Immune complexes, rheumatoid factors, and cellular immunological parameters in patients with giant cell arteritis

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SUMMARY Circulating immune complexes were found in 2 of 15 patients with giant cell arteritis (GCA) by using a solid phase C1q enzyme linked immunoabsorbent assay (ELISA). The prevalence in controls was 5%. Rheumatoid factor could be demonstrated in 2 out of 27 patients and in 11% of the controls by using a similar ELISA technique. The prevalence of T cells in blood was similar in 25 patients with GCA and in controls. The blood lymphocyte blastogenic response to the mitogens, phytohaemagglutinin, concanavalin-A, and pokeweed mitogen did not differ in 25 untreated patients compared with controls. Stimulation of lymphocytes by arterial homogenates was tested in 8 patients. In no case could a significant stimulation be obtained. We conclude that immune complexes and rheumatoid factors are present in the same low frequency in GCA patients as in the normal population, and that the studied parameters of cellular immunity appear to be normal.

Several authors have suggested that immunological mechanisms may be involved in the pathogenesis of giant cell arteritis (GCA).¹⁻⁴ Our group has found that serum levels of IgG and total serum complement, as well as factors C3 and C4, are slightly elevated in this disease.⁵ Other workers have demonstrated immunoglobulin and anti-immunoglobulin (rheumatoid factors) in the arterial wall,^{6,7} findings which were interpreted as indicating either deposition of immune complexes or the occurrence of antigen-antibody reactions within the vessel wall.

Cell mediated immune mechanisms have also been proposed as being involved in the pathogenesis of GCA. Hazleman *et al.*⁸ found that blood lymphocytes from patients with GCA in vitro showed a higher transformation response to artery antigen than lymphocytes from controls, a finding which has been questioned by others, however.^{9,10}

In this study the presence of circulating immune complexes and rheumatoid factors (RF) was investigated by 2 enzyme linked immunoabsorbent assays (ELISA). We have also studied the prevalence

of T cells in peripheral blood and the reactivity of blood lymphocytes to different mitogens and to arterial wall homogenate.

Materials and methods

PATIENTS

The studies were performed on untreated GCA patients during the years 1975-8 at the Department of Internal Medicine III, Sahlgren's Hospital, and at the Department of Infectious Diseases, East Hospital, Göteborg. A temporal artery biopsy was performed on all patients. If the biopsy showed mononuclear cell infiltration and destruction of the internal elastic membrane, the patients were regarded as biopsy-positive. Biopsy-negative patients were included if certain clinical criteria, defined earlier, were fulfilled.¹¹ The patients were classified by symptoms at diagnosis into 4 clinical groups: temporal arteritis, combined temporal arteritis and polymyalgia rheumatica, polymyalgia rheumatica, and general symptoms only.¹² The mean age was 71.6 years (range 58-85 years). Not all the immunological tests were available during the entire period of study, and the number of patients studied with the different tests therefore varies.

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CONTROLS

People aged 70 were selected at random from a gerontological and geriatric population study in Göteborg¹³ and used as controls.

IMMUNE COMPLEXES

Determination of immune complexes by ELISA was performed as previously described.¹⁴ Polystyrene tubes were coated by incubation with a solution containing 50 ng purified C1q in 0.5 ml of 0.05 M phosphate buffered saline, pH 7.2, for 3 hours at 37°C. After rinsing away the excess of C1q, 0.5 ml of the serum samples was added in 10-fold serial dilutions. Incubation was performed by gently shaking the tubes at 37°C for 3 hours. After a further rinsing rabbit antihuman C1q (kindly provided by Dr Bengt Johansson, Lund, Sweden) was added to the tubes to cover unoccupied C1q antigenic determinants. The tubes were incubated for another 3 hours at 37°C. After a further rinsing rabbit antisera against human IgG, IgA, and IgM (Dakopatts AS, Copenhagen, Denmark), conjugated with alkaline phosphatase, were added. After incubation on a roller drum for 16 hours at room temperature and subsequent rinsing the remaining enzyme activity was determined by adding para-nitrophenyl phosphate (Sigma Chemical Company, Saint Louis, Missouri, USA) 1 mg/ml in 1 M diethanolamine buffer, pH 9.8, containing 0.0011 M MgCl₂. The enzymatic reaction was interrupted after 100 minutes by addition of 0.1 ml 3 M NaOH, and the light absorption at 405 nm was registered. Optical densities (OD) were recorded for 1:10 dilutions of the tested serum samples and compared with the OD obtained for a pool of positive sera.¹⁵ Sera giving an OD higher than 25% for IgG, 35% for IgA, and 75% for IgM of the OD for the reference pool of sera were regarded as positive. All dilutions and rinses were done with 0.05 M phosphate buffered saline, pH 7.2, containing 0.5% Tween 20, except for the coating procedure, where Tween 20 was excluded.

RHEUMATOID FACTORS

Polystyrene tubes were coated by incubation with 10 µg bovine serum albumin (BSA) in 0.5 ml 0.15 M phosphate buffered saline for 2 hours at room temperature. After rinsing, rabbit anti-BSA serum diluted in 1/5000 in PBS-Tween was added. After further rinsing, the serum sample to be tested, diluted 1:100 or 1:1000, was added and the presence of bound human immunoglobulins was determined as described above for the C1q immunosorbent test. The results were expressed as the OD at 405 nm for 1/100 dilution of the tested samples in per cent of the OD of a pool of sera containing rheumatoid

factor. Values above 7% for IgG, 6% for IgA, and 7% for IgM were considered as positive.¹⁵

PREPARATION OF LYMPHOCYTES

Lymphocytes were prepared from fresh heparinised venous blood samples by centrifugation on Metrizoate-Ficoll (Lymphoprep, Nyegaard) as described by Böyum.¹⁶ After washing, the cells in the intermediary phase were suspended in minimal essential medium (MEM) and used for lymphocyte stimulation tests with various mitogens and arterial antigens (see below).

MITOGENS

Phytohaemagglutinin (PHA) (Wellcome Ltd, Beckenham, Kent, England) was used in a final concentration of 1:100. Concanavalin-A (con-A) (Miles-Yeda Ltd, Rehovot, Israel) was used in a final concentration of 75 µg/ml. Pokeweed mitogen (PWM) (Grand Island Biological Co, Paisley, Scotland) was used in a final concentration of 1:10.

Arterial antigens were prepared from aortic tissues obtained from a 20-year-old man who was killed in an accident and from a 50-year-old woman who had died from a brain tumour. The tissues were frozen within 3 and 12 hours respectively after death and stored at -70°C. After thawing, the tissue was first cut into small pieces with scissors, dispersed in an equal volume of PBS, then finely dispersed in an Omnimixer. The mixture was centrifuged at 23 500 g (14 000 rpm) for 30 minutes. The clear supernatant was stored frozen in suitable portions at -20°C until used. Another piece of aortic tissue was treated as described above, but after grinding in the Omnimixer the tissue homogenate was subjected to freeze-pressing in an X-press.¹⁷ The freeze-pressed material was centrifuged at 23 050 g for 30 minutes and the clear supernatant frozen and stored at -20°C. The sediment was suspended in an equal volume of MEM, and after division into suitable portions it was frozen and stored at -20°C. All 3 antigen preparations were used undiluted and diluted 1:10, 1:100, and 1:1000 in lymphocyte stimulation tests.

LYMPHOCYTE STIMULATION TESTS

Lymphocyte stimulation was performed according to the routine procedures of the immunological laboratory. Briefly, washed lymphocytes were suspended in Mishell-Dutton medium¹⁸ supplemented with 10% pooled human AB serum, 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 mM HEPES buffer, and adjusted to a concentration of 2×10^6 cells. Cultivations were performed in Linbro Tissue Culture grade microtitre plates at 37°C in air containing 5% CO₂. 10 µl of the mitogens or arterial antigen preparations were

added to 0.2 ml of the lymphocyte suspensions. Each mitogen and the different antigen dilutions were tested in triplicate. 1 μ Ci 3 H-thymidine (Radiochemical Centre, Amersham, Bucks) was added to each well on the third day of culture for PHA and con-A and on the sixth day for PWM and arterial antigens. After further incubation overnight the cultures were harvested, and the incorporated

radioactivity was determined by liquid scintillation counting. The stimulation index is given as the ratio between means for triplicates obtained in cultures with and without mitogens or antigens.

T LYMPHOCYTES

Owing to a change of routine laboratory procedures during the course of this investigation T cells were quantitated by 2 different methods during different phases of the study. ANAE (α -naphthyl acetate esterase) staining was performed on peripheral blood as described by Mueller *et al.*¹⁹ The number of ANAE-positive cells is given as a percentage of the total number of white blood cells. E rosettes were determined as described by Jondal *et al.*,²⁰ and the number of T cells is given as a percentage of the number of lymphocytes.

STATISTICS

Pitman's nonparametric permutation test was used for the statistical analysis of possible differences between patients and controls in the prevalence of T cells and the results of lymphocyte stimulation tests.

Results

Immune complexes containing IgM were found in serum from 2 out of 15 patients studied (13%) and in 5 of the 94 controls (5%). None of the patients had immune complexes containing IgG or IgA. Rheumatoid factors (RF) were studied in 27 patients. In 1 patient RF of all 3 immunoglobulin classes studied were demonstrated, whereas 1 patient had RF of the

Fig. 1 Prevalence of T cells in patients with GCA and controls. O = temporal arteritis, Δ = temporal arteritis and polymyalgia rheumatica, ∇ = polymyalgia rheumatica, □ = general symptoms. Filled symbols represent biopsy-positive cases.

Fig. 2 Stimulation indexes for lymphocytes in blood from patients with GCA and controls obtained by phytohaemagglutinin (PHA), concanavalin-A (con-A), and pokeweed mitogen (PWM). O = temporal arteritis, Δ = temporal arteritis and polymyalgia rheumatica, ∇ = polymyalgia rheumatica, □ = general symptoms. Filled symbols represent biopsy-positive cases.

IgG class only. In the control group 10 individuals out of 94 (11%) had RF of IgG and IgM classes and 7 (7%) out of 94 had RF of the IgA class.

The prevalence of T cells was determined in 17 patients by the ANAE technique and in 8 patients by the E rosette technique. The distributions of T cells among patients and controls are shown in Fig. 1. No statistically significant difference between the patients and the control group was revealed.

Lymphocyte stimulation tests with PHA, con-A, and PWM were performed on 25 patients. Fig. 2 gives a graphic illustration of the results obtained. There were no significant differences between patients and controls for any of the studied mitogens.

Lymphocyte stimulation with arterial homogenates was performed on 8 patients. In no instance was the incorporation of ^3H -thymidine significantly increased in samples exposed to the arterial homogenate compared with nonexposed lymphocytes.

Discussion

Light and electron microscopic studies of the temporal artery in patients with GCA have revealed destruction of the internal elastic membrane with accumulation of mononuclear cells and giant cells.^{21, 22} These observations form the basis of the hypothesis that immunological mechanisms participate in the damage to the arterial wall. This hypothesis has later been supported by findings of immunoglobulins in the arterial wall, first reported by Sauerbruch *et al.*²³ and later confirmed by other workers.^{6, 24} Waaler *et al.*⁷ have also demonstrated anti-immunoglobulin (rheumatoid factor) in temporal artery biopsies from patients with GCA. In a recent study by Plouvier *et al.*²⁵ immunoglobulins were demonstrated in the temporal artery not only of patients with GCA but also of patients with other collagen diseases. Others, however, have failed to find immunoglobulin in artery biopsies.²⁶ The finding of immunoglobulin in the temporal artery has been interpreted as being due to either deposition of circulating immune complexes or antigen-antibody reactions in the arterial wall. One aim of this study was therefore to demonstrate the presence of circulating immune complexes.

However, it was impossible to demonstrate circulating immune complexes in GCA patients more often than in controls by the ELISA technique employed. Our findings therefore do not support the hypothesis that circulating immune complexes participate in the pathogenesis of GCA. However, there are certain difficulties involved in the demonstration of circulating immune complexes, and many methods have been developed for this purpose. Recently published results from an extensive

collaborative study²⁷ showed that different methods give varying results in different diseases. As the method used in this study gives a high frequency of positive results for immune complexes containing IgG and IgA in patients with systemic lupus erythematosus and for immune complexes containing IgG in patients with rheumatoid arteritis,¹⁵ we conclude that immune complexes of this type are not present in sera from GCA patients. However, this does not exclude the possibility of demonstrating complexes with other properties by other techniques. Recently Papaioannou *et al.*²⁹ found raised levels of immune-complex-like material in sera from patients with active GCA using the Raji cell radioimmunoassay.

The prevalence of rheumatoid factor in serum from patients with GCA has been reported to be as low as in serum from healthy persons of the same age when the Waaler-Rose test was used.²⁸ The findings of anti-immunoglobulin activity, mainly of IgA type, in the arterial wall reported by Waaler *et al.*⁷ stimulated us to use the ELISA in order to search for rheumatoid factors of different Ig classes in serum. With this test rheumatoid factors can be demonstrated in a high percentage of patients with rheumatoid arthritis.¹⁵ We conclude that the prevalence of rheumatoid factor of any Ig class in blood from GCA patients is not higher than in blood from randomly selected individuals of the same age.

The finding of mononuclear cell infiltration in the damaged artery, together with the finding of Hazleman *et al.*⁸ that lymphocytes in peripheral blood can be stimulated by arterial antigen in patients with GCA, has suggested that cell mediated immune mechanisms may be involved in GCA. Our results indicate that the prevalence of T cells in blood is not significantly different in a group of GCA patients as compared with a group of healthy people of the same age. The reactivity to the mitogens PHA, con-A, and PWM does not seem to differ between untreated GCA-patients and controls. Our results are in accordance with those of Papaioannou *et al.*,⁹ who found a normal prevalence of E rosette forming cells and a normal uptake of thymidine in GCA patients after stimulation by PHA, PPD, mumps, and candida antigens.

We have not been able to reproduce the results of Hazleman *et al.*⁸ concerning lymphocyte stimulation by arterial antigen in GCA patients. Two other groups^{9, 10} have also studied the proliferation of lymphocytes exposed to arterial wall homogenate without finding any difference between GCA patients and controls.

The conclusion we draw from the present study is that the immunological parameters we have investi-

gated in patients with GCA seem to be normal as compared with a population sample of the same age. The aetiology and pathogenesis of this disease are still not understood. The light and electron microscopic findings of accumulation of mononuclear cells round the destroyed internal elastic membrane and the rapid beneficial effect of corticosteroids warrant further immunological studies on patients with GCA.

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