Immunological and histological study of temporal arteries

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SUMMARY Sixty-four temporal arteries were studied. 36 were from patients with clinically active temporal arteritis or polymyalgia rheumatica; 22 showed histological changes of temporal arteritis, 12 of which were in an active stage. 28 arteries, none of which showed histological changes, were taken at necropsy or from patients with unrelated disease.

Extracellular immunoglobulin and complement deposition was seen in the artery biopsies showing active arteritis and in 1 of the 10 biopsies with inactive arteritis. There was no immunoglobulin or complement deposition in the 14 patients with clinically active temporal arteritis and/or polymyalgia rheumatica, but with a normal artery biopsy. Patients with clinically active temporal arteritis were more likely to have a positive biopsy. Our results support the suggestion that the immune deposition is concurrent with an active histologically proven arteritis. Immunofluorescent examination does not appear to be a better diagnostic test than histological examination.

There is an increasing tendency to consider temporal arteritis and polymyalgia rheumatica as closely related conditions and it is difficult to maintain a practical distinction between them by clinical or histological criteria (Hazleman, 1976). Patients originally suffering from polymyalgia rheumatica have later had symptoms of cranial arteritis and in a number of patients with typical myalgia and no symptoms from the temporal region, biopsies have shown arteritic changes. Fauchald et al. in 1972 found that 40% of patients with myalgia alone had a positive biopsy and of those with a proven arteritis 18% did not have myalgia.

However, diagnosis may prove difficult and despite a typical pattern of musculoskeletal symptoms and the presence in many of significant systemic features, there is often considerable delay before diagnosis—a mean of 6.2 months in one study (Mowat and Hazleman, 1974). Although the erythrocyte sedimentation rate is usually greatly raised in almost all patients and can be regarded as a key diagnostic test, it can be normal in patients with active disease proven by biopsy (Bruijn, 1967; Roux, 1954). Temporal artery biopsy is therefore a most important diagnostic procedure; however, the arteritis is not uniform and the small section of temporal artery taken at biopsy may well be normal.

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Liang et al. (1974) demonstrated certain patterns of immunoglobulin deposition in the temporal arteries of patients with histologically positive temporal arteritis. However, only 3 patients with a positive biopsy were studied. It was therefore felt that a further study was necessary, the aims being (1) to ascertain the incidence of arteritis in 64 temporal artery biopsies; (2) to assess the patterns of immunoglobulin and complement deposition; and (3) to establish the diagnostic value of immunofluorescence.

Histology
The temporal artery biopsy was divided into two, half being processed in the routine histology laboratory. Biopsies with abnormal histology were classified as being either in an active or inactive stage. Active arteritis: showing infiltration with lymphocytes, plasma cells, and multinucleate giant cells in the media, and a disrupted internal elastic lamina (IEL). Inactive arteritis: showing a swollen and disrupted IEL with thickened intima but lacking an inflammatory cell infiltrate.

Patients
Temporal arteries were biopsied in 36 patients presenting with symptoms of polymyalgia rheumatica or temporal arteritis over an 18-month
period. Disease activity was assessed at presentation and graded 1–4 (mild–active).

Diagnosis of polymyalgia was based on clinical criteria (Dixon, 1969; Hunder et al., 1969). The clinical details are summarised in Table 1. Diagnosis of temporal arteritis was supported in all cases by a temporal artery biopsy. All patients had a negative test for rheumatoid factor. None of the patients studied was on corticosteroids at the time of biopsy, though 3 patients in each group had previously been treated.

Seventeen biopsies were from patients with an unrelated pathology (Table 2). 11 control arteries were obtained at necropsy from patients who had died of unrelated disease (4 carcinomatosis, 3 chronic bronchitis, 4 myocardial infarction) with no previous evidence of temporal arteritis.

Table 1  Clinical details of patients presenting with symptoms of polymyalgia rheumatica and temporal arteritis

<table>
<thead>
<tr>
<th></th>
<th>Active</th>
<th>Inactive</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>12</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>Range</td>
<td>65-75</td>
<td>61-79</td>
<td>59-80</td>
</tr>
<tr>
<td>Sex</td>
<td>M 5</td>
<td>2 F 3</td>
<td></td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td>13</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>before biopsy (m)</td>
<td>1-72</td>
<td>1-96</td>
<td>1-36</td>
</tr>
<tr>
<td>Headache</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Visual symptoms</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Myalgia</td>
<td>5</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Normal artery on clinical examination</td>
<td>5</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10-5</td>
<td>12-3</td>
<td>12-5</td>
</tr>
<tr>
<td>Range</td>
<td>6-6-13-2</td>
<td>10-8-13-9</td>
<td>9-3-14-8</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>96</td>
<td>83</td>
<td>58</td>
</tr>
<tr>
<td>Mean</td>
<td>26-138</td>
<td>7-130</td>
<td>16-136</td>
</tr>
</tbody>
</table>
| *Certain features atypical of polymyalgia rheumatica. Symptoms settled within 3 months with no recurrence.

Table 2  Diseases in controls

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral myalgia*</td>
<td>5</td>
</tr>
<tr>
<td>Transient ischaemic attacks</td>
<td>4</td>
</tr>
<tr>
<td>Polyrteritis nodosa</td>
<td>3</td>
</tr>
<tr>
<td>Cervical spondylitis</td>
<td>3</td>
</tr>
<tr>
<td>Polyneuritisis</td>
<td>1</td>
</tr>
<tr>
<td>Carcinoma of larynx</td>
<td>1</td>
</tr>
<tr>
<td>Post mortem</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
</tr>
</tbody>
</table>

Method

Immunofluorescence was carried out before the histology results were known. The arteries were washed in phosphate-buffered saline (PBS) and placed on a 12 mm diameter circular millipore membrane (Millipore 0.22 μm pore size). The membrane was then inserted into a 5 ml polytube, orientated so that transverse sections of artery would be obtained, and covered with a 7% (w/v) gelatin in 0.9% sodium chloride containing 0.05% sodium azide. The tubes were dipped in liquid nitrogen for 90 seconds, capped, and placed at −20°C for storage. 5 μm cryostat sections were cut at several levels and placed on glass microscope slides, which had been washed overnight in concentrated nitric acid. The sections were rapidly air dried, fixed for 10 minutes in 4% formaldehyde in PBS freshly prepared from paraformaldehyde (Glauert, 1965), and washed in PBS (30 minutes). Sections were treated with 50 μl of reagent, incubated in a moist chamber (30 min), and then washed (30 min). Slides were rinsed in distilled water before mounting under coverslips in Trisglycerol (9 parts glycerol, 1 part 0.2 M Tris buffer pH 8.7).

Ethidium bromide (5–50 μg/ml in 5% EDTA), which selectively stains nuclei, was used as a counterstain to assist location of the fluorescent material. The sections were examined using a standard incident light fluorescence microscope system. Photographs were taken using a high speed Ektachrome ASA 160 (daylight) film.

REAGENTS

Direct immunofluorescence was carried out using specific antibodies to human IgG, IgA, IgM, and the third component of complement all labelled with fluorescein isothiocyanate (FITC) (Dako).

Indirect immunofluorescence was carried out using F(ab')2 preparations of rabbit antiserum to human IgG F(ab')2, normal rabbit serum IgG and FITC-labelled pig antiserum to rabbit IgG F(ab')2, prepared by the method of Poole et al. (1976). Before treatment of the sections, the divalent F(ab')2 was reduced to monovalent Fab' by incubation for 30 minutes in 10 mM L-cysteine (Sigma) in PBS. The smaller Fab' molecule more easily diffuses into the tissue. Staining and washing was carried out in 5 mM L-cysteine in PBS. Unlabelled reagents were used in blocking tests to confirm specificity of the reaction.

All reagents were tested by immunoelectrophoresis to check their specificity, and appropriate controls were carried out in each of the arteries tested. Where necessary an initial incubation with
normal pig or sheep serum was used to reduce non-specific binding of the FITC-labelled antisera to the tissue.

Results

Three distinct patterns of immunofluorescent staining were evident: (a) specific fluorescence in the media, (b) fluorescent stain on or adjacent to the IEL, and (c) 'nonspecific' background stain.

**MEDIA FLUORESCENCE**

Specific fluorescent stain was seen in the media of all patients with active histology and in one patient with inactive histology (Fig. 1). The stain was granular, varied from minute specks to larger discrete areas of fluorescence, and was not distinctly located around the nucleus. The deposits tended to be elongated parallel to the circumference of the artery and were often close to the IEL. In three arteries deposition was along the media/adventitia junction alone. In some of the sections showing this staining in the media, similar fluorescence could be observed in both the intima and adventitia of the same section.

Fig. 2 shows the number of patients showing media fluorescent staining with each of the direct immunofluorescent reagents; most arteries showed more than one class of immunoglobulin stain. The C3 component of complement was seen in all arteries that contained immunoglobulin in a similar distribution to the immunoglobulin. The presence of immunoglobulin in the intima alone was not regarded as evidence for a positive fluorescent lesion as this was seen in normal arteries, and was probably due to diffusion of immunoglobulin from the blood.

The results using the indirect method were essentially the same as those using the direct method.

**ELASTIC FLUORESCENCE**

Linear fluorescence was demonstrated along both sides of the IEL and capping the natural breaks in the lamina (Fig. 3). This staining pattern occurred in 3/12 patients with active histology, in 2/10 with inactive histology, and in 3/17 patients with normal histology and unrelated pathology. For this reason we find this staining pattern inconclusive, although it was one which Liang et al. (1974) regarded as significant as it was found in 3/3 patients with histologically active disease, in 4/12 patients with polymyalgia but normal histology, and in 0/10 controls. The criteria for inclusion in the polymyalgia group may well be different in the two studies.

**'NONSPECIFIC' BACKGROUND STAIN**

The background stain was seen in both controls and arteritis patients and could be attributed to the permeability of the vessel wall to immunoglobulin and other components of the blood increasing with age. The staining could be reduced by prior incubation with normal serum, but in some cases it was quite intense and could have masked the more granular immunoglobulin stain of the media. Table 3 summarises the distribution of fluorescent staining patterns in the histology groups studied.

Fig. 1 Medial fluorescence in a patient with clinically active temporal arteritis. The internal elastic lamina is seen in the lower left corner. Nuclei are weakly stained with ethidium bromide. × 320.
Immunological and histological studies of temporal arteries

Figs. 4 and 5 correlate clinical features, namely presence of arteritis or myalgia, and an estimate of clinical activity with the histological findings. Fig. 4 shows that 50% of patients with arteritis complained of myalgia. However, in patients with myalgia but a negative biopsy there were 3 with clinical symptoms suggestive of arteritis. Patients with active arteritis on biopsy tended to have more severe symptoms (Fig. 5), whereas patients with inactive arteritis tended to fall between these two extremes. Although it seems that there is a correlation between the

Table 3  Distribution of fluorescent staining patterns correlated with histology

<table>
<thead>
<tr>
<th>Histology</th>
<th>Symptoms of PMR</th>
<th>n</th>
<th>IEL fluorescence</th>
<th>Specific media fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active arteritis</td>
<td>+</td>
<td>12</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Healed arteritis</td>
<td>+</td>
<td>10</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal (PM arteries)</td>
<td>−</td>
<td>17</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Normal (PM arteries)</td>
<td>−</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

IEL = internal elastic lamina.
severity of symptoms and the activity of arteritis on biopsy, it should be noted that serial sections of a biopsy often show the presence of both active and inactive changes.

In no patient were autoantibodies detected and the SCAT was negative. Immunoglobulin levels fell within the normal range and there was no difference between groups.

Discussion

These studies confirm those of Liang et al. (1974) and Sauerbruch et al. (1973) and suggest that immunoglobulins are involved in the disease process of temporal arteritis. But Horowitz et al. (1977) carried out immunofluorescent staining in 12 temporal artery biopsies and detected no immunoglobulin staining, however only one biopsy showed definite arteritis. It is not possible to state whether the patterns we observed result from the passive deposition of immune complexes or from the combination of specific antibodies with antigens.

The finding of immune deposits in affected tissue is commonly thought to indicate immune-complex induced disease. However, immune deposits may be found in tissue in other situations where there is little else to suggest immune-complex disease (Sutherland et al., 1974). Conversely, the absence of immune deposits may not rule out immune complex disease, because immune complexes are removed by granulocytes within hours after injecting antigen into the skin of a sensitised animal (Cochrane et al., 1959). Immune reactants are frequently absent from vessel walls of patients with chronic vasculitis, although they can be identified during the acute necrotising stage (Conn et al. 1972).

Our results can not be compared with the immunological and histological study of Waaler et al., 1976, because in their study sections fixed in 4% formalde-hyde showed no activity—a treatment used in preparing our sections. Our results are similar to those of Liang et al. (1974), but we detected no specific stain within the cytoplasm, and think the stain is in an extracellular position, nor do we attach such diagnostic significance to staining of the IEL. This was present in 3 patients who had certain features atypical of polymyalgia rheumatica. These symptoms resolved within 3 months and on follow-up there has been no recurrence over one year.

Some recent observations have suggested a possible immunological basis for polymyalgia rheumatica and temporal arteritis. Infiltration of the IEL by mononuclear cells occurs (Parker et al., 1972). Raised circulatory immunoglobulins, especially IgM, are found in some patients (Bacon et al., 1975). Also, circulating immunoblasts have been described (Eghtedari et al., 1976). Recently increased sensitivity of peripheral blood lymphocytes to human arterial and muscle has been reported using the lymphocyte transformation test (Hazleman et al., 1975) and the histocompatibility antigen HLA B27 was significantly more common in patients with polymyalgia rheumatica and temporal arteritis (Hazleman et al., 1977).

Histological examination of the temporal artery in an active region shows inflammatory cells and sometimes giant cells together with swelling and disruption of the IEL. When present giant cells are often closely associated with the IEL and may show elastic fragments in their cytoplasm (Parker et al., 1972). Histological changes also occur in temporal arteries with advancing age and they differ only in degree from those seen in temporal arteritis. It is sometimes difficult to distinguish between the two (Ainsworth et al., 1961). However, an inflammatory cell infiltrate does not occur except in relationship to large plaques of atherosclerosis. Diagnosis may also be complicated by the presence of skip lesions. These were identified in 28% of patients with temporal arteritis in a recent study (Klein et al., 1976). They found foci of arteritis as short as 330 μm in length in an otherwise normal biopsy. In addition, 65% of the patients with skip lesions had temporal arteries completely normal to palpation, compared to only 23% of those with continuous involvement. It should be noted that patients with skip lesions do not have a more benign disease.

There is a close relationship between arteritis and the amount of elastic tissue present in the arterial wall (Wilkinson and Russell, 1972). Temporal arteritis may arise as an immune reaction to the IEL which has been damaged by advancing age (Hazleman et al., 1975). Certainly a widespread vasculitis would explain many of the features of polymyalgia rheumatica and the close clinical relationship is again borne out in this study.

Our results support the idea that immune deposition is concurrent with an active arteritis. However, the immunofluorescent study of temporal arteritis does not appear to be a more sensitive guide to disease state in our hands than other methods currently used.

We thank the Arthritis and Rheumatism Council for continued support; and are grateful to the physicians at Addenbrooke's Hospital who kindly referred cases, and to the Department of Ophthalmology for many of the temporal artery biopsies. We thank Dr R. Poole and Miss R. Hembry for helpful advice about immunofluorescence techniques.
References


