Differential Stimulation of Mouse Lymphoid Cells by Phytohemagglutinin and Pokeweed Mitogen (35410)

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Data from *in vivo* and *in vitro* exposure of lymphoid cells to the plant mitogens phytohemagglutinin (PHA) and pokeweed mitogen (PWM), suggest a possible specificity of action related to the dichotomous nature of the lymphoid system. Histochemically and morphologically the cells formed in response to PWM are similar to plasma cells and plasmablasts (*i.e.*, rough surfaced endoplasmic reticulum, well-developed Golgi apparatus and an eccentric nucleus with a distinctive chromatin distribution), as well as to less differentiated lymphoblasts (1–3). On the other hand, cells responding to PHA show structural similarities mainly to the less differentiated lymphoblasts.

Barker *et al.* (4) reported the development of plasmacytosis after systemic exposure to PWM. *In vitro* stimulation of lymphoid cells from patients with a variety of immunologic dysfunctions (5) suggests that differences in response reflect deficiencies of different populations of lymphoid cells. Using cells from mice with either lymphomatous or myelomatous disease, Dent (6) reported different responses upon exposure to PHA or PWM. Meuwissen *et al.* (7) have shown that lymphoid cells from bursectomized and from normal chickens respond well to PHA. However, they found a markedly decreased response to PHA when the lymphoid cells came from thymectomized birds, as has been reported for other species (8).

These reports, considered in the perspective of the division of the lymphoid system into thymus-dependent and nonthymus-dependent populations of cells, can be interpreted as indicating that PHA selectively stimulates the thymus-dependent portion of the lymphoid system, whereas PWM stimulates proliferation of the nonthymus-dependent components. To test this interpretation, the *in vitro* responses to PHA and PWM were measured for spleen cells from normal mice and from thymectomized, lethally irradiated mice reconstituted with isogeneic bone marrow (TIR). Morphologically the TIR animals show reconstitution of the nonthymus-dependent lymphoid elements, whereas the thymus-dependent elements are not replenished (unpublished data).

Female (C57 $\times$ A)F_1 mice were thymectomized at 1 month of age. Approximately 1 month later they were exposed to 1000 R of X-rays and reconstituted by infusion of 10^7 isogeneic bone marrow cells. Approximately 6 months later they were sacrificed by cervical dislocation and their spleens were removed aseptically. Cells from the spleens of two TIR mice or of two intact age control mice were pooled and suspended by passage through a sterile 50-mesh screen, for each of three experiments, performed on three separate occasions. One million mononuclear cells were placed in each culture tube with 2 ml of medium consisting of Earle's MEM supplemented with 20% inactivated fetal calf serum and 50 μg of erythromycin, 200 μg of streptomycin, and 1.5 μg sodium bicarbonate/ml. To each of three tubes of cells was added either 0.01 ml of PHA-M (Difco) reconstituted with phosphate buffered saline (PBS); or 0.01 ml of PBS (control); or 0.05 ml of PWM (Gibco) reconstituted with water; or 0.05 ml of water (control). These

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TABLE I. Spleen Cell Response of (C57 × A)F_1 Mice to PHA and Pokeweed Mitogen.

		Intact		Thymectomized[a]	
	Expt. no.	(cpm)[b]	(sp. inc.)[c]	(cpm)[b]	(sp. inc.)[c]
PBS control	1[d]	998	1.0	1311	1.0
	2	842	1.0	364	1.0
	3	399	1.0	386	1.0
PHA-M	1	2347	2.4	769	0.5
	2	1343	1.5	145	0.3
	3	900	2.2	218	0.6
H_2O control	1	764	1.0	652	1.0
	2	788	1.0	378	1.0
	3	395	1.0	293	1.0
PWM	1	6414	8.3	2371	3.6
	2	2060	2.6	1083	2.9
	3	1723	4.4	754	2.6

[a] Thymectomized–irradiated (1000 R)–reconstituted with isogeneic bone marrow.

[b] Mean counts per minute of three tubes.

[c] Specific incorporation = cpm/(control cpm).

[d] Each experiment consisted of a pool of two TIR spleens and a pool of two intact age control spleens.

sets of tubes were incubated at 37° with 5% CO_2. Three days later, 0.5 μCi of tritiated thymidine was added to each culture tube. Approximately 24 hr later the cells were washed twice with Hank's balanced salt solution, and the cell pellet precipitated with 5% cold trichloroacetic acid (TCA). The precipitate was then washed with methanol and digested with 0.2 ml of 0.1 *N* sodium hydroxide. Ten ml of scintillation cocktail consisting of 4 g of Butyl-PBD (Beckman), 100 mg of POPOP (Packard), and 100 ml of Bio-Solv. BBS-3 (Beckman)/1000 ml of scintillation grade toluene (Beckman) was added to each tube 2 hr prior to counting in a Beckman LS 150 counter.

The specific incorporation of tritiated thymidine by spleen cells from intact animals in response to PHA was significantly greater than unity, whereas the specific incorporation of thymidine by similarly incubated spleen cells from thymectomized-irradiated-reconstituted animals was less than one (Table I). In contrast, the specific incorporation of thymidine by spleen cells incubated with PWM was greater than unity for either TIR or intact animals.

We interpret these results as indicating that the so-called nonspecific mitogens PHA and PWM do have stimulatory specificity; that PHA acts upon thymus-dependent lymphoid cells, whereas PWM stimulates lymphoid cells of the nonthymus-dependent system.

Summary. The *in vitro* response to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) of lymphoid cells from thymectomized, irradiated, bone marrow reconstituted (TIR) mice was compared to that of intact age controls. TIR spleens, lacking cells of the thymus-dependent compartment, responded only to pokeweed mitogen, whereas spleens from intact donors responded to both PHA and PWM. These results suggest that PHA stimulates thymus-dependent cells; whereas PWM stimulates cells of the nonthymus-dependent compartment.

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