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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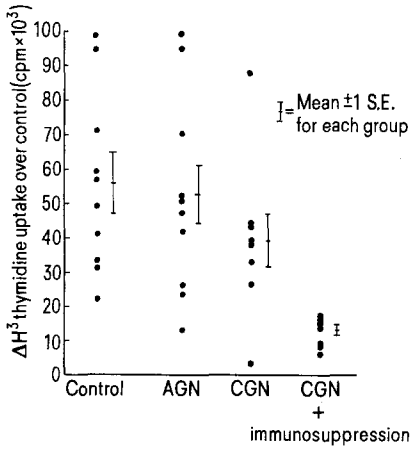

Fig. 1. PHA response in patients with glomerulonephritis with and without immunosuppression. AGN, acute glomerulonephritis; CGN, chronic glomerulonephritis.

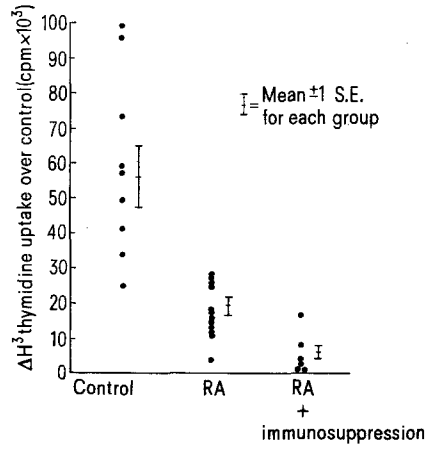

Fig. 2. PHA response in rheumatoid arthritis (RA) with and without immunosuppression.

Results. Patients with RA had a lower PHA response than controls (Figure 1). Immunosuppressed patients with RA demonstrated an even lower response than RA patients. These differences were significant at the 95% level (paired *t*-test).

Lymphocytes from patients with AGN responded in the same manner as the control group (Figure 2). The response of lymphocytes from CGN patients was slightly lowered but this difference was not statistically significant.

Immunosuppressed CGN patients showed a much lower response than controls, AGN and CGN groups at the 99% level (paired *t*-test). No correlation was observed between the lowered response and the mode of immunosuppression used.

Discussion. This study demonstrates that patients with RA have a significantly decreased lymphocyte response to PHA whereas patients with AGN and CGN do not differ significantly from controls. The consistent decrease in lymphocyte response in patients on immunosuppressive agents is especially important in light of the recent literature suggesting an increase in cellular immunity to certain antigens in renal disease[7]. The effect of immunosuppression can be observed as a decrease in lymphocyte response to PHA. This test may be of value in monitoring patients on immunosuppressive therapy[8].

Résumé. La transformation lymphocytaire en phytohemagglutinine est étudiée chez des individus normaux, aussi bien que chez des malades souffrant de glomérulonéphrites aiguës et chroniques ainsi que de polyarthrite rhumatoïde. La réponse des glomérulonéphritiques fut conforme aux contrôles, les polyarthritiques eurent des réponses affaiblies. La transformation lymphocytaire est fortement réduite chez les patients sous immunosuppression.

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Stem Cells in the Peripheral Blood of Rauscher Leukemic Mice

Stem cells circulate in the peripheral blood of mice[1] and can easily be detected by the spleen colony assay[2] review see [3]). Recently it has been shown that stem cells in the spleen and the bone marrow, measured as colony-forming units (CFU), are affected by the Rauscher virus[4,5] but no experiments have been performed with peripheral blood cells in any virus-induced leukemia to my knowledge. In this report, data are given for CBA mice infected with Rauscher virus.

Groups of 4–7 female mice (8–10 weeks old) were bled from the retro-orbital sinus 2, 5 or 13 days after infection and 0.2–0.35 ml of blood were injected into lethally irradiated (800 R) isogeneic recipients (10 per group). 23 days after infection, blood from a single mouse with erythroblastosis was taken, diluted with saline and 0.2 ml containing 0.04 ml of blood was injected. Blood from 2

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