Suppression of Graft-Versus-Host Reaction by Phytohemagglutinin

The graft-versus-host (GVH) reaction occurs when competent cells are transplanted to recipients which are unable to reject them, and contain histocompatible antigens which are absent from the donor. This reaction, which occurs when allogeneic cells are injected into new-born animals, results in the development of a wasting syndrome in the nurslings referred to as 'runt disease'[1-3].

Phytohemagglutinin (PHA), a known inducer of in vitro blastogenesis of small lymphocytes[4,5] has more recently been studied for its effects in immune response in animals. While there have been some reports of immune response enhancement[6,7] studies by CALNE[8] in dogs submitted to kidney homografts and studies by SPREAFICO and LERNER[9] and in this laboratory (unpublished) on the immune response of mice to sheep erythrocytes have demonstrated marked depression with PHA administration prior to immunization or grafting. Similar work by ELVES[10] on the homograft reaction and by ST. PIERRE[11] on skin allograft survival in mice have indicated significant immunosuppression with PHA. In the present study, the GVH reaction in new-born mice was employed as a model to investigate the immunopotential of spleen cells derived from PHA-treated animals.

NZB mice, 2–4 months old, employed as donors in these experiments came from a colony which has been maintained in this laboratory since the receipt of the strain in January 1966, from W. HALL, University of Otago, Dunedin, New Zealand. The Swiss strain and Balb/c pregnant mice were obtained from Simonsen Laboratories, Gilroy, California.

A volume of 0.25 ml containing 1 mg PHA purified by the method of RIGAS[12] was injected i.p. into donor mice. 2 days later the mice were sacrificed and spleen cell suspensions prepared in medium 199 by the method of BILLINGHAM and BRENT[1]. New-born Balb/c mice were injected i.p. with 0.1 ml of this suspension containing $6–8 \times 10^6$ cells. Control groups included spleen cell suspensions from untreated NZB and Swiss strain mice. A further control included injection of new-born Balb/c with medium 199 only. In in vitro experiments a suspension of NZB spleen cells was incubated with 5 μg/ml PHA for 60 min at 37 °C. Following incubation cells were washed twice in medium 199 and 0.1 ml containing 6×10^6 cells were injected i.p. into new-born Balb/c mice. Runting was assessed by growth retardation as compared with untreated litter-mates over a 20-day period.

The results summarized in the Table show that i.p. administration of 1 mg PHA to young adult NZB or Swiss mice results in marked suppression of the GVH reaction following spleen cell injection from these animals into new-born Balb/c recipients. None of 24 Balb/c recipients of spleen cells from PHA-treated NZB mice developed a GVH reaction in contrast to 22/31 which came down with a wasting syndrome following injection of spleen cells from untreated NZB mice. Similarly, in the case of Swiss donor mice, PHA treatment 2 days prior to sacrifice and harvesting of spleen cells resulted in significant inhibition of the GVH reaction.

Suppression of the GVH reaction in nursling Balb/c mice was also achieved by incubation of NZB donor spleen cells with PHA for 1 h at 37 °C just prior to inoculation. This incubation period was previously shown[13] to be sufficient to induce transformation in cultures of human lymphocytes. As noted in the Table all animals receiving in vitro PHA-treated spleen cells evidenced inhibition of the GVH reaction. This would indicate that the effect of PHA is on the donor cells themselves.

Pretreatment with PHA a few days before administration of antigen has been observed generally to have an immunodepressive effect[8,9,11]. PHA is a well-known stimulator of mitosis in culture and has been demonstrated to have a comparable effect in vivo[6]. In mice, significant spleen cell proliferation, as judged by increase in spleen cell counts, splenic weight and in the proportion of cells engaged in DNA synthesis and mitosis, was observed to commence within 2 days following PHA administration and to reach a peak at 3 days[14-16]. Qualitatively, also, changes induced in the splenic lymphocytes in vivo simulated changes observed following the addition of PHA to cultures, most characteristically the transformation of small lymphocytes to large blast-like cells[14-16].

Mature small lymphocytes initiate an immune response to foreign antigens by enlarging and dividing. The mechanism of the immunologic defect of splenic lymphocytes from PHA-treated animals may reside in the inability of these cells to proliferate in response to such stimuli. This could develop from the circumstance that spleen cell transformation and proliferation would have already reached a near maximum in the donor animal pretreated with PHA; these splenic cells might then be unable to mount an effective GVH reaction. In this connection, proliferative response could be defective in terms of the metabolic capabilities of the responding cells. SPREAFICO[9]

Incidence of 'runt disease' in Balb/c mice injected at birth with spleen cells derived from untreated and PHA-treated donors

Experimental group	Donor strain	Incidence of runt disease
Untreated	NZB	22/31[a] (70%)
Treated with PHA	NZB	0/24 (0%)
Cells incubated with PHA	NZB	0/18 (0%)
Untreated	Swiss	4/7 (57%)
Treated with PHA	Swiss	1/15 (6.7%)
Medium 199 Control	–	0/27 (0%)

[a] No. of mice showing runting syndrome during first 20 days of life over the No. of experimental animals.

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has reported finding greatly increased in vivo incorporation of thymidine into the DNA of mouse spleen cells after injection of PHA. In view of the inverse relationship observed between the antibody-forming and DNA-synthesizing capacity of immune cells[17] cellular synthetic activities instigated by PHA could prove to be incompatible with productive antibody.

Studies done by GOWANS[18] showed that injected spleen cells rapidly changed into large pyroninophilic cells which then proceeded to divide in the host. Similar morphological results were obtained in vitro by HIRSCHHORN[19] with PHA and with specific antigen. The failure to produce a GVH reaction with PHA-stimulated spleen cells in vivo and in vitro in the present experiments suggests that cells once stimulated, even non-specifically, lose their ability to respond to a second stimulus with host antigens. Thus blast cell transformation may be connected with immunologic inactivation of the lymphoid cells, a 'sterile activation', as it were, through the mediation of PHA.

Since the GVH reaction is achievable only through immunocompetent cells, the findings here would suggest that blastogenesis may result in the production of cells no longer competent to initiate this reaction. Further studies in our laboratory will attempt to explore the possibility that specific blast transformation may also be productive of a cell incompetent to induce the GVH reaction[20].

Zusammenfassung. Mit Phytohämagglutinin behandelte Milzzellen erwachsener Mäuse, übertragen auf neugeborene Balb/c-Mäuse, lösen die erwartete «Runt-disease» nicht aus. Versuche mit Milzzellsuspensionen werden diskutiert.

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The Effect of L-Thyroxine on the Absorption of Calcium and Strontium

It is now becoming increasingly clear that in experimental animals the absorption of calcium and strontium is under metabolic control at least at 2 levels in the gastro-intestinal tract. Firstly by the calcium-specific active transfer process located in the duodenum[1] and secondly, by a metabolically dependent block which limits the passage of calcium and strontium from the lumen of the lower small intestine[2,3]. Both these processes are dependent on oxidative phosphorylation and can be inactivated by certain metabolic inhibitors[3,4].

The thyroid hormones have been shown to uncouple oxidative phosphorylation[5] and a number of manifestations of impaired calcium metabolism have been reported in cases of human hyperthyroidism[6–9]. In vitro studies of calcium transfer across everted duodenal sacs prepared from triiodothyronine- or thyroid stimulating hormone-treated rats showed a marked depression of the duodenal active transport of calcium[10]. This observation led to the suggestion that calcium absorption may be depressed in human hyperthyroidism. In this report it is shown that the overall absorption of both calcium and strontium is in fact increased in rats treated with thyroxine.

The experimental procedures have been described in detail elsewhere[3,11]. Female rats of the highly inbred August strain, aged 6–8 weeks, were given 1 mg L-thyroxine s.c. for 3 days. After fasting overnight the rats were given a single oral dose of 0.5 μCi$^{47}CaCl_2$ plus 0.5 μCi$^{85}SrCl_2$ and killed 7 h later. Absorption was estimated from the amount of each nuclide retained in the whole body less the amount retained in the gastro-intestinal tract.

The results presented in the Table show that in thyroxine-treated rats there is an increased absorption of both calcium and strontium. It is suggested that this increased absorption results from inhibition of the metabolic block in the small intestine, due to uncoupling of oxidative

Effect of L-thyroxine on the absorption of calcium and strontium by the rat

Treatment	Percentage of dose absorbed from G.I. tract in 7 h	
	Calcium-47	Strontium-85
None	[a] 56.6 ± 2.3 (13)	26.7 ± 1.0 (22)
L-Thyroxine 1 mg/d for 3 days	77.6 ± 2.2 (8)	46.2 ± 3.0 (17)

No. of animals in brackets. [a] Mean ± standard error of the mean.

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