Macrophages and Dendritic Cells Constitute a Major Subpopulation of Cells in the Mouse Dermis

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Macrophages and dendritic cells (DC) in tissues with close contact to the environment are of essential importance in host defense and are therefore present in sizeable numbers. Therefore, it is surprising that mononuclear phagocyte populations of the dermis have rarely been investigated in a quantitative manner. In this study, we examined mouse dermal skin immunophenotypically and related the observed numbers of observed cells to the total number of nucleated cells. These analyses show that about 70% of all dermal cells represent CD45$^+$ leukocytes. The vast majority of these cells ($\sim$60% of total) expresses the mononuclear phagocyte markers mMGL (ER-MP23), F4/80 and CD11b. In addition, these cells show avid phagocytic capacity and thus are identified as dermal macrophages. Different subpopulations can be defined using markers such as sialoadhesin, ER-HR3 and mSIGN-R1 (ER-TR9). Interestingly, MHC class II expression differs significantly between dermal cells from ear *versus* back skin. Moreover, we have identified small populations of dermal DC and migrating Langerhans cells (together $\sim$10% of total). In summary, our findings show that mononuclear phagocyte populations form the majority of dermal cells and thus have been clearly underestimated so far.

Key words: connective tissue cells/fibroblasts/phagocytes
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Mononuclear phagocytes, i.e. macrophages and dendritic cells (DC), are crucial initiators and regulators of both innate and adaptive host defense responses. As a consequence, especially tissues in close contact with the environment possess sizeable populations of mononuclear phagocytes. In the skin, different populations of such sentinel cells have been described: in the suprabasal layer of the epidermis, where Langerhans cells (LC) build up a network with their dendrites, and in the dermis, where interstitial dermal dendritic cells and dermal macrophages reside between extracellular matrix components. Dermal mononuclear phagocytes appear to be important for skin immunity, as they are highly competent in inducing an immune response without involvement of epidermal LC (Streilein, 1989; Allan *et al*, 2003). Surprisingly enough, dermal mononuclear phagocyte populations have never been investigated in a quantitative manner compared to the total amount of dermal cells. Therefore, we performed immunofluorescence double stainings on mouse ear and back skin sections using different macrophage and DC markers. To be able to compare the amount of stained cells to the total amount of dermal cells, we counterstained the sections with DAPI to identify all nucleated cells. Thereafter, we assessed the phenotype of 200–400 dermal nucleated cells, excluding cells in skin appendages.

Abbreviations: DC, dendritic cells; LC, Langerhans cells

Results and Discussion

The starting point in our analyses was the reactivity of the anti-mononuclear phagocyte antibody ER-MP23 (Jansen *et al*, 1994; Leenen *et al*, 1994). This antibody recognizes an epitope on mMGL (murine macrophage galactose/*N*-acetylgalactosamine-specific C-type lectin; Oda *et al*, 1988; McKnight and Gordon, 1998), also known as DC-ASGPR (dendritic cell asialoglycoprotein receptor, Valladeau *et al*, 2001), and has been described previously to label connective tissue macrophages strongly (Jansen *et al*, 1994; Leenen *et al*, 1994). Staining of skin sections for this marker revealed that a majority of all dermal cells expressed it (55% $\pm$ 3% in back skin and 58% $\pm$ 4% in ear skin, resp.). To ascertain that this number did not originate from dermal fibroblasts expressing mMGL, we performed double stainings for mMGL and CD45, the pan leukocyte marker. As shown in Fig 1*A*, all mMGL-positive cells also stained positively for CD45. In addition, around 14% of all back dermal cells were single positive for CD45, whereas 31% expressed neither of both markers. A similar result was obtained in the ear skin dermis.

To analyze further the nature of mMGL-positive cells, we performed double stainings for mMGL and other macrophage markers: F4/80, CD11b, MOMA-1/sialoadhesin, ER-HR3, ER-TR9/mSIGN-R1 and MHC class II. The staining of F4/80 and mMGL virtually coincided (Fig 1*B*); 63% of all dermal cells were counted as double positive cells, 1% as single mMGL-positive cells, 2% single F4/80-positive cells and 34% as double negative cells in the back dermis.

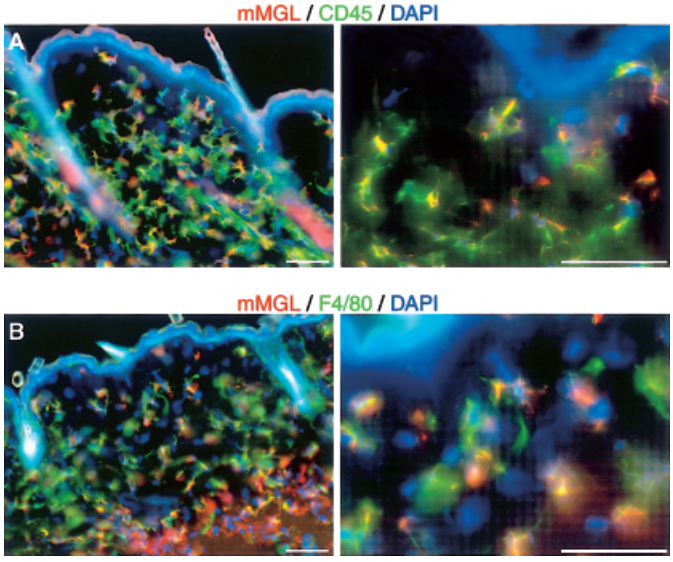

Figure 1
The majority of dermal interstitial cells express mononuclear phagocyte markers. Back skin sections were stained for mMGL / ER-MP23 (red) and CD45 (green) (*A*) or mMGL (red) and F4/80 (green) (*B*). Sections were counterstained with DAPI, showing nuclei in blue. (*A*) About 60% of all dermal cells express mMGL. All mMGL-positive cells also express CD45. About 10% of the CD45-positive cells are mMGL-negative. (*B*) Staining for mMGL and F4/80 virtually coincided. Note the gradient of increasing F4/80 expression toward the epidermal side, which could be observed occasionally. *Scale bar* = 50 μm for all pictures.

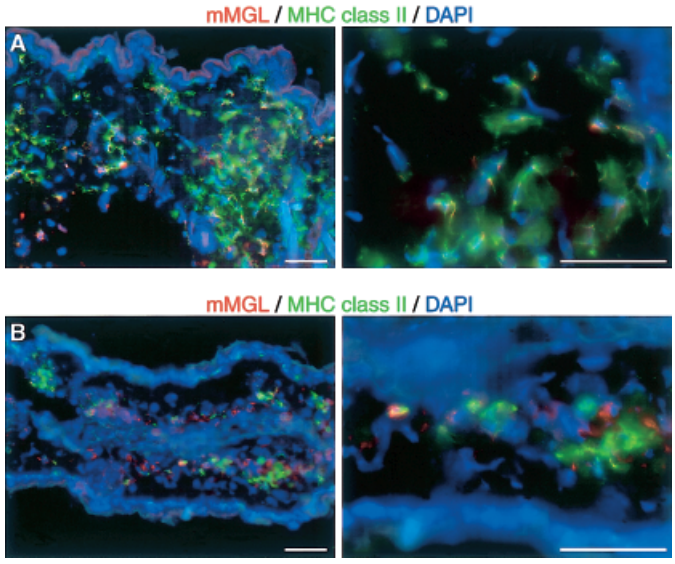

Figure 2
Dermal macrophages from different sites show differential MHC class II expression. Back skin sections (*A*) and ear skin sections (*B*) were stained for mMGL (red) and for MHC class II (green). Sections were counterstained with DAPI. (*A*) In the back skin, virtually all mMGL-positive cells coexpress MHC class II. (*B*) Similar to the back skin, the ear dermis contains about 60% interstitial cells expressing mMGL. However, only about one third of these cells coexpresses MHC class II molecules. These mMGL/MHC class II double-positive cells are arranged in clusters. *Scale bar* = 50 μm for all pictures.

CD11b was, as F4/80, expressed on almost all mMGL-positive cells (Fig S1, online; 55% double positive, 1% single mMGL-positive, 4% single CD11b-positive and 40% double negative cells). The 4% single CD11b-positive cells presumably represent dermal mast cells. MOMA-1 was found to bind a subpopulation of dermal phagocytes, in particular those cells laying deeper in the dermis (Fig S1, online; 23% double positive, 39% single mMGL-positive, 2% single MOMA-1-positive, 37% double negative). ER-HR3, with still unknown binding epitope, likewise stained a significant subpopulation of mMGL-positive cells throughout the dermis (Fig S1, online; 42% double positive, 18% single mMGL-positive, 5% single ER-HR3-positive, 35% double negative). ER-TR9, which recognizes mSIGN-R1 (Kang *et al*, 2003), has been found to stain a small percentage of cells in the dermis (6%–8%; Fig S1, online). For all these markers, similar numbers have been obtained comparing back skin to ear skin dermis. When we compared the staining of dermal cells of the two sites for MHC class II, we found that virtually all mMGL-positive cells in the back dermis expressed this marker (58% double positive, 3% single mMGL-positive, 4% single MHC class II-positive, 35% double negative; Fig 2*A*). In marked contrast, only a minor subpopulation of all mMGL-positive cells expressed MHC class II in the ear dermis (20% double positive, 40% single mMGL-positive, 2% single MHC class II-positive, 38% double negative; Fig 2*B*). To explain this divergence, we speculate that differences in the structure of the dermis at different locations (subdermal adipose tissue *vs* cartilage) create different microenvironments that differentially influence the expression of the MHC class II molecules.

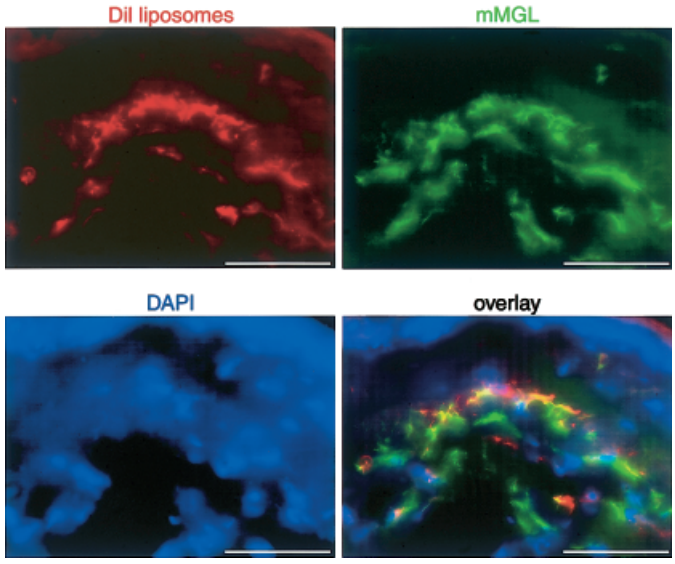

Figure 3
mMGL-positive dermal cells are phagocytic. DiI-labeled liposomes (red label) were injected into ear dermis. Four hours later, animals were euthanized, ears cut off, frozen, sectioned, and stained for mMGL (green). Note that the vast majority, but not all mMGL-positive cells, have taken up liposomes, whereas mMGL-negative cells and keratinocytes remained free of labeling. *Scale bar* = 50 μm for all pictures.

To seek further confirmation regarding the nature of mMGL-positive dermal cells, we made use of the fact that macrophages are the only connective tissue cells showing avid phagocytic capacities. Therefore we injected DiI-labeled liposomes, which previously have been shown to target phagocytic cells exclusively (Buiting *et al*, 1993), into the ear

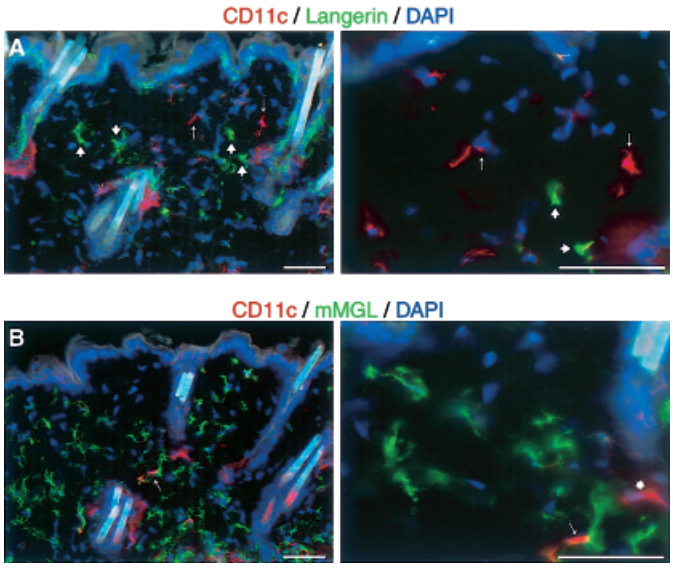

Figure 4
Dermal DC constitute a small, distinct mononuclear phagocyte population. Back skin sections were stained for CD11c (red) and Langerin (green) (*A*) or CD11c (red) and mMGL (green) (*B*). Sections were counterstained with DAPI. (*A*) About 7% of all dermal cells express CD11c but does not express Langerin. These cells thus represent genuine DDC (marked with thin arrows). Note that the CD11c staining is too weak to let CD11c/Langerin-double-positive mLC (marked with thick arrows) appear yellow. (*B*) Double staining for CD11c and mMGL shows that most CD11c-positive cells coexpress mMGL (arrow). Thus, most if not all DDC express mMGL. The red labeled cell marked with an asterisk is part of a sebaceous gland and displays autofluorescence rather than specific labeling. *Scale bar* = 50 μm for all pictures.

Table I. Frequencies of different cell populations in the mouse dermis, related to total nucleated cells; the quantification is derived from findings in both ear and back skin

Cell type	Mean percentage in the dermis	CD45	Langerin	CD11c	mMGL
Dermal macrophages (DMϕ)	50	+	–	–	+
Dermal DC (DDC)	7	+	–	+	6%–7%
Migrating LC (mLC)	4	+	+	+	1%–2%
Non-BM-derived interstitial cells	29	–	–	–	–
Other BM-derived cells (mast cells, T cells)	10	+	–	–	–

dermis. Thereafter we killed the mice, made sections of the treated ears and stained for mMGL. As shown in Fig 3, we indeed found that the vast majority of mMGL-positive cells had taken up DiI-labeled liposomes. However, a few mMGL-positive cells as well as all mMGL-negative cells remained free of DiI-fluorescence, indicating the specificity of this procedure. Therefore, we conclude that mMGL-positive cells not only express mononuclear phagocyte markers but also possess phagocytic capacity, thus identifying them as dermal macrophages unequivocally.

To evaluate the presence of DC in the dermis, we stained back and ear sections for CD11c. To distinguish dermal DC from migrating LC that are on their way from the epidermis to skin-draining lymph nodes, we performed a double labeling for Langerin, which specifically stains LC (Valladeau *et al*, 2002). As shown in Fig 4, we indeed found a subpopulation of $CD11c^+$ $Langerin^-$ dermal DC (7%), while even fewer cells represented migrating LC, which all expressed CD11c (4% $CD11c^+$ $Langerin^+$). DC-SIGN is a specific marker for dermal DC in the human situation (Turville *et al*, 2002). Therefore, we wondered whether ER-TR9 (mSIGN-R1) would recognize dermal DC specifically in the mouse. Double stainings for ER-TR9 and CD11c revealed that ER-TR9 recognized a small subset of dermal APC (6%–8%), but that it did not uniquely stain $CD11c^+$ cells (Fig S1, online and data not shown).

To summarize, we have shown that mononuclear phagocytes constitute the major population of nucleated cells in the mouse dermis where they amount to about 60% of all dermal cells (Table I). Previous studies have quantified dermal mononuclear phagocyte subsets for mice (Duraiswamy *et al*, 1994) and for humans (Meunier *et al*, 1993) by flowcytometric analyses of dermal cell suspensions. In contrast to our findings, these authors concluded that dermal macrophages and dermal DC only composed around 5% of all dermal cells. The discrepancy between their findings and ours lies most probably in the difference of the used methods, as we stained and counted cells *in situ* in the non-denaturated dermis. Clearly, during the digestion procedure needed to obtain a dermal cell suspension, markers either may have been destroyed by enzymatic digestion, as is known to happen with the CD1 molecules, or the procedure may have released fibroblasts preferentially. We further tried to strengthen our findings by staining and counting fibroblasts specifically, but were not successful due to the unavailability of suitable markers. However, the concept that most dermal interstitial cells are indeed mononuclear phagocytes is well supported as we found similar percentages for dermal mononuclear phagocytes using several markers (F4/80, CD11b, mMGL, CD68 (data not shown) and MHC class II in the back dermis). Furthermore, this high frequency is confirmed by the CD45 pan-leukocyte labeling. Taken together, we feel that the size of the dermal mononuclear phagocyte population has been clearly underestimated so far. These cells probably play crucial roles in skin inflammatory and immune responses and they should be taken into account when the role of the dermis is studied in steady state and inflammatory conditions.

Materials and Methods

Immunofluorescence double stainings on mouse (C57BL/6) ear and back skin sections have been performed essentially as described (Stoitzner *et al*, 2003). Details can be obtained from the online supplementary materials.

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Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23427/JID23427sm.htm

Figure S1

Dermal macrophages show different degrees of heterogeneity. Back skin sections were double-stained for mMGL (red) and other macrophage markers in green: CD11b (A), MOMA-1 (B), ER-HR3 (C), mSIGN-R1/ER-TR9 (D). Sections were counterstained with DAPI. (A) All mMGL-positive cells coexpress CD11b. Separate CD11b single-positive cells may represent dermal mast cells, or occasional monocytes, granulocytes or activated T cells, (B) Staining for MOMA-1 divides DMϕ into two subpopulations: cells lying deeper in the dermis express MOMA-1, whereas cells directly underneath the epidermis do not. (C) ER-HR3 also divides DMϕ into two subpopulations, but with no clear localization pattern. A small number of ER-HR3 single-positive cells presumably represents mLC. (D) mSIGN-R1/ER-TR9 labels a small subpopulation of DMϕ and DDC. The two arrows mark two mMGL/mSIGN-R1 double-positive cells. Scale bar = 50 μm for all pictures.

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