Mast cells in pathological and surgical scars

T W Beer, H Baldwin, L West, P J Gallagher, D H Wright

Abstract

Aim—To investigate the role of mast cells in surgical and pathological scar reactions by their identification and quantification using immunohistochemistry.

Methods—Surgical scars and pathological scar reactions were stained immunohistochemically for tryptase to identify mast cells. These were quantified in the scar tissue and surrounding dermis. Statistical analyses were performed to test the hypothesis that mast cell numbers were different in the varying types of scar reaction.

Results—A significant difference was found between the mean number of mast cells in periocular scars compared with keloids, hypertrophic scars, and surgical scars from other sites ($p < 0.05$). No significant difference was found in mast cell numbers between the other scar types either within the lesions or surrounding dermis. There were significantly more mast cells in the dermis than in the scar tissue itself, except for the small group of periocular scars. The ratio of mast cells in the lesion compared with the dermis was not significantly different between the scar types, except for the periocular scars.

Conclusions—Mast cell numbers are similar in and around keloid, hypertrophic, and surgical scars. The increased number of mast cells at periocular scar sites was contrary to expectation since keloids are rare at this site. Absolute mast cell numbers may not be an accurate measure of tissue concentrations of active mast cell products. Further comparisons between immunological characteristics of keloid and periocular scars may elucidate specific immunological abnormalities of keloid scars, and this has implications for the development of immunotherapy.

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In adult skin significant dermal damage is repaired by the deposition of connective tissue forming a scar. Fetal skin has greater powers of regeneration and healing without scar formation can occur.¹ The majority of scar reactions produce an appropriate amount of connective tissue to fill the dermal defect, but in certain instances excessive fibrous tissue may be deposited leading to the formation of a hypertrophic scar or keloid. These types of pathological scar reactions have many recognised associations and predisposing factors, but the cellular events involved in their formation are not fully established. It has been suggested that mast cells may have an important role in the

formation of pathological scar reactions, possibly through the action of IgE.² Studies have shown that keloids may be associated with increased tissue histamine levels and increased serum IgE.³ Mast cells interact with fibroblasts and are pivotal in the early stages of fibrotic reactions such as fibrosing alveolitis, scleroderma, and wound healing.⁴ The liberation of mast cell contents, which include tryptase and histamine, is partly mediated by IgE. Keloid scars are frequently sited on the head and neck, but are rare lesions of the eyelids.⁵⁻⁷ In this study cutaneous mast cells were identified by immunohistochemistry using an antibody to mast cell tryptase and quantified in order to investigate the hypothesis that these cells have an important role in the formation of pathological and physiological scar reactions.

Methods

Paraffin embedded blocks were retrieved from the archives of Southampton University Hospital Trust. All cases were reviewed on haematoxylin and eosin stained sections and categorised as hypertrophic scars ($n = 10$) or keloids ($n = 12$) by their clinical and histological features according to recognised criteria.⁸⁻¹⁰ In brief, keloids are well circumscribed, raised lesions that may extend beyond the site of original skin damage. By contrast, hypertrophic scars remain confined to the site of skin injury and are less likely to recur. Histologically, keloids are characterised by nodular fibroplasia with hypocellular dermal collagenisation. Hypertrophic scars are more cellular lesions and lack the "glassy" collagen associated with keloids. Tissue from healing scars was obtained from wider surgical excisions following primary malignant melanoma resection "surgical scars" ($n = 24$). Only cases with no evidence of recurrent/residual melanoma were used. Cases of periocular healing scars, "periocular scars", ($n = 10$) were obtained prospectively, and all were derived from non-pathological scar tissue excised after previous surgery at the same site. This was related to revision blepharoplasty for non-neoplastic conditions.

The ages of the surgical scar subjects were between 24 and 86 years (median 48.0), with nine women and 15 men. All lesions were sited on the leg, trunk, or face. Hypertrophic scar patients were aged from 14 to 66 years (median 29.0), with four women and six men. The scars were located on the head, neck, upper limb, and one case was from the foot. Keloid scars were located on the head, neck, upper trunk, and upper limb in subjects of 11-74 years of age (median 34.5); five patients were male and seven female. The periocular scars were derived from the lower or upper eyelid in

Department of
Histopathology,
Southampton
University Trust
Hospital,
Southampton
SO16 6YD
T W Beer
L West
P J Gallagher
D H Wright

Southampton Eye
Unit, Southampton
University Trust
Hospital,
Southampton
SO16 6YD
H Baldwin

Correspondence to:
Dr T W Beer, Department of
Histopathology, Royal
Hospital Haslar, Gosport,
Hampshire, PO12 2AA.

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Table 1 Mast cell numbers in the four scar types and surrounding dermis

	Mast cell numbers per mm ²		Scar age (days)
	Lesion	Dermis	
Surgical scars	58.1	104.4	33
	80.6	241.9	37
	36.3	164.4	28
	56.3	58.1	32
	35.6	58.8	23
	111.9	188.1	32
	26.3	76.9	37
	36.9	115.6	8
	15.6	96.9	26
	58.1	113.1	49
	51.9	141.3	30
	143.8	124.4	83
	58.1	116.9	49
	116.3	142.5	13
	29.4	76.3	26
	39.4	122.5	36
	12.5	87.5	32
	153.8	175.0	46
	39.4	91.9	21
	32.5	64.4	50
Hypertrophic scars	21.3	100.6	36
	96.3	171.9	35
	47.5	66.9	35
	61.9	106.3	29
	40.3	38.8	
	47.7	208.2	
	77.3	136.8	
	64.7	214.5	
	109.5	269.2	
	62.5	120.0	
Keloid scars	164.4	85.0	
	60.6	76.3	
	40.6	69.4	
	58.8	158.8	
	21.8	122.0	
	81.7	134.2	
	121.3	131.3	
	55.8	208.2	
	61.8	103.2	
	61.9	135.6	
Periocular scars	126.3	226.9	
	81.9	200.0	
	40.6	206.3	
	40.6	120.6	
	24.4	96.3	
	53.1	133.1	
	28.1	*	
	199.7	*	
	216.7	*	
	212.3	*	
	190.1†	*	
	298.8†	*	
	10.0	121.3	
	246.3	295.0	
	117.5	*	
	132.5	101.3	

*Insufficient material for assessment.

†These samples from the left and right lid of the same patient.

patients between 28 and 80 years (median 57.0), one male and eight females. Samples from the left and right eyelids of one subject were used (cases 5 and 6).

IMMUNOHISTOCHEMISTRY

Five µm paraffin block derived sections from each case were stained immunohistochemically for mast cell tryptase using the following method.

Table 2 Mean concentration of mast cells in the scars and adjacent dermis

	Mean number of mast cells per mm ² (SD)		Ratio mast cells lesion: dermis
	Lesion	Dermis	
Keloid scars	64.3 (33.6)	151.5 (45.5)	0.42
Surgical scars	59.2 (39.74)	116.9 (45.9)	0.51
Hypertrophic scars	72.6 (38.0)	137.7 (74.5)	0.53
Periocular scars	165.2 (92.7)	172.5 (106.5)	0.96

The cut sections were dried overnight at 37°C. These were then dewaxed and dehydrated. Endogenous peroxidase activity was inhibited by treatment with 0.5% hydrogen peroxide in methanol for 10 minutes. The slides were next rinsed in water for 5 minutes. Trypsin proteolysis was then used, immersing the prewarmed slides in pre-prepared 0.1% trypsin solution at 37°C. Proteolysis was stopped by placing the slides in cold water for 5 minutes. Before application of the primary antibody it was necessary to treat the sections with an avidin biotin blocking kit (Vector, USA) since mast cells tend to bind avidin/streptavidin non-specifically. The neat avidin solution was applied for 20 minutes before rinsing in TRIS buffer and the application of neat biotin solution for 20 minutes. This was rinsed using two 2 minute cycles and then a solution of 10% fetal calf serum in DMEM containing 1% bovine serum albumin was applied for 20 minutes. This was drained and the primary antibody, AA1 to tryptase¹¹ (donated by Dr Andrew Wall, Southampton General Hospital) was applied at a dilution of 1/100 for 30 minutes at room temperature. AA1 is a mouse monoclonal antibody of isotype IgG1, kappa. The slides were then washed in TRIS buffer using three 5 minute cycles and then biotinylated sheep antimouse immunoglobulin was applied (Amersham, UK) for 30 minutes. This was washed off with TRIS in three 5 minute cycles and then the streptavidin/biotin complex (Dako, UK) was applied. This was washed off in TRIS buffer using three 5 minute cycles. The DAB substrate was next applied for 10 minutes (Sigma Fast DAB tablets, Sigma, UK) and then the slides were rinsed in TRIS buffer before being washed in running water for 5 minutes. Slides were counterstained using Harris's haematoxylin for 2 minutes before dehydration, clearing and mounting in DPX.

CELL COUNTING

Areas of scar tissue were identified on the haematoxylin and eosin stained sections and the corresponding haematoxylin and eosin area identified on the immunohistochemically stained sections. The number of cells with distinct brown immunostaining in the cytoplasm were counted, as well as defined granular foci of positive staining considered to represent degranulated mast cells as previously described.¹² An eyepiece graticule (Graticules Ltd, UK) was used to ensure that cells were not missed or counted twice. Ten fields were counted from the superficial to the deep area of the scar. Cells were counted at a magnification of 250 times using a Lietz Dialux 20 microscope. The field area was 0.160 mm² and from 10 consecutive fields the number of mast cells per mm² was calculated. The number of dermal mast cells were counted in a similar way. Mast cells were counted in 10 microscopic fields in the dermis immediately adjacent to the scar, up to a depth of 0.4 mm from the base of the overlying epidermis. The number of dermal mast cells per mm² was then calculated.

Figure 1 Mast cells strongly reactive for tryptase within a hypertrophic scar. (Immunohistochemistry for tryptase, original magnification $\times 400$.)

Figure 2 Mast cells of dendritic morphology which were identified in the dermis of a number of cases. (Immunohistochemistry for tryptase, original magnification $\times 400$.)

STATISTICAL ANALYSES

One way analysis of variance and pairwise comparisons were used to compare the mean mast cell counts between the different scar groups. This was performed for counts within the scar tissue and in the surrounding dermis. The ratio between mast cell numbers in the scar and surrounding dermis was also calculated and one way analysis of variance used to establish any differences between the groups for this ratio. In the group of surgical scars following malignant melanoma removal the relation between the number of mast cells and age of the scar was determined.

Results

Mast cells were readily identified in all the scars and in the surrounding dermis (Tables 1 and 2). The morphology of the cells varied from rounded to dendritic (Figs 1 and 2) and in some cases scattered defined extracellular clusters of positive staining granules were interpreted as marking the sites of recently degranulated mast cells. Only two of the eyelid scars included sufficient dermis surrounding the scar reaction to allow full analysis.

A significant difference ($p < 0.05$) was found between the mean number of mast cells in periocular scars (mean 165.2 (SD 92.7)) com-

Figure 3 Mast cells in surgical scars related to age of scar.

pared with keloids (64.3 (33.6)), hypertrophic scars (72.6 (38.0)), and surgical scars (59.2 (45.9)).

No significant difference was found in mean mast cell numbers between the other scar types either within the lesions or surrounding dermis. There were significantly more mast cells in the dermis than in the scar tissue itself, except for the small group of periocular scars. The ratio of mast cells in the lesion compared with the dermis was not significantly different between the scar types, except for the periocular scars (Table 2). Here there were similar numbers of mast cells in the lesions and adjacent dermis with a ratio of 0.96, but the numbers in this group were small.

A positive correlation ($r = 0.41$, $p < 0.05$) was found between mast cell concentration and scar age for the group of surgical scars (Fig 3). There were insufficient data on scar age to examine the correlation for other scar types. No correlation was seen between mast cell concentration and patient age for any of the scar types.

Discussion

Mast cell and fibroblast interaction is important in the early stages of wound healing,⁴ fibrosing alveolitis, and scleroderma.¹³ Evidence for their role in pathological scar reactions is limited and often conflicting. Mast cells have been suggested as important factors in the pathogenesis of multiple¹⁴ and solitary¹⁵ cutaneous dermatofibromas.

Mast cells can be subdivided according to the nature of the enzymes they produce. The subgroup producing tryptase and chymase (M_{TC}) have been found predominantly in skin, breast, axillary lymph node, and the submucosa of the bowel.¹⁶ Cutaneous mast cells are almost exclusively of type M_{TC} . Tryptase is a neutral protease with a number of physiological roles. These include inactivation of fibrinogen causing reduced fibrin deposition, highly specific protein cleavage, and activation of prostromelysin (MP III) which modulates collagenase activity and tissue turnover.¹⁶ The monoclonal antibody AA1 is specific for tryptase¹¹ facilitating the classification of mast cells in the skin and allowing identification of sites of recent mast cell degranulation. This immunohistochemical method is more sensitive and specific than toluidine blue staining¹²

which has been used in most previous studies of mast cells. All types of mast cells are positive with toluidine blue but recently degranulated mast cells may not be identified by this method.¹²⁻¹⁷

Adolescents prone to keloid or hypertrophic scar formation are more likely to display allergic symptoms suggesting that hypersensitivity plays a role in the development of these types of scar.³ Antigen stimulated release of IgE may cause mast cell degranulation, release of histamine, and subsequent heparin liberation with increased collagen synthesis.¹⁸ These events, coupled with the angiogenic effects of macrophage products, could lead to keloid vascularisation and growth.³ Interestingly, African Americans have relatively high levels of atopy¹⁹ and are also more prone to keloid formation.¹⁰

Tissue histamine levels are elevated in keloids which may promote abnormal collagen cross links² and production of increased amounts of soluble collagen.³ Several authors have suggested that increased numbers of mast cells may be identified in keloid scars.²⁰⁻²¹ These studies, however, mostly lack methodological data and are far from definitive. Other workers, using immunohistochemistry for tryptase and toluidine blue, found similar numbers of mast cells in keloid scars and normal skin, although the numbers studied were very small (three keloid scars).¹² It has been stated that hypertrophic scars show greater numbers of mast cells than mature cutaneous scars¹⁷⁻²² and that a decrease in mast cells is seen when pressure is applied to a hypertrophic scar,¹⁷ an intervention known to reduce the severity of hypertrophic scarring.

In this study we found that mast cell numbers were greater in normally healing eyelid scars than in keloids, hypertrophic scars, and surgical scars. Increased numbers were also observed in apparently normal dermis surrounding keloid, hypertrophic scars, and surgical scars. Mast cell numbers were seen at similar levels within normally healing surgical scars, hypertrophic scars, and keloids.

In most keloids, hypertrophic scars, and surgical scars there were approximately twice as many mast cells in apparently normal dermis surrounding the scar as in the lesion itself. This ratio showed no consistent change with time in surgical scars, although there was a positive correlation between the duration of the surgical scar and the lesional mast cell concentration (Fig 3). It has been previously suggested that mast cell numbers increase in connective tissue as healing progresses.²³ This may also be so during the "active" phase of scar formation, but there is some evidence to indicate that mast cell numbers decrease in aging keloids²⁴ and solitary dermatofibromas.¹⁴ Finding mast cells in greater numbers in dermis surrounding scars suggests that as the surgical scars progress, so their mast cell complement approaches that of normal dermis. It would be valuable to quantify mast cells in perilesional and entirely

normal dermis from another skin site to ascertain if any increase in dermal mast cells occurs surrounding scars even when lesional mast cells are not increased.

Mast cell numbers are not of value to distinguish between keloid and hypertrophic scars histologically. Finding increased mast cells in periocular scars was contrary to expectation in light of literature linking mast cells with keloids and the rarity of keloids in the eyelid.

The precise role of mast cells in cutaneous scar reactions remains unclear, but absolute mast cell numbers may not accurately reflect tissue concentrations of active mast cell products. Further comparisons between the immunological characteristics of cutaneous scar reactions are warranted and may lead to specific immunotherapy to ameliorate or prevent pathological scar reactions.

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