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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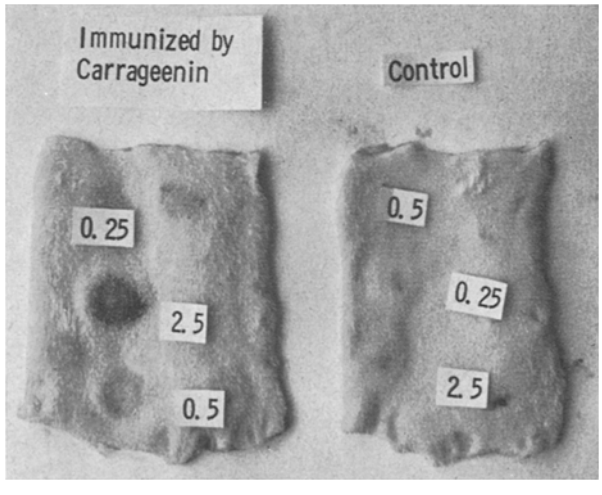
Induction of Delayed Hypersensitivity by Carrageenan

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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Amount of carrageenan used for sensitization was 25 μg to 1.5 mg. The 25 μg (0.05 ml of 0.05%) of it was enough for sensitization. A single injection of 1 mg of carrageenan sensitized the animals intensively. Approximately 40 guinea-pigs sensitized with 1.5 mg carrageenan (0.05 ml of 0.5% to 6 sites) gave an intensive reaction without exception. A dose of 250 μg of carrageenan was used for elicitation. However 25 μg of it was able to elicit a definite reaction. The carrageenan was dissolved by means of dialysis for 3 days against distilled water. The dissolved carrageenan had the same ability to induce and elicit the hypersensitivity reaction. Several kinds of carrageenan supplied by Marine Colloids, USA and by Nittō-Kaisō, Tokyo, had not a strong ability to induce hypersensitivity to them. While the extracts of Chondrus Ocellatus harvested from Korea gave an intensive reaction.

We performed systemic passive transfer experiments by peritoneal exudate cells with the usual method. Cell donors were sensitized by 1.5 mg carrageenan. 3 weeks later $1–1.5 \times 10^8$ peritoneal exudate cells were obtained from 4–6 donors and transferred to a normal animal. A dose of 250 μg of carrageenan was injected to the recipient and 4 normal animals. In 3 of 4 experiments a clear skin reaction appeared on the following day only in the recipient. From these data, the reaction described above is considered to be a typical delayed-type hypersensitivity.

It is well known that polysaccharides including carrageenan[2,3] have antigenicity and that some polysaccharides have an important role in delayed hypersensitivity. Purified polysaccharides isolated from Nocardia was reported to elicit a delayed hypersensitivity in guinea-pigs sensitized by living Nocardia with adjuvant[4]. However, it has not been reported that polysaccharides or polysaccharide-polypeptide complexes including carrageenan easily induced and elicited delayed hypersensitivity. Moreover there are only a few laboratory models of delayed-type hypersensitivity that can be induced easily and intensively as this model. Since the carrageenan used appeared to have chemically simple structure, we hope that this model will be helpful to analyze the antigenicity in delayed hypersensitivity reaction.

Zusammenfassung. Nachweis einer Sensibilisierung des Meerschweinchens durch intradermale Injektion von Carrageenan, was zu «passiv übertragbaren» Spätreaktionen führte.

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Lymphocyte Responses to Phytohemagglutinin in Rheumatoid Arthritis and Glomerulonephritis and the Effects of Immunosuppression[1]

It has been recognized that both cellular and humoral immune mechanisms play a role in many forms of immunological mediated tissue injury. Although immunosuppressive agents may have a variable beneficial effect in some of these diseases, the drugs are associated with life threatening complications due to immunodeficiency[2–4]. Thus knowledge of the status of these immunologic parameters in disease and in monitoring of patients on immunosuppressive drugs is of great importance. The response of cultured lymphocytes to the non-specific mitogen phytohemagglutinin (PHA) is used as a quantitative measure of lymphocyte function and one index of cell mediated immunity[5]. This study reports the results of lymphocyte transformation to PHA in rheumatoid arthritis and glomerulonephritis with and without immunosuppressive therapy.

Patients and methods. Four groups of patients were used. Group I. Acute glomerulonephritis (AGN) – 10 patients. All patients in this group had acute proliferative glomerulonephritis and impaired renal function which resolved spontaneously. None of these patients were azotemic at the time of the study and none were on immunosuppressive therapy.

Group II. Chronic glomerulonephritis (CGN). These patients all had morphological evidence of chronic glomerulonephritis. None were azotemic at the time of the study. II A) 8 patients – no immunosuppressive therapy. II B) 7 patients – all on immunosuppressive therapy at the time of the study.

Group III. Rheumatoid arthritis (RA). All patients met the criteria of the American Rheumatology Society for the diagnosis of RA. III A) 13 patients – no immunosuppression. III B) 6 patients – all on immunosuppressive therapy at the time of the study.

Group IV. Control patients – 10. These were normal healthy adults taking no medication at the time of the study.

Immunosuppression was accomplished with Prednisone 1–2 mg/kg body weight/day and/or azathioprine (Immuran – Burroughs Welcome, Tuckahoe N.Y.) 2.5–4 mg/kg body weight/day. Some patients received cyclophosphamide (Cytoxan) 2.5 mg/kg body weight/day.

Peripheral blood lymphocytes were cultured with and without PHA according to the method of OPPENHEIM et al.[6] except that fetal calf serum was used rather than homologous serum. Blast cell transformation was measured by incorporation of H^3 thymidine into cells at 72 h. Results were expressed as net increase in counts per min of PHA stimulated cultures over control cultures of the same patient.

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