Organization of the Monocyte/Macrophage System of Normal Human Skin

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Monocytes and macrophages are known to be important for a variety of functions; however, whereas epidermal Langerhans cells have been studied in great detail, few data are available for the dermal monocyte/macrophage system. Therefore we investigated the density, distribution, and phenotype of dermal macrophages in normal human skin using a panel of monoclonal antibodies for single and double labeling.

We demonstrate here that within normal human dermis macrophages reside with a remarkable density. Principally, these cells exhibit the phenotype of the phagocytic macrophage system (CD11c+, KiM8+), whereas members of the immune phagocyte system (CD11c+, KiM8−) are absent from normal dermis with the exception of a few Langerhans cells in the papillary body. Within the dermal phagocytic macrophage system we uncover an unexpected phenotypical and morphologic heterogeneity, which correlates with the tissue localization.

This study provides a basis for investigating the participation and change of the dermal macrophage system in cutaneous disorders. *J Invest Dermatol 95:83–89, 1990*

Over the last 20 years, numerous studies have established the importance of the epidermis as an immunologically active organ [1–4]. Langerhans cells take up antigens penetrating the epidermis, and, after migration to the regional lymph nodes via the dermal lymphatics, present them to unprimed or primed T cells. Clonally expanded primed T cells are then available for migration to the site of antigen exposure. A second macrophage-related epidermal-cell population characterized by the phenotype OKM5+, OKM1−, CD1a− occurs in several skin diseases [5,6] and has been shown to be a potent stimulator in autologous mixed lymphocyte reactions [7,8]. Keratinocytes are capable of secreting a wide array of cytokines and can express class II antigens on their surface [9]; thus they are able to amplify epidermal immune mechanisms.

We have currently detailed knowledge concerning the fate of antigens penetrating the epidermis from the outside; however, antigens entering directly the dermis—for instance, during septicemia or deep intracutaneous injuries—do not enter into contact with the epidermal immune system primarily. Therefore it seems likely that other cells are responsible or at least integrated in the generation and maintainance of the dermal immune response. Probable candidates are the cells of the dermal monocyte/macrophage system, but little attention has been paid to this population.

During the last decades, knowledge about macrophages has been widely expanded. The classical concept, based on the investigations of Metchnikoff, suggested that the function of macrophages is defined by the uptake and destruction of foreign material. Further studies revealed many additional properties, such as the production of mediators and lymphokines as well as antigen presentation to T cells [10–13]. In addition, it has become clear that macrophages are heterogeneous according to a variety of parameters like localization [14–16], state of maturation or activation [17,18], and function [19].

Macrophage heterogeneity can be studied by several means. Functional assays are useful, when the isolation of cells is possible. This is the case for peritoneal and pulmonary macrophages as well as macrophages derived from peripheral blood monocytes. When these cells are exposed to appropriate stimuli (IFN, TNF, etc.), they undergo significant changes in phenotype and functional properties (adherence, antigen presentation, cytotoxicity) [20–22].

Today, numerous monoclonal antibodies (MoAb) directed against surface and cytoplasmatic molecules of macrophages are available; many of these molecules have been studied functionally. Such antibodies can be used in the analysis of the dermal macrophage system. In the present study, we have evaluated localization and density of cells expressing macrophage-associated antigens; we demonstrate that normal human dermis contains numerous macrophages far outnumbering those localized within the epidermis, and give evidence for an unexpected heterogeneity.

MATERIALS AND METHODS

Biopsies Punch biopsies of clinically normal skin from diverse anatomical sites were obtained from eight healthy volunteers after local anaesthesia. Age of patients varied between 20 and 57. Biopsies were snap frozen in liquid nitrogen and stored at −80°C. Prior to immunostaining, 6-μm cryostat sections were fixed in acetone for 10 min.

Single Labeling Sections were stained with a panel of antibodies (Table I) using a three-step peroxidase technique as described in detail elsewhere [24]. Briefly, fixed sections were sequentially incubated with MoAb, peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark) and peroxidase-conjugated goat

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Abbreviations:
aMLR: autologous mixed lymphocyte reaction
IFN: interferon
MoAb: monoclonal antibody
TNF: tumor necrosis factor
TSP: thrombospondin

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Table I. MoAb Used in This Study

MoAb	CD	MW	Comments	References
KiM6[a]	68	110	Pan-macrophage antibody reacting with macrophages but not lymphocytes, granulocytes or fibroblasts. Increasing antigen densities per cell during differentiation of monocytes into macrophages. Located intracytoplasmatically at the outer cell membranes of lysosoms.	[23–26]
KiM8[a]		30,32	Strongly reacts with all macrophages. Only exception: does not react with starry sky macrophages.	[24]
KiM1[a]	11c	95	Strongly reactive with tissue macrophages in all tissues except Kupffer cells of the liver and macrophages of splenic red pulp. Reactive with IDC. Not reactive with granulocytes. Reactive with some activated lymphocytes.	[24, 27, 28]
LeuM5[b]	11c	95	As KiM1, but weakly reactive with granulocytes.	[29–35]
KiM4[a]			Strongly reactive with follicular dendritic reticulum cells.	[28, 36]
LeuM3[b]	14		Reacts with monocytes, macrophages, and activated granulocytes. CD14 antigen is located on chromosome 5 in close relation to genes, encoding for mediators and growth factors. Expression of CD14 is down-regulated on macrophages by IFN-gamma, whereas HLA-DR is upregulated.	[30, 37]
OKM5[c]	36	88	Recognizes the thrombospondin receptor, present on platelets, monocytes, some macrophages, and several tissue components. A subpopulation of OKM1−/OKM5+ monocytes are highly potent T-cell stimulators	[6, 7, 8, 30]
OKM1[c]	11b	35	α-chain of C3bi-receptor (CR3) on monocytes, macrophages, and granulocytes.	[34, 38–40]
Leu6[b]	1a	49	Present on epidermal Langerhans cells, some dermal dendritic cells, some IDC, thymocytes.	
OKT6-Fitc	1a	49	As Leu6.	
LeuM1[b]	15		Present on monocytes, granulocytes, activated lymphocytes. Reed-Sternberg cells, few tissue macrophages.	[36, 41]
Mac387[d]			Expressed by the majority of macrophages within most tissues.	[42]
HLA-DR, -DQ, -DP (-Fitc)[b]			Class II molecules.	
RFD1[e]			Dendritic cells.	[43]
Dako-LC[d]	45	180–220	Bone-marrow–derived cells.	

[a] Kindly provided by Dr. Radzun, University of Kiel, Department of Pathology, FRG.
[b] Becton-Dickinson.
[c] Ortho.
[d] Dakopatts.
[e] Dr. Poulter, Department of Immunology, Royal Free Hospital, London.

anti-rabbit IgG (Medac, Hamburg, FRG). Peroxidase activity was visualized with 0.06% diaminobenzidine (Walter, Kiel, FRG) and hydrogen peroxide. Counterstaining was performed with hemalaun.

Double Labeling For immunohistochemical double staining the sections were first stained by a three-step peroxidase technique as mentioned above, followed by the detection of the second antigen using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method as previously described [44].

Briefly, peroxidase-stained sections were sequentially incubated with the MoAb, rabbit anti-mouse Ig (Dakopatts), APAAP-complex (alkaline phosphatase from Sigma, Munich, FRG; anti-AP kindly provided by Dr. Radzun, Department of Pathology, University of Kiel, FRG). Incubations with rabbit anti-mouse Ig and APAAP-complex were performed twice. Alkaline-phosphatase activity was visualized using naphthol-AS-phosphate and fast blue after blocking of endogeneous alkaline-phosphatase activity with levamisol (Sigma). Appropriate controls were performed to exclude cross reactivities between the secondary antibody of both staining steps.

Immunofluorescence double labeling was performed as follows: sections were incubated with the first MoAb followed by tetramethylrhodamine isothiocyanate- (TRITC) conjugated goat anti-mouse antibody (Medac) and fluorescein isothiocyanate- (FITC) conjugated second MoAb. Three 5-min washes with phosphate-buffered saline (PBS) were performed after each staining step.

Evaluation The density of positive cells within the upper and the lower dermis was determined using a Zeiss ocular grid. The data were then calculated for an area of 0.1 mm^2 of tissue section. Staining intensity was evaluated using immunocytochemical methods.

RESULTS

CD68 Anti-CD68 (KiM6) staining intensity of dermal macrophages was only moderate, in contrast to strongly positive, single monocytes in intravascular localization. The morphology of CD68+ dermal macrophages varied from oval to spindle-shaped, and some showed a more or less dendritic morphology. Endothelial cells weakly expressed the KiM6 antigen.

The density of CD68+ cells was significantly higher in the upper dermis than in the lower dermis (Fig 1). CD68+ cells were predominantly localized in the perivascular zone, where the cells also showed the highest KiM6 antigen expression.

Weakly CD68+ macrophages were also present within the papillary body, often lining the dermo-epidermal junction with a typical flat-shaped morphology. Similar cells were also present around the dermal adnexae, in particular near sweat glands and hair follicles.

A consistent finding within all biopsies was the absence of KiM6-positive cells from the epidermis and hair follicles.

CD14 Using LeuM3 MoAb, we found that the distribution and density of CD14 was similar to that of KiM6 (Fig 1); double-staining experiments confirmed this, as we found an almost complete

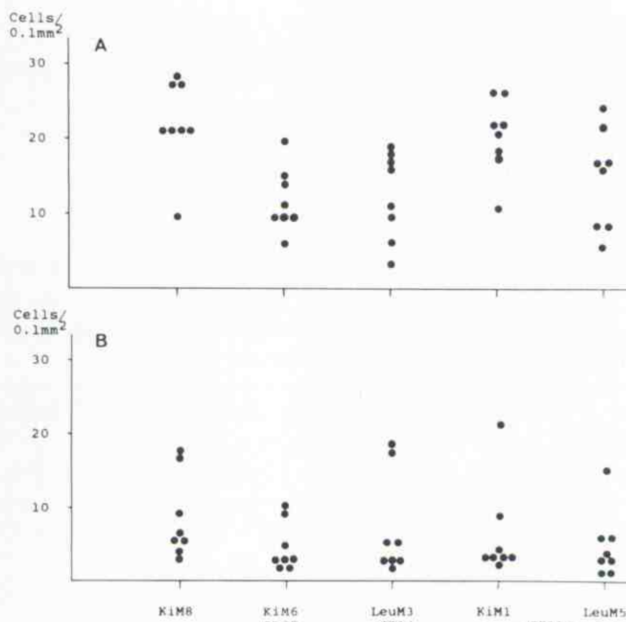


Figure 1. Macrophage density (positive cells/0.1 cm²) within the upper *(A)* and lower *(B)* dermis. Note the different densities for the two anti-CD11c antibodies.

coexpression of both antigens. Epidermal Langerhans cells were CD14−.

CD11b CD11b expression (OKM1 MoAb) was found on a considerable number of dermal macrophages, and gave a similar staining to KiM6. No epidermal cells showed CD11b expression. The staining intensity of CD11b+ cells was rather low.

KiM8 The staining pattern of KiM8, another broadly reacting anti-macrophage antibody, differed from KiM6. The density of KiM8+ macrophages was significantly higher (Fig 1), particularly within the connective tissue between the blood vessels. Positive cells were scattered all over the dermis (Fig 2A and 2B), with decreasing density towards the lower dermis. Double labeling with KiM6 and KiM8 revealed that all KiM6 cells coexpressed KiM8, while there was an additional population positive only for KiM8. These KiM6−/KiM8+ macrophages were localized in the lower dermis and showed a typical, large, and slightly dendritic appearance. As with KiM6, KiM8 did not recognize any epidermal cells.

KiM6+ and KiM8+ cells were double stained with anti-HLA-DR, -DQ, -DP and anti-CD1a (OKT6). About 70% of KiM6 and KiM8+ macrophages coexpressed HLA-DR, -DQ, and -DP antigens; staining intensity was weaker for DQ and DP as compared to DR. Remarkably, none of the KiM6+ or KiM8+ cells expressed CD1a. It should be mentioned here that blood vessels in normal skin — in particular, arteriols — also expressed HLA-DR and DQ. The expression of class II molecules on blood vessels was restricted to the upper dermis.

CD11c We have investigated the expression of CD11c on dermal cells using the antibodies KiM1 and LeuM5. An interesting diversity in the staining patterns of both antibodies was observed in normal skin.

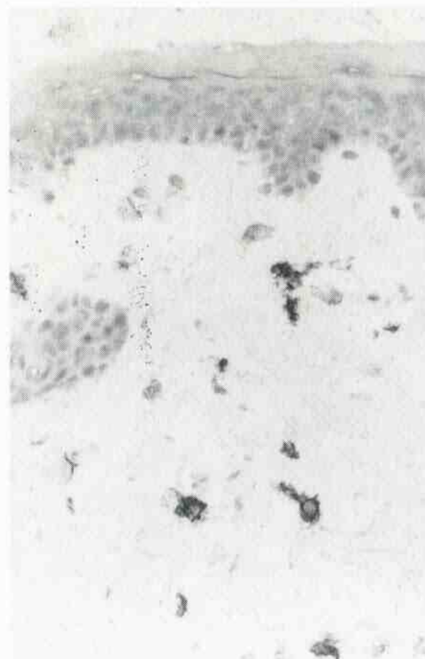
The density and distribution of KiM1+ cells is most similar to that of KiM8+ cells. Apparently all macrophages are intensively stained. Langerhans cells are negative or show extremely weak reactivities.

In contrast to KiM1, LeuM5+ cells were clearly confined to the perivascular area, the papillary body, and were present around the appendages (in particular, hair follicles) (Fig 3). Especially small lymphoid infiltrates occasionally present in normal skin were highly enriched with moderately dendritic LeuM5+ cells. Also, unlike KiM1, LeuM5 recognized epidermal Langerhans cells in varying degrees. This was confirmed by immunofluorescence double labeling with OKT6. Dermal LeuM5+/CD1a+ cells gave a stronger LeuM5 staining than those within the epidermis. Most KiM1+ or LeuM5+ cells showed a rather homogeneous staining of the whole cytoplasm compatible with a possible intracellular localization of the antigen.

The differences in the reaction patterns of the two CD11c MoAb KiM1 and LeuM5 were further investigated by immunohistochemical double staining. All macrophages in the papillary body and around blood vessels were KiM1/LeuM5 double positive. Intervascular macrophages were stained with KiM1, but were consistently LeuM5 negative.

CD36 We investigated the expression of CD36 on dermal macrophages using the antibody OKM5. Their density could not be determined, because it was sometimes impossible to differentiate

A



B

Figure 2. *A:* KiM8+ macrophages are distributed all over the dermis with decreasing densities towards the lower dermis. *B:* Strongly KiM8+ macrophages within the connective tissue between the blood vessels.

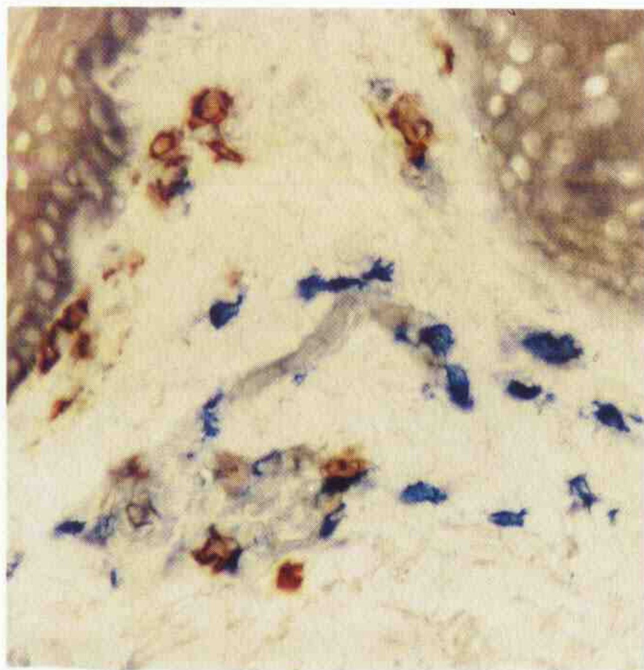


Figure 3. Double staining of CD11c with LeuM5 (brown) and KiM1 (blue). Perivascular and papillary body macrophages are double positive, whereas macrophages away from blood vessels are only stained with KiM1 (blue).

between macrophages and endothelial cells. The majority of macrophages that could be identified unequivocally, however, expressed the CD36 molecule. Most CD36+ cells were closely associated with blood vessels of the upper dermis, exhibiting strong staining of the cell membrane.

Further attention was paid to a population of OKM5+ cells, which is located within the basal layer of the epidermis or at the dermo-epidermal junction (Fig 4). Many of these cells were moderately dendritic; dendrites were always directed towards the epidermis. None of the antibodies used in this study was reactive with any of the OKM5+ cells in this area. Their density, highly variable in different specimens, did not correlate with the density of dermal macrophages.

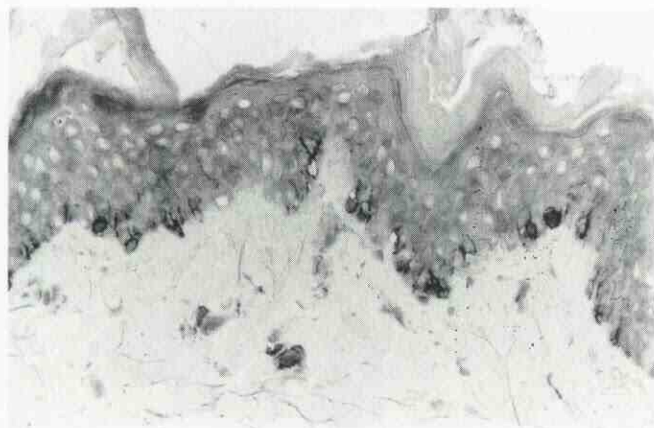


Figure 4. CD36+ cells at the dermo-epidermal junction. Note that dendrites are always directed into the epidermis. Some dermal CD36+ macrophages are also present.

CD1a CD1a+ Langerhans cells were present mostly within the suprabasal epidermis. Additionally, few CD1a+ cells occurred within the upper dermis, predominantly within the papillary body and near blood vessels. In contrast to the highly dendritic morphology of epidermal Langerhans cells, dermal CD1a positive cells were only rarely dendritic, sometimes even exhibiting a round morphology. Expression of KiM6, KiM8, and LeuM3 was never found on either epidermal or dermal CD1a+ cells. In contrast, CD11c (LeuM5 > KiM1) was variably expressed on CD1a+ cells; remarkably, CD1a+ dermal cells were more strongly stained by anti-CD11c than epidermal Langerhans cells.

Macrophage-Related Antigens, Absent from Dermal Macrophages in Normal Skin The following macrophage-related antigens were either extremely weakly expressed or even absent on normal dermal macrophages: CD15, Mac387, KiM4. Very few cells could be detected by RFD1.

CD45 Double staining of CD45 and KiM1, KiM6, and KiM8 antigens, demonstrated that the three antibodies of the KiM-series react exclusively with CD45-positive cells. Fibroblasts are not stained with KiM1, KiM6, or KiM8.

DISCUSSION

Our results show that there is a surprising density of macrophages per section area, especially within the upper dermis of normal human skin. Their density within the upper dermis is roughly about 30% of the density of Langerhans cells within the epidermis. However, if the absolute numbers of Langerhans cells and macrophages are compared, macrophages of the upper dermis clearly outnumber epidermal Langerhans cells, thus underlining their importance for the integrity of human skin. It remains to be analyzed how their number and density change when acute and chronic inflammatory skin diseases, or malignant cutaneous disorders, are present.

The morphology and phenotype of macrophages in the skin allows the differentiation of at least three types of macrophages (Fig 5). The first morphologic type is a rather flat, spindle-shaped macrophage which lines the blood vessels in the upper dermis and sometimes also the epidermis and adnexal structures (Fig 5). A second frequently observed morphologic type is a moderately dendritic macrophage, which extends dendrites between the few perivascular lymphocytes; sometimes these moderately dendritic cells also appear in the papillary body. Both types of macrophages exhibit a similar phenotype which can be summarized as Ki-M8+, Ki-M6+, CD11c+, and CD14+; additionally these macrophages express class II antigens. The third morphologic type of macrophages is found in the connective tissue between the blood vessels (intervascular macrophages) and is located throughout the entire dermis (Figs 5 and 6). These macrophages represent rather large cells, show a large cytoplasm with sometimes poorly defined borders, and are variably dendritic. In contrast to the other two morphologic types, these macrophages are not recognized by the LeuM5 antibody.

Finally, there are few Langerhans cells appearing within the papillary body, which are not as dendritic as those within the epidermis, but more often show a round-shaped morphology.

One of the major problems in the investigation of macrophage densities within tissues is the lack of specific pan-macrophage markers, which recognize all macrophages independent of their state of activation, and do not cross-react with other components such as hemopoetic cells, fibroblasts, endothelium, etc. A variety of MoAb, previously characterized as "macrophage specific," now appear to react with leukocyte adhesion molecules (CD11b, CD11c) or other nonrestricted antigens (CD14 on neutrophils) [30,31,35,38].

Of all antibodies investigated in this study, KiM8 had the broadest reactivity with dermal macrophages (Fig 1). Thus, KiM8 seems to be the appropriate antibody when investigating normal skin macrophages. Despite the broad reactivity of KiM8 with dermal macrophages, both Langerhans cells and the OKM5+ dendritic cells at the dermoepidermal junction do not express the KiM8 antigen; the nature of the latter remains to be further clarified.

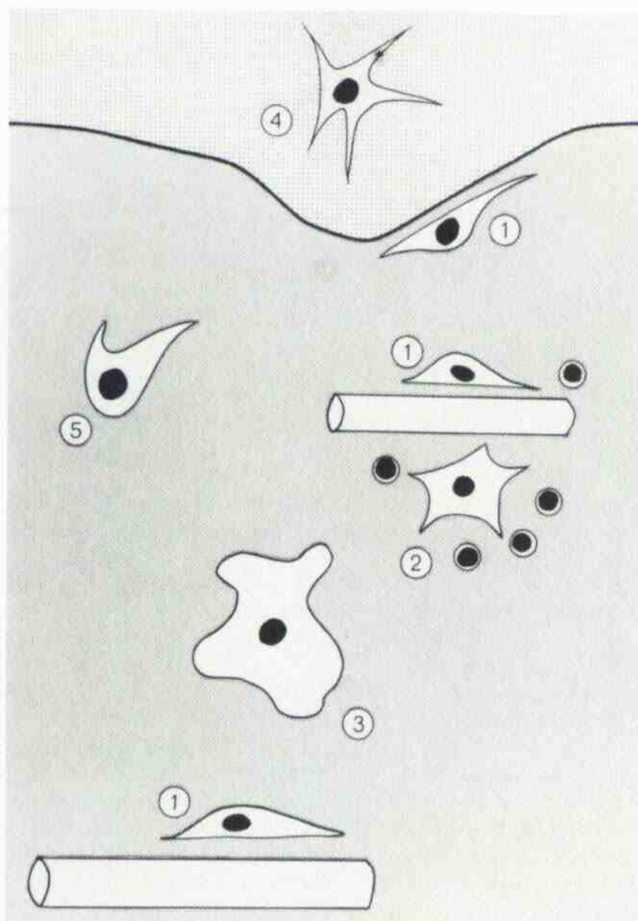


Figure 5. Distribution of morphologically and phenotypically distinct subsets of dermal macrophages 1: flat shaped cells, lining the blood vessels, epidermis and adnexal structures. 2: moderately dendritic, perivascular cells; similar cells are also present in the papillary body. 3: large intervascular macrophages in the connective tissue between the blood vessels. 4: highly dendritic epidermal Langerhans cells. 5: rarely dendritic dermal Langerhans cells.

In extracutaneous tissue, KiM8 recognizes all macrophages such as alveolar macrophages or von Kupffer cells in the liver [24]. The notable exceptions to KiM8 expression in the monocyte/macrophage family are the immune accessory cells, such as Langerhans cells, and, in the lymph node, both interdigitating reticulum cells in the T-cell areas and dendritic reticulum cells in the B-cell areas.

Therefore, Radzun and coworkers proposed to distinguish between the phagocytic macrophage compartment expressing the KiM8 antigen and other macrophage antigens like CD11c, and the immune macrophage compartment which fails to express the KiM8 antigen [24]. If this interpretation is transferred to the monocyte/macrophage system of the skin, in normal human skin there would exist only one type of immune macrophage, namely, the Langerhans cell. In the dermis, apart from a few Langerhans cells in the papillary body, all macrophages belong to the phagocytic macrophage compartment.

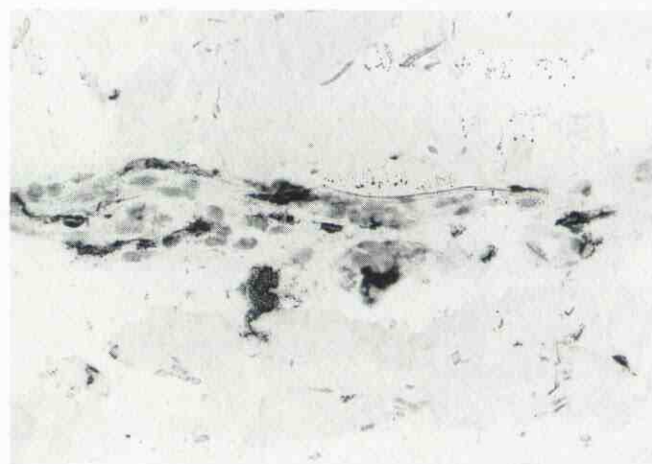


Figure 6. Flat-shaped, KiM8+ cells lining a dermal blood vessel. Similar cells are often lining the epidermis or adnexal structures. A single strongly KiM8+ tissue macrophage is also shown.

Several macrophage antibodies showed a strong reactivity with macrophages around the dermal blood vessels and those in the papillary body, whereas they had only a low (KiM-6, LeuM3) or even absent (LeuM5) reactivity with macrophages localized within the connective tissue between the blood vessels (intervascular macrophages) (Table II). We suggest that some of the antigens, which are all expressed on blood monocytes [23,30,35], are still retained in a perivascular localization, probably due to recent immigration into the skin, whereas they show a low expression or even disappear when macrophages stay within the skin for longer periods.

A minor subset of blood monocytes (OKM5+/OKM1−) has been shown to be an extraordinarily potent stimulator in the autologous mixed lymphocyte reaction (aMLR) in the absence of antigen [7]. OKM5 has become of recent interest in dermatologic research, since Cooper et al have isolated a population of OKM5+/OKM1−/DR+/DQ+/CD45+/CD1a-epidermal melanophages, which occur after UV exposure of normal skin and have antigen-presenting properties similar to those of the above-mentioned monocyte subset [6,30]. OKM5+ cells could not be isolated by these authors from non-irradiated normal epidermis.

We clearly show that in normal skin a considerable number of strongly OKM5+ cells with variable dendritic morphology is present at the dermo-epidermal junction, directing their dendrites towards the epidermis (Fig 4). However, these cells differ strikingly from the melanophages described by Cooper and co-workers in that they are DR-/DQ-/CD45-. This raises questions regarding the lineage and function of these cells. There is evidence that these cells are non-macrophages because except for CD36, they lack all macrophage-associated antigens so far investigated, and even appear not to be bone-marrow-derived (CD45-). Our data confirm similar results of Volk-Platzer and co-workers [45], who additionally showed

Table II. Reactivity of MoAb with Dermal Macrophages in Different Localizations and with Epidermal Langerhans Cells[a]

	KiM8	KiM6	LeuM3	KiM1	LeuM5	OKM5	OKM1	LeuM1
Perivascular cells	++	++	++	++	++	++	++	−
Papillary body	++	+	+	++	++	+	+	−
Intervascular Macrophages	++	+	+	++	−	+	+	−
Epidermal Langerhans cells	−	−	−	−[b]	+[c]	−	−	−

[a] ++, strong; +, weak; −, negative.
[b] In some specimens, a few Langerhans cells were weakly KiM1+.
[c] Percentage of LeuM5+ cells varied from 30 to 100% in different specimens.

by ultrastructural studies that these cells are morphologically different from melanocytes and melanophages (Stingl, personal communication).

CD36 functionally acts as the receptor for the extracellular matrix protein thrombospondin, which is produced by a variety of cells including endothelial cells, macrophages, and fibroblasts [46]. Keratinocytes, which express CD36 under certain conditions, attach and spread on TSP-coated dishes [47,48]. This can be inhibited by anti-TSP [48]. Because TSP is located in basement membranes [46], it can be speculated that the OKM5+ cells at the dermo-epidermal junction are attached to the epidermal basement membrane because of their strong expression of the thrombospondin receptor.

Most of the macrophages in the dermis express the CD36 molecule and thus resemble in their phenotype the majority of blood monocytes which express CD36 as well as CD11b [7]. Because monocytes have been shown to be important antigen-presenting cells both in allogeneic and autologous mixed leucocyte reactions, one can anticipate that dermal macrophages are capable of these functions as well. This is supported indirectly by recent observations of Sontheimer [49], who isolated a potent antigen-presenting macrophage population localized in the perivascular tissue of normal human dermis.

CD11c (150 kD) is one of three α-chains belonging to the β_2-integrin-family (leucocyte-integrin family), and is expressed together with the β_2-chain (CD18, 95 kD) as p150,95 on the cell surface [29,30,32,34]. All leucocyte integrins have been shown to play a role in the adhesion of leucocytes to endothelial cells, thereby enabling them to enter into various tissues [38]. Recent findings demonstrate that, apart from macrophages, the CD11c molecule may also be expressed on activated lymphocytes, on granulocytes, and in some B-cell lines [30,31]. Surprisingly, in our study we found different results when we used two different antibodies (KiM1 and LeuM5) against the CD11c molecule (Fig 3). On the one hand, LeuM5 recognizes Langerhans cells much better than Ki-M1 does; on the other hand, intervascular macrophages lacked LeuM5 reactivity but clearly were stained when using the Ki-M1 antibody. This differential reactivity with two CD11c antibodies, both of which have been clustered by the leucocyte typing conference III, may indicate a micro-heterogeneity in this molecule probably due to differential glycosylation of its extracellular portion [35]. As all perivascular macrophages express the LeuM5 antigen, whereas intervascular macrophages do not, it seems likely that during the residence of such macrophages within the skin for long periods, a modification of the CD11c molecule occurs, resulting in a loss of LeuM5 reactivity, although KiM1 reactivity remains unchanged. In contrast, Langerhans cells obviously express more CD11c molecules containing the epitope recognized by the LeuM5 antibody than the epitope recognized by Ki-M1. This possible modification of the CD11c molecule requires further investigation.

The CD11c molecule, which is expressed intracytoplasmatically within immature monocytes, undergoes a change in its glycosyl components before it is expressed on the surface of mature monocytes and macrophages [35]. Our finding that many of the intervascular macrophages within normal skin express CD11c predominantly within the cytoplasm, gives evidence that the CD11c molecule is not required for cell/cell interactions of resting macrophages.

It is well known that monocytes and macrophages change their phenotype upon activation and during maturation [43,50]. Antibodies have been raised against antigens, which are absent in monocytes but strongly expressed on most macrophages within tissues [51]. Other antibodies stain macrophages occurring preferentially in the down-regulatory phase of inflammation [16], while some are associated with acute inflammatory processes [52]. A variety of de novo expressed antigens have been shown to play a role in specific functions, for example, antigen presentation (i.e. HLA-DQ) and iron uptake (transferrin receptor) [53]. Examples of molecules like CD11c or CD45 being modified during activation or maturation also exist [33].

Unexpectedly, macrophages within normal skin did not express the Mac387 antigen, although they are reported to be Mac387+ in all other tissues [42]. Also, in many skin diseases Mac387 strongly stains macrophages in great numbers (staining of diseased skin was performed as a positive control). It will be interesting to study whether differential antigen expression of normal skin macrophages, as compared to other tissues, can be observed also with other monoclonal antibodies.

MoAb LeuM1 recognizes CD15 on granulocytes and activated monocytes but on few tissue macrophages [41]. Hofman and coworkers have shown that within the lymph node LeuM1+, cells are preferentially associated with the interfollicular area, which harbors the interdigitating reticulum cells [36]. We found that LeuM1 does not stain macrophages within normal skin, whereas positive cells can be detected within skin diseases. This finding supports our suggestion that normal skin does not contain cells from the immune macrophage compartment. Whether LeuM1+ cells within skin diseases phenotypically resemble interdigitating cells has to be further clarified.

The results presented in this study demonstrate that there is a large number of macrophages localized within normal human dermis. Although there is a moderate heterogeneity within these macrophages as to their morphology and their phenotype, they are all characterized by coexpression of CD11c (KiM1) together with Ki-M6 and Ki-M8 antigens. This phenotype has been shown to be the hallmark of the phagocytic macrophage compartment and contrasts with the phenotype of the T-cell – dependent immune macrophage compartment which lacks Ki-M6 and Ki-M8 antigens, but also expresses CD11c. The only member of this immune macrophage compartment within normal human skin seems to be the epidermal and dermal Langerhans cell. Based on this study we will be able to analyze the role of dermal macrophages in various cutaneous diseases, and to investigate whether, under certain conditions, cells belonging to the immune macrophage compartment also can be observed within the dermis.

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