy of acute leukemia. *Cancer Res.* 29:1574, 1969.
9. Khan, A., and Hill, J. M.: Allergische Hypersensitivität durch L-asparaginase. *Int. Arch. Allergy* 40:463, 1971.
10. Killander, A., Gahrton, G., Dohlwitz, A., et al.: The significance of asparaginase antibodies during treatment of acute leukemia. First Meeting European Division International Society Haematology, Milan, 1971, (abstract) p. 164.
11. Oettgen, H. F., Stephenson, P. A., Schwartz, M. K., et al.: Toxicity of *E. coli* L-asparaginase in man. *Cancer* 25:253, 1970.
12. Peterson, R. S., Handschumacher, R. E., and Mitchell, M. S.: Immunological responses to L-asparaginase. *J. Clin. Invest.* 30:1080, 1971.
13. Pinsky, C. M., Mitchell, S., Oettgen, H. F., and Schwartz, M. K.: Immune reactions to L-asparaginase in man. *Proc. Am. Assoc. Cancer Res.* 11:63, 1970.
14. Whelan, H. A., and Wriston, J. C., Jr.: Purification and properties of asparaginase from *Escherichia coli* B. *Biochemistry* 8:2386, 1969.
15. Wide, L.: Radioimmunoassays employing immunosorbents. *Acta Endocrinol. (Suppl.)* 42:207, 1969.
16. Wide, L.: Solid phase antigen-antibody systems. In *Radioimmunoassay Methods*, K. E. Kirkham and W. M. Hunter, Eds. Edinburgh-London, Livingstone, 1971; p. 405.
17. Wide, L., Axén, R., and Porath, J.: Radioimmunosorbent assay for proteins. Chemical couplings of antibodies to insoluble dextran. *Immunochemistry* 4:381, 1967.
18. Wide, L., Bennich, H., and Johansson, S. G. O.: Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* 2:1105, 1967.
19. Zyk, N.: Modification by homologous antibodies. *Biochim. Biophys. Acta* 302:420, 1973.