

## A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation

C.E.H. GRATTAN, T.B. WALLINGTON\*, R.P. WARIN, C.T.C. KENNEDY  
AND J.W. BRADFIELD†

Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW, \* Regional Blood Transfusion Centre, Southmead and † Department of Pathology, University of Bristol, U.K.

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### SUMMARY

Seven of twelve autologous sera from patients with chronic idiopathic urticaria re-injected intradermally produced a weal at the site of injection. There was no response in 19 control subjects. Patients showing a positive response had a shorter duration of disease and shorter duration of spontaneous weals, and their urticaria was less likely to be exacerbated by pressure. There was some serological evidence of circulating immune complexes in both positive and negative responders to autologous serum, but only two showed complement abnormalities. When six of the serum-positive patients were re-tested after one year, five still showed a positive response with their original stored serum, but only two, whose disease remained active, were positive when challenged with freshly drawn serum, suggesting that a serum mediator is only present when the urticaria is active. A marked neutrophil infiltrate was seen within and around small dermal blood vessels at the injection site in the majority of urticaria patients but this appearance did not correlate with weal formation. In control subjects the cellular response was mild and mainly mononuclear.

The aetiology of chronic urticaria is poorly understood. Studies by Monroe *et al.* (1981) and Russell Jones *et al.* (1983) have shown a spectrum of histological changes ranging from mild perivascular infiltrates, through dense mixed mononuclear or neutrophil infiltrates, to overt necrotizing vasculitis with leukocytoclasia, fibrinoid changes in the vessel walls and erythrocyte extravasation. Many of the patients with histological features of necrotizing vasculitis showed evidence both of immune complexes in the circulation and of complement consumption. Some also had cryoglobulinaemia.

Whitsed & Penny (1971) showed that autologous plasma and isolated cryoglobulins

re-injected intradermally produced vasculitic lesions in a patient with vasculitis and cryoglobulinaemia. In preliminary studies we noted that some patients with chronic urticaria also produce a weal at the site of re-injection of their own serum, suggesting that active material is present in the circulation and can be detected through its effect on the skin, even though it may not be evident in serological tests. We have attempted to define this response clinically and histologically in patients with chronic urticaria, using parallel serological tests for immune complex formation, complement activation and cryoglobulin production to attempt to correlate these with the skin test response. Skin testing was repeated after one year to establish whether skin test reactivity altered with changing disease activity.

#### METHODS

Twelve patients (four male, eight female) with chronic idiopathic urticaria of more than six weeks' duration presenting to clinics at the Bristol Royal Infirmary were studied. All gave written informed consent. Patients less than 16 years old and those with symptomatic dermatographism or other predominantly physical urticaria were excluded. Patients' mean age at presentation was 46 years (range 24–78). Nineteen healthy control subjects (9 male, 10 female; mean age 40.8 years, range 20–81) were also skin tested with their own serum and nine were biopsied. The urticaria patients were admitted overnight after stopping antihistamine therapy for at least 48 h. A full history and examination were undertaken, with particular reference to evidence of atopy, vasculitis or connective tissue disease.

Blood was taken for full blood count, plasma viscosity, immunoglobulins A, G and M, IgE (PRIST, Pharmacia), immunoelectrophoresis, liver function tests, HB<sub>s</sub>Ag, cryoglobulins, standard autoimmune profile, C3, C4, CH<sub>50</sub>, alternative pathway and immune complex tests which included fluid phase C1q binding, anti-complement activity (ACA), platelet aggregation titre (PAT) and quantitation of protein precipitated by 2% polyethylene glycol 6000 (PEG) (Haeney, 1981).

Blood from both patients and controls was allowed to clot for 30 min in sterile glass bottles and centrifuged at room temperature. Serum was withdrawn using aseptic technique and one or more 0.1 ml aliquots re-injected intradermally into volar forearm skin with a 27 gauge needle, the remainder being stored at -20°C. Sterile normal saline was injected into the other forearm at the same time as a within-patient control.

Biopsies were taken from paired serum and saline skin tests in all the patients and nine of the 19 controls between 90 and 135 min from the time of injection, using a 4 mm punch under lignocaine with adrenaline local anaesthesia. Specimens were divided, half being fixed in 10% formal saline for routine paraffin processing and half snap frozen in liquid nitrogen for direct immunofluorescence using fluorescein isothiocyanate labelled antisera to IgG, A, M, C3 and fibrin (Dako Laboratories, High Wycombe, Berkshire, U.K.).

Histology was assessed by two independent observers without reference to the subjects' identity or group. The quantity of cellular infiltration was graded into three categories, mild, moderate or marked, and the predominant cell type was recorded as neutrophil predominant, mononuclear cell predominant or mixed. The presence of eosinophils and distribution of the infiltrate was also noted.

Skin tests were repeated in each patient with the same serum, and using serum drawn during varying phases of urticarial activity. Measurements of weal size and the surrounding erythema were made immediately after injection, after 1 h and after 2 h and at variable intervals for up to 24 h, measuring only skin tests that had not been biopsied. The maximum and minimum

diameters of both weal and confluent erythema were measured with a millimeter scale and their means recorded.

Patients were classified as serum positive if their own serum produced a weal within 2 h of intradermal re-injection, provided that the weal size was at least 5 mm greater than the saline control and there was a difference of at least 10 mm in the diameter of the surrounding erythema ( $W \geq C + 5$  and  $E \geq C + 10$ ). The skin tests were repeated one year later in six of the subjects who were serum positive, using the patient's fresh serum and also the serum that had been stored from the original skin test.

## RESULTS

### *Skin tests and clinical characteristics*

Seven of the 12 patients had a positive skin test. Their main clinical characteristics are summarized in Table 1, which shows that the serum-positive patients had a shorter duration of disease and shorter duration of spontaneous weals, and were less likely to give a history of exacerbation with pressure. None of the 19 control subjects had a positive skin test.

The effects of re-injection with serum drawn at different phases of urticarial activity and at follow-up one year later are shown in Table 2. This demonstrates that the skin test remains positive irrespective of the activity of urticaria when the serum is drawn and it only converts to negative as the disease remits. Similarly, in the serum-negative group the skin tests remained consistently negative whether serum was drawn during an eruption of weals or at a time when there was no urticaria. The difference in response to stored serum from 1984 and fresh serum taken at follow-up in 1985 is illustrated for patient 2 in Figure 1.

Positive skin tests were often evident within 30 min and were usually maximal between 1½ and 2 h. They remained positive for an average of 8 h (range 4–13 h) in the four serum-positive patients who were followed. Ten patients who were re-injected with the same serum between 1½ and 19 h later (mean 14½ h) all showed the same response as that shown to the initial skin test.

TABLE 1. Clinical characteristics of 12 urticaria patients with serum-positive and serum-negative response to autologous serum

|                            | Serum-positive | Serum-negative      |
|----------------------------|----------------|---------------------|
|                            | <i>n</i> = 7   | <i>n</i> = 5        |
| Age (years): range         | 24–78          | 36–62               |
| mean                       | 42             | 51                  |
| Sex: M:F                   | 1:6            | 3:2                 |
| Duration of disease: range | 4–36 months    | 15 months–29 years  |
| mean                       | 13 months      | 12 years, 10 months |
| Duration of weals (h)      |                |                     |
| mean and (range): minimum  | 2.4 (0.5–6)    | 6 (2–10)            |
| maximum                    | 23 (6–36)      | 36 (24–48)          |
| History of exacerbation    |                |                     |
| with pressure              | 3              | 4                   |
| Personal history of atopy  | 1              | 0                   |
| Family history of atopy    | 2              | 0                   |

TABLE 2. Characteristics of skin test response in serum-positive patients

| Patient | Sex | Age | Initial assessment                  |        |       | Follow-up at one year                  |                       |       |
|---------|-----|-----|-------------------------------------|--------|-------|----------------------------------------|-----------------------|-------|
|         |     |     | State of urticaria when serum drawn |        |       | Activity of urticaria                  | Re-injection of serum |       |
|         |     |     | Erupting                            | Stable | Clear |                                        | Original              | Fresh |
| 1       | F   | 31  | +                                   | N/R    | +     | Almost clear<br>No treatment           | -                     | -     |
| 2       | F   | 30  | N/R                                 | +      | +     | Sporadic<br>anti-H <sub>1</sub> p.r.n. | +                     | -     |
| 3       | F   | 32  | +                                   | +      | N/R   | Active<br>Regular anti-H <sub>1</sub>  | +                     | +     |
| 4       | M   | 78  | +                                   | +      | N/R   | Clear<br>No treatment                  | +                     | -     |
| 5       | F   | 64  | N/R                                 | +      | +     | Sporadic<br>anti-H <sub>1</sub> p.r.n. | +                     | +     |
| 6       | F   | 32  | N/R                                 | N/R    | +     | Clear<br>No treatment                  | +                     | -     |
| 7       | F   | 23  | N/R                                 | +      | N/R   | Lost to follow-up                      |                       |       |

+ = serum-positive skin test.

- = serum-negative skin test.

N/R = not recorded.

anti-H<sub>1</sub> = antihistamines.

Three serum-negative patients developed late weals at saline skin test sites, and two also developed weals at the site of serum injection between 8 and 24 h from the time of injection.

#### Laboratory investigations

All patients were HB<sub>s</sub>Ag negative. There was some evidence of immune complex formation in both serum-positive and serum-negative groups. Three patients had elevated C1q binding but, of these, only the one patient who was serum negative also had a raised PAT. PEG was elevated in five of the six patients who were assayed, including one serum-positive patient who had raised C1q binding. ACA was normal in all patients. Two patients in the serum-positive group showed abnormalities suggesting activation of the classical complement pathway. Cryoglobulins were elevated in one patient from each group. The anti-nuclear factor was greater than 1:10 but less than 1:80 (homogeneous IgG) in two serum-negative and one serum-positive patient. Total serum IgE was above the normal range (up to 81 kU/l) in four serum-negative patients (639, 116, 143 and 165 kU/l respectively) and one serum-positive patient (529 kU/l). IgG was elevated in one serum-negative subject, IgA in two and IgM in two. IgA was also raised in one serum-positive subject and IgM in three. All other biochemical and haematological tests were normal except for the plasma viscosity which was raised in two serum-negative and one serum-positive patient.

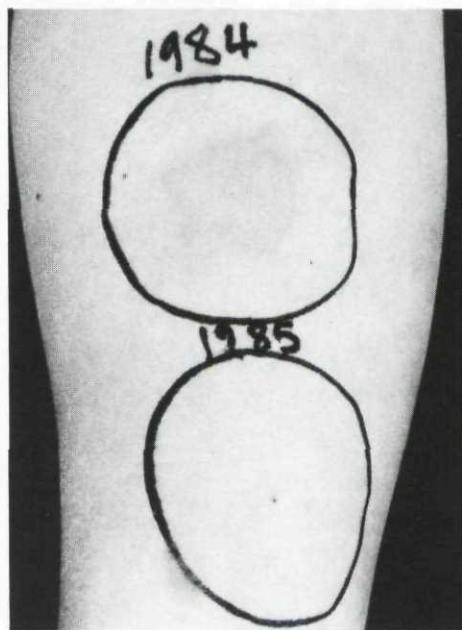


FIGURE 1. Reaction to re-injection of stored (1984) and fresh (1985) autologous serum.

#### *Histology and direct immunofluorescence*

Five of the seven serum-positive and four of the five serum-negative patients showed a marked neutrophil perivascular infiltrate in the serum-injected sites (Table 3). This neutrophil reaction was much less common (two of nine) and less severe in the control subjects. Reactions to saline were dominated either by mononuclear cells or by a mixed infiltrate. Eosinophils were seen in

TABLE 3. Histological grading of infiltrate in serum and saline-injected skin (normal biopsies not tabulated)

|                                    |          | Serum                     |       |                            | Saline                    |       |                            |
|------------------------------------|----------|---------------------------|-------|----------------------------|---------------------------|-------|----------------------------|
|                                    |          | Neutrophil<br>predominant | Mixed | Mononuclear<br>predominant | Neutrophil<br>predominant | Mixed | Mononuclear<br>predominant |
| Serum-<br>positive<br><i>n</i> = 7 | mild     | —                         | —     | —                          | —                         | 3     | 3                          |
|                                    | moderate | 2                         | —     | —                          | —                         | —     | —                          |
|                                    | marked   | 5                         | —     | —                          | —                         | —     | —                          |
| Serum-<br>negative<br><i>n</i> = 5 | mild     | —                         | —     | —                          | —                         | 1     | 1                          |
|                                    | moderate | —                         | 1     | —                          | —                         | 1     | 2                          |
|                                    | marked   | 4                         | —     | —                          | —                         | —     | —                          |
| Controls<br><i>n</i> = 9           | mild     | —                         | 2     | 1                          | —                         | 2     | 5                          |
|                                    | moderate | 2                         | —     | 2                          | —                         | —     | —                          |
|                                    | marked   | —                         | —     | —                          | —                         | —     | —                          |

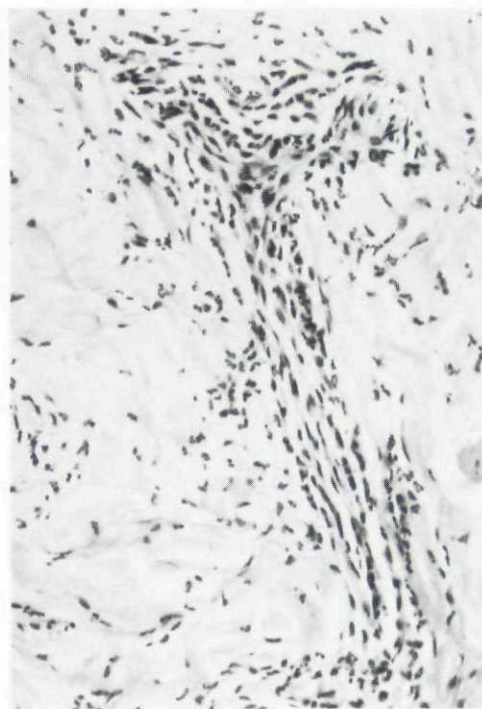


FIGURE 2. Neutrophil infiltration within and around a dermal venule in serum injected skin. (H & E; original  $\times 213$ ).

some serum-positive and some serum-negative patients in both serum and saline-injected skin but were rare in control subjects by comparison. The infiltrates were always perivascular, involving vessels at all levels of the dermis, but were also seen when the inflammatory changes were intense in collagen and around skin appendages. Migration of neutrophils through vessel walls was commonly observed when the infiltrate was marked (Figs 2 and 3) but leukocytoclasia, erythrocyte extravasation and fibrinoid changes in the vessel walls were not seen.

Direct immunofluorescence showed weak dermal deposition of fibrin in one serum-positive patient, weak perivascular staining with IgM in another and weak perivascular fibrin in a third, but was negative in all saline-injected biopsies and control subjects.

#### DISCUSSION

Our studies show that the serum of some patients with chronic urticaria contains a mediator which will produce a wheal on re-injection into their skin. The concept that the presence or absence of this mediator correlates with disease activity is strongly supported by the persistence of a positive skin test in five out of six of the patients re-tested with their stored serum after a year, particularly as two patients became serum negative after their urticaria had cleared. The two patients who continued to show a positive response with fresh serum also had continuing disease activity.

The nature of this circulating mediator remains uncertain. There was some serological evidence of immune complex formation in our patients, although in all but one the evidence was

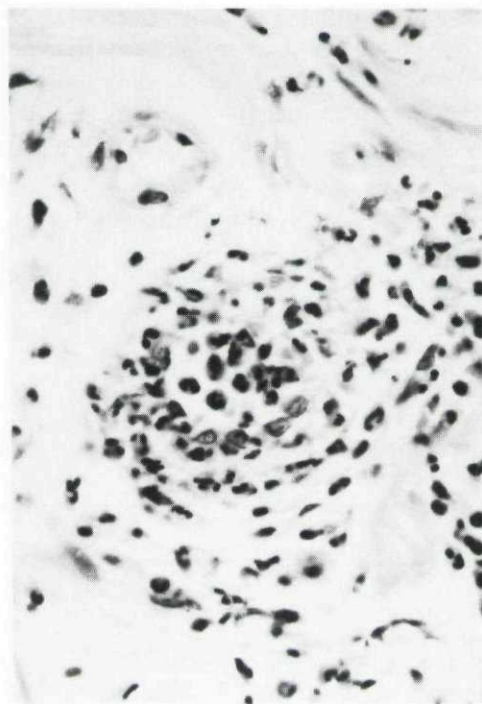


FIGURE 3. Detail of neutrophil perivascular infiltrate. (H & E; original  $\times 465$ ).

slight and did not correlate with skin test reactivity. Only two patients showed evidence of complement consumption. The absence of unequivocal evidence of immune complex formation may be due to insensitivity of the detection systems used, and does not provide formal evidence of their absence from the circulation. Peters and Winklemann (1985) found no evidence of complement abnormalities in a series of 10 patients with urticaria who showed the histological picture of neutrophil infiltration within and around blood vessels of spontaneous weals. They suggested that this appearance may imply the presence of physical urticaria rather than vasculitis.

Reports that symptomatic dermographism may be mediated by IgE (Aoyama, 1971; Newcomb & Nelson, 1973) suggest a possible role for an immunoglobulin-mediated response. However, as the positive skin test response is produced by serum from the same individual it is unlikely to result from IgE 'autosensitization' and subsequent mast cell degranulation. Horiko and Aoki (1984) described the successful passive transfer of IgM-mediated wealing in three patients with symptomatic dermographism and also showed that one patient reacted with a weal to his own serum. They speculated that the IgM was causing direct or antibody-mediated damage to the mast cell. However, the time course and duration of the weals was much shorter than in our series, arising in 15 min and disappearing by 30 min, in contrast to the positive responses in our patients which were usually maximal between 90 and 120 min and lasted for around 8 h.

Whilst the presence of a positive skin test provides evidence of an inflammatory serum mediator, it is too early to be sure whether it also carries prognostic significance in clinical practice. The similar incidence of neutrophil infiltration in serum-positive and negative skin

tests and the relatively arbitrary definition of the criteria for a positive response emphasize that the distinction between serum-positive and serum-negative patients is not absolute. However, the longer duration of spontaneous weals, disease activity and the more frequent history of exacerbation with pressure in the serum-negative patients suggest that they may belong to a distinct clinical sub-group, which can be identified by the skin test response.

#### ACKNOWLEDGMENT

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