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CELL-MEDIATED IMMUNITY TO CARTILAGE PROTEOGLYCAN IN RELAPSING POLYCHONDRITIS

D. A. RAJAPAKSE AND E. G. L. BYWATERS

MRC Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks, and the Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12

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SUMMARY

The presence of cell-mediated immunity and the concurrent absence of humoral immunity to human cartilage proteoglycan are demonstrated in two cases of relapsing polychondritis compared with nine control subjects with other rheumatic diseases. Two *in vitro* tests have been applied to study each type of immunity.

INTRODUCTION

Relapsing polychondritis, though uncommon, is an important disease because hyaline cartilage and other tissues containing chondromucoprotein seem to be primarily affected. Its study may therefore throw light on other connective tissue diseases. Many attempts to demonstrate specific biochemical (Verity, Larson & Madden, 1963; Ziff, Gribetz & LoSpalluto, 1960), haematological (Moskowitz *et al.*, 1970), metabolic (Thomas *et al.*, 1960) or genetic (Arundell & Hasevick, 1960) abnormalities have failed. The presence of antibodies to cartilage (Hughes *et al.*, 1972) and the occurrence of increased lymphocyte transformation with cartilage constituents suggestive of cell-mediated immunity to it (Herman & Hess, 1971; Herman & Dennis, 1973) have been reported recently by two independent groups. In each study, the same abnormality was reported to occur in some of the control subjects with other rheumatic diseases as well. We here report the absence of humoral immunity by double diffusion and immunofluorescent staining methods but the exclusive presence of cell-mediated immunity to human laryngeal proteoglycan—a chemically purer form of cartilage antigen—by the macrophage migration inhibition method and the lymphocyte transformation test in two patients and its absence in a variety of control subjects.

METHODS AND MATERIALS

Subjects

Two patients with relapsing polychondritis were available for these studies. The clinico-

Correspondence: Dr Donald A. Rajapakse, MRC Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire.

pathological details of the first under the care of one of us (EGLB) are being published elsewhere. The second case was under the care of Dr R. E. Church of the Rupert Hallam Department of Dermatology, Sheffield University, who most kindly allowed us access to the patient. He has already published (Church, 1971) the details of the second case.

Nine control subjects consisted of three with rheumatoid arthritis, two with Stills disease, one with sarcoidosis, one with polyarthritis, one with ankylosing spondylitis and one with rheumatic fever.

The macrophage migration inhibition test

The macrophage migration inhibition test was performed as previously described (Rajapakse & Glynn, 1970) with slight modifications. Macrophages induced in guinea-pigs—a sample of the batch showed no skin reaction with proteoglycan, the test antigen—by intraperitoneal injection of sterile liquid paraffin were harvested, separated and washed. 20×10^6 of these macrophages were mixed with 3×10^6 (equivalent to 15%) human peripheral blood lymphocytes which had been specially prepared earlier. The cell mixture or macrophages alone were suspended in 0·5 ml of medium (normal Parker 199 + 15% inactivated foetal calf serum + 5% of 4·4% sodium bicarbonate + 100 i.u. Penicillin/ml + 40 μg streptomycin/ml) or medium containing either 10 μg/ml or 100 μg/ml of human laryngeal proteoglycan (most generously supplied by Dr Helen Muir of the Kennedy Institute). Capillaries were filled, spun to pack, cut at the cell/fluid interphase and two such portions were implanted in a migrating chamber. Either duplicate or triplicate chambers were set up for each cell suspension. At the end of 24 hr and then 48 hr of incubation at 37°C, the fans of migrations were projected, traced on filter paper, cut and weighed. The percentage migration was calculated as follows:

$$\frac{\text{The mean area of migration in the antigen-containing capillaries in mg} \times 100}{\text{The mean area of migration in the corresponding antigen-absent capillaries in mg}}.$$

The lymphocyte transformation test

The lymphocyte transformation test was done by culturing $1\text{–}2 \times 10^6$ human peripheral blood lymphocytes in the presence of a previously titrated dose of the antigen, namely 20 μg of proteoglycan or 0·1 ml of freshly prepared phytohaemagglutinin M (Difco) in 5 ml of medium identical to that used in the migration experiments. Duplicate tubes were put up for PHA and the antigen, apart from duplicate control cultures for each material. The PHA and its control cultures were harvested on the 4th day and the antigen and its control tubes on the 5th day 24 hr after a pulse of tritiated thymidine. Additional control tube(s) to verify the effectiveness of DNA extraction where the thymidine was added at the time of harvesting the final tubes were included (zero tube(s)).

The DNA extraction was preceded by two washes of the cells in buffered saline and precipitation of the protein with trichloracetic acid. The cells were then washed with 44% dimethyl sulphoxide and finally ethanol. A sodium hydroxide lysate of the final residue was transferred to counting vials containing 10 ml of Bray's fluid. The degree of radioactivity was counted against a blank (Bray's fluid only) for background activity and a standard (one with known activity). The degree of transformation was expressed as a ratio of the radioactivity of the stimulated cultures to its own control cultures after deducting the zero values.

Immunofluorescent staining

Drs G. Loewi, G. Johnson and Mr D. Kingston each separately carried out the immunofluorescent staining for us using as substrate respectively human articular cartilage, human foetal cartilage and pig's foot cartilage. A standard double sandwich technique was employed by them.

Immunodiffusion

A double diffusion with serum and the proteoglycan was carried out in an agar immunodiffusion plate (Hyland Laboratories).

RESULTS

Table 1 shows the percentage migration in the two patients and the eight control subjects in

TABLE 1. The percentage macrophage migration inhibition in relapsing polychondritis and control subjects using human laryngeal cartilage proteoglycan as antigen

Amount of antigen:	0 μg/ml	10 μg/ml	100 μg/ml
Subject	Mean percentage migration*	Mean percentage migration	Mean percentage migration
Macrophages 20×10^6 (100%) no human lymphocytes			
J.L. = 4 } Patients	(100%) 100%†	(85%) 83%	(68%) 63%
J.M. = 8 } Patients	(100%) 100%	(99%) 103%	(92%) 109%
K.H. = 2 } Controls	(100%) 100%	(85%) 83%	(68%) 63%
G.P. = 5 } Controls	(100%) 100%	(116%) 120%	(115%) 122%
D.B. = 3 } Controls	(100%) 100%	(116%) 120%	(115%) 122%
J.T. = 6 } Controls	(100%) 100%	(116%) 120%	(115%) 122%
L.H. = 7 } Controls	(100%) 100%	(99%) 103%	(92%) 109%
V.S. = 9 } Controls	(100%) 100%	(99%) 103%	(92%) 109%
L.L. = 10 } Controls	(100%) 100%	(96%) 85%	(123%) 94%
A.P. = 11 } Controls	(100%) 100%	(96%) 85%	(123%) 94%
Macrophages 20×10^6 (100%) plus human lymphocytes 3×16^6 (15%)			
J.L = 4 } Patients	(100%) 100%	(25%) 26%	(20%) 21%
J.M. = 8 } Patients	(100%) 100%	(34%) 31%	(24%) 24%
K.H. = 2 } Controls	(100%) 100%	(73%) 66%	(75%) 69%
G.P. = 5 } Controls	(100%) 100%	(85%) 84%	(84%) 73%
D.B. = 3 } Controls	(100%) 100%	(121%) 133%	(117%) 116%
J.T. = 6 } Controls	(100%) 100%	(106%) 142%	(89%) 96%
L.H. = 7 } Controls	(100%) 100%	(93%) 88%	(87%) 83%
V.S. = 9 } Controls	(100%) 100%	(111%) 97%	(96%) 89%
L.L. = 10 } Controls	(100%) 100%	(126%) 128%	(117%) 106%
A.P. = 11 } Controls	(100%) 100%	(120%) 140%	(120%) 105%

* This percentage is calculated on the mean of four to six migrations.

† The figures appearing in brackets are the 24-hr results, while those appearing unbracketed are the 48-hr results.

the presence of 10 μg/ml and 100 μg/ml of human laryngeal proteoglycan. The 48-hr results are essentially similar to the 24-hr bracketed results, validating the inhibition. The figures around 100% or above as seen in the upper half of the table indicate the absence of a direct effect of the antigen in the strengths used on the migrating population of cells. Similarly where cell mixtures were employed, the extent of migrations around 100% or above show the virtual absence of any effect on the control subjects. However the inhibition of macrophage migration in the two patients with both strengths of antigen is striking.

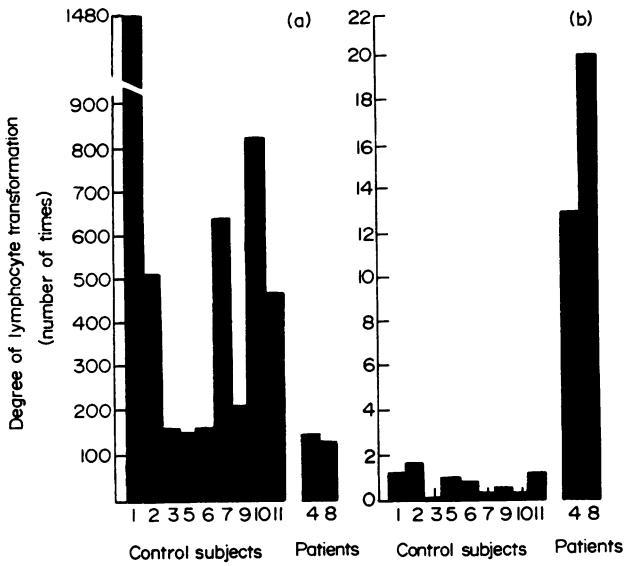

FIG. 1. Histogram of lymphocyte transformation in relapsing polychondritis. The amount of tritiated thymidine uptake with (a) 0·1 ml of phytohaemagglutinin M and (b) with 20 μg human laryngeal cartilage proteoglycan, expressed as a ratio of stimulated cultures to their control cultures to indicate the degree of lymphocyte transformation.

The histogram (Fig. 1) indicates the degree of lymphocyte transformation with PHA and 20 μg of proteoglycan. PHA shows marked but varied stimulation supporting the validity of the experiments. The two patients in contrast to the control subjects show increased transformation with the proteoglycan.

Neither the immunofluorescent stainings nor the immunodiffusions were positive, pointing to the absence of antibodies against cartilage or proteoglycan in the sera of the patients.

DISCUSSION

The inhibition of macrophage migration supplemented by the increased lymphocyte transformation by the human laryngeal cartilage proteoglycan indicates the presence of cell-mediated immunity to it in the two patients with relapsing polychondritis compared with the nine control subjects with other rheumatic diseases. Concurrently, the negative immunofluorescent staining and immunodiffusion point to the absence of antibodies (humoral immunity) to cartilage or its proteoglycan. These two patients thus seem to manifest, at least

in the circulating blood, exclusively cell-mediated immunity to a chemical compound of body's own tissue, namely cartilage proteoglycan.

Hughes' group (Hughes *et al.*, 1972) reported positive immunofluorescent staining of foetal cartilage in two out of three cases of relapsing polychondritis and weakly in twelve out of twelve cases of rheumatoid arthritis and none in the other thirty-two control subjects. We, like other previous workers (Herman & Hess, 1971; Herman & Dennis, 1973; Dolan, Lemmon & Teitelbaum, 1966; Pearson, Kline & Newcomer, 1960; Jensen, 1962; Potter, Duthie & Alexander, 1962; Strobel & Seifert, 1961; Quinn & Cerroni, 1957; Menkes *et al.*, 1970), have failed to demonstrate any humoral immunity in these two cases. The discrepancy could be resolved only by further studies of fresh cases. Herman's group (Herman & Hess, 1971; Herman & Dennis, 1973) similarly obtained increased lymphocyte transformation with a variety of unpurified cartilage and chondrocyte fractions in three out of three cases of polychondritis and in nine out of twelve cases with rheumatoid arthritis and in one with gouty arthritis but they did not test with the macrophage migration inhibition technique which of course is a conclusive indicator of cell-mediated immunity. The authors suggest the possibility of the operation of cell-mediated immunity in the perpetuation of the disease. As most of their control subjects had similar transformation, it seems reasonable to exclude the so-called non-specific stimulatory effect of the materials used to transform the lymphocytes.

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