In Vivo Effects of Pokeweed Mitogen on Mouse Spleen Cells[1] (34630)

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(Introduced by R. J. Watson)
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Phytohemagglutinin (PHA), an extract of the red kidney bean *Phaseolus vulgaris,* has been shown to induce blastic transformation and mitosis of peripheral blood lymphocytes *in vitro* (1, 2). Studies of its *in vivo* effects have been limited. Its intravenous administration to mice produced changes in the splenic (3) and mesenteric (4) small lymphocytes that are similar to the changes observed in *in vitro* PHA-stimulated lymphocyte cultures.

A second mitogen, pokeweed mitogen (PWM), has been recently extracted from the plant *Phytolacca americana* (5). Farnes *et al.* (6) found that the *in vitro* effect of PWM on human peripheral lymphocyte cultures was similar to that of PHA. Schwarz (7) reported similar findings with rat lymphocyte cultures. Chessin *et al.* (8), on the other hand, described three distinct cell types in cultures of human lymphocytes stimulated with PWM. They were small and medium-sized lymphocytes, blast cells similar to those seen in PHA-stimulated lymphocyte cultures, and an intermediate cell type of unique features. On light and electron microscopy the latter was characterized by an eccentric nucleus with prominent perinuclear clear zone, a well-developed Golgi apparatus, and granular endoplasmic reticulum, with numerous ribosomal aggregates (9). These cells were similar to the "plasmablasts" or "plasmacytoid lymphocytes."

Reports of accidental systemic exposure of humans to PWM have appeared in the literature (10, 11). In these cases, plasma cells and their precursors, as well as mitotic figures, were found in the peripheral blood for periods up to 2 weeks after the exposure. The *in vivo* effects of PWM on the lymphopoietic system of normal laboratory animals has not, however, been reported.

The present study was undertaken to determine the *in vivo* effect of PWM on mouse spleen and peripheral blood cells. The results indicated that the cellular changes induced in the spleen after intravenous injection of PWM were similar to those induced by PHA. In addition, the absolute number of plasma cells also showed an increase. However, these changes were not reflected in the peripheral blood, and the differential cell count in the peripheral blood remained unchanged.

Materials and Methods. Animals. Male CBA/T6 mice, 3–9 months of age and weighing 18–30 g were housed in groups of 3–5 to a cage and fed a standard diet.

Pokeweed mitogen (PWM). PWM (Grand Island Biological Company, Grand Island, New York) was reconstituted with sterile normal saline and kept at —20° until used. The erythroagglutinating activity of the PWM was found to be minimal, and no attempt was made to adsorb it. A dose of 3.5 – 4 mg protein in a volume of 0.4 – 0.7 ml, was injected into the tail vein of each mouse.

Colchicine. One milligram of Colcemide (CIBA Pharmaceutical Products, Inc., Summit, N.J.) was dissolved in 2.5 ml of sterile distilled water and injected intraperitoneally in a dose of 0.01 ml/g body weight, 2 hr prior to the sacrifice of the animals.

Spleen cell suspensions. Spleens were weighed and placed in siliconized test tubes containing Eagle's minimum essential medium. Cell suspensions were prepared by using

[1] This investigation was supported by U.S. Public Health Service Grant 5R01 AM 10710-03.

[2] Recipient of Career Scientist Award, Health Research Council of City of New York Contract I-330.

Teflon hand homogenizer and gently expressing the splenic contents (12). The cells were then further dispersed by drawing through a Pasteur pipette and a 23-gauge hypodermic needle.

Total and differential spleen cell counts. Spleen cell counts were done in a standard hemacytometer; the total number of nucleated cells per spleen was calculated. Differential smears were made with a 2-0 hair brush (13). They were stained either with benzidine (14) and hematoxylin or with Wright's stain. The former was used to evaluate the pronormoblasts and normoblasts; the latter, for the differentiation of mitotic figures, blast cells, mature plasma cells, granulocytes, and lymphocytes. Slides were read independently by two observers. Absolute counts of various cell types were calculated and were expressed as millions per spleen.

Hematologic studies. Two hours prior to sacrifice, blood samples were obtained from individual mice by bleeding through the retro-orbital sinus. Hematocrit (Hct), white blood count (WBC), and differentials were done according to standard hematological techniques (15).

Study plan. All mice were weighed at the beginning of the experiment and then divided into groups of five or six, comparable in age and weight. Five mice (control group) which did not receive PWM or saline were sacrificed at the onset of the experiment. The rest of the animals were injected with either intravenous PWM (19 animals) or sterile saline (4 animals). Nineteen mice receiving PWM were sacrificed in groups of four or five, on Day 1, 3, 5, and 7. A saline-injected animal was sacrificed concomittantly. Since the results from saline-injected and control animals were similar, in the final analysis they were combined.

Two hours prior to sacrifice, peripheral blood was obtained for hematological studies, animals were weighed, and given intraperitoneal colchicine. After death, the spleens were removed, weighed, and the total number of nucleated cells and differentials were calculated.

Results. Toxicity to PWM. The intravenous administration of PWM in doses of 3.5 – 4 mg was found to be nontoxic to the animals studied. The total volume of fluid was well tolerated, and only one animal was lost immediately after the injection.

Effect of the PWM on whole body and spleen weights. One to three days after the administration of PWM all mice lost an average of 1.5 g of body weight. Animals did not appear to be sick, and by Day 5 all showed a net weight gain. Saline-injected animals gained weight steadily.

There was an initial steady increase in the spleen weights of animals injected with PWM. This weight gain reached its peak on Day 3, remained stationary through Day 5, and returned to normal Day 7. Changes in the relative splenic weights (spleen weight in milligrams per body weight in grams) closely paralleled the changes in the absolute weights (Fig. 1). In contrast, the relative splenic weights of saline-injected animals showed no gain.

Effect of PWM on spleen cell proliferation. The increase in the absolute and relative spleen weights was closely paralleled by an appreciable rise in the total number of nucleated spleen cells (Fig. 1). The biphasic curve described by Gamble (3) in relation to the nucleated cell proliferation in the spleens of PHA-treated mice was not observed in our studies.

Absolute counts of various nucleated cell types also showed increases closely reflecting the changes in the total number of nucleated spleen cells. The first cell type to show an increase was the blast cell. The rise in their total number was sharp and sudden on the first day after PWM injection. It remained high up to Day 5 and then dropped sharply to normal levels between Day 5 and Day 7 (Fig. 2). Criteria used for defining blast cells were the same as those adopted by others in studies involving PHA and human cells (16). The changes in the mitotic figures closely followed those of the blast cells (Fig. 2). The absolute number of normoblasts, lymphocytes, and mature plasma cells all reached their maximum values by Day 3, showed a drop by Day 5, and returned to

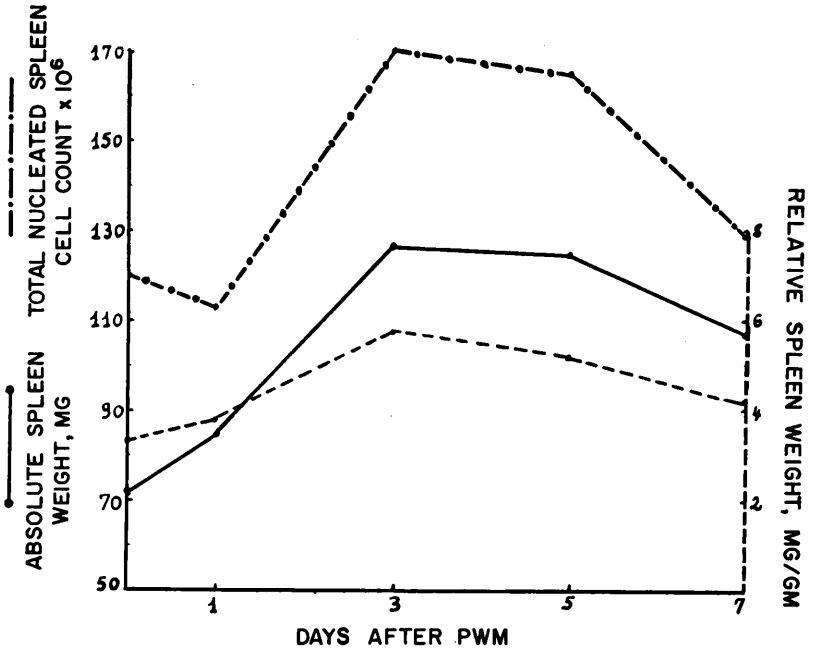

FIG. 1. Absolute spleen weights, total nucleated spleen cell counts, and relative spleen weights of control and PWM-injected mice. The first point on each graph represents average values for control and saline-injected animals. Subsequent point represent average values for 4 or 5 PWM-injected animals.

pretreatment levels by Day 7 (Fig. 3). No consistent patterns could be demonstrated for granulocyte counts.

Effect of PWM on peripheral blood. No significant change was observed in the hematocrit. There was a transient drop in the total white blood cell count on Day 1 (from an average of $13.5 \times 10^3/mm^3$ to $5.7 \times 10^3/mm^3$) with complete recovery by Day 3. In contrast to the findings in the peripheral blood of humans who were accidentally exposed to PWM, the peripheral blood differentials of the test animals did not show any plasmablasts, mature plasma cells, or mitotic figures.

Discussion. The marked changes observed in the absolute and relative spleen weights of mice given PWM indicate that in addition to its *in vitro* effects, PWM induced *in vivo* changes. The principal change was the striking increase in the total number of nucleated cells in the spleen during the first 3 – 5 days after the injection of the PWM. In the course of the first 24 hr the principal cell type to contribute to the increase in the number of nucleated cells was the blast cell. The sharp rise in their absolute numbers was probably the result of the direct effect of PWM on splenic lymphocytes. This was followed by rapid cell division as evidenced by an increase in the number of mitotic figures. Both blast cells and mitotic figures showed a synchronous development during Days 3 – 5 and returned to pretreatment values by Day 7.

From Day 3 onward the absolute total number of lymphocytes and normoblasts also increased. The former can be attributed to the division and maturation of the blast cells; the latter is not so readily explainable. The proliferation of splenic normoblasts after the intravenous injection of the PHA has been attributed to the stimulatory role of PHA on a wide variety of cell types. A similar explanation has been offered for its beneficial effect in some cases of human aplastic anemia (17). No direct effect of PWM on erythropoiesis has been reported, and in the present study no changes in the

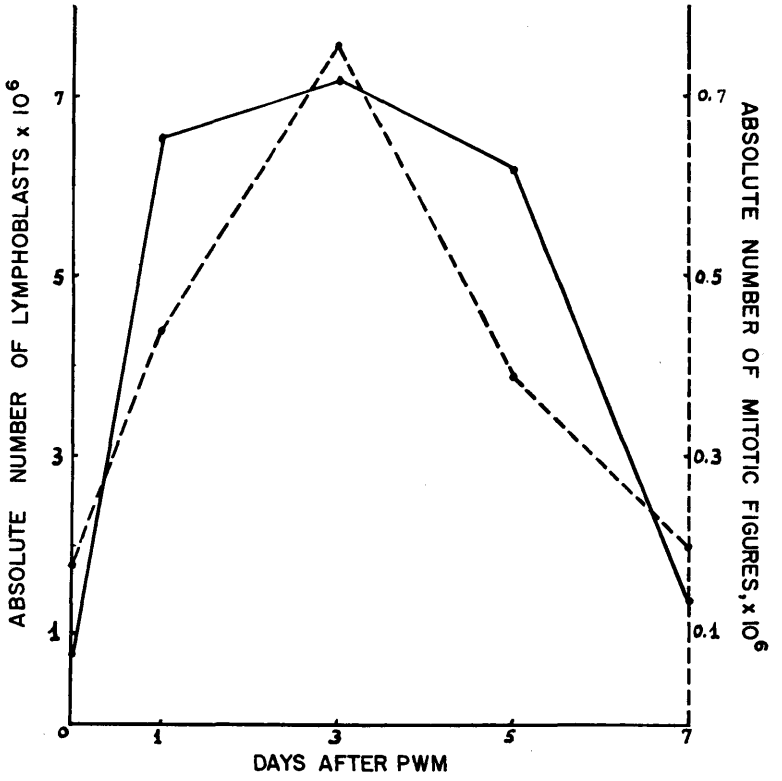

FIG. 2. Absolute numbers of lymphoblasts (—) and mitotic figurse (---) of control and PWM-injected mice. The first point represents the average value for control and saline-injected animals. Subsequent points represent average values for 4 or 5 PWM-injected animals.

hematocrit values were observed, in spite of the fact that no attempt was made to adsorb the minimal erythroagglutinating effect of PWM. A possible explanation is that the rise in the number of normoblasts is due to an influx of these cells from other areas of the body.

An interesting and important finding is the marked increase in the total number of mature plasma cells in the spleen. Plasma cells have already been described in the peripheral blood of humans who accidentally ingested PWM (11) and in human lymphocyte cultures stimulated with it (8). The origin of these cells is not known. The fact that a great number of plasma cells was found in the spleen of PWM-stimulated mice point to the spleen as the possible site of their origin. However, this rise was not followed by their appearance in the peripheral blood.

A still more pressing question is the immunologic competence of the blast and plasma cells observed after PWM stimulation. Tao (18) reported that lymph node cells from rabbits previously sensitized to human chorionic gonadotropin or bovine plasma albumin could be stimulated to give an anamnestic response after a short (2-hr) exposure to PHA in an *in vitro* tissue culture system. Forbes (19) obtained antibody production against thyroglobulin when he cultured human peripheral lymphocytes from a patient with Hashimoto's disease and stimulated them with PHA. Others (20) failed to elicit a nonspecific anamnestic response when an antigen-sensitized system was exposed to PHA. The finding of mature plasma cells in addition to the blast cells in the spleen of PWM-stimulated mice provides a stronger cellular basis for production of appreciable amounts of γ-globulin. Whether this γ-globulin is specific and whether animals previously sensi-

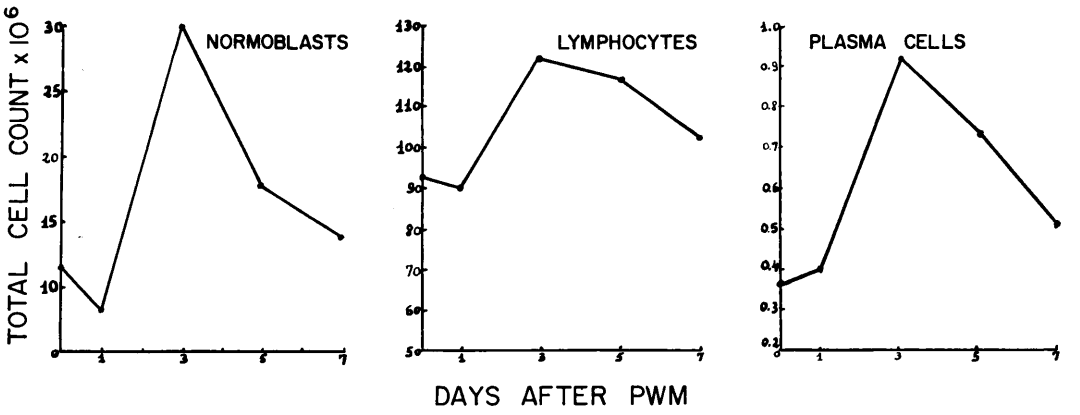

FIG. 3. Absolute counts of spleen cell types of control and PWM-injected mice. The first point on each graph represents average values for control and saline-injected animals. Subsequent points represent average values for 4 or 5 PWM-injected animals.

tized to a specific antigen will be stimulated to make specific antibody on exposure to PWM remains to be seen.

Summary. The intravenous injection of PWM to mice produced *in vivo* changes in the spleen similar to those produced by intravenous injection of PHA. These changes were a marked increase in the weight of the spleen, along with an increase in the number of nucleated cells, lymphoblasts, and mitotic figures. In addition, PWM also produced a marked increase in the total number of plasma cells. However, these cells did not appear in the peripheral blood, and the effect of PWM on it was limited to a transient leukopenia.

The technical assistance of Mrs. Barbara Kartzinel is gratefully acknowledged.

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Received Sept. 9, 1969. P.S.E.B.M., 1970, Vol. 133.