

Mast cells and T lymphocytes in chronic urticaria

R. J. BARLOW, E. L. ROSS*, D. M. MACDONALD*, A. KOBZA BLACK and M. W. GREAVES

*St John's Institute of Dermatology, UMDS, St Thomas's and *Guy's Hospitals, London, UK*

Summary

Mast cells are considered to be the primary effector cells in urticaria but it is possible that lymphocytes contribute to the formation of weals by secreting histamine releasing factors. The aim of this study was to examine the population of mast cells and to quantify the T cell subsets and their activation status in delayed pressure urticaria (DPU), chronic idiopathic urticaria and normal controls. Three biopsies were obtained from each of four patients with chronic idiopathic urticaria but not DPU. Three biopsies were taken from each of 13 patients with DPU, from a combination of unchallenged skin and at 0, 2, 6, 24, 48 and 120 h after weighted steel rods (diameter 1.5 cm) had been applied to the thighs. Three biopsies were similarly obtained from each of four normal controls before an identical pressure challenge and at 6, 24 and 48 h afterwards. The chloracetate esterase stain was used to demonstrate mast cells and an alkaline phosphatase anti-alkaline phosphatase immunohistochemical technique to assess the phenotypic and activation characteristics of the T cell infiltrate. The mast cell count did not differ significantly between unchallenged skin from DPU patients and normal controls. Following a pressure challenge to the DPU patients, the number of stainable mast cells decreased significantly to a level comparable with that in spontaneous weals of chronic idiopathic urticaria. Investigation of T cell subsets showed a preponderance of CD4+ cells over CD8+ cells. There was no evidence of T cell activation in chronic idiopathic urticaria or DPU when compared with normal controls. These data support the view that mast cell degranulation occurs in chronic idiopathic urticaria and suggest that it may also play a role in the pathogenesis of DPU. There was no evidence that lymphocyte activation occurs in either condition.

Clinical and Experimental Allergy, Vol. 25, pp. 317–322. Submitted 28 January 1994; revised 16 August 1994; accepted 14 October 1994.

Introduction

Chronic idiopathic urticaria may occur in isolation or may be associated with one or more of the urticarias elicited by physical stimuli [1,2]. Delayed pressure urticaria (DPU) is one of the physical urticarias and is characterized by persistent and often painful swellings which arise several hours after pressure has been applied to the skin [3].

The primary effector cell in idiopathic urticaria is considered to be the mast cell [4] and its release of

histamine to result in the formation of weals. The inflammatory infiltrate in both DPU [5] and idiopathic urticaria [6,7] includes mononuclear cells which may contribute to weal formation by secreting histamine releasing factors [8]. *In vitro* studies have demonstrated that supernatants from cultured human mononuclear cells can elicit histamine release from basophils [9] and also that T lymphocytes can induce the proliferation of murine mast cell clones [10]. In turn, rat mast cell degranulation has been shown to result in chemotaxis of rat lymphocytes [11].

The lymphocytic infiltrate in most inflammatory skin diseases is characterized by a preponderance of CD4+ over CD8+ T cells [12]. In addition to demonstrating their phenotypes, it is important to establish whether the

Correspondence: Dr R. J. Barlow, St John's Institute of Dermatology, UMDS, St Thomas's Hospital, Lambeth Palace Road, London SE1 7EH, UK.

lymphocytes are expressing markers of activation by antigen. Activation of T lymphocytes occurs in stages which are characterized by the appearance of specific cell surface antigens [13]. One of the earliest of these is the interleukin-2 receptor (IL-2R), the density and duration of which indicates the magnitude of T cell clonal proliferation and the consequent T cell immune response [14]. This is followed at 48–72 h by the expression of the transferrin receptor [15] and then by the T10 antigen [15]. The major histocompatibility complex molecule, human lymphocyte antigen (HLA)-DR is the last activation marker to appear and signifies the ability of T cells to present antigen [16]. In chronic urticaria, most of the mononuclear cells in the infiltrate have been reported to be activated helper-inducer T cells [17]. Likewise, a study of two pressure weals in DPU showed that 'nearly all' of the lymphocytes were activated T cells [18].

In order to test the hypothesis that activated lymphocytes contribute to the formation of urticarial weals, we have examined the population of mast cells in patients with both chronic idiopathic urticaria and DPU and have quantified the immunoregulatory T cell subsets and their activation status.

Patients and methods

Thirteen patients (three men, age range 21–49 years) with DPU were studied. All had pressure-related symptoms and a positive response to a pressure challenge test [19]. For the purposes of this study, pressure weals were induced using 1.5 cm diameter steel rods, weighted to between 2.5 and 3.5 kg and applied to the subject's thighs for 20 min [20]. Three 4 mm punch biopsies were taken from each patient so that a total of eight specimens was obtained from unchallenged skin, six from skin immediately after the pressure challenge and thereafter, six at 2 h, nine at 6 h, six at 24 h and two each at 48 and 120 h. None of the patients were taking medications other than antihistamines and these were discontinued 3 days before the pressure challenge. Spontaneous weals which lasted approximately 6 h were also biopsied in each of four patients (one man, age range 28–71 years) with chronic idiopathic urticaria but not DPU. Four normal controls (two men, age range 22–66 years) were subjected to an identical pressure challenge. Three biopsy specimens were obtained from unchallenged skin and three each at 6, 24 and 48 h after pressure.

The biopsies were performed under local anaesthesia (without adrenalin) and covered in cryoprotectant (Tissue-Tek, Miles Inc, USA) before immersion in melting isopentane and storage in liquid nitrogen. Tissue

specimens were cut into 5 μ m sections which were stained for mast cells using a conventional chloracetate esterase stain [21]. Sections were also stained immunohistochemically for T lymphocytes and for cells expressing HLA-DR. In addition, biopsies from DPU patients were stained for T cell subsets and for expression of interleukin 2-receptor (IL2-R), T9 (transferrin receptor) and T10. Prior to staining, frozen sections were air-dried, fixed in acetone (5 min) and washed in tris-buffered saline. They were then immersed in 20% normal swine serum to block non-specific binding before incubation for 60 min with the primary antibodies listed in Table 1.

Immunolabelling was performed using rabbit anti-mouse bridging antibodies conjugated with alkaline phosphatase (1:10 dilution, 30 min) (Dako Ltd, High Wycombe, UK) followed by incubation with murine alkaline phosphatase anti-alkaline phosphatase (APAAP) complexes (1:20 dilution, 30 min) (Dako Ltd, High Wycombe, UK). Alkaline phosphatase activity was demonstrated using Fast Red development (15 min) before sections were counterstained with Mayer's Haemalum and mounted in aqueous medium. Control sections were processed identically except that 20% normal swine serum was substituted for primary antibody.

Assessment of histological sections was undertaken 'blind' by two independent observers. Cells were counted in a central column of each section which was two high power fields ($\times 400$) in width and which spanned the depth of the dermis. The mean cell count per high power field was established for each section and the median count then calculated at each time interval after pressure. Median counts were compared with unchallenged skin, with normal controls and with chronic idiopathic urticarial weals. Statistical analysis was performed using the Mann-Whitney *U*-test for comparing unpaired, non-parametric data.

Results

Of the patients with DPU, all except one of the six biopsied at 2 h and one of the nine biopsied at 6 h had developed pressure-induced weals at these times. All of the patients who were biopsied at 24 h had pressure weals. One patient developed a weal immediately after pressure was applied and one of the patients biopsied at 48 h still showed a weal at this time. Neither patient biopsied at 120 h had any clinically apparent abnormality at the pressure challenge sites (Table 2). None of the normal controls developed delayed weals in response to pressure though two had erythematous annular patches at 6 h.

Table 1. Primary antibodies used in the study

Antibody	Specificity	Dilution	Source
<i>T cell subsets</i>			
Anti Leu 1	T cell antigen (CD5)	1:20	Becton Dickinson Immunocytometry Systems, USA
Anti Leu 2a*	T suppressor-cytotoxic cell antigen (CD8)	1:25	Becton Dickinson Immunocytometry Systems, USA
Anti Leu 3a*	T helper-inducer cell antigen (CD4) monocytes/macrophages	1:10	Becton Dickinson Immunocytometry Systems, USA
<i>T cell activation markers</i>			
Anti IL2-R*	IL2-R (CD25)	1:20	Dako Ltd, Denmark
OKT9*	T9 (Transferrin receptor)	1:10	Ortho Diagnostic Systems, USA
OKT10*	T10	1:10	Ortho Diagnostic Systems, USA
Anti HLA-DR	HLA-DR antigen	1:10	Becton Dickinson Immunocytometry Systems, USA

* DPU patients only.

Histopathological findings

The time course patterns of the inflammatory cell infiltrate and of the T lymphocyte activation markers are summarized in Tables 3 and 4.

DPU patients The number of mast cells demonstrable with the chloracetate esterase reaction decreased significantly following a pressure challenge ($P < 0.05$, 6 h vs unchallenged skin) and was comparable with that in idiopathic weals (Fig. 1). At 48 and 120 h the median number per high power field had returned to baseline levels.

The number of T cells in unchallenged skin was significantly higher than that in unchallenged skin from normal controls ($P < 0.05$). At 24 h after pressure, there was an increase in the T cell infiltrate which was not statistically significant compared with unchallenged skin. The density of CD4+ cells exceeded the density of total lymphocytes and did not change significantly. All sections showed a preponderance of CD4+ cells over CD8+ T cells. No significant changes were seen in the median numbers of cells expressing the T cell activation markers IL2-R, T9 and T10. The number of cells expressing HLA DR did not increase from baseline levels which were similar to those in unchallenged skin from normal controls.

Normal controls The number of mast cells demonstrated with chloracetate esterase did not change

significantly following a pressure challenge (Fig. 1). Similarly, there was no change in the number of T lymphocytes or of HLA DR+ cells after pressure.

Chronic idiopathic urticaria The number of stainable mast cells in idiopathic weals was comparable to that seen in DPU patients at 6 and 24 h after a pressure challenge (Fig. 1). The infiltrate of T lymphocytes was intermediate between that of the DPU patients at 6 and at 24 h after pressure.

Discussion

Mast cells are understood to be the primary effector cells in urticaria [4] and have previously been reported to be significantly increased in number in chronic idiopathic urticarial weals as compared with normal controls [6]. We have not been able to confirm this finding. One possible explanation is that we biopsied weals of approximately 6 h duration whereas the 'most recent fully developed urticarial lesion' was biopsied in the previous study. Newer weals are likely to contain fewer degranulated mast cells and a correspondingly higher number would be demonstrable by chloracetate esterase staining. The higher mast cell counts reported previously could also have resulted from selecting zones of infiltration whereas the present study was an attempt at assessing the mast cell population in the dermis as a whole.

Table 2. Clinical appearance of the skin to be biopsied in relation to the time after pressure challenge

Patient	Unchallenged	Time after pressure challenge (h)					
		0	2	6	24	48	120
1		W 15 mm	W 18 mm	W 15 mm			
2	N		W 14 mm	W 42 mm			
3	N	N	W 15 mm				
4	N	N			W 34 mm		
5	N			W 30 mm			N
6		N	W 16 mm		W 18 mm		
7	N			E 18 mm	W 30 mm		
8			W 15 mm	W 22 mm	W 20 mm		
9	N			W 18 mm		N	
10	N			W 30 mm	W ?mm		
11				W 16 mm		W 6 mm	N
12	N		E 20 mm		W 80 mm		
13	N	E 44 mm		W 44 mm			

N. Clinically normal. E. Erythema only. W. Erythematous weal.

Table 3. Time course pattern of the infiltrate of mast cells and of T lymphocytes, T cell subsets and T cell activation markers (median numbers/hpf) in patients with DPU, normal controls (NC) and chronic idiopathic urticaria controls (UC)

	Mast cells			Pan T cells (CD5)			CD4+ cells	CD8+ cells
	DPU	NC	UC	DPU	NC	UC	DPU	DPU
Unchallenged	3.65	4.90	2.37	4.73	1.23	5.06	8.79	0.77
0 h	4.41	—	—	2.35	—	—	7.48	0.56
2 h	3.96	—	—	2.88	—	—	8.86	0.99
6 h	2.16*	2.67	—	1.73	2.25	—	5.81	0.50
24 h	2.46	4.27	—	8.25	2.05	—	11.08	0.83
48 h	3.39	3.82	—	4.10	1.64	—	16.15	0.60
120 h	4.67	—	—	2.69	—	—	3.15	3.15

* $P < 0.05$, vs unchallenged skin (Mann-Whitney U -test).

Table 4. Time course pattern of the infiltrate of mast cells and of T lymphocytes, T cell subsets and T cell activation markers (median numbers/hpf) in patients with DPU, normal controls (NC) and chronic idiopathic urticaria controls (UC)

	IL2-R DPU	OKT9 DPU	OKT10 DPU	DPU	HLA DR NC	UC
Unchallenged	0.00	0.99	0.00	26.27	32.90	26.88
0 h	0.00	3.80	0.00	23.00	—	—
2 h	0.00	2.59	0.00	27.32	—	—
6 h	0.05	2.15	0.00	32.30	23.39	—
24 h	0.20	5.03	0.00	24.25	25.90	—
48 h	0.06	1.08	0.00	34.08	22.50	—
120 h	0.18	6.60	0.00	53.44	—	—

Mast cells have also been postulated to play a role in DPU [22]. It has been shown previously in patients with DPU that intradermal injection of the mast cell degranulating agent, compound 48/80 elicits weals which both clinically [23,24] and histologically [24] resemble those induced by pressure. In addition, mast cell numbers have been reported to be increased in early (4–6 h) [18,25] but not late (20–24 h) pressure-induced weals in DPU [18]. In the present study, the number of demonstrable mast cells in unchallenged skin did not differ significantly in DPU patients from that of normal controls but showed a significant decrease 6 h after pressure. At this time, the level was similar to that in spontaneous weals in chronic idiopathic urticaria in which mast cell degranulation is understood to occur. It is possible therefore that mast cell degranulation is a factor in the development of pressure related weals and that the later decrease in

mast cells is caused by degranulation with resultant loss of their staining properties. In asthma, the propensity of mast cells to degranulate may be more important than the actual number [26], and it is possible that this may also be the case in DPU.

The median count of T lymphocytes in unchallenged skin in DPU patients was significantly higher in the DPU patients than in the normal controls. The reason for this is not clear but may be explained by the recent finding that the vascular endothelial cell adhesion molecules E-selectin and Intercellular Adhesion Molecule-1 (ICAM-1) are upregulated in some biopsies of non-lesional skin in DPU [27]. Both E-selectin [28] and ICAM-1 [29] act as adhesion receptors for T lymphocytes and their increased expression in unchallenged skin may reflect the inflammatory nature of DPU.

In this study, CD4+ T cells predominated over CD8+ T cells as has been reported previously in chronic urticaria [17,18] and other inflammatory dermatoses [12]. We were unable to confirm that T cell activation occurs in spontaneous weals in chronic idiopathic urticaria or that it occurs after pressure in patients with DPU or in normal controls. In the DPU patients, the number of cells expressing HLA-DR showed no increase after a pressure challenge and did not differ significantly from normal controls or from idiopathic urticarial weals.

We have recently shown that E-selectin is upregulated in both chronic idiopathic urticaria and DPU and that there is close similarity between the inflammatory cell infiltrate in idiopathic urticarial weals of approximately 6 h duration and in induced DPU weals at 6 h after pressure [27]. Induction of E-selectin can be elicited by degranulation of mast cells [30] and the data from the present study suggest that mast cells may be involved in DPU as they are in idiopathic urticaria. In both conditions the process appears to be independent of activated T lymphocytes.

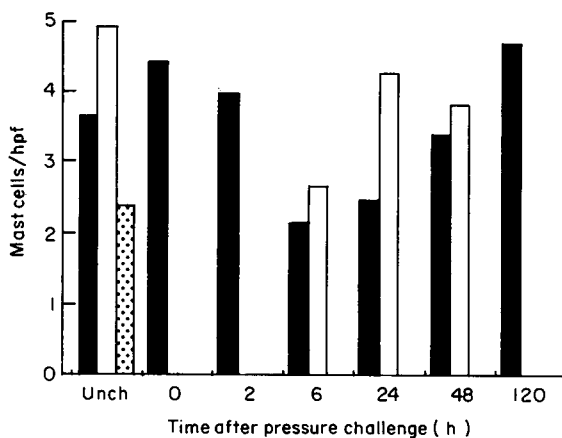


Fig. 1. Histogram showing the population of mast cells, in patients with DPU compared with idiopathic weals (UC) and pressure challenged sites from normal controls (NC). ●. Mast cells (DPU). □. Mast cells (NC). ▨. Mast cells (UC). Unch. Unchallenged.

Acknowledgements

Dr Barlow was supported by the Dunhill Trust, the Kieckhefer Foundation and the Eleanor Naylor Dana Charitable Trust. We are grateful to Fiona Warburton (Department of Public Health Medicine, St Thomas's Hospital) for her help with statistical analysis of the data.

References

- Doeglas HMG, Bleumink E. Plasma protease inhibitors in chronic urticaria. In: Champion RH, Greaves MW, Kobza Black A, Pye RJ, eds. *The Urticarias*. Edinburgh: Churchill Livingstone, 1985: 59–69.
- Champion RH. Urticaria: then and now. *Br J Dermatol* 1988; 119:427–36.
- Dover JS, Kobza Black A, Milford Ward A, Greaves MW. Delayed pressure urticaria. *J Am Acad Dermatol* 1988; 18:1289–98.
- Schwartz LB. Mast cells and their role in urticaria. *J Am Acad Dermatol* 1991; 25:190–203.
- Sussman GL, Harvey RP, Schocket AL. Delayed pressure urticaria. *J Allergy Clin Immunol* 1982; 70:337–42.
- Natbony SF, Phillips ME, Elias JM et al. Histologic studies of chronic urticaria. *J Allergy Clin Immunol* 1983; 71:177–83.
- Lever WF, Schaumberg-Lever G. Urticaria. In: *Histopathology of the skin*. Philadelphia: JB Lippincott, 1989: 152–3.
- Kaplan AP. Urticaria: The relationship of duration of lesion to pathogenesis. *Allergy Proc* 1990; 11:15–8.
- Thuesen DO, Speck LS, Lett-Brown MA, Grant JA. Histamine-releasing activity (HRA). Production by mitogen- or antigen-stimulated human mononuclear cells. *J Immunol* 1979; 123:626–32.
- Nable G, Galli SJ, Dvorak AM et al. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. *Nature* 1981; 291:332–4.
- Center DM. Identification of rat mast cell-derived chemoattractant factors for lymphocytes. *J Allergy Clin Immunol* 1983; 71:29–35.
- Smolle J. Mononuclear patterns in the skin. *Am J Dermatopathol* 1988; 10:36–46.
- Alcover A, Ramarli D, Richardson NE et al. Functional and molecular aspects of human T lymphocyte activation via T3-Ti and T11 pathways. *Immunol Rev* 1987; 95:5–36.
- Cantrell DA, Smith KA. Transient expression of interleukin 2 receptors. *J Exp Med* 1983; 158:1895–911.
- Hercend T, Ritz J, Schlossman SF, Reinherz EL. Comparative expression of T9, T10, and 1a antigens on activated human T cell subsets. *Hum Immunol* 1981; 3:247–59.
- Brown MF, Cook RG, Van M, Rich RR. Cloned human T cells synthesize 1a molecules and can function as antigen presenting cells. *Hum Immunol* 1984; 11:219–28.
- Mekori YA, Giorno RC, Anderson P, Kohler PF. Lymphocyte subpopulations in the skin of patients with chronic urticaria. *J Allergy Clin Immunol* 1983; 72:681–4.
- Czarnetzki BM, Meentken J, Kolde G, Brocker E. Morphology of the cellular infiltrate in delayed pressure urticaria. *J Am Acad Dermatol* 1985; 12:253–9.
- Lawlor F, Kobza Black A, Milford Ward A et al. Delayed pressure urticaria, objective evaluation of a variable disease using a dermatographometer and assessment of treatment using colchicine. *Br J Dermatol* 1989; 120:403–8.
- Illig L, Kunick J. Klinik und Diagnostik der physikalischen Urticaria. *Hautarzt* 1969; 20:167–78.
- Leder L.-D. The chloracetate esterase reaction. *Am J Dermatopathol* 1979; 1:39–42.
- Ryan TJ, Shim-Young N, Turk JL. Delayed pressure urticaria. *Br J Dermatol* 1968; 80:485–90.
- Davis KC, Mekori YA, Kohler PF, Schocket AL. Late cutaneous reactions in patients with delayed pressure urticaria. *J Allergy Clin Immunol* 1986; 73:810–2.
- Mekori YA, Dobozi BS, Schocket AL et al. Delayed pressure urticaria histologically resembles cutaneous late-phase reactions. *Arch Dermatol* 1988; 124:230–5.
- Kobza Black A, Dover JS, Greaves MW. The histological features of induced delayed pressure urticaria. *Br J Dermatol* 1987; 116:427–8.
- Litchfield TM, Lee TH. Asthma: Cells and cytokines. *J Asthma* 1992; 29:181–91.
- Barlow RJ, Ross E, MacDonald DM, Kobza Black A, Greaves MW. Adhesion molecule expression and the inflammatory cell infiltrate in delayed pressure urticaria. *Br J Dermatol* (in press).
- Pickler LJ, Kishimoto TK, Smith CW et al. ELAM-1 is a mechanism for skin-homing T cells. *Nature* 1991; 349:796.
- Dustin ML, Springer TA. Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J Cell Biol* 1988; 107:321–31.
- Klein LM, Lavker RM, Matis WL, Murphy GF. Degranulation of human mast cells induces an endothelial antigen central to leukocyte adhesion. *Proc Natl Acad Sci USA* 1989; 86:8972–6.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.