



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Dendritic cells in cutaneous lupus erythematosus: a hint to the pathogenesis of lesions

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Dendritic cells in cutaneous lupus erythematosus: a hint to the pathogenesis of lesions / M. MORI; N. PIMPINELLI; P. ROMAGNOLI; E. BERNACCHI; P. FABBRİ; B. GIANNOTTI. - In: HISTOPATHOLOGY. - ISSN 0309-0167. - STAMPA. - 24:(1994), pp. 311-321.

Availability:

This version is available at: 2158/320389 since:

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

Dendritic cells in cutaneous lupus erythematosus: a clue to the pathogenesis of lesions

M. MORI, N. PIMPINELLI, P. ROMAGNOLI*, E. BERNACCHI†, P. FABBRI† & B. GIANNOTTI

*II Dermatology Clinic, *Department of Human Anatomy and Histology, Section 'E Allara', and †Institute of Dermatology and Venereology, University of Florence, Florence, Italy*

Date of submission 23 July 1993

Accepted for publication 1 November 1993

MORI M., PIMPINELLI N., ROMAGNOLI P., BERNACCHI E., FABBRI P. & GIANNOTTI B.

(1994) *Histopathology* 24, 311-321

Dendritic cells in cutaneous lupus erythematosus: a clue to the pathogenesis of lesions

In view of the critical role of dendritic cells in immune mediated skin diseases, we have investigated the membrane antigen patterns and ultrastructure of cutaneous dendritic cells in eight patients with chronic discoid lupus erythematosus and five with subacute cutaneous lupus erythematosus. In the lesional epidermis, the expression of HLA-DR antigens by epidermal dendritic cells was reduced, as compared with perilesional, clinically normal skin. In addition, only few CD1a+ dendritic cells (Langerhans' cells), along with some CD11c+ and CD14+ cells (presumable precursors of Langerhans' cells), were found in atrophic areas of lesional epidermis. In contrast, the number of Langerhans' cells in non-atrophic areas of lesional epidermis was similar to that in perilesional skin. On electronmicroscopy, epidermal Langerhans' cells appeared depleted of organelles and dendrites and contained tubuloreticular inclusions. In the lesional dermis, both CD1a+ and CD36+ dendritic cells were found, associated with CD4+ and CD8+ T-cells, respectively. Moreover, CD11c+ and CD14+ cells were found around capillaries in the papillary dermis on electronmicroscopy. Indeterminate cells (dendritic cells with features of Langerhans' cell lineage, but apparently without Birbeck granules) and dendritic macrophages were found, associated with lymphocytes and mast cells. No cells with intermediate/transitional features between these two dendritic cell types were found. Conversely, peculiar dendritic cells—with short and blunt dendrites and cytoplasm containing many flat, rough cisternae, moderately well developed Golgi apparatus and no lysosomes—were found in the same location as the CD11c+ and CD14+ cells identified by light microscopy. These findings might be interpreted as follows: 1 the alterations in cytological differentiation and expression of functionally meaningful molecules by epidermal Langerhans' cells in cutaneous lupus erythematosus lesions suggest an impairment of their immunological efficiency; 2 in the lesional dermis of cutaneous lupus erythematosus, a CD4+ T-cell/CD1a+ dendritic cell-based, delayed-type immune response is possibly modulated by a suppressor T-cell circuit in which CD36+ dendritic cells may act as accessory cells; 3 CD11c+ and CD14+ cells with peculiar ultrastructure are possible precursors of both CD1a+ indeterminate cells and CD36+ dermal dendrocytes in the dermis.

Keywords: chronic discoid lupus erythematosus, subacute cutaneous lupus erythematosus, dendritic cells, Langerhans' cells, electronmicroscopy

Introduction

Skin lesions are a major finding in lupus erythematosus. In particular, two major forms of lupus erythematosus—with specific clinical, histological, and immunopatho-

logical features—predominantly or exclusively affect the skin and are grouped together as cutaneous lupus erythematosus: chronic discoid lupus erythematosus and subacute cutaneous lupus erythematosus^{1,2}. The pathogenesis of skin lesions in lupus erythematosus, and in particular the intimate relationships between UV-sensitivity and immunological injury, have still to be clarified². The deposition of immunoglobulins and

Address for correspondence: Dr M. Mori, Clinica Dermosifilopatica II, Università degli Studi di Firenze, Via della Pergola 58, 50121 Firenze, Italy.

complement components at the dermo-epidermal junction is a useful marker of lupus erythematosus, but this process is not likely to play an important and direct role in the pathogenesis of skin lesions, because immunoglobulins and complement deposits are usually also found in perilesional, clinically normal skin^{1,2}. The hypothesis of T-cell mediated cytotoxic damage of UV-modified basal keratinocytes is much more likely¹, and fits well with the finding of CD8+ T-cells³⁻⁸ and γ/δ T-cells⁹ closely apposed to damaged basal keratinocytes. In addition, the possibility of an anti-DNA antibody-dependent cellular cytotoxic (ADCC) reaction against UV-damaged basal keratinocytes, possibly mediated by natural killer cells, has been suggested by several authors¹⁰⁻¹².

The possible role of a functional impairment of epidermal Langerhans' cells in the pathogenesis of cutaneous lupus erythematosus has been suggested by *in vivo* studies, on the basis of their morphological alterations (reduction of dendriticity) and diminished expression of membrane ATPase activity and CD1a and HLA-DR antigens¹³⁻¹⁵. However, it is unclear how a functional impairment of Langerhans' cells would lead to the histological and clinical picture of the disease. In addition, the possible significance of other types of dendritic cells of the immune system has not been investigated so far. Therefore, we studied the antigenic and ultrastructural features of cutaneous dendritic cells in patients with cutaneous lupus erythematosus (eight with chronic discoid and five with subacute cutaneous lupus erythematosus). The study aimed to evaluate the possible relationship between different micro-environments (epidermis *v.* dermis, atrophic *v.* non-atrophic epidermis).

possible relevance of the different types of skin dendritic cells in the pathogenesis of cutaneous lupus erythematosus lesions.

Materials and methods

We studied 13 patients (six males, seven females; age range 18-51 years, median 34 years). Eight patients had chronic discoid lupus erythematosus, with no clinical or laboratory signs of extracutaneous disease; five patients had subacute cutaneous lupus erythematosus (two with psoriasiform pattern, three with annular polycyclic pattern), with no clinical signs of extracutaneous involvement. The diagnoses were established according to the clinical, histological and immunopathological criteria proposed by Sontheimer and co-workers¹. Subacute cutaneous lesions were preferentially distributed

on the trunk, shoulders and upper limbs, while chronic discoid lesions were typically located on the face, neck or scalp areas. Subacute cutaneous lesions were characteristically non-scarring and non-indurated, a feature particularly useful for differentiation between the early lesions of the two forms of lupus erythematosus¹⁶. Serologically, anti-nuclear antibodies were not found in chronic discoid lupus erythematosus patients. In the five subacute cutaneous lupus erythematosus patients, four patients had anti-nuclear antibodies, five had anti-Ro/SSA antibodies and one had anti-La/SSB antibodies as well.

Biopsies were taken under local anaesthesia from lesional and perilesional skin. Each tissue specimen was in part formalin-fixed and paraplast-embedded for routine histological examination, in part embedded in ornithine carbonyl transferase (OCT) (Tissue Tek, Miles Scientific, Naperville, IL, USA), snap frozen, and stored at -80°C until sectioning and preparation for routine immunofluorescence and immunohistochemistry.

Cryostat sections (6 μ m thick) were air dried, fixed in acetone for 10 min, and processed according to direct immunofluorescence and indirect peroxidase¹⁷ and alkaline phosphatase-anti-alkaline phosphatase (APAAP) methods¹⁸. Normal human lymph nodes were stained in parallel as positive controls. Sections incubated with an irrelevant, isotype-matched mouse antibody were used as negative controls. The step section method was used to evaluate results; serial sections of each tissue specimen were carefully evaluated by two of us (N.P. and M.M.). For quantitative analysis, the stained cells were counted in five consecutive sections

the number of stained cells overlying 100 basal cells. Only cells whose nuclei were contained in the plane of the section were considered. The results were scored independently by the two observers, and the resulting figures were averaged.

The antibodies used and their specificities¹⁹ are listed in Table 1. To rule out the possibility that CD36 staining was due to vascular staining, related to the strong expression of thrombospondin receptor by platelets, serial sections were incubated with biotin-conjugated *Ulex europaeus* agglutinin-I (UEA-I), a marker for endothelial cells, and processed according to an indirect immunoperoxidase technique¹⁷.

In addition, part of the biopsies from six patients were in part fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 mol/l cacodylate buffer, pH 7.4, followed by 1% OsO₄ in 0.1 mol/l phosphate buffer, pH 7.4, and

Table 1. Monoclonal antibodies used in this study

	Cluster designation	Source	Specificity ^{19,23}
T11	CD2	CC	Peripheral T-cells (E-rosette receptor)
T3	CD3	CC	Peripheral T-cells
T4	CD4	CC	Helper T-cells
OKT8	CD8	OD	Suppressor/cytotoxic T-cells
OKT6	CD1a	OD	Langerhans' and related cells
HLA-DR	—	BD	MHC class II molecules
CD16	CD16	DP	NK cells
NK	CD56	DP	NK cells
α/β TCR	—	E.B.*	α/β T-cell receptor
gamma/delta TCR	—	E.B.*	Gamma/delta T-cell receptor
anti-f.XIIIa	—	E.B.†	Dermal dendrocytes
OKM5	CD36	OD	Dermal dendrocytes (thrombospondin receptor)
CL-203.4	CD54	S.F.‡	ICAM-1
MIM24	CD11a	DP	LFA-1 (α subunit)
OKM1	CD11b	OD	β 1 integrin (monocyte macrophages)
LeuM5	CD11c	BD	β 1 integrin (monocyte-macrophages)
MIM23	CD18	DP	LFA-1 (β subunit)
LeuM3	CD14	BD	Monocytes

CC, Coulter Clone, UK; BD, Becton & Dickinson, Mountain View, CA, USA; OD, Ortho Diagnostic Systems, Raritan, NJ, USA; DP, Dakopatts, Denmark.

* Anti- α/β and -gamma/delta T-cell receptor; generous gift of Professor E.Berti, Milan, Italy.

† Anti-factor XIIIa (embedded tissue); generous gift of Professor E.Berti, Milan, Italy.

‡ Generous gift of Dr S.Ferrone, New York, USA.

embedded in Epon 812. Sections were stained with uranyl acetate followed by bismuth subnitrate or lead citrate and observed in Elmiskop I and 102 electron-microscopes, at 80 kV.

IDENTIFICATION AND CLASSIFICATION OF DENDRITIC CELLS

Anti-CD1a monoclonal antibody was used to identify the most reliable antigenic marker of dendritic cells of Langerhans' lineage. Antibody to S-100 protein was not used in this study because S-100 protein is less specific (the marker is shared by Langerhans' cells and melanocytes), less sensitive (especially in frozen material) therefore possibly disturbing the correct quantitative evaluation of dendritic cells of Langerhans' lineage. Strictly speaking, only cells containing Birbeck granules should be considered Langerhans' cells; the identification of all epidermal (and dermal, when present) CD1a+ cells as Langerhans' cells is an approximation, which is not generally accepted. CD1a+ dendritic cells not containing Birbeck granules should be more correctly interpreted as immediate precursors of Langerhans' cells, the so-called indeterminate cells²⁰, at least in the dermis. Indeed, the development of Birbeck granules is clearly related to the epidermal micro-environment^{20,21}.

and the absence of these peculiar cytoplasmic inclusions may be related to a too short homing in the epidermis or to morphological and/or functional alterations of the epidermis. Because of the above considerations, we have considered all CD1a+ dendritic cells as of Langerhans' cells lineage, and have defined them as Langerhans' cells in the epidermis and indeterminate cells in the dermis.

Anti-CD36 (thrombospondin receptor) and anti-factor XIIIa were used to identify the most reliable dendritic cells markers of so-called dermal dendrocytes, a specific subset of CD1a- CD11b- dendritic cells with ultrastructural features of dendritic macrophages. They are mostly perivascular in location, are identified in both the dermis²²⁻²⁵ and the lamina propria of the oral mucosa²⁶. These cells, found in increased numbers in various inflammatory dermatoses²³ are thought to play a role in the activation of suppressor circuits²⁷, by analogy with the CD36+, CD1a- dendritic cells which appear in the skin following UV irradiation^{28,29}.

Results

HISTOLOGY

A mononuclear cell infiltrate was found in the dermis and at the dermo-epidermal junction in all cases. The



Figure 1. Chronic discoid lupus erythematosus, lesional skin. The severely atrophic epidermis is devoid of CD1a staining cells. CD1a+ dendritic cells are present in the papillary dermis, in perivascular clusters (arrow). $\times 1440$.

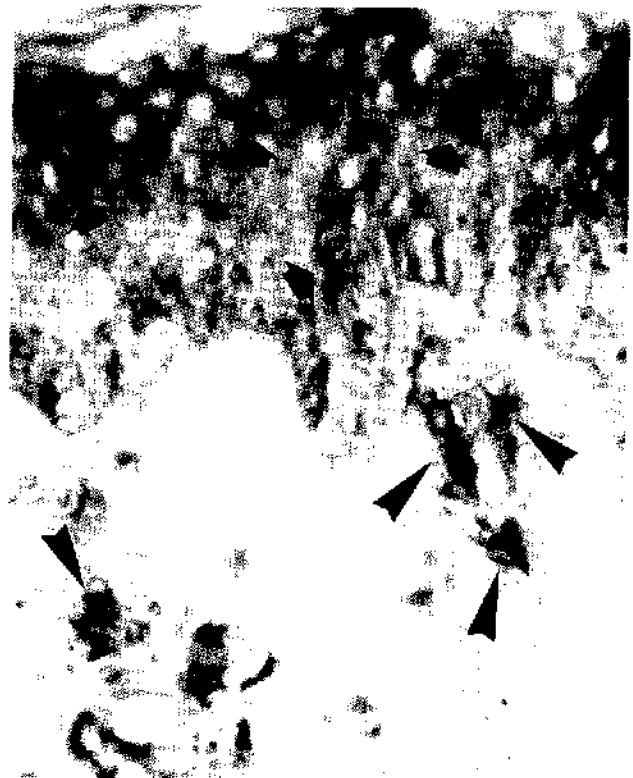


Figure 2. Chronic discoid lupus erythematosus, lesional skin. CD1a+ dendritic cells are shown both clustered in the dermis (arrowheads) and within non-atrophic epidermis (arrows). $\times 720$.

infiltrate was generally denser and deeper in chronic discoid than in subacute cutaneous lupus erythematosus, especially when comparing lesions of similar age. Hyperkeratosis of variable degree and periadnexal infiltration were constant findings in chronic discoid lupus erythematosus, while they were occasionally present in subacute cutaneous lupus erythematosus. Epidermal atrophy of variable degree—either associated with or independent of hyperkeratosis—was found in both forms of lupus erythematosus, even though it was obviously more marked in late chronic discoid lesions.

DIRECT IMMUNOFLOUORESCENCE

Lesional skin

Six of eight patients had IgG and/or IgM, and eight of eight had C3b staining at the dermo-epidermal junction, with a particulate ('lupus band') pattern. In subacute cutaneous lesions, we always found IgG and/or IgM and/or C3b particulate staining at the dermo-epidermal junction; we never observed particulate IgG epidermal staining, a pattern described recently and considered typical of subacute cutaneous lupus erythematosus¹⁶.

Perilesional skin

No immunoreactivity was found in any of the chronic discoid lupus erythematosus patients. In subacute cutaneous lupus erythematosus patients, IgG and/or IgM and/or C3b particulate staining was found at the dermo-epidermal junction. In no patient did we observe IgG particulate epidermal staining.

IMMUNOHISTOCHEMISTRY AND ELECTRONMICROSCOPY

In all cases, the cutaneous infiltrate consisted of T-cells and cells of monocyte-macrophage lineage (dendritic and non-dendritic). The results of the immunohistochemical and electronmicroscopic studies were substantially uniform among different patients, independent of lesion morphology (clinical and histological), distribution (sun-exposed *v.* non-exposed areas), direct immunofluorescence staining pattern, and serological profile of the patients.

Epidermis

In *lesional epidermis*, mainly CD8+ T-cells were found in the basal cell layers. Concerning CD1a+ dendritic cells, clear-cut differences were found—even in the same



Figure 3. Subacute cutaneous lupus erythematosus, lesional skin. HLA-DR positive cells infiltrate the dermo-epidermal junction. Note the virtual absence of HLA-DR+ dendritic cells in the suprabasal layers of epidermis. $\times 1440$.

specimen—between atrophic and non-atrophic areas. The number of CD1a+ dendritic cells/100 basal cells, and their dendriticity, were found to be inversely related to the degree of histologically assessed epidermal atrophy (Figure 1), without any relevant difference between subacute cutaneous and chronic discoid lupus erythematosus. In non-atrophic areas, the number of CD1a+ cells was similar to that found in perilesional epidermis (Figure 2). More importantly, we observed a clear-cut reduction in the number of HLA-DR+ dendritic cells as compared to CD1a+ dendritic cells (Figure 3), which suggests a reduced expression of HLA-DR antigen by epidermal CD1a+ dendritic cells. CD54 (ICAM-1)+ dendritic cells were not found in lesional epidermis. CD11c+ and CD14+ cells, round to bluntly dendritic in shape, were found in atrophic areas only, mainly in the basal layer (Figure 4). CD36+ or factor XIIIa+ dendritic cells were not identified in the epidermis. In this respect, cutaneous lupus erythematosus lesions seem to be different from other inflammatory dermatoses^{24,27,30}—including some photo-induced ones^{27,30}—in which such cells have been described in the epidermis. Keratinocytes stained for HLA-DR only seldom and focally, and never for CD54.



Figure 4. Chronic discoid lupus erythematosus, lesional skin. CD14+ cells are shown in both atrophic epidermis (arrow) and clustered in perivascular areas in the dermis (arrowheads). $\times 720$.

On electronmicroscopy, we found intra-epidermal mononuclear cells, characterized by poorly developed organelles, including some smooth tubules and vesicles, relatively small Golgi apparatus, few mitochondria, exceptional primary lysosomes but no Birbeck granules, and few and small indentations of the cell surface. The number of organelles and the degree of dendrite development varied amongst these cells, but were less than in fully developed Langerhans' cells (Figure 5). Many of these cells contained tubuloreticular inclusions (Figure 6). Lymphocytes infiltrating the epidermis were found to be either independent of other mononuclear cells or in contact with cells richer in organelles and in surface indentations. Due to the small sample size available for electronmicroscopy, it was impossible to check for differences between atrophic and non-atrophic areas.

In *perilesional epidermis*, the numbers of CD1a+ and of HLA-DR+ dendritic cells were similar, and similar to those found in clinically normal skin of healthy subjects³¹. CD54+ dendritic cells, although very sparse, were almost constantly observed here. CD11c+ and/or CD14+ cells were not found. No clear-cut differences



Figure 5. Chronic discoid lupus erythematosus, lesional skin. A mononuclear cell with a smooth surface in the epidermis is shown. The cytoplasm contains some rough and smooth endoplasmic reticulum, a small Golgi apparatus (arrowheads) and a few primary lysosomes. $\times 14\ 000$.



Figure 6. Chronic discoid lupus erythematosus, lesional skin. A dendritic cell within the epidermis contains a tubuloreticular inclusion (arrow). This cell did not contain Birbeck granules in the plane of section. The stars indicate keratinocytes. $\times 28\ 000$.

were found between subacute cutaneous lupus erythematosus (non-sun-exposed) and chronic discoid lupus erythematosus (sun-exposed) perilesional skin. The electronmicroscopic appearances were those of normal epidermis, including the presence of fully developed Langerhans' cells, with typical Birbeck granules.

Dermis

In *lesional dermis*, T-cells and dendritic cells were constantly found. Among infiltrating T-cells, the CD4/CD8 ratio varied from 3:1 to 2:1. An overwhelming CD8+ T-cell population was occasionally found in older lesions. CD1a+ dendritic cells were always found (Figures 1 and 2); their number and distribution roughly correlating with those of CD4+ T-cells. Both round and dendritic cells were strongly HLA-DR+ (Figure 3). CD54+ cells were found mainly in perivascular clusters, almost always colocalized with CD11a+ and CD18+ cells. Numerous CD11c+ cells and variable numbers of CD14+ cells, round to bluntly dendritic in shape, were constantly found in a perivascular location (Figure 4).

CD36+ dendritic cells and factor XIIIa+ dendritic cells were numerous (and in some specimens very numerous) in a perivascular location, and some of them were also found scattered between collagen bundles. CD11b+ cells were variable in number, and mainly dispersed among collagen bundles. The number of HLA-DR+ perivascular dendritic cells was roughly similar to that of CD36+ dendritic cells plus CD1a+ dendritic cells. No differences were found between subacute cutaneous and chronic discoid lupus erythematosus.

On electronmicroscopy we found clusters of indeterminate cells (dendritic cells rich in smooth endoplasmic reticulum and Golgi apparatus and with some primary lysosomes, but devoid of Birbeck granules, except in one case), macrophages (rich in secondary lysosomes and residual bodies), mast cells and lymphocytes. Tubuloreticular inclusions were found in all cases within many dendritic cells in the dermis, but not within macrophages. In some dendritic cells of one patient we found also cylindrical confronting cisternae (Figure 7). Besides the above described dendritic cells, further ones with

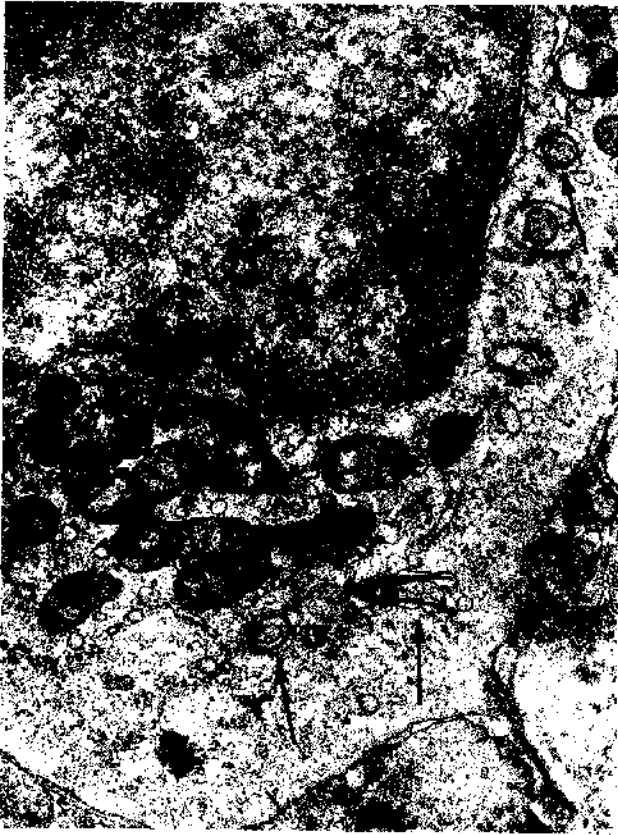


Figure 7. Chronic discoid lupus erythematosus, lesional skin. A mononuclear cell in the dermal infiltrate contains many mitochondria, scanty smooth and rough endoplasmic reticulum and cylindrical confronting cisternae (arrows). $\times 17\,000$.



Figure 8. Chronic discoid lupus erythematosus, lesional skin. Two cells with irregular cell projections are present. The cytoplasm contains some flat cisternae of rough endoplasmic reticulum and an inconspicuous (cell at bottom) to relatively well developed (cell on top, arrowhead) Golgi apparatus. $\times 8000$.

unique features were found in the papillary dermis, around blood capillaries and sometimes clustered with other cell types. These cells contained many narrow cisternae of rough endoplasmic reticulum, very few smooth vesicles and tubules and a relatively small Golgi apparatus (Figure 8).

In the *perilesional dermis*, we found a dendritic cell pattern similar to that observed in the clinically normal skin of healthy subjects^{32,33}. CD1a+ dendritic cells were observed in a perivascular location and were very rare. CD11b+ cells, CD11c+ cells, and CD14+ cells were found mainly dispersed among collagen bundles, while CD36+ and factor XIIIa+ dendritic cells were almost exclusively observed in a perivascular location. No significant differences were found between subacute cutaneous lupus erythematosus (non-sun-exposed) and chronic discoid (sun-exposed) perilesional skin. The electronmicroscopic findings were those of normal dermis, including a few dendritic macrophages around blood capillaries.

Discussion

Our results provide new information concerning the features of dendritic cells in cutaneous lupus erythematosus lesions. For the sake of clarity, we will first discuss our findings separately for the epidermis and dermis, and then try to use these findings as the basis for a hypothesis on the pathogenesis of cutaneous lupus erythematosus lesions.

Epidermis

Our results show that in cutaneous lupus erythematosus lesions the number and dendrite development of epidermal Langerhans' cells are inversely related to the degree of histologically assessed epidermal atrophy and are not reduced in non-atrophic areas. Therefore, the previously claimed overall reduction in the number of epidermal Langerhans' cells¹¹⁻¹⁵ is possibly related to the average figure resulting from both atrophic and non-atrophic areas.

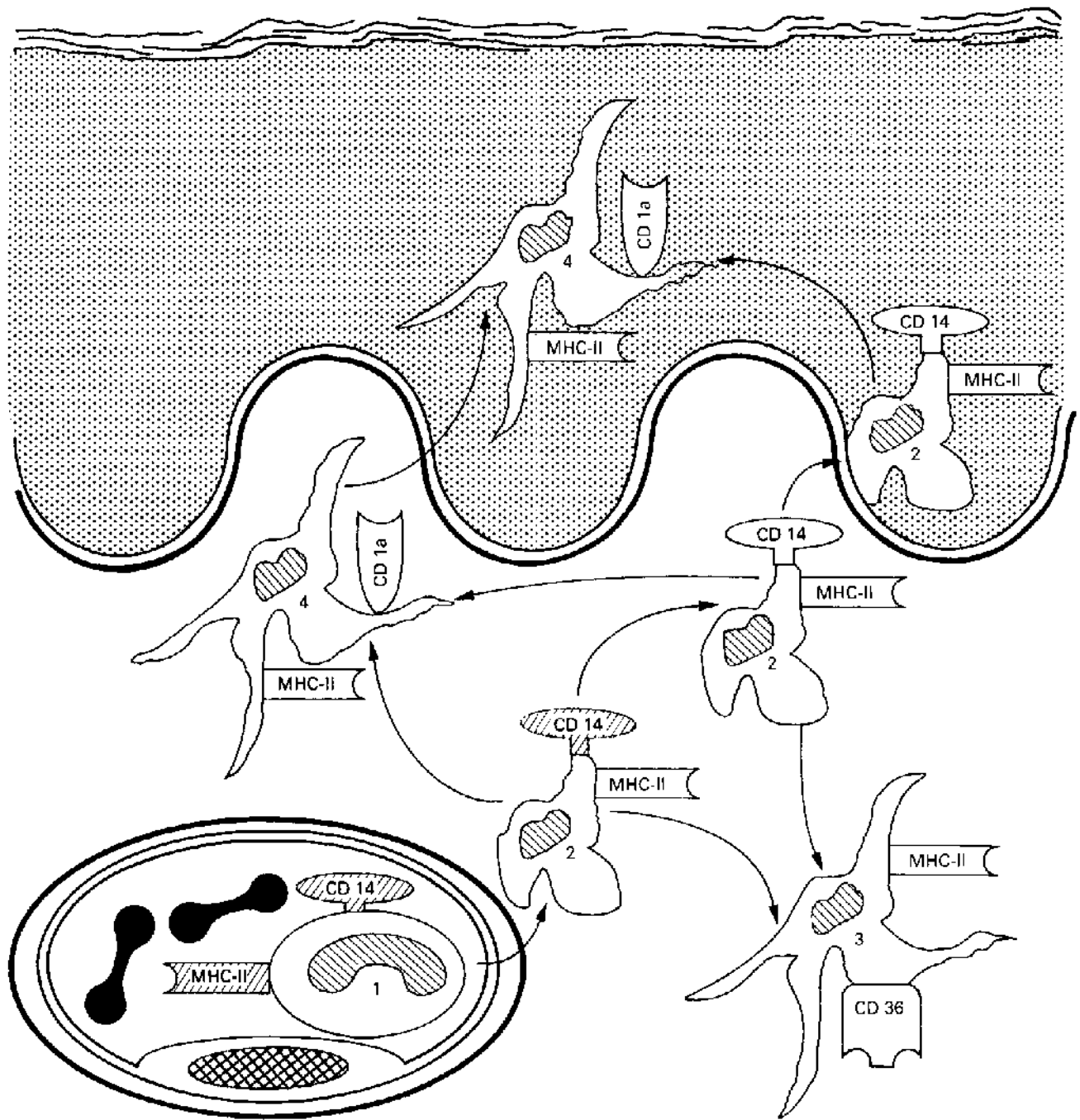


Figure 9. Diagram illustrating a possible differentiation pathway of skin dendritic cells. **1** Circulating precursors of skin dendritic cells are monocytoid cells, which weakly express HLA-DR and CD14 antigens. **2** Blunt dendritic cells in the dermis and, in some instances, in the basal epidermal layer show enhanced expression of HLA-DR and, when the recruitment occurs slowly as in certain chronic dermatoses (including cutaneous lupus erythematosus), CD14 antigens. They contain prominent rough and smooth endoplasmic reticulum and Golgi apparatus, few primary lysosomes and variably numerous endocytic pits and vesicles. **3** Dermal dendrocytes (dendritic macrophages) differentiate in the dermis by expressing CD36 antigen and developing many primary and secondary lysosomes. **4** Indeterminate cells differentiate in the dermis, or directly in the epidermis, by expressing CD1a antigen, and fully acquire the features of Langerhans' cells (developing Birbeck granules) in the epidermis. In normal skin, indeterminate cells differentiate rapidly and, as a rule, in the dermis; therefore, blunt dendritic CD14+ cells are rarely detectable in the dermis, and not at all in the epidermis. Epidermal atrophy, and other conditions of altered epithelial micro-environment, would hamper the terminal differentiation of Langerhans' cells in the epidermis, so that CD14+ monocytoid precursors are recognizable.

Electronmicroscopically, we found epidermal dendritic cells with features of incomplete differentiation (little dendrite development, few Birbeck granules); by immunohistochemistry, we found epidermal CD11c+ and CD14+ cells restricted to atrophic areas. A degree of similarity exists between the findings in atrophic epidermal areas of cutaneous lupus erythematosus and the lesional epithelium of oral hairy leucoplakia³⁴, a peculiar disease of immunodeficient patients characterized by Epstein-Barr virus-related changes. In both lesions, we found a reduced number of CD1a+ dendritic cells and the presence of CD14+ monocytic precursors on immunohistochemistry, and incomplete cytological differentiation of dendritic cells on electronmicroscopy.

Another interesting finding is that the HLA-DR+/CD1a+ dendritic cell ratio in lesional epidermis was always lower than in perilesional epidermis, where the HLA-DR expression by CD1a+ Langerhans' cells did not seem to be affected as compared to the normal skin of healthy subjects. These findings resemble those in the atrophic central part of lupus erythematosus-like lesions experimentally induced in MRL/lpr mice, where a decreased density of Ia+ and ATPase+ dendritic cells is observed^{35,36}.

On the basis of the light and electronmicroscopic evidence, we would like to conclude that the terminal differentiation and expression of functionally meaningful molecules of epidermal dendritic cells is clearly disturbed in cutaneous LE lesional skin. The alteration of the epidermal micro-environment (i.e. atrophy) influences even more heavily the terminal differentiation of epidermal dendritic cells.

Dermis

In the lesional dermis, indeterminate cells (CD1a+ dendritic cells with ultrastructural features of Langerhans' cells, but apparently without Birbeck granules) were constantly observed in lesional skin, as expected, in close association with CD4+ T-cells. According to a careful evaluation in serial sections, both CD1a+ dendritic cells and CD4+ T-cells strongly expressed HLA-DR antigens, as is typical of T-cell mediated reactions. Dermal dendrocytes²² (CD36+ and factor XIIIa+ cells with electronmicroscopic features of dendritic macrophages) were also present and numerous in lesional dermis, both in perivascular locations (very numerous) and scattered between collagen bundles (sparse). Their number was consistently higher in lesional than in perilesional skin; in the latter, they were found in number and distribution (i.e. perivascular) similar to those described in normal skin²²⁻²⁵ and oral mucosa²⁶. The significance of the observed increase in the number of dermal dendrocytes is currently not known; they may

serve as accessory cells for suppressor T-cell circuits, as suggested by others^{27,29}. In this case, the increase in the number of CD36+ dermal dendrocytes paralleling that of CD1a+ indeterminate cells might hint at a balance between these two cell types in the regulation of local immune responses.

Perivascular CD11c+ cells and CD14+ cells were constantly found in lesional skin. In the same location, we found cells with unique features on electronmicroscopy. The antigenic profile and structure of these monocytoïd cells and their location, as a rule close to capillaries, lead to the proposal that these cells are possible precursors to both CD1a+ indeterminate cells and CD36+ dermal dendrocytes (Figure 9). In our opinion, this hypothesis is supported by the fact that cells with antigenic or ultrastructural features intermediate between indeterminate cells and dermal dendrocytes were never found in this study.

A picture similar to that described above was found in the papillary dermis of specimens taken from early lesions of acute graft-versus-host disease³⁷ and erythema multiforme (data to be published), diseases characterized by a rapid increase in the number of cutaneous CD1a+ and CD36+ dendritic cells. In these conditions, however, cells around blood capillaries weakly express CD14 antigen. It might be hypothesized that there could be a single precursor of indeterminate cells and dermal dendrocytes, which would express CD14 intensely when the recruitment and intradermal differentiation occur slowly (as in cutaneous lupus erythematosus or lichen planus), and express this antigen weakly when these processes occur rapidly as in graft-versus-host disease and erythema multiforme; this hypothesis (Figure 9) would be in line with the finding that circulating progenitors of dendritic cells express CD14 weakly.³⁸

On the basis of our findings, we would like to propose one possible interpretation for the pathogenesis of lupus erythematosus lesions: 1 a delayed-type immune response, driven by CD1a+ dendritic cells which in turn stimulate CD4+ T-cells, occurs in the dermis and at the dermo-epidermal junction, possibly following UV-induced basal keratinocyte damage; 2 this response is possibly modulated by a suppressor CD8+ T-cell reaction—CD36+ dendritic cells may act as accessory cells for this circuit; 3 progenitors of dendritic cells enter the dermis and presumably differentiate continuously within lupus erythematosus lesions; 4 the cell-mediated immune response arising from the dermis might be responsible for further keratinocyte damage, leading to an alteration of the epidermal micro-environment. When severe, this alteration would, in turn, cause the observed impairment of Langerhans' cells differentiation within the epidermis.

Acknowledgements

The authors gratefully thank Mr G.Orlandi (Institute of Dermatology and Venereology), Mr D.Guasti and Mrs L.Calosi (Department of Human Anatomy and Histology) for excellent technical assistance. This research was supported by the Italian Ministry of University, Science and Technology (University funds, 60% and 40%) and National Research Council (grant no. 93.00402.CT04).

References

- Sonthheimer RD, Rothfield N, Gilliam JN. Lupus erythematosus. In Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF eds. *Dermatology in General Medicine*. New York: McGraw-Hill, 1987; 1816-1834.
- Norris DA, Lee LA. Pathogenesis of cutaneous lupus erythematosus. *Clin. Dermatol.* 1985; 3: 20-35.
- Bjerke JR. Subpopulations of mononuclear cells in lesions of psoriasis, lichen planus and discoid lupus erythematosus studied using monoclonal antibodies. *Acta Derm. Venereol. (Stockh.)* 1982; 62: 477-483.
- Andrews BS, Schenk A, Barr R, Friou G, Mirick G, Ross P. Immunopathology of cutaneous human lupus erythematosus defined by murine monoclonal antibodies. *J. Am. Acad. Dermatol.* 1986; 15: 474-481.
- Bos JD, Emsbroek JA, Krieg SR. T-cell subsets and Langerhans cells in cutaneous lupus erythematosus. In MacDonald DM ed. *Immunology, Vasculitis and Autoimmune Disease*. London: Butterworths, 1984; 261-266.
- Synkowski DR, Provost TT. Characterization of the inflammatory infiltrate in lupus erythematosus using monoclonal antibodies. *J. Rheumatol.* 1983; 10: 920-924.
- Kouchiyama A, Oka D, Ikei H. T cell subsets in lesions of systemic and discoid lupus erythematosus. *J. Cutan. Pathol.* 1985; 12: 491-499.
- Viljaranta S, Ranki A, Kariniemi AL, Nieminen P, Johansson L. Distribution of natural killer cells and lymphocyte subclasses in Jessner's lymphocytic infiltration of the skin and in cutaneous lesions of discoid and systemic lupus erythematosus. *Br. J. Dermatol.* 1987; 116: 831-838.
- Volc-Pikatzer B, Anegg B, Milota S, Plekl W, Fischer G. Accumulation of gamma/delta T-cells in chronic cutaneous lupus erythematosus. *J. Invest. Dermatol.* 1993; 100: 84s-91s.
- Nataf PG, Tan EM. Experimental skin lesions in mice resembling systemic lupus erythematosus. *Arthritis Rheum.* 1973; 16: 579-589.
- LeFeber WP, Norris DA, Ryan SR et al. Ultraviolet light induces binding of antibodies to select nuclear antigens on cultured human keratinocytes. *J. Clin. Invest.* 1984; 74: 1545-1550.
- Aiba S, Yoshie O, Tomita Y. Cross-reactivity of murine monoclonal anti-DNA antibodies with human and murine skin: a possible pathogenetic role in skin lesions of lupus erythematosus. *J. Invest. Dermatol.* 1989; 93: 739-745.
- Sonthheimer RD, Bergstresser PR. Epidermal Langerhans cell involvement in cutaneous lupus erythematosus. *J. Invest. Dermatol.* 1982; 79: 237-243.
- Bergroth V, Kontinen YT, Johansson E. Langerhans cells in SLE skin. *Scand. J. Rheumatol.* 1985; 14: 411-416.
- Shiohara T, Moriya N, Tanaka Y et al. Immunopathologic study of lichenoid skin diseases: correlation between HLA DR positive keratinocytes or Langerhans cells and epidermotropic T cells. *J. Am. Acad. Dermatol.* 1988; 18: 67-74.
- David-Bajar KN, Bemion SD, DeSpain JD, Colitz LE, Lee LA. Clinical, histologic, and immunofluorescent distinctions between subacute cutaneous lupus erythematosus and discoid lupus erythematosus. *J. Invest. Dermatol.* 1992; 99: 251-257.
- Iisu SII, Raine L, Fanger H. Use of avidin-peroxidase-complex (ABC) in immunoperoxidase techniques. A comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* 1981; 29: 1149-1153.
- Cordell JL, Folini B, Erber WN, Stein H, Mason DY. Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal antialkaline phosphatase (APAAP complexes). *J. Histochem. Cytochem.* 1984; 32: 219-226.
- Knapp W, Darken B, Rieber P et al. CD antigens 1989. *Am. J. Pathol.* 1989; 135: 420-421.
- Romagnoli P, Pimpinelli N, Lotti T. Dendritic cells in the skin and oral mucosa. *Ital. Gen. Rev. Dermatol.* 1991; 28: 49-79.
- Bani D, Moretti S, Pimpinelli N, Giannotti B. Differentiation of monocytes into Langerhans cells in human epidermis. An ultrastructural study. In Thivolet J, Schmitt D eds. *The Langerhans Cell*. London: J.Libbey Eurotext Ltd, 1988. coll. INSERM 172; 75-83.
- Headington JT. The dermal dendrocyte. In Callen JP, Dahl MV, Goltz LF et al. eds. *Advances in Dermatology*. Chicago: Year Book Medical Publishers, 1986; 1: 159-171.
- Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ, Headington JT. Characterisation of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br. J. Dermatol.* 1989; 121: 421-431.
- Soyer HP, Smolle J, Kerl H. Distribution patterns of the OKM5 antigen in normal and diseased human epidermis. *J. Cutan. Pathol.* 1989; 16: 60-65.
- Headington JT, Cerio R. Dendritic cells and the dermis: 1990. *Am. J. Dermatopathol.* 1990; 12: 217-220.
- Pimpinelli N, Borgognoni L, Riccardi R et al. CD36(OKM5)+ dendritic cells in the oral mucosa of HIV- and HIV+ subjects. *J. Invest. Dermatol.* 1991; 97: 537-542.
- Weiss JS, James WD, Cooper KD. Melanophages in inflammatory skin disease demonstrate the surface phenotype of OKM5+ antigen-presenting cells and activated macrophages. *J. Am. Acad. Dermatol.* 1988; 19: 633-641.
- Cooper KD, Neises GR, Katz SI. Antigen-presenting OKM5+ melanophages appear in human epidermis after ultraviolet irradiation. *J. Invest. Dermatol.* 1986; 86: 363-370.
- Baadsgaard O, Fox DA, Cooper KD. Human epidermal cells from ultraviolet light-exposed skin preferentially activate autoreactive CD4+ 2H4+ suppressor/inducer lymphocytes and suppressor/cytotoxic CD8+ lymphocytes. *J. Immunol.* 1988; 140: 1738-1744.
- Lisby S, Ralfkiaer E, Hansen ER, Vejlsgaard GL. Keratinocyte and epidermal leukocyte expression of CD36 (OKM5) in benign and malignant skin diseases. *Acta Dermatol. Venereol. (Stockh.)* 1990; 70: 18-22.
- Ashworth J, Turbill ML, Mackie R. The distribution and quantification of the Langerhans cell in normal human epidermis. *Clin. Exp. Derm.* 1986; 11: 153-158.
- Bos JD, Kapsenberg ML. The skin immune system (SIS). Its cellular constituents and their interaction. *Immunol. Today* 1986; 7: 235-240.
- Weber-Matthiesen K, Sterry W. Organization of the monocyte/

- macrophage system of normal human skin. *J. Invest. Dermatol.* 1990; **95**: 83-89.
34. Riccardi R, Pimpinelli N, Borgognoni L *et al*. Morphology and membrane antigen of non-lymphoid accessory cells in oral hairy leukoplakia. *Hum. Pathol.* 1990; **21**: 897-904.
 35. Kanauki H, Furukawa F, Imamura S. Characterization of cutaneous infiltrates in MRL/lpr mice monitored from onset to the full development of lupus erythematosus-like skin lesions. *J. Invest. Dermatol.* 1991; **96**: 478-483.
 36. Kanauki H, Furukawa F, Horiguchi Y, Horio T, Imamura S. Distribution of Langerhans cells in skin lesions of MRL/l mice. *Arch. Dermatol. Res.* 1988; **280**: 327-331.
 37. Pimpinelli N, Romagnoli P, Bosi A *et al*. Dendritic cells in the skin after allogeneic bone marrow transplantation. *Eur. J. Dermatol.* 1993; **3**: 310-317.
 38. Thomas R, Davis LS, Lipsky PE. Isolation and characterization of human peripheral blood dendritic cells. *J. Immunol.* 1993; **150**: 821-834.