FEATURE OF MONONUCLEAR HUGGING IN VITILIGO

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Summary

Histomorphological study on 48 cases of vitiligo was carried out. The main finding in our serial section study was the demonstration of mononuclear hugging at the borders of the progressive vitiliginous macules, with normally pigmented skin. The mononuclear cells were almost exclusively small lymphocytes. They were found at the dermo-epidermal junction and also intraepidermally. A selective absence of melanocytes and melanin in the basal layer of the epidermis was the striking feature at the site of mononuclear hugging. That these mononuclear cells are possibly responsible for selective destruction of melanocytes (auto-destruction) and that vitiligo is perhaps a slow reacting auto-immune disorder of delayed hypersensitivity type, have been suggested on histological grounds.

Vitiligo has been subjected to extensive medical research with the subsequent emergence of several suggestions, postulations and hypothesis to explain its aetiology. Pertinent suggestions put forward are the genetic theory,[1] nutritional, metabolic disturbances and copper deficiency[2,3], hormonal imbalance, etc. More recently tropho-neurosis and neurogenic hypothesis have been suggested by Lerner[4] and Breathnach et al [5,6], since some authorities believe that melanocytes develop from nerves. Furthermore, we find literature suggesting auto-immune basis in its aetiology. [7,1]. Lastly the melanocyte self-destruction hypothesis[8] is added to the list i.e. a defect in the natural mechanism concerned with the successful elimination of toxic melanin precursors.

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Material and Methods

48 cases of vitiligo were subjected to histopathological study. The cases were divided as per the four clinical stages i.e. active, quiescent, improving, and segmental or zosteriformis. The number of cases included in each clinical stage and their sex distribution are shown in Table 1. The age limit of the cases included in this study was from 5 to 67 years. The following criteria were employed to recognise the different clinical stages of vitiligo. A case was

TABLE 1
Vitligo patients in each clinical stage

Clinical stages	No. of cases	Sex distribution Male	Female
Active	20	13	7
Quiescent	10	7	3
Improving	12	5	7
Zosteriformis	6	2	4
	48	27	21

recognised to be in 'active' stage, when the vitiliginous patches showed clinical signs of progress in size, had an ill-defined margin i.e. the whiteness merging imperceptively with the surrounding skin at the border. In a 'Quiescent' stage the lesion was found to remain stationary in size over a considerable period of time ; the border being hyperpigmented. The cases in the 'improving' stage showed signs of decrease in size of the lesion with repigmentation. The segmental zosteriformis variety was recognised as segmental emergence of white patches having a unilateral distribution (Table 2). However, the division

TABLE 2
Clinical Criteria for recondition of stages of Vitiligo

Stages of Vitiligo	Clinical features
Active :	(i) New lesions developing. (ii) Lesions increasing in size. (iii) Border ill defined.
Quiescent :	(i) No new lesion developing (ii) Lesion stationary in size. (iii) Border hyperpigmented and well defined.
Improving :	(i) Lesions decreasing in size. (ii) No new lesions developing. (iii) Border defined and signs of spontaneous repigmentation (follicular and peripheral).
Zosteriformis :	(i) Unilateral distribution of lesions. (ii) Lesions increasing in size. (iii) Border ill defined.

of cases into active, quiescent and improving was based on the predominent number of lesions of a particular stage in a patient of vitiligo ; because patients having lesions predominently of one stage did have in other parts of the body, few lesions showing features of other stages. Accordingly, biopsy was done from that type of vitiliginous patch which constituted the maximum number of the same type of lesions in a particular patient. The site for biopsy was chosen from a vitiliginous patch which was free from clinical signs of inflamation or infiltration. Further it was ensured that the cases included in the series did not receive any prior treatment for vitiligo (either systemic or topical therapy) or had any evidence of dermatoses or irritation.

Biopsies were taken from the margin of a vitiliginous patch by a punch biopsy needle having 7 m.m. diameter. Each circular piece of skin, thus obtained, contained a portion of the normal skin as well as the vitiliginous skin which was bisected into 2 equal halves each half representing both vitiliginous and normal skin portion. One half was subjected to DOPA processing and stained by H. & E. Each paraffin block was subjected to exhaustive serial sectioning at 5 μ thickness. Special stains to demonstrate basal lamina[10] and mast cells[11] were employed on serial sections. About 60-90 serial sections (including special stains) on each case were examined.

Results

In this serial section study, the method described above enabled us on every section to examine the vitiliginous and the adjacent normal skin portion, as well as the border area and appreciate the relative changes between normal and vitiliginous skin. The pigment and the clear cells in the basal layer were reduced or absent in the vitiliginous skin. Reduced or absent DOPA reaction was also a conspicuous feature (Fig. 1). The border between vitiliginous and normal skin revealed collection of mononuclear cells at the dermo-epidermal junction (Fig 2). These mononuclear cells were found to be predominently small lymphocytes with occasional histiocytes lying amongst them. They were situated in close proximity with the basal layer cells (Fig 3) and sometimes lying intra-epidermally i.e. within the basal lamina

Fig. 1 DOPA positive basal layer of the normal skin. But the adjacent vitiliginous skin on the left side shows complete DOPA negative basal layer (DOPA original magnification × 100).

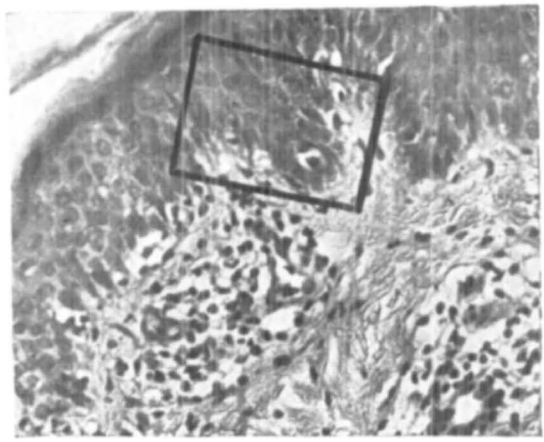

Fig. 2 Mononuclear hugging at the dermo - epidermal junction between the vitiliginous (left side) and the normal skin portion (right side). The normal skin contains melanin and clear cells in the basal layer which are absent in the vitiliginous skin. The mononuclear cells predominently small lymphocytes. Some of these cells are also lying intraepidermally. (Haemotoxylin and Eosin, original magnification × 100).

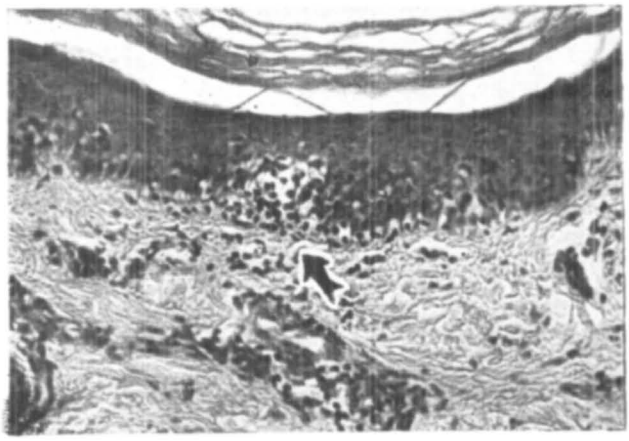

Fig. 3 The mononuclear cells exclusively small lymphocytes are lying intraepidermally (arrow) at the border between the vitiliginous (left side) and the normal skin (right side). The dermis underneath contains blood vessels with endothelial hyperplasia and perivascular infiltrates. (Haematoxylin and Eosin, Original magnification × 100).

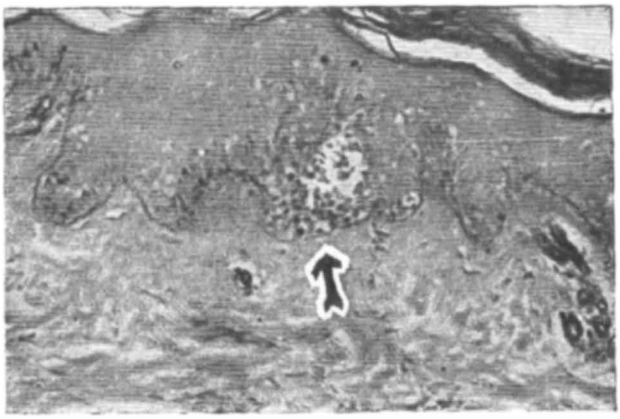

Fig. 4 The border area between the vitiliginous (right side) and the normal skin shows mononuclear cells lying within the basal lamina (arrow) (Per-iodic Acid Schiff, original magnification × 100).

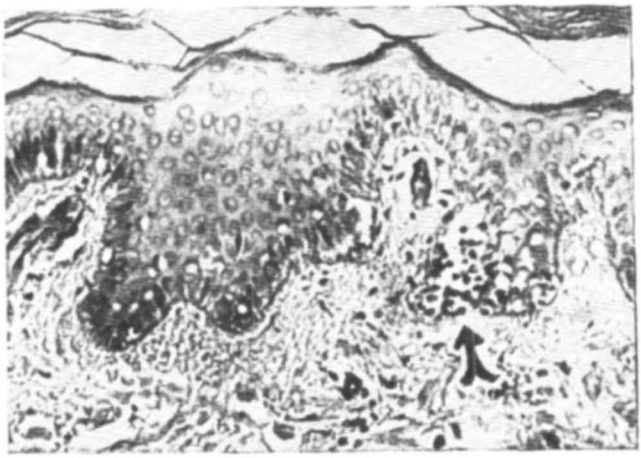

Fig. 5 The basal lamina is seen to be broken up at the site of mononuclear hugging (arrow) (Periodic Acid Schiff, original magnification × 250).

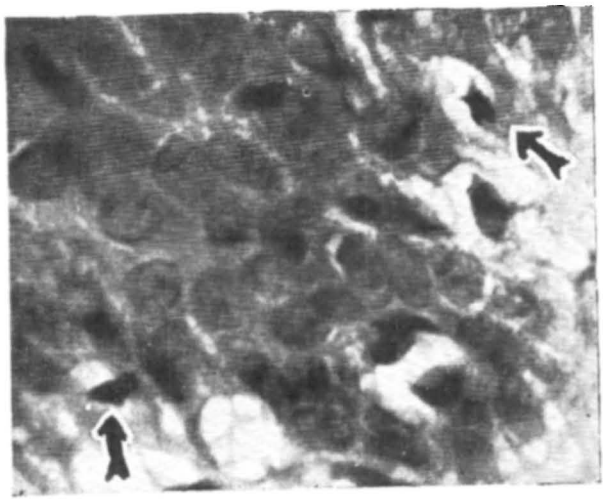

Fig. 6 (Enlarged view of the area marked in Fig. 2) The clear cells in the basal layer on the normal skin portion in close proximity to mononuclear hugging are undergoing mitosis (arrow) (Haematoxylin and Eosin, Original magnification × 450).

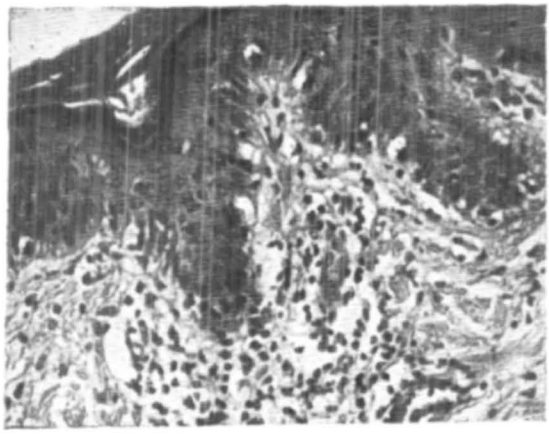

Fig. 7 A normal skin portion shows a microscopic area of depigmentation at the site of mononuclear collection around the rete ridge whereas the basal layer on either side contains clear cells and pigment (Haematoxylin and Eosin, original magnification × 100).

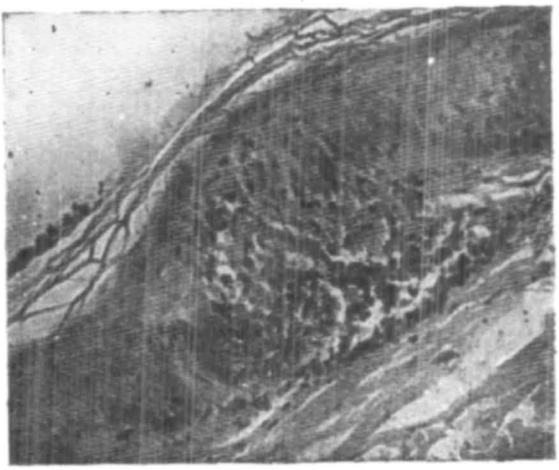

Fig. 8 A normal skin portion shows a small area of DOPA negative basal layer at the site of mononuclear hugging. The basal layer on either side is DOPA positive (DOPA, original magnification × 100).

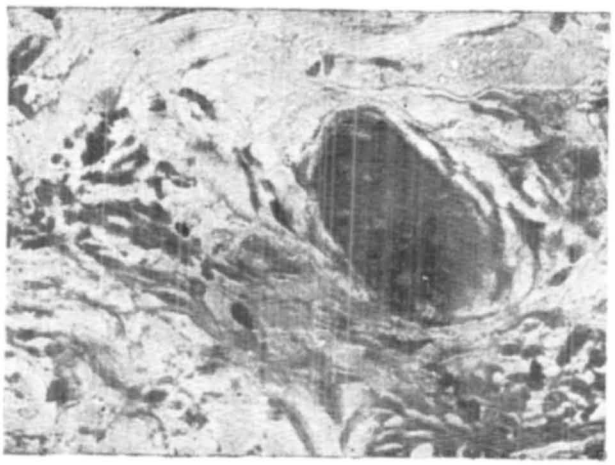

Fig. 9 Among the perivascular infiltrates, large number of mast cells are seen (darkly stained cells). (Azure-A, Original magnification × 250).

(Fig 4). On serial section examination with PAS stain, the line of cleavage or break in the basal lamina could also be observed (Fig 5). It was further observed that there was no pigment bearing cells or basal clear cells (melanocytes) towards the depigmented side (vitiliginous portion) of the mononuclear cell collection whereas on the pigmented side (normal skin portion) pigment bearing keratinocytes and the basal clear cells were seen as usual (Fig 2). The clear cells in the basal layer of the normal skin, lying in close vicinity of the mononuclear cell collection were found to be undergoing mitosis (Fig 6). This mononuclear cell colletcion, composed of predominently small lymphocytes with occasional histiocytes (at places exclusively small lymphocytes), shall hereafter be referred to in the text, as "mononuclear hugging" irrespective of the site at which they have appeared.

It must be mentioned that the features of mononuclear hugging were small microscopic areas of cellular interaction. If they were observed in one section, it was difficult to find their trace beyond the successive fifth or sixth serial section.

Besides the mononuclear hugging at the border of the vitiliginous patch, such tiny foci of hugging against the basal cell layer in the normal skin (which was considerably far from the vitiliginous area) were seen to produce depigmentation (Fig 7), and DOPA negative reaction of the basal Layer (Fig 8).

The dermal blood vessels under the sites of mononuclear hugging were conspicuous with prominent endothelial cells and perivascular infiltrates (Fig 3) composed of lymphocytes, histiocytes and occasional plasma cells. Among these infiltrates large number of mast cells were found as demonstrated by Azure-A stain (Fig 9).

The feature of mononuclear hugging at the border of the vitiliginous skin was invariably found in active and zosteriformis varieties, and was a less frequent finding in quiescent and improving ones (Table 3).

TABLE 3

Incidence of Mononuclear hugging in each clinical stage of Vitiligo

Clinical stages	Number of cases showing mononuclear hugging	Number of cases studied
Active	17	20
Quiescent	6	10
Improving	6	12
Zosteriformis	6	6
	35	48

Comments

The main finding in the present study is the demonstration of mononuclear hugging at the border of the vitiliginous patch with the normal skin. These mononuclear cells are predominently small lymphocytes. They are found at the dermo-epidermal junction and also intra-epidermally within the basal lamina as shown with PAS stain (Fig 4). It is further seen that they come to lie intra-epidermally by producing a cleavage in the basal lamina (Fig 5). This feature demonstrates the fact that the mononuclear cells perhaps need to establish a close contact with the basal layer. The contiguous basal keratinocytes, on either side of the mononuclear hugging, which constitute the larger cell population of the basal layer, do not show any histological abnormality except that on the vitiliginous side the keratinocytes do not contain melanin granules in their cytoplasm. The very change that is evident, is the absence of melanocytes and melanin on the affected side (vitiliginous portion) in contrast to their normal presence on the unaffected side (normal skin portion). It is, therefore, considered that the mononuclear cells at the border of the vitiliginous patch are possibly

concerned with the melanocytes and the melanin only. In the absence of a preceding clinical history of inflammation in the form of erythema, pain, swelling or itching and in the lack of previous systemic or topical therapy as treatment for vitiligo; the feature of mononuclear hugging cannot be considered as a secondary response to any nonspecific tissue damage or irritation. Further, their selective localisation at the border of a vitiliginous patch as tiny collections of almost exclusively small lymphocytes, is unlikely a pattern of tissue reaction seen in dermatitis. Thus we believe that the mononuclear cells are perhaps a specific tissue reaction and in view of their selective concern with the melanocytes and melanin, as discussed above, it is possible that they are specifically directed against the melanocytes only, leading to their selective destruction and the consequent disappearance of melanin from the epidermis. The finding of increased number of mitotic figures among the clear cells on the unaffected side in close vicinity of the mononuclear hugging (Fig 6) is further suggestive of the fact that since the attack on melanocytes continues at the border, the melanocytes on the healthy side manifest cellular unrest and undergo mitosis which is indicative of an attempt on the part of the survived melanocytes to meet the loss. From the foregoing discussion it appears logical to suggest that the mononuclear hugging at the border of a vitiliginous patch is perhaps directly responsible for the selective destruction of melanocytes and the subsequent elimination of melanin from the epidermis, thereby producing the depigmented patch of vitiligo. Thus, the increased incidence of mononuclear activity at the border in patients with active and zosteriformis varieties (Table 3) is natural because of the progressive nature of the lesions.

In view of the monomorphic cell composition (almost exclusively small lymphocytes) in mononuclear hugging, occurving invariably at the border of the growing vitiliginous lesions establishing a close contact with the target cell (melanocytes) and producing selective destruction of melanocytes (auto-destruction), we go further to suggest that this cellular interaction may perhaps be considered as a type of auto-immune delayed (cell mediated) hypersensitivity reaction. But this suggestion needs further work to demonstrate that the small lymphocytes involved in this tissue reaction are specific thymic derived cells (T-cell).

Similarly, mononuclear hugging against the basal layer has also been demonstrated around rete ridge (Fig 7) producing depigmentation in that site. Besides depigmentation, DOPA negativity of the basal layer has also been observed in areas of mononuclear hugging (Fig 8). But all these sites of involvement were parts of the normal skin and when the biopsy was taken, no apparent depigmentation on naked eye examination was visible over this portion of the skin. The mechanism of depigmentation in these sites can be explained in the similar way as it happens at the border of a vitiliginous patch. Their presence under normal skin portion, is suggestive of the fact that probably mononuclear hugging against the basal layer brings about depigmentation over a microscopic area of a normally pigmented skin and with the passing of time, when the depigmented area enlarges, it becomes visible to the naked eye as a vitiliginous patch.

One can appreciate now that the cellular activity between the melanocytes and the specific lymphocytes are tiny microscopic foci of interaction which is not sufficient to produce any detectable clinico-morphological change in the form of erythema, swelling or pain in a vitiliginous patch. The slow reacting auto-immune process perhaps

starts over a small area and then continues at the border of the lesion, thus manifesting a gradual increase in the size of the initial focus through months.

The increased number of mast cells in the perivascular location might be a result of chemotactic influence on these cells due to liberation of chemical substances i.e, lymphokines, from the site of cellular interaction.

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