Resumen. El grado mayor de proliferación de las RE células después del tratamiento de endotoxina no fué afectado por la cantidad de fósforo del régimen de alimentación pero 1.0% del fósforo abatió el thymidine incorporado dentro del DNA de la médula órea.

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Splenic Cellular Responses in vitro After Tumour-Immunization

Some committed small lymphocytes continue their immunological role, with rapid transformation to proliferating cells in the event of re-exposure to the initially sensitizing antigen[1]. Lymphocytes may be stimulated mitotically or transformed by phytohemaggutinin (PHA)[2]. Although the full significance of such stimulation is not known, the reactivity of lymphocytes to PHA has been used as one of the parameters for evaluating the immune status of individuals carrying or immunized to tumours[3-5]. We have described an induction of active anti-tumour immunity to a transplantable methylcholanthrene induced sarcoma with Sendai virus-fused cells in A/Jax mice[6]. In the studies reported here we examined cellular response of spleen lymphocytes from immunized mice with and without addition of PHA in cultures.

Materials and methods. Spleens were removed from mice and physically dissociated to produce a suspension of single cells. Cells were grown in Eagle's minimum essential medium with 5% heat inactivated normal rabbit serum and 1% glutamine. Antibiotics were added to the medium. PHA-P (Difco Laboratories, Detroit, Michigan) was added to some cultures at dilutions of 1:25 and 1:250 from the stock. Triplicate cultures of 2×10^6 cells (2 ml) were prepared for each group in glass tubes with loosely fitted caps. ^{3}H-thymidine was purchased from the New England Nuclear Corporation, Boston, Mass., with a specificity of 6.7 C/n*M* in sterile deionized water. The cells were incubated at 37°C in a humified atmosphere of 5% CO_2 in air.

The incorporation of tritiated thymidine by the cells in culture was estimated according to the method described by DENT[7]. The results were calculated as counts per min (cpm) per culture.

Results and discussion. Splenic cell preparations were studied from 5 immune mice and 5 normal control mice of comparable age. The immune mice had been given 3 separate fused-cell immunizing doses and were resistant to at least one tumour challenge[6]. The results are given in the Table. There was good agreement between the individual radioactivity levels within triplicates, of the order of 5–10%. However, a wide range of variations in the radioactivity was found in individuals within each group, especially among those from immune mice. The data was therefore transformed to logs to stabilize the variance and achieve additivity. As a result of this transformation, the standard deviation varies directly as the mean.

The control spleen cell culture, without adding PHA, showed a low level of radioactivity. By contrast, immune spleen cells exhibited a high rise in radioactivity, this being 23 fold greater than the control (the difference is significant at 1% level). These observations indicate that a high degree of spontaneous DNA synthesis of lymphocytes occurred in the immune mice. This is compatible with an ongoing immune response in the immunized animals in which viable tumour cells were injected 15 days

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Tritiated thymidine incorporation by normal and immune A/Jax mouse spleen cells with or without PHA stimulation in culture

Experimental group	^{3}H-thymidine incorporation (mean cpm/culture ± S.E.			Ratio of incorporation	
	Unstimulated	PHA stimulated		PHA 1:25 stimulated/ unstimulated	PHA 1:250 stimulated/ unstimulated
		1:25	1:250		
Control	95 ± 17 (2.45 ± 0.22)[a]	4,933 ± 706 (3.74 ± 0.07)	1,932 ± 261 (3.46 ± 0.08)	51.9	20.3
Immune	2,211 ± 1,548 (2.95 ± 0.28)	7,248 ± 1,877 (3.80 ± 0.72)	4,884 ± 896 (3.65 ± 0.10)	3.3	2.2
Ratio of incorporation (immune/control)	23.3	1.3	2.5		
Difference (*t*-value)	3.45[b]	0.95	3.31[c]		

In these experiments, 2×10^6 spleen cells were cultured in 2 ml of medium with or without PHA for 48 h of culture. 2 μc ^{3}H thymidine in 0.2 ml was added for the final 5 h of culture. Results are expressed as the mean counts per min (cpm) of 5 animals in each group having triplicate samples for each spleen in culture. The triplicates in each spleen culture have less than 10% variation in counts. [a] Figures in parenthesis indicate mean cpm/culture ± S.E. after $\log_{10}$ (X) transformation. [b] Significant at 1% level; [c] Significant at 5% level.

before the test. Furthermore, this in vitro finding seems to be in accord with lymphocytosis which is assumed to be intimately involved with tumour specific rejection[8].

The response to stimulation by PHA was tested in spleen cells from immune and control animals. In control spleen cells, ^{3}H-thymidine incorporation was increased more than 50-fold with the optimal dose (1:25)[9] and more than 20-fold with the lower concentration of PHA. In contrast, the equivalent figures for cells from the immune animals at two different PHA levels were only about 3.3 and 2.2 times the levels found in unstimulated immune spleen cell culture. A significant difference between the immunized and control groups was demonstrated at 5% level in response to 1:250 PHA, but not to 1:25 PHA stimulation. The absence of significant response of spleen cells from the immunized animals to an optimal concentration of PHA could be attributed to failure of immune cytotoxic lymphocytes to respond to PHA as demonstrated by MacLennan and Harding[10].

A recent report dealing with PHA reactivity of spleen cells from tumour-bearing mice and immunized mice, indicated that there was no difference from normal for the latter but less reactivity than normal for the tumour-bearing animals[11]. The results of that investigation with regard to immunized mice are different from ours. In their experiment, the 'immunized' mice, in which the tumours were removed 4 weeks previously had not been challenged with viable tumour cells, while the multinucleate-cell immunized mice used in our study had been re-exposed to viable tumour cells 15 days before initiation of the cultures. As mentioned earlier, an ongoing immune response together with specific tumour rejection in these immunized mice appeared to have occurred at the time of the test[12].

Résumé. L'incorporation de ^{3}Htdr, en l'absence de PHA, a obtenu un niveau élevé chez les animaux immunisés. Les cellules témoins montrèrent une radioactivité faible, à moins qu'elles n'aient été stimulées par la PHA. Les cellules spléniques des souris immunisées ne présentèrent aucune stimulation significative par l'utilisation d'une dose optimum de PHA.

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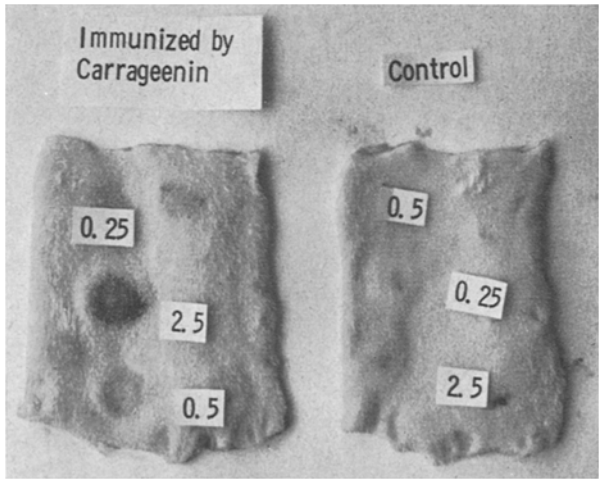
Induction of Delayed Hypersensitivity by Carrageenan

Carrageenan is a long-chain polymer of sulfated galactose units that can be extracted from marine plants. Carrageenan is used widely, not only in pharmaceutical, cosmetic and dairy industries, but also as a substance to produce acute and chronic inflammation in the animal experiment[1]. We found that an intensive hypersensitivity reaction to certain carrageenan was induced in guinea pigs by a single injection of the carrageenan. The reaction was considered to be a typical delayed-type reaction.



Delayed hypersensitivity to carrageenan. Intensive skin reaction was elicited by carrageenan ($2.5–0.25 \times 10^{-4}$ g) in the sensitized guinea-pigs (left) but not in nonsensitized one (right).

Random-bred male albino guinea-pigs weighing 300–500 g were sensitized by carrageenan. Carrageenan was supplied from Burtonite Co. Ltd., New Jersey, USA as 1K. CP. V-40-E (sample of the carrageenan will be supplied from the authors by request). The carrageenan contained about 1.5% polypeptides (as measured by the Lowry method) as some other kinds of carrageenan. For sensitization 0.05 ml of 1–0.05% carrageenan suspension in saline was injected intradermally to 1–6 sites of the right dorsal skin. 2 to 3 weeks later skin tests were done. A volume of 0.05 ml of 0.5–0.05% of carrageenan suspension was injected to the left dorsal skin of the sensitized animals. Nonsensitized animals served as controls. They were depilated with a barium sulfide-starch paste before the injection. 4 to 5 guinea-pigs were used in each group.

Within 5 h after the challenge, skin reactions were weak and revealed no differences between sensitized and nonsensitized animals. However, an intensive erythema appeared on the following day only in the sensitized animals (Figure). The reaction was frequently associated with a pale area at the centre, bleeding, and induration. It persisted, at a somewhat lower intensity, through 72 h. The skin reaction was characterized histologically, after 48 and 72 h, by a formation of granuloma composed mainly of lymphoid cells and with scattered giant cells. The lesion produced by carrageenan in normal guinea pigs was nonspecific with infiltrations of polymorphonuclear cells predominantly and mononuclear cells in lesser degree. The sensitization to carrageenan persisted at least through 1 to 9 weeks after the injection.

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