Brief Report

Use of Phytohemagglutinin to Stimulate Hematopoiesis in Humans

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PHYTOHEMAGGLUTININ (PHA), a mucoprotein extract of the bean Phaseolus vulgaris, has been shown to have mitosis-stimulating effect on cells in tissue culture. Although cells from the peripheral blood of normal human do not usually proliferate in tissue culture, the addition of PHA to such cultures causes the initiation of intense mitotic activity among cells of the lymphocytic and monocytic series.

Administration of PHA to rats by differing routes and dose schedules causes no measurable change in hematocrit, WBC, percentage of lymphocytes in peripheral blood, serum globulin concentration, or histologic pattern of the liver and spleen.

Many studies have demonstrated the presence in the bone marrow of a pluripotent stem cell which is morphologically indistinguishable from the lymphocyte. Because of the presence of lymphocytes which could have pluripotency in the marrow of patients with aplastic anemia, Humble elected a trial of PHA in humans with this disease. Initial reports showed a 100 per cent response rate in four patients with marrow hypoplasia secondary to known causes. Because of this report, the following preliminary study was undertaken.

This report includes the results of four courses of PHA therapy in three patients: two with aplastic anemia and one with myelofibrosis. While it is recognized that one might question the inclusion of two diseases which probably differ in their pathogeneses, we felt this was justified in the present study because all patients satisfied a criterion suggested by the studies mentioned above. Marrow specimens from all 3 patients were examined to insure that large numbers of lymphocytes and reticulum cells were present. We then felt that mitogenic potency could be demonstrated regardless of the basic cause of marrow depression.

Patient #1, H. W., a 49-year-old white male, was admitted to the North Carolina Baptist Hospital with a 3-month history of fatigue, blurred vision, headache, ecchymoses and dependent petechiae. There was no history of known exposure to marrow toxins. Physical examination was within normal limits except for pallor, petechiae and ecchymoses of the skin. Laboratory data included: hemoglobin, 7.6 Gm./100 ml.; reticulocytes, 4 per cent.
platelets, 38,000/cu. mm., and WBC, 2800, with 38 per cent lymphocytes, 14 per cent monocytes, 30 per cent polymorphonuclear leukocytes, 8 per cent band forms, 3 per cent metamyelocytes, 2 per cent myelocytes, 2 per cent promyelocytes, and 2 per cent myeloblasts. Bone marrow aspiration yielded no marrow particles and an open marrow biopsy revealed almost complete marrow aplasia with only scattered lymphocytes and reticulum cells present. After following the blood counts for several days to insure stability, a course of PHA was given. The patient was given 50 mg. intravenously daily (as per Humble) for 7 days. Repeated blood counts during and after this therapy showed no significant change (fig. 1). Prednisone therapy was subsequently given with stabilization of the hemoglobin level but no improvement in platelets or leukocytes. At no time were "proliferative lymphocytes" as described by Humble noted in the peripheral blood nor in a second marrow aspirate.

Patient #2, C. S. R., a 67-year-old white male farmer, was admitted to this hospital for evaluation of a "blood condition." There was a 2-month history of spontaneous scleral hemorrhages, recurrent epistaxis, weakness and headaches. The family physician had administered prednisone, 45 mg. daily, for 3 weeks without response. There was no known toxic drug or chemical exposure. The patient’s father died of a "blood condition," requiring 10 to 12 transfusions annually for the last 20 years of his life. Except for purpura involving the forehead, eyes, mouth and extremities, the physical examination was within normal limits. The hemoglobin was 8.6 Gm./100 ml.; hematocrit, 26 vol. per cent; reticulocytes, 1.3 per cent; platelets, 11,000/cu. mm., and WBC, 3300, with 74 per cent lymphocytes, 4 per cent monocytes, 16 per cent neutrophiles, 5 per cent band forms, and 1 per cent metamyelocytes. Bone marrow aspiration yielded hypocellular marrow fragments with sparse erythroid and megakaryocytic elements. After 16 days of prednisone therapy at 60 mg. daily, no response was noted. At this time a 5-day course of PHA (50 mg./day) was given without reaction. A bone marrow aspiration performed 4 days following cessation of PHA therapy showed slight increase in erythroid activity. Because of this possible improvement, a second 5-day course of PHA was given. Seven days after the termination of treatment there was still no other evidence of improvement. Androgen therapy was started at this time, also without response, and the patient expired of pulmonary hemorrhage 3 weeks later. The details of the clinical course are showed in figure 2. One can see readily that the response noted was equivocal at best.

Patient #3, P. C. B., a 53-year-old white male carpenter, had been seen at this hospital for 2 years with a histologically verified diagnosis of myelofibrosis. Previous therapy had included adrenal corticosteroids and androgenic steroids with only slight response. Physical

Fig. 1.—Clinical course of patient #1 (H. W.) treated with phytohemagglutinin.
Fig. 2.—Clinical course of patient #2 (C. S. R.) during treatment with phytohemagglutinin.
Fig. 3—Clinical course of patient #3 (P. C. B.) during treatment with phytohemagglutinin.
abnormalities noted at the most recent admission included pallor, scattered ecchymoses, and massive hepatosplenomegaly. Hemoglobin was 7.5 Gm./100 ml., hematocrit, 23 vol. per cent; reticulocytes, 0.9 per cent; platelets, 340,000/cu. mm. and WBC, 3100, with 40 per cent polymorphonuclear leukocytes, 2 per cent eosinophiles, 1 per cent basophiles, 51 per cent lymphocytes, and 6 per cent monocytes. Bone marrow biopsy showed myelo-fibrosis with scattered nests of lymphocytes and reticulum cells. A course of 14 days of PHA therapy was given with no reaction or beneficial response noted. A repeat bone marrow biopsy 5 days after cessation of therapy showed no change in the histological pattern noted earlier. Details of the clinical course are recorded in figure 3.

In summary, treatment of three patients with marrow hypoplasia yielded no significant benefit in any. The only exception is the slight increase in erythroid precursors in the marrow of patient #2 (C. S. R.). The vagaries of marrow sampling are such that we are unwilling to attach a label of significance to this finding in the absence of concomitant clinical improvement.

DISCUSSION

Humble\textsuperscript{6} reported his original four cases in more detail with an additional two cases treated in similar fashion. All six of these patients showed some evidence of marrow stimulation, apparently in response to PHA therapy. It is of interest that two of these six patients died subsequent to therapy despite the fact that a “significant” response was observed. Another point of interest is the fact that all patients had marrow suppression due to known causes. None of them had “idiopathic aplastic anemia.”

Fleming\textsuperscript{7} described a single case of “idiopathic hypoplastic anemia” which responded transiently to prednisone therapy. Treatment with PHA for 7 days evoked no response.

Although Humble\textsuperscript{5,6} described only minor “allergic” reactions in patients treated with PHA, studies of the human pharmacology of this agent are nonexistent. Therefore, the study of Nirins and Marshall\textsuperscript{6} is doubly interesting in that they found doses of 100 to 300 mg./Kg. consistently killed rats and mice. Although toxicity in humans has been of minor degree, and doses used in humans have been much smaller, this finding indicates that there may be an upper limit to human tolerance of the drug and that toxicity could well be lethal.

Six patients with “idiopathic aplastic anemia” were treated by Retief, Wassermann and Hofmeyer,\textsuperscript{9} using the program of Humble. No response was noted in 4-6 weeks as measured by standard hematologic parameters. These workers raise the question of whether patients with marrow suppression due to a specific cause (e.g., drug) may respond to the stimulus of PHA while those with “idiopathic aplasia” may be incapable of responding. Indeed, it seems likely that the spontaneous remission rate of the former might logically be expected to be higher than that of the latter.

Baker and Oliver\textsuperscript{10} recorded the case of a 58-year-old female with marrow depression treated with PHA. Therapy was followed by cessation of need for transfusions but no other objective effect was noted.

The most recent report is that of Gruenwald et al.,\textsuperscript{11} who described three patients treated with PHA. One of these had “idiopathic marrow aplasia” and did not respond to therapy. The remaining two had marrow hypoplasia due
to drugs. One responded as shown by increased marrow cellularity and decreased transfusion requirements. The other did not respond until "after high doses of corticosteroids were added to the PHA."

**Conclusions**

Although the reports by Humble were enthusiastic and impressive, responses obtained by others attempting PHA therapy have been considerably less frequent and of lesser magnitude. The responses of our own patients, those of Retief et al., Fleming, and Gruenwald et al. seem to indicate that patients with "idiopathic aplastic anemia" are less likely to respond to PHA therapy, if at all.

Several areas remain to be explored. The first of these is a realistic assessment of the toxicology of PHA. Further studies should be tempered in their enthusiasm by the knowledge that large doses of this compound are lethal in rats and mice. Secondly, Humble's patients were routinely treated with chlorpheniramine. Since this compound is known to have some effect on the marrow and since patients so treated have shown the best responses, studies of possible interaction between PHA and chlorpheniramine are needed. Finally, attempts should be made to accumulate sufficiently large groups of patients, undiluted by other therapies (e.g., prednisone) to obtain a true estimate of the effectiveness of this agent. Particular attention should be given to patients with and without known causes for their marrow suppression.

**Summary**

PHA therapy was administered to three patients with bone marrow suppression, two with "idiopathic aplastic anemia" and one with myelofibrosis. One patient with aplastic anemia showed increased marrow erythroid activity; the others had no response. Review of the available data on PHA therapy suggests that states of marrow depression related to known etiologies may respond more readily than idiopathic states. Further studies are needed before definitive conclusions can be drawn as to the true efficacy of this agent.

**Summario in Interlingua**

Phytohemagglutinina esseva administrate therapeuticamente a tres patientes con suppression de medulla ossee, i.e., duo con "idiopathic anemia aplastic" e un con myelofibrosis. Un del patientes con anemia aplastic reageva per un augmentate activitate erythroide del medulla; in le altere, nulle responsa eseva notate. Un revista del accessibile datos concernente therapia a phytohemagglutinina suggere que statos de depression medullari relationate a etiologias cognoscite responde plus prestemente que statos idiopathic. Studios additional es requisite ante que un conclusion definitive pote esser formulare relative al ver efficacia de iste agente.

**References**

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