Immunological studies in frozen shoulder

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SUMMARY Serum immunoglobulin levels were determined in 25 patients with frozen shoulder and in 25 age- and sex-matched controls. Serum IgA levels were significantly reduced ($P < 0.001$) in the patients with frozen shoulder and remained so after clinical recovery. Lymphocyte transformation to phytohaemagglutinin in 21 patients also showed significant depression ($P < 0.01$). These results support the suggested immunological pathogenesis of this condition.

The frozen or painful stiff shoulder is a common clinical problem. (Hazleman, 1972). The onset is commonly spontaneous, but can be associated with a variety of conditions including trauma, myocardial infarction, hemiplegia (Mattingley, 1960), and tuberculosis (Johnson, 1959). Other associated disorders reported include Dupuytren's contracture and thyroid disease (Meulengracht and Schwartz, 1952) and diabetes (Bridgman, 1972).

Frozen shoulder usually causes disability for 6-24 months. It is said to be self-limiting; however, careful shoulder measurement has shown that 20% of the total range of movement may be permanently lost, even though the patient may deny disability (Clarke *et al.*, 1975). Little is known of the pathogenesis of this condition. It has been suggested that frozen shoulder is precipitated by degeneration in the supraspinatus tendon (Simmonds, 1949) and is associated with chronic inflammation of the shoulder capsule and sometimes of the subacromial bursa (Simmonds, 1949; Nevaizer, 1945). The vulnerability of the supraspinatus tendon has been explained on the basis of microvascular studies which show a constant relatively avascular area close to the insertion of this tendon into the head of the humerus (Rathburn and Macnab, 1970). Experiments on tendons of rabbits (Macnab, 1973) suggest that the capsulitis of frozen shoulder may be a type IV autoimmune reaction to the damaged supraspinatus tendon. These findings led us to assess immunological function in patients with frozen shoulder.

Materials and methods

Twenty-five patients with frozen shoulder were studied. All complained of severe shoulder pain for at least one month, particularly at night, so as to awaken them and to prevent them lying on the affected shoulder. In addition there was generalised restriction of movement, both active and passive, particularly at the glenohumeral joint, with reduction of external rotation by at least 50%. These criteria were chosen to exclude lesions of the rotator cuff, bicipital tendinitis, and subacromial bursitis. Cervical spondylosis as a cause of shoulder pain and polyarthritis were excluded on clinical grounds. Patients who fulfilled the above criteria and who on careful history and examination failed to show any associated disease were included in the study.

The mean age was 56 years, range 40-70 years. There were 16 women and 9 men. Onset of symptoms was spontaneous in 20 patients and followed minor trauma but not falls in 5. 5 patients had had a previous episode of frozen shoulder affecting the other shoulder in each case. Patients were first seen an average of 4.1 months after the onset of frozen shoulder, range 1-9 months. Therapy consisted of analgesics only.

The following investigations were performed on each patient: full blood count and erythrocyte sedimentation rate, Rose-Waaler, antinuclear antibody, urinalysis, chest and shoulder x-rays.

IMMUNOGLOBULIN ASSAY

Immunoglobulin levels of serum samples from patients when they first presented and from 25 healthy age- and sex-matched controls were measured using single radial immunodiffusion against specific antisera. Repeat estimations were

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made on 10 of the patients and their controls an average of 5½ months after clinical recovery (range 2–11 months). Serum IgM and IgG levels were measured using laboratory poured agar plates, Dakopatt antiserum, and pooled normal serum as controls and Diffugen standards. Serum IgA levels were measured using Tripartigen plates (Behringwerke) and Behringwerke standards for the initial readings, and Diffugen standards for the repeat estimations. The plates were diffused for over 72 hours and the precipitin ring diameters were measured to within 0.1 mm. A control serum was run on every plate and the coefficient of variation of the results for each immunoglobulin class was <11%.

LYMPHOCYTE TRANSFORMATION

In 21 further patients assessment of lymphocyte proliferation to phytohaemagglutinin (PHA) was assessed. The mean age was 63.5 years (range 49–81 years). There were 18 females and 3 males. Healthy age- and sex-matched controls were assessed at the same time.

PREPARATION OF LYMPHOCYTES

Four ml of Dextran 240 was added to 20 ml heparinised blood and allowed to stand at 37°C for 30 minutes. The white cell rich supernatant was layered on to 2 ml of Ficoll Hypaque and centrifuged at 400 *g* for 20 minutes. The lymphocyte rich layer was removed and washed twice with buffered Eagle's medium and cultured in medium supplemented with 10% calf serum, penicillin, and streptomycin.

MEASUREMENT OF DNA SYNTHESIS

Cultures containing 1×10^6 lymphocytes/ml were maintained at 37°C for 72 hours, with varying concentrations of PHA ranging from 1000 µg to 2 µg. DNA synthesis was estimated by measuring the incorporation of tritiated thymidine. 1 µCi tritiated thymidine was added 4 hours before termination of the culture.

At the end of the incubation period 2% acetic acid in normal saline was added to lyse red cells and the tubes spun at 1300 *g* for 10 minutes at room temperature. The cells were then washed twice in 5% trichloroacetic acid, spun, and washed with absolute alcohol, and the final residue allowed to dry. To redissolve the precipitated DNA, 0.3 ml 0.1 N sodium hydroxide was added and a 0.2 ml aliquot of this solute was added to 10 ml scintillation fluid. The activity in each sample was assessed in a liquid scintillation counter.

Results

Lymphocyte counts were normal. 4 patients had ESR (Westergren) levels above 20 mm/h, but below 30 mm/h. Urinalysis was normal. Rose-Waaler and antinuclear antibodies were negative. Chest x-rays were normal apart from one which showed a healed primary complex. Shoulder x-rays showed minor degenerative changes in 6, the rest were normal.

There was no significant difference between the patients with frozen shoulder and controls in the levels of IgG and IgM (Fig. 1). However, there was a reduction in serum IgA levels in the patients with frozen shoulder compared with the controls ($P < 0.001$). Repeat IgA estimations after recovery on 10 patients and their controls showed that the patients' IgA levels remained depressed compared with controls. However, both groups gave results approximately 0.5 g/l higher than the previous readings. In retrospect, it is likely that this discrepancy was due to the change in the standard used and not to any real change in the relative IgA levels between the patients and their controls. This was confirmed by plotting the recovery levels for both groups against the levels at onset. Fig. 2 shows that the slopes of the regression lines (which would

Fig. 1 Immunoglobulin levels in 25 patients with frozen shoulder and in 25 age- and sex-matched controls. Shaded area represents laboratory normal range.

have to be different if the two groups behaved differently) are almost identical and that both regression lines are only transposed vertically. This was also confirmed statistically by comparing the slopes and intercepts of the two respective lines. This transposition represents a change which is therefore common to both groups and must have been caused by the change of laboratory standards.

A dose-response curve was obtained for the stimulation by PHA of lymphocytes (Fig. 3). The PHA response of the patients' lymphocytes was reduced compared with the controls and the significance reached $P < 0.01$ at 32 μg PHA.

Discussion

Serum IgA levels were low in our patients with

Fig. 2 Frozen shoulder. Comparison of disease and recovery IgA levels.

Fig. 3 Frozen shoulder. PHA (phytohaemagglutinin) dose/response curves.

frozen shoulder and remained so after recovery. The significance of this is uncertain. Selective deficiency of IgA, < 0.05 g/l, is the commonest isolated immune deficiency, and occurs in 1:500 (Hobbs, 1968) to 1:700 (Bachmann, 1965) of otherwise normal individuals. The most commonly associated disorders are the autoimmune disorders (Ammann and Hong, 1971) including rheumatoid arthritis, systemic lupus erythematosus, pernicious anaemia, thyroiditis, and chronic active hepatitis.

Selective IgA deficiency has therefore been extensively studied (Ammann and Hong, 1971), but there are few reports of simple depression of IgA as seen in our patients. Simple depression of serum IgA has been described in 50% of patients with 'idiopathic' warm-type haemolytic anaemia (Blajchman *et al.*, 1969). It also occurs in myasthenia gravis (Behan *et al.*, 1976) in which impairment of thymus-derived lymphocytic function has also been reported (Simpson *et al.*, 1976).

IgA production is closely related to effective thymic control (Lancet, 1975). Neonatal thymectomy in the mouse and the rat results in reduced IgA levels (Arnason *et al.*, 1964). T cell function decreases with age (Toh *et al.*, 1973) as mirrored by a decreasing response of lymphocytes to PHA (Pisciotta *et al.*, 1967), and it has been suggested that a pre-existing deficiency in the cellular immune system, associated with impaired expression of cell-mediated immunity, predisposes the patient to the development of autoimmune disease (Allison *et al.*, 1971). This immune deficiency may permit an autoimmune inflammatory reaction in the shoulder after supraspinatus tendon degeneration, thus explaining why this condition occurs in the middle aged and elderly, can be bilateral and recurrent, and may respond to corticosteroids.

We have previously reported an increased incidence of HLA B27 in 38 patients with frozen shoulder (42%) (Bulgen *et al.*, 1976). This study has now been extended and 21 of 58 patients (36%) have this tissue antigen.

The classification of shoulder disorders is confused largely because little is known of their pathogenesis. Our studies so far support the hypothesis that frozen shoulder may be the result of a localised autoimmune reaction, and that some patients may have a genetic predisposition to develop it. Further studies are under way, both to correlate these findings with the clinical course and to extend these studies to other shoulder syndromes.

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