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Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare

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SUMMARY

Using an antibody to S100 protein, the number of dendritic cells above the basal layer in the epidermis was assessed in necrobiosis lipoidica and granuloma annulare. A statistically significantly higher number of these cells was found within the epidermis in necrobiosis lipoidica compared with granuloma annulare and normal skin. The numbers were similar to those seen in sarcoidosis and tuberculous reactions in the skin, which raises the possibility of an immune pathogenesis for necrobiosis lipoidica.

Although the clinical appearances of granuloma annulare and necrobiosis lipoidica are quite distinct,[1] histologically the two conditions have similar features. Both show a variable degree of collagen degeneration within the dermis, with an associated histiocytic inflammatory reaction. This reaction takes the form of a palisade of histiocytes, some of which may be epithelioid, with occasional multinucleate giant cells.[2,3] Langerhans cells form a population of dendritic cells that are widely distributed throughout the skin, in the stratified squamous mucosal epithelia, lymph nodes and lymphatic vessels draining the skin. They are found in the largest numbers, however, within the epidermis where they function as antigen-presenting cells. Their density within the epidermis is similar in most body sites, except for the palms and soles where the numbers of cells are significantly lower.[4]

Langerhans cells were first identified by Paul Langerhans, in 1868, using a gold-chloride technique. They may also be demonstrated using a variety of methods including membrane ATPase, monoclonal antibody to the T6 antigen, and ultrastructurally by the presence of Birbeck granules. A subset of these cells can be identified using an antibody to S100 protein.[5] This subset of dendritic S100 protein positive cells within the epidermis has been counted in biopsies from patients with necrobiosis lipoidica and granuloma annulare.

METHODS

Fifteen biopsies were taken, and of these seven were of necrobiosis lipoidica and eight granuloma annulare. The tissues were formalin fixed and paraffin embedded. Staining with

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antibody to S100 protein was performed using a standard peroxidase antiperoxidase technique. The sections were trypsinized before application of primary antibody (D.A.K.O. Rabbit anti-cow S100 Code Z311 lot 026) at a final dilution of 1:100. Control sections of normal skin adjacent to non-neoplastic/non-inflammatory lesions, and of normal skin removed at autopsy (20 cases) were used. The numbers of basal cells overlying the intradermal lesion were counted (500–1000 cells), and the number of epidermal dendritic S100 positive cells lying above them were counted. Using this method, S100 positive, basal melanocytes were excluded. Counts were expressed as the number of epidermal dendritic S100 positive cells per 200 basal cells.

Student's unpaired *t*-test was used to assess statistical differences between necrobiosis lipoidica, granuloma annulare, and the control group.

RESULTS

All the biopsies were of predominantly lesional skin. There was no disparity between the histological and clinical diagnoses in any of the cases. All showed the recognized features of collagen degeneration, some having palisaded granulomas. In the majority, the full thickness of the biopsy that included the lower reticular dermis was involved by the lesions.

Dendritic cells were seen positively stained, lying between keratinocytes, within the epidermis (Fig. 1). There was an increase in numbers of epidermal dendritic S100 positive cells in both granuloma annulare (mean 8·0) and necrobiosis lipoidica (mean 24·5), compared with normal skin controls (mean 3·7) (see Table 1). The numbers were higher in necrobiosis lipoidica than granuloma annulare ($0{\cdot}001 > P > 0{\cdot}0005$); but there was no statistical difference between granuloma annulare and the control group ($0{\cdot}15 > P > 0{\cdot}10$).

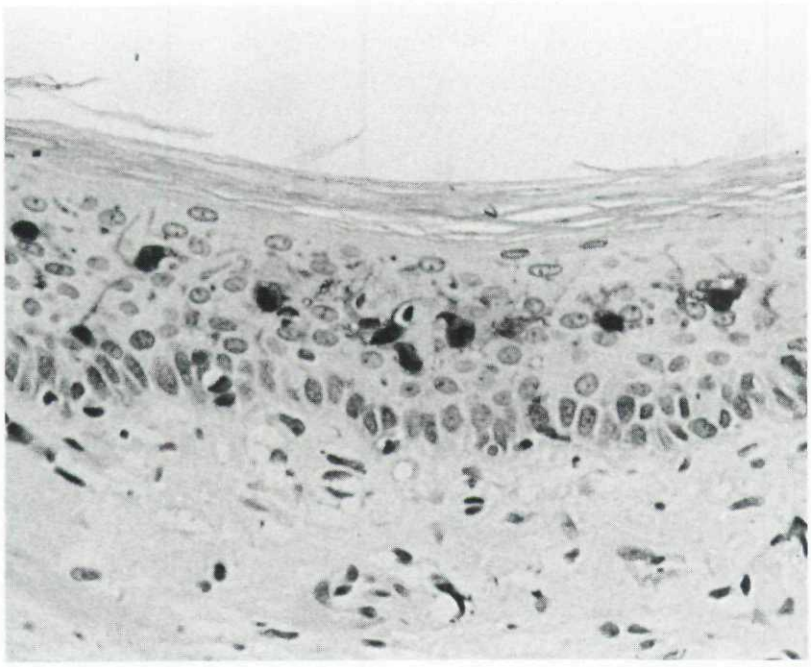

FIGURE 1. Dendritic S100 protein positive cells seen within the epidermis in a case of necrobiosis lipoidica (× 204).

TABLE 1. Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare

	Dendritic S100 positive cells per 200 basal cells		
	Granuloma annulare $n=8$	Necrobiosis lipoidica $n=7$	Normal skin $n=20$
	4·8	17·9	—
	8·8	30·7	—
	11·9	22·0	—
	9·2	20·8	—
	4·9	33·2	—
	6·3	20·4	—
	13·2	26·2	—
	5·1		
Mean	8·0	24·5	3·7
Range	4·8–13·2	17·9–33·2	1·6–5·8
SD	3·3	5·7	1·2

Of the seven patients with necrobiosis lipoidica one had diabetes mellitus and the number of S100 positive dendritic cells within this patient's biopsy was in the same range as the remaining six. None of the eight patients with granuloma annulare were known to be diabetic.

DISCUSSION

Although clinically different, the similarity of the histological features of granuloma annulare and necrobiosis lipoidica raises the possibility of a common pathogenesis. We have determined the dendritic S100 protein positive subset of the Langerhans cell population in the epidermis in these two conditions. There was a significantly higher number of S100 positive dendritic cells within the epidermis, above the basal layer, in necrobiosis lipoidica compared with both normal skin and granuloma annulare. In the latter the numbers of cells were elevated, but not to a significant level above normal. This suggests that the pathogenesis of the two conditions may be different. We have found that the numbers of S100 positive epidermal dendritic cells are elevated to a similar level to that seen in necrobiosis lipoidica in sarcoidosis, positive Kveim test biopsies, tuberculous leprosy and other tuberculous reactions (unpublished data). A cell-mediated, type IV delayed-hypersensitivity reaction is thought to be common to all these conditions. The increased numbers of S100 and dendritic cells within the epidermis in necrobiosis lipoidica may be a reflection of a similar pathological process.

The widely accepted view is that necrobiosis lipoidica is a result of collagen degeneration with a subsequent histiocytic inflammatory reaction.[2] However, the initiating stimulus to degeneration remains unknown. An immune complex vasculitis has also been proposed; direct immunofluorescence demonstration of IgM and C3 in vessel walls, and fibrinogen within necrobiotic areas, lends some support to this theory;[6,7] however, other workers have found these to be present only rarely.[8] There is also little histological evidence of a vasculitis, the vessels showing only a mild degree of intimal thickening. The association of necrobiosis lipoidica with

diabetes mellitus has led to the suggestion that the changes may be a result of diabetic microangiopathy.[9] Vascular changes, however, are often absent, and the affected vessels are usually larger than those involved in diabetic microangiopathy. Only one of our cases had diabetes mellitus.

In granuloma annulare the primary event is also thought to be collagen degeneration. Electron microscopy shows degeneration of elastic and collagen fibres in necrobiotic areas.[10,11] Vascular changes are few.[10] A cell-mediated immune reaction has been implicated with the demonstration of increased numbers of T helper/inducer lymphocytes within the granulomatous infiltrate, an increased number of OKT6 positive Langerhans cells within epidermis and dermis, and increased HLA-DR antigen and interleukin 2 receptor expression.[12] The increased numbers of S100 positive cells in granuloma annulare lends some support to these findings, but the numbers are higher in necrobiosis lipoidica and other known type IV delayed hypersensitivity reactions.[5] In conclusion, an increase in the number of epidermal dendritic S100 positive cells above the basal layer, as well as implicating a cell mediated immune-reaction, may provide an adjunct to histological means of distinguishing between necrobiosis lipoidica and granuloma annulare.

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