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Altered cellular immunity and suppressor cell activity in patients with primary retinitis pigmentosa

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SUMMARY A group of 54 patients with primary retinitis pigmentosa were studied and the following findings are described: response of lymphocytes to stimulation by phytohaemagglutinin (PHA); response of lymphocytes to stimulation by xenogenic retinal extract; distribution of T and T-active lymphoid populations; total suppressor activity induced by concanavalin-A (con-A). The results obtained showed a reduction in the response to PHA ($p<0.05$), a positive response of 26/45 ($p<0.001$) to stimulation by retinal extract, a reduction in the T and T-active lymphoid populations ($p<0.01$), ($p<0.01$), and a diminished total suppressor activity induced by con-A ($p<0.01$). This alteration of immune cellular responses and the diminished suppressor activity in the group of patients, in comparison with a control group of healthy subjects, seems to indicate the existence of an immune process of disregulation (autoimmunity?) in the pathology of primary retinitis pigmentosa.

The term 'primary retinitis pigmentosa' (PRP) coined more than 60 years ago by Collins[1] denotes what we know today to be a genetically determined ocular alteration affecting approximately 0·5% of the world population.[2] Its pathogenesis is as little known now as it was when the disease was first described. Rahi wrote recently: The degeneration of the pigment epithelium and photoreceptors of the retina, which is the hallmark of primary retinitis pigmentosa (PRP), is still without a satisfactory explanation. . . . For want of a better explanation an autoimmune hypothesis has been proposed, but the evidence is largely indirect and not entirely persuasive.[3]

So far as indirect evidence is concerned we may cite the description of autoantigens located in the photoreceptors of guinea-pigs[4] and characterised as soluble (S) and insoluble or particulate (P). These can produce P retinal antigen, antibodies, but not delayed response or ocular dystrophies, and, soluble retinal (S), antibodies, delayed response, and disease when injected into homologous animals.[4] Rahi[5] has demonstrated the antigenicity of the photoreceptors of bovine retina in rats and rabbits.

Phylogenetically closer are the observations of Wong *et al.*,[6] who found that injecting photoreceptors of fresh retinas of rhesus monkeys into the interscapullar zone of 5 normal animals of the same species brought about the specific inflammatory destruction of its outer segments, with secondary alteration of the retina and the pigmentary epithelium layer.

Other evidence is supplied by Fessel,[7] who in 1962 found high levels of IgM and rheumatoid factor in 6 out of 10 patients with PRP, and by Rahi,[8] who also found high levels of IgM in a group of 52 patients with PRP.

Char *et al.*,[9] however, gave more direct evidence of the rise in cytotoxic activity of lymphocytes in patients with PRP (17 out of 20) in relation to cells from a line of retinoblastoma derived from photoreceptors. Their findings led these authors to affirm the existence of an immune cellular component in the pathogenesis and pathophysiology of the pigmentary degenerations of the retina.

Finally in 1977 Rocha and Antunes[10] raised the question of possible autoimmune origin of PRP after studying a group of patients with this disease (immunoglobulins, T-active lymphocytes, B lymphocytes, and inhibition of migration to normal retinal extracts). They discovered an alteration of the immunological parameters of the patient group in relation to the control group.

All the studies mentioned so far, together with the knowledge that an autoimmune process may be

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triggered off by an alteration in suppressor activity,[11] prompted us to undertake the studies reported here on a group of 54 patients with PRP. We studied the response of lymphocytes to stimulation by a mitogen (PHA) and by a retinal extract of mouse, DBA/2J; the distribution of the T and T-active lymphoid populations; and the total suppressor activity induced by concanavalin-A (con-A).

Materials and methods

Patients. A group of 54 patients (35 males and 19 females) aged between 20 and 56 with PRP were studied. The principal symptoms included loss of field of vision, arteriolar narrowing, pigment accumulations, poor adaptation to light or darkness, and abolished electroretinogram. After the diagnosis the immunological study was carried out.

Controls. The control group consisted of a 100 healthy subjects (free from ocular alterations and immunological abnormalities of whatever origin—asthma, atopic dermatitis, food and medicament provoked allergies, autoimmune disease, etc.). They were subjected to the same examinations as the patient group with PRP with the exception of stimulation by retinal extract (see results).

Isolation of lymphoid suspension. 25 ml of blood was drawn from each patient and control person by sterile venous puncture. The sample was defibrinated, diluted 1:1 with phosphate buffered saline (PBS), and deposited on a gradient of Ficoll-metrizoate (d= 1078). After centrifugation for 20 minutes at 600 *g* the cell layers were collected, washed 3 times with PBS, and the viability was studied before being resuspended in Roswell Park Memorial Institute solution (RPMI) 1640 and adjusted to: 1×10^6 and 4×10^6 cells/ml.

Preparation of retinal extracts (RE). Retinas from mouse DBA/2J were homogenised, ultrasonicated, and ultrafiltered using Sartorious No. 1234 membranes (Sartorious Membranfilter, GMPH, Göttingen, Germany) excluding molecules of fewer than 50000 daltons. The material obtained was adjusted to 100 μg/ml, sterilised by filtration, and diluted in culture medium until a concentration of 10 μg/ml was achieved.

Stimulation by PHA and RE. Aliquots of the lymphocyte suspensions (0·1 ml) adjusted to 1×10^6 cells/ml were distributed in different wells of a microtest plate with a flat bottom (Nunc, Denmark). 0·1 ml of a solution of PHA-M (Gibco) in culture medium RPMI 1640 at 2 concentrations: 5 and 10μg/ml or 0·1 ml of a concentration of RE at 10 μg/ml were added to some of the wells. Then the plates were incubated at 37°C in a humid atmosphere of 5% CO_2 for 92 hours, and 18 hours before the completion of the culture 0·2 μCi of thymidine ^3H was added per well. The cells were collected on a glass fibre filter with a cell collector (Skratron AS) and then transferred to a vial with 5 ml of scintillation liquid, which was read on a Beckman counter model 7000. The results were obtained in counts per minute (cpm), and the response index (RI) was expressed by dividing the means obtained in cpm in the presence of PHA or RE and the control wells.

Distribution of T and T-active lymphoid populations. To count the number of T lymphocytes aliquots of a suspension of lymphocytes (4×10^6 cells/ml) and sheep red blood cells (SRBC) in PBS at 1% (approx. 200×10^6 cells/ml) were mixed. After incubation for 5 minutes at 37°C they were centrifuged for 5 minutes at 200 *g* and left for the night at 4°C. The readings were carried out on a haemocytometer. 200 cells were counted (by 2 technicians) and a lymphocyte showing 3 or more adhering erythrocytes was considered to be positive. For the study of the T-active lymphocytes 0·1 ml of lymphocyte suspensions (4×10^6 cells/ml) and 0·1 ml of inactivated fetal bovine serum absorbed by SRBC were mixed. After incubation for 60 minutes at 37°C 0·1 ml of SRBC suspension was added to the mixture, which was centrifuged immediately (200 *g* for 5 minutes) before being gently resuspended. The readings were carried out as in the case of the T lymphocytes.

Study of the total suppressor activity induced by con-A. 2 ml of lymphocyte suspension (1×10^6 cells/ml) with 30 μg con-A/1×10^6 cells *(suppressor cells)* and 2 ml only in culture medium *(control cells)* were incubated for 24 hours at 37°C in 5% CO_2. The remaining cells were distributed in wells of a microtest plate (Nunc, Denmark) at a concentration of 1×10^5 cells/well *(responder cells)*. After a period of 24 hours the control and suppressor cells were washed, incubated with mitomycin-C (100 μg/tube) for 30 minutes at 37°C. They were then washed 3 times and resuspended in the original volume with RPMI 1640 with 20% serum. 1×10^5 suppressor cells were added to some of the responder cells, and 1×10^5 control cells (concentration for each well) were added to the rest of the cells, and then PHA-M (Gibco) at the concentration of 10μg/1×10^6 cells was added to half of the wells with responder cells. After 72 hours of culture 0·2 μCi of thymidine ^3H was added to each well, and the procedure followed as in the case of stimulation by mitogens described above.

The total suppressor activity was expressed as a percentage by this formula:

$$1-\frac{\text{(responder cells + suppressor cells with PHA)} - \text{(responder cells + suppressor cells without PHA)}}{\text{(responder cells + control cells with PHA)} - \text{(responder cells + control cells without PHA)}}\times 100$$

Statistical study. The results obtained from the different determinations were compared with those

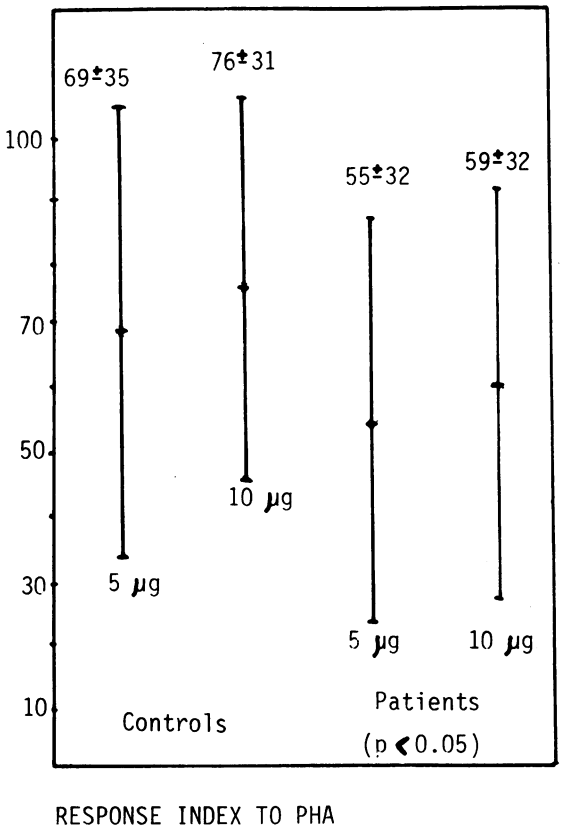

Fig. 1 *Mean values and deviations of the RIs of 100 controls and the 54 patients with PRP.*

of the control group by the Mann-Whitney U test and Student's *t* test. For the study of sensitisation to the RE the χ^2 test was used.

Results

Fig. 1 shows the mean values and the deviations of the response indexes (RIs) of the 100 controls to the stimulation with 5 and 10 µg/ml of PHA: 69±35 and 76±31. However, the RIs of the 54 patients with PRP were 55±32 and 59±32. There was a statistically significant difference ($p<0.05$) in the lowest RI registered in comparison with the values of the control group.

Fig. 2 shows the positive and negative RIs of the 45 patients with PRP (the study was not carried out on 9 patients) when the lymphocytes were stimulated by 10 µg/ml of RE. An RI of 2 or higher was considered positive (26 positive responses and 19 negative responses to RE). In the control group, however, of the 21 subjects studied only 2 obtained an RI of 2 or higher (positive), the remaining 19 being negative ($\chi^2=13.99$, $p<0.001$).

Fig. 3 shows the mean values and deviations of the T lymphoid population of the control group (60±8·3) and of the patients with PRP (50·4±10·1), ($p<0.01$). As in the case of the T-active population (25±13·9 in controls and 22·8±6·2 in patients) there was a statistically significant difference ($p<0.01$).

Finally, Fig. 4 shows the mean values and deviations of total suppressor activity induced by con-A. In the control group it was 34±13·9 and in the group of patients with PRP 13·1±23·2 ($p<0.01$).

Discussion

The report, initially in guinea-pigs, of the possibility of the production of autoimmune ocular diseases with retinal extracts,[12] the subsequent demonstration of specificity (retina specific) of the retinal antigen,[13]

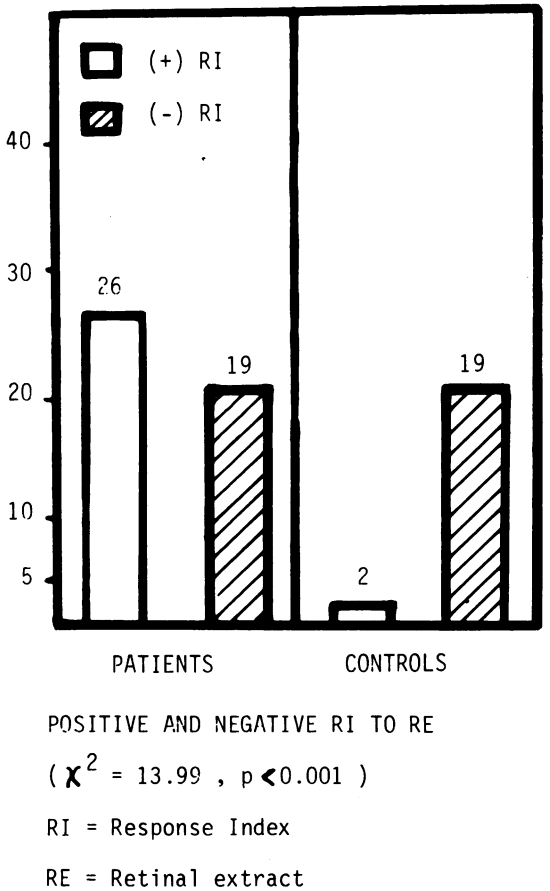

Fig. 2 *Positive and negative RIs of 45 patients with PRP and 21 subjects of the control group when stimulated by RE.*

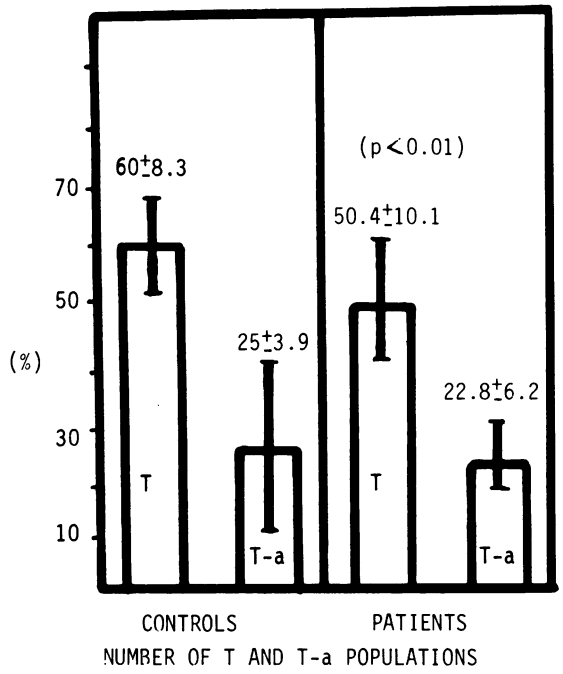

Fig. 3 *Mean values and deviations of the T lymphoid population of the control group and of the patients with PRP.*

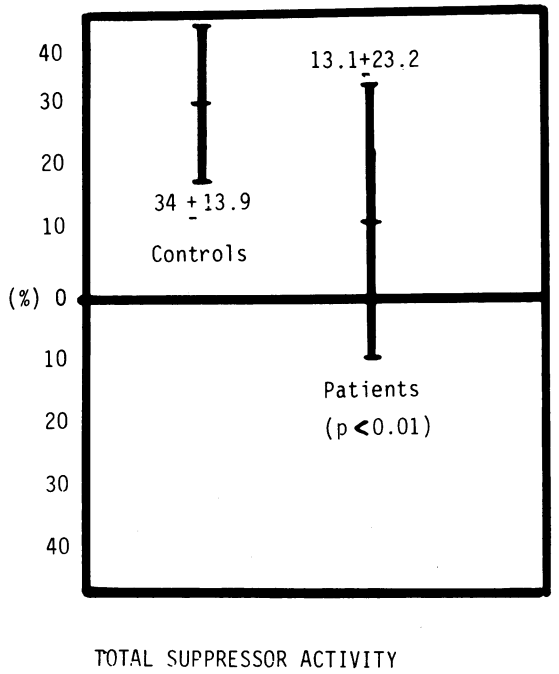

Fig. 4 *Mean values and deviations of total suppressor activity in the control group and in the group of patients with PRP.*

and its localisation in the layer of the photoreceptor cells[14] have led some authors to consider the hypothesis of a possible relationship (so far as the autoimmune pathogenesis is concerned) between these and other experimental models and some human retinopathies.[15] This hypothesis was reaffirmed some years later in the conclusion of an immunological and immunogenetic study in man[10] which declared that the retina should be included in immunopathology. PRP could be included in the same group.

So far as the number of T-active lymphocytes are concerned Rocha and Antunes[10] found in a normal population 26±3·5, while the mean and deviations in patients with PRP were 22·2±8·1; whereas we obtained the following values: 25±13·9 in the control group and 22·8±6·2; ($p<0·01$) in the patient group. However, the number of controls and patients in the study by the Rocha and Antunes was unstated.

A direct comparison of the numbers of T lymphocytes cannot be made since Rocha and Antunes[10] did not determine it. They studied B instead of the T lymphocytes. There was no significant variation between healthy subjects and patients with PRP when studying the B population.

In our case the number of T lymphocytes was also lower in the group of patients with PRP than in the control group. There was a statistically significant difference ($p<0·01$), though a controversy exists about the role the T lymphoid population might play in some abnormal immune states in which the relevance of the study of the active subpopulation has been demonstrated.[17]

So far as stimulation by RE is concerned, our findings show a positive response in 26/45 in the patient group as against 2/21 in the control group ($p<0·001$). Although Rocha and Antunes[10] carried our their study with homologous retinas and by another technique, namely, lymphocyte inhibitory factor (LIF), the results are not too disparate, since they found a 34/50 relation in the group of patients and 0/40 in the control group.

The stimulation by PHA was lower in the patient group than in the control group to both the mitogenic concentrations used ($p<0·05$). The fall in the response to the mitogen might be explained by the fact that the PHA is a general marker of the T population.[18] The response to the PHA is not a lineal function of the said population, and, since not all T lymphocytes respond to stimulation by a mitogen, the response is only an initial screening test of the T function.

The results obtained in the study of total suppressor activity induced by con-A were markedly lower in the group of patients with PRP (13·1±23·2) than in the

control group (34±13·9), ($p<0·01$). The fall in the suppressor activity is described in other classic autoimmune processes such as the SLE[19] and juvenile rheumatoid arthritis,[20] where the loss of the 'normal' level of suppression will result in the presence of autoantibodies and the nonfunctioning of the immunological regulation network.

The discovery of altered immune cellular response together with the reduction in suppressor activity in PRP seem to indicate the existence, at least with our methods of a process of immunological disregulation in this disease. And this may have some relevance if, as Leopold[21] states, some ocular disorders in particular individuals might benefit from measures that increase the competence of the immune mechanism where it is low or absent and reduce it in others where cellular immunity might be harmful.

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