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REVIEW

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The role of dendritic cells in cutaneous immunity

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Abstract This article reviews the role of dendritic cells in cutaneous immunity. Langerhans cells (LC) found in the epidermis are the best-characterized dendritic cell population. They have the ability to process antigen in the periphery, transport it to the draining lymph nodes (DLN) where they are able to cluster with, and activate, antigen-specific naive T cells. During migration LC undergo phenotypic and functional changes which enable them to perform this function. There are other less well-characterized dendritic cells including dendritic epidermal T cells, dermal dendrocytes and dermal 'LC-like' cells. Although there is no evidence that dendritic epidermal T cells (DETC) can present antigen or migrate to lymph nodes, they do influence the intensity of cutaneous immune responses to chemical haptens. Antigen-presenting cells (APC) in the dermis may provide alternative routes of antigen presentation which could be important in the regulation of skin immune responses. Therefore, dendritic cells are vital for the induction of immune responses to antigens encountered via the skin. LC are particularly important in primary immune responses due to their ability to activate naive T cells. The faster kinetics of secondary responses, and the ability of nonprofessional APC to induce effector function in previously activated cells, suggest that antigen presentation in the DLN may be less important in responses to previously encountered antigens. In these seondary responses, dendritic and nondendritic APC in the skin may directly induce effector functions from antigen-specific recirculating cells.

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Dendritic cells in the skin

The concept that the skin has its own associated immune system was first proposed in 1978 [1]. Langerhans cells (LC) are a constituent part of the skin immune system (SIS), and are found regularly spaced throughout the epidermis, forming a semicontinuous network via long dendritic processes. LC are defined by their dendritic morphology and the presence of a unique intracytoplasmic organelle, the Birbeck granule [2]. In addition to the appearance of LC, they are distinguished by their phenotype, being the only population in normal epidermis to express MHC class II (a molecule involved in the presentation of exogenous antigen to T cells). They originate from bone marrow progenitors, as indicated by their CD45 expression, but are not T-cell derived since they lack the CD3 marker [3]. Human LC express CD1a and stain faintly with anti-CD1c (Table 1) [4]. CD1 molecules show similarities to MHC class I, and may be involved in the presentation of antigen to γ/δ T cells.

Another population of dendritic cells (DC) in the skin is the dendritic epidermal T cell (DETC) which comprise 1–2% of the epidermal cells in rodents [5, 6]. They are bone-marrow-derived ($CD45^+$), belong to the T-cell lineage ($Thy\text{-}1^+$ and $CD3^+$), and express the γ/δ T-cell receptor (TCR) (Table 1). DETC do not express CD4 or CD8 antigens [5], or MHC class II [3]. Their T-cell lineage is confirmed by evidence that the numbers of DETC are significantly reduced in athymic nude mice and that they proliferate in vitro when stimulated with a mixture of concanavalin A and interleukin-2 (IL-2) [5]. γ/δ T-cells are interesting because they recognize a limited set of epitopes including heat shock proteins (HSP), MHC-like molecules and mycobacterial antigens [7]. However, because HSP are produced by both bacterial and mammalian cells, and are highly conserved in both, it has been sug-

Table 1 Phenotype of dendritic cells in the skin (✓ expression, × no expression)

Surface marker	Murine LC	Human LC	DETC	Dermal dendrocyte	Dermal 'LC-like'
MHC class II	✓ [114, 115]	✓ [116]	× [7]	✓ [10]	✓ [12]
MHC class I	✓ [115]				✓ [12]
CD45	✓ [117]	✓ [118]	✓ [5]		✓ [12]
CD3	× [119]		✓ [7]		× [12]
Thy-1	× [119]		✓ [120, 121]		
γδ TCR	× [117]		✓ [122]		
ICAM-1 (CD54)	✓ [79]	✓ [123]		× Induced by IFNγ [124]	
ICAM-2 (CD102)		× [125]			
ICAM-3 (CD50)		✓ [65]			
LFA-1 (CD11a/CD18)	$CD11a^{-}/CD18^{+}$ [61]				
MAC-1 (CD11b/CD18)	✓ [119]	✓ [126]		× [124]	$CD11b^{-}$ [12]
p150/95 (CD11c/CD18)	✓ [127]	✓ [126]			✓ [12]
LFA-3 (CD58)		✓ Weak [128]			
α1 (VLA-1 with β1 chain)		✓ α1β1 weak on 40% LC [129] × [130]			
α2 (VLA-2- with β1 chain)		✓ α2β1 weak on 40% LC [129] × [130]			
α3 (VLA-3 with β1 chain)		✓ α3β1 weak on 40% LC [129] × [130]			
α4 (VLA-4 with β1 chain)	✓ [58]	✓ Weak [128] ✓ α4β1 [129]			
α5 (VLA-5 with β1 chain)		✓ α5β1 [129] × α5β1 [65]			
α6 (VLA-6 with β1 chain)		✓ Weak [128] ✓ α6β1 [129]			
β1		✓ [130]			
CD1a		✓ [4]		✓ [124]	✓ [131]
CD1b		✓ [4]			
CD1c		✓ Weak [4]			✓ [131]
CD44	✓ [58]				
Heat-stable antigen	✓ [132]				
E-cadherin	✓ [55]	✓ [133]			
FcγRI (CD64)					
FcγRII (CD32)	✓ [117]				✓ [131]
FcγRIII (CD16)	✓ [117]				
FcεRI		✓ [134]			
FcεRII (CD23)	× [117]	✓ [134]			
εBP (MAC-2)		✓ [134]			
CD80 (B7-1)	× [93, 94]	× [95]			
CD86 (B7-2)	✓ Weak [93] × [65]				
Factor XIII				✓ [10]	

gested that γ/δ T cells may be involved in autoimmune responses [7]. It has also been proposed that DETC are involved in the induction of tolerance [8], and that the ratio of DETC to LC in the epidermis influences the intensity, but not the duration, of sensitization in mice [9].

A third type of DC present in the skin is the dermal dendrocyte. These cells have been characterized to some extent by their phenotype (Table 1). In human skin they are factor XIII $antigen^{+}$, sometimes express class II antigen [10] and are $CD1a^{+}$, $CD1b^{+}$ and $CD1c^{+}$ [11]. Factor

XIII is involved in scab formation, crosslinking fibrin with structural proteins, which leads to the conclusion that dermal dendrocytes may be of importance in wound healing. Dermal dendrocytes surrounding the microvasculature in the skin have been called veil cells [10]. Both populations share a common phenotype, but while dermal dendrocytes show both 'dendritic' and 'fibroblast-like' morphology, veil cells are thought to show only 'fibroblast-like' morphology [10]. It has been suggested that dermal dendrocytes are immature precursors of LC. If LC are continuously being replaced by blood-borne bone-marrow-derived DC precursors, then veil cells which are concentrated around the microvasculature, may be DC precursors entering the skin. Besides the dermal dendrocytes, there are other cells in the dermis of mice which express MHC class II, with one subset showing an LC-'like' phenotype (Table 1) [12].

Cutaneous immune responses

Contact hypersensitivity (CH) responses are commonly used as a model to test an animal's or a human's ability to mount a cutaneous immune response. To induce contact sensitivity, the experimental animal is sensitized to a specific hapten by painting a solution containing the chemical onto the skin. Haptens are small molecules which by themselves are nonimmunogenic, but which can act as epitopes when bound to a protein 'carrier'. In the skin haptens acquire immunogenicity by binding to endogenous proteins, which results in the generation of a hapten/protein-specific immune response. Approximately 5 days after the original hapten sensitization, the animal is challenged with a subinflammatory concentration of hapten which within 24 h leads to the generation of a cell-mediated inflammatory response at the site of application. The site of challenge is usually the ears or the foot pad, as it is easy to quantitate the inflammatory reaction by measuring the increase in ear or footpad thickness. Delayed hypersensitivity (DH) responses are broadly similar, but they differ from CH in that the antigen is not a hapten and is administered intradermally. DH inflammatory reactions are characterized by the infiltration of mononuclear cells into the dermis with less cellular infiltration into the epidermis compared with CH responses.

Evidence suggesting that Langerhans cells play an important role in cutaneous immunity

The first clues that LC are involved in cutaneous immunity came from experiments examining contact sensitivity responses in mice. When haptens were applied through sites naturally deficient in LC expression, such as the hamster cheek pouch epithelium and mouse tail skin, specific unresponsiveness to hapten challenge was observed [13, 15]. It was noted that a regime of UVB treatment could artifically deplete LC from normal skin sites [13]. Four consecutive doses of UVB radiation (100 J/m^2 per day) caused a highly significant depletion of ATPase$^+$ epidermal LC in mice [13]. Haptens applied to UVB-treated skin induced hapten-specific unresponsiveness on subsequent exposure at an unirradiated site [13]. The ability of UVB to induce unresponsiveness was dependent on the strain of mouse used, leading to the classification of UVB 'susceptible' and 'resistant' strains. The evidence for this is not as strong as it once was, since resistant strains show significant reduction of CH responses when mice are sensitized with optimal doses of hapten via irradiated skin [16].

These results led to the hypothesis that the suppression of CH is directly related to loss of the epidermal LC. However, there are a number of observations which suggest that other factors are involved also. Firstly, LC are depleted from the epidermis of both susceptible and resistant mouse strains following low-dose UVB treatment [17]. However, resistant mice are able to generate responses to haptens encountered through the depleted skin. Thus, there may be other antigen-presentation pathways available for cutaneous immunity, a concept which is explored more fully later in this review. Secondly, specific tolerance is generated to haptens applied through irradiated skin. This was demonstrated in mice first sensitized to a hapten through skin naturally deficient in LC, or irradiated skin, which showed suppressed immune responses even after they were sensitized again through normal skin prior to challenge [13]. Tolerance generated in this way is associated with the induction of regulatory/suppressor T cells which can transfer suppression of CH [18] and DH responses [19]. Antigen presentation may be taking place but there are likely to be differences in the presentation of antigen encountered via skin depleted of LC (at least in susceptible strains). Thirdly, the immunosuppression generated by UVB is not always confined to skin sites depleted of LC. To examine the local and systemic effects of UVB, mice were exposed to a suppressive dose of UVB, and a contact sensitizer was then painted on to the irradiated site or a distant unirradiated site [20]. When the sensitizer was painted on the skin immediately after the last irradiation, immunosuppression was limited to haptens applied directly to the irradiated site. However, 3 days after the last exposure, there was systemic immunosuppression to haptens applied via unirradiated skin. This evidence suggests that UVB exerts systemic as well as local effects on cutaneous immunity, possibly via soluble mediators.

Antigen processing in the epidermis

The first stage in the recognition of antigen by the immune system is the processing of that antigen by antigen presenting-cells (APC). Processing refers to the internalization and degradation of antigen into immunogenic peptide fragments that can be presented with MHC molecules; it takes place intracellularly in acidified endosomes/lysosomes, although the exact compartment where peptides and MHC class II associate has not been fully defined.

Freshly isolated murine LC are able to process and present the protein ovalbumin (OVA) in its native form, to an OVA peptide-specific MHC-restricted T-cell hybridoma in vitro, an ability which is lost during culture [21]. LC taken from the epidermis and cultured for 72 h are much less efficient at processing native OVA [21]. Similarly, the ability to process antigen from *Leishmania major (L, major)* amastigotes is found in freshly isolated murine epidermal LC, but is lost after 12 h in culture [22]. Haptens such as nickel are able to bind MHC class II-associated peptides directly [23], suggesting that antigen processing may not be required for all forms of contact sensitization.

As LC have phagocytic activity when freshly isolated, it seems likely that epidermal LC have the capacity to process native antigen in vivo. In contrast, like cultured LC, murine lymph node DC are generally poorly phagocytic, although freshly isolated splenic DC show phagocytic activity for zymosan and latex beads [24]. Prior to the work on freshly isolated LC, the lack of phagocytic activity in lymphoid DC led to speculation about the identity of the cell population that processed antigen in the epidermis with the suggestion that keratinocytes may provide this function [25, 26]. Since freshly isolated LC are capable of phagocytosis, it seems unlikely that epidermal LC would require a source of peptides. However, processing by keratinocytes may affect the immune response, depending on whether the peptide fragments are degraded, released into the extracellular matrix or presented on the surface of keratinocytes. Although keratinocytes do not express MHC class II molecules constitutively, they can be induced to do so by interferon-γ (IFNγ) [27]. MHC class II-bearing keratinocytes can provide accessory function for T cells that have been stimulated previously with superantigens or anti-CD3 monoclonal antibody [27]. Keratinocytes are therefore equipped to play an important role in secondary, but not primary, immune responses.

Birbeck granules: markers of antigen processing?

The endocytosis of the CD1 molecule on human LC has been demonstrated using immunogold-labelled anti-CD1 monoclonal antibody and electron microscopy [28, 29]. LC were incubated at various temperatures. At 4° C there was diffuse labelling over the entire cell surface. When the temperature was raised to 15° C, the cell surface labelling was concentrated in clathrin-coated pits and there was internalization of the label in endosomes. At 37° C lysosomes were labelled, and isolated labelled Birbeck granules were visible in the cytoplasm. Another study has examined the endocytosis of Ia antigen by Birbeck granule-like structures in murine DC [30]. Gold-labelled Ia molecules were internalized within Birbeck granules when LC were incubated at 21° C. After 30 min the Birbeck granules disappeared, and the gold label was associated with lysosomes and vacuoles, with aggregates of gold particles on the cell surface. The experiments suggest that Birbeck granules may be important in receptor-mediated endocytosis and intracellular antigen processing. However, there is some evidence that Birbeck granules may not be an absolute requirement for the functional activity of LC. A healthy male subject whose LC lack Birbeck granules has been identified [31]. The LC were present in normal numbers in the skin and expressed CD1a and MHC class II. Functionally, the LC were normal as measured by their ability to induce CH responses in vivo and alloresponses in vitro.

Induction of LC migration

To induce a primary immune response, antigen processed by epidermal cells has to be transported to the local draining lymph node (DLN), where competent antigen-bearing APC can stimulate MHC-restricted proliferation and differentiation of antigen-specific T-cell clones. There is good evidence, that will be presented later in this section, that antigen-bearing LC are able to transport antigen to the DLN. Certainly, LC are capable of migration out of the skin. A number of stimuli cause a loss of LC from the epidermis including exposure to low-dose UVB radiation and skin painting with contact sensitizers [14]. Depletion of murine epidermal LC using these treatments results in a subsequent accumulation of LC and/or DC in the lymph nodes draining the treated site [32, 33], which has been attributed to an influx of DC from the skin. However, skin painting with haptens causes a smaller influx of DC into lymph nodes that drain nonsensitized sites, in addition to inducing an increase in DC numbers in DLNs [34]. This suggests that sensitization may induce systemic LC migration from untreated skin sites. Alternatively, the systemic effects may be due to entry of non-LC-derived DC into lymph nodes.

Keratinocytes provide the necessary microenvironment of the epidermis by producing cytokines which are thought to play an important role in LC migration and differentiation. In response to various stimuli, including UVB radiation and contract sensitizers, keratinocytes express a wide variety of cytokines [35]. Using PCR, it has been shown that topical exposure of mice to contact sensitizers results in increased epidermal mRNA for IL-1α, IL-1β, GM-CSF, TNFα, macrophage inflammatory protein-2 (MIP-2), interferon-induced protein-10 (IP-10) and MHC class II [36, 37]. Tolerogens and chemical irritants also induce an increase in epidermal mRNA for a number of these cytokines [37].

The induction of IL-1β by contact sensitizers is interesting. The earliest change in cytokine mRNA expression in the epidermis is IL-1β, found 15 min after hapten application [37]. This precedes TNFα mRNA expression which is found after 30 min [37]. Depletion of epidermal cell subsets revealed that IL-1β activity is mainly restricted to LC [36]. When IL-1β is injected intradermally, it causes similar changes in cytokine production to those that occur after sensitization, with increased expression of mRNA for IL-1α, MIP-2, IL-10, TNFα and MHC class II, while intradermal injection of IL-1α or TNFα does not affect the cytokine pattern [38]. In addition, a neutralizing

antibody to IL-1β is able to block sensitization [38], suggesting that LC-derived IL-1β is an important initiation signal for the induction of contact sensitization.

The role of TNFα in the migration of LC from the epidermis is controversial. TNFα is induced in human keratinocytes after exposure to UVB radiation [39] and its expression is upregulated by sensitizing chemicals [36]. Intradermal injection of TNFα causes an accumulation of DC in the DLN of mice [40] and a decrease in epidermal LC numbers [41]. Accumulation of DC in the DLN induced by UVB irradiation, contact sensitizers and contact irritants is blocked by pretreatment with neutralizing antibodies to TNFα [42, 43]. Evidence suggests that TNFα may also mediate migration of DC out of the intestine, measured by counting numbers of DC in lymph collected from the thoracic duct of mesenteric-lymphadenectomized rats [44]. Injection of 50 mg endotoxin caused an 8–15 fold increase in DC numbers in the lymph 10–15 h after injection. As with the epidermis, an injection of neutralizing antibody directed against TNFα into these rats prior to the endotoxin abrogated the effects of TNFα. However, in contrast to these results, TNFα has been suggested to act on LC by immobilizing them in the epidermis, as an intradermal injection of TNFα prior to sensitization prevented hapten-induced loss of LC from the epidermis, and so blocked the induction of CH [45].

Other mediators may be involved in reducing the number of LC in the epidermis. One, which may be specific to UVB-induced depletion, is *cis*-urocanic acid (*cis*-UCA). *Trans*-UCA is found in the stratum corneum of the epidermis and is isomerized to *cis*-UCA by UVB irradiation. The *cis*-form is more soluble, being found transiently in the serum of mice after irradiation [46]. *Cis*-UCA mimics some UVB-induced effects on the immune system and causes depletion of LC from the epidermis of mice. The latter effect seems to be specific, as the reduction is abrogated by prior administration of a monoclonal antibody to *cis*-UCA [47]. However, unlike UVB radiation, *cis*-UCA applied topically to the skin of mice does not induce an accumulation of DC in the DLN [33].

The most compelling evidence for epidermal LC migrating to the DLN after antigen challenge, is shown in a model using nu/nu BALB/C mice grafted with skin from C3H mice [48]. When the BALB/c mice were contact sensitized with fluorescein isothiocyanate (FITC) through the graft tissue, the cells binding FITC in the DLN were found to be derived from the C3H graft. Isolated FITC-binding cells from the DLN of BALB/c mice were able to induce a CH response in C3H but not in BALB/c mice. In the same study it was found that at least some of the FITC-binding cells found in the DLN contained Birbeck granules, a feature used to identify epidermal LC. Therefore, it can be concluded that some APC in the DLN are derived from cells in the skin and are LC in origin. However, in another study there were very few donor MHC class II^+ cells in lymph nodes draining allogeneic skin grafts [49]. Also, FITC painted on to allogeneic grafts could be found associated with recipient MHC class II^+ cells in the DLN, suggesting that some of the FITC reaches the lymph node without binding donor APC in the epidermis [49].

FITC has proved to be a useful hapten in migration studies. It can be painted on to the skin of a mouse and FITC-bearing DC can be visualised in the local DLN. Increasing the dose of FITC increases both the number of DC in the DLN [50] and also the amount of FITC displayed on the surface of the DC [51]. Treatment of mice with monoclonal antibody to MHC class II depletes Ia^+ cells in the spleen and lymph nodes but not Ia^+ LC in the skin [52]. Skin painting of these mice with FITC resulted in the appearance of Ia^+ FITC bearing cells in the lymph nodes that could stimulate an FITC-specific hybridoma in an MHC class II-restricted manner [52].

Although FITC rapidly associates with proteins in vivo, it has been claimed that FITC an move freely to lymph nodes without necessarily binding to LC in the epidermis. After ear painting, FITC found in the DLN is associated predominantly with interdigitating dendritic cells (IDC). Therefore, free FITC entering lymph nodes would have to bind IDC with a high affinity. Although FITC is popular because it can be visualized, other antigens have also been used to study migration. *L. major* amastigotes, administered intradermally, induce migration of DC from the skin to the DLN. DC isolated from these nodes were immunostimulatory for *L. major*-specific T cells [22]. In other studies in sheep and cattle, the afferent lymph veiled cells (ALVC) draining a site challenged intradermally with antigen were collected and used to induce antigen-specific proliferation [53, 54]. ALVC are discussed below where this evidence is reviewed more fully.

Mechanisms of LC migration

Although much is unknown about the mechanism of LC migration, it is likely that adhesion molecules play a role. It has been postulated that the migration signal acts to alter the phenotype of the epidermal LC, causing them to exit from the epidermis. Recent evidence has shown that LC bind to keratinocytes via E-cadherin and that the expression of this molecule is downregulated on LC during culture (Table 2) [55]. Since keratinocytes are the predominant cell population in the epidermis [56], E-cadherin could tether LC in the epidermis and may influence the morphology of LC in the epidermis. Cytokine signals, which could include TNFα and GM-CSF, may induce the downregulation of E-cadherin, weaken adhesive bonds between LC and keratinocytes, and allow migration of LC from the epidermis. ICAM-1, and to a lesser extent LFA-1, may have roles in the migration of LC to local lymph nodes. Intravenous injection of monoclonal antibodies directed against these molecules caused a reduction in the numbers of $FITC^+$ Ia^+ DC found in lymph nodes after FITC skin painting, and an inhibition of the induction of CH [57]. Molecules that have been ascribed roles in the homing and recirculation of cell populations, are upregulated on LC during culture. There is increased expression of the surface molecules CD44 and α4 integrin (the

Table 2 Phenotype of various dendritic cell populations in comparison with freshly isolated LC [↑ expression upregulated (compared with LC), ↓ expression downregulated (compared with LC), ✓ expression, × no expression]

Surface marker	Murine DC	Human DC
MHC class II	↑ Lymph node DC [78] ↑ Cultured LC and spleen DC [119] ↑ Spleen DC [61]	↑ Cultured LC [135]
MHC class I	↑ Spleen and thymic DC [115] Spleen DC [61]	↑ Cultured LC [135]
CD45		✓ Blood DC [136] ✓ Blood DC and lymph node DC [118]
ICAM-1	↑ Lymph node DC [79]	✓ Blood and tonsil DC [125]
ICAM-2		✓ Weak blood and tonsil DC [125]
ICAM-3		✓ [65]
LFA-1	✓ Spleen DC [137]	✓ Blood DC [136]
MAC-1	↓ Spleen DC and cultured LC [119]	✓ Weak on blood DC [136]
P150,95	✓ Spleen DC [137] ✓ Cultured LC [127]	✓ Blood DC [136] ✓ 25% of spleen DC [126]
LFA-3		✓ Blood DC [136|
CD1a		× Blood DC [136]
CD1b		✓ Weak on blood DC [136]
CD1c		× Blood DC [136]
CD44	↑ Cultured LC [93]	✓ [65]
CD40	↑ Cultured LC [93]	✓ Cultured LC [138]
Fc Recs	↓ Cultured LC [93]	↓ Cultured LC [138]
CD80 (B7-1)	↑ Cultured LC [93]	↑ Cultured LC [95]
CD86 (B7-2)	↑ Cultured LC [93]	✓ $CD34^+$ progenitors cultured in GM-CSF and TNFα [99]
E-cadherin	↓ Skin-associated lymph node DC [138]	↓ Cultured LC [133]

α-chain of LPAM-1/VLA-4) and ICAM-1 [58]. CD44 is a transmembrane glycoprotein with a molecular weight around 85 kDa [59]. Evidence points to this molecule being involved in cell recirculation by binding specific carbohydrate residues on high endothelial venules [60]. VLA-4 has two ligands, VCAM-1 a cell adhesion molecule induced on endothelium by inflammatory mediators, and the extracellular matrix protein, fibronectin [60]. Adhesive interactions between these molecules on differentiated LC and their ligands may play a role in the migration of LC to the DLN.

Differentiation of LC

The cytokines produced by keratinocytes after treatment with haptens or UVB are important in the differentiation of LC into lymphoid DC. Differentiation of murine LC in vitro requires the presence of GM-CSF, produced by contaminating keratinocytes [61]. Culturing highly purified murine LC in GM-CSF increases their ability to stimulate mixed lymphocyte reactions (MLR) and LC purified from bulk epidermal cell cultures, where contaminating keratinocytes provide cytokines, are also good stimulators of MLR [62]. This ability is not only due to improved viability and increased Ia expression [62]. If LC are cultured in the presence of both GM-CSF and IL-1, a twofold enhancement in their capacity to stimulate MLR is induced compared with LC cultured in GM-CSF alone [62]. Further evidence of a role for GM-CSF in the differentiation/maturation of LC is provided by the inability of LC from unprimed mice to induce an immune response to a tumour-associated antigen, unless the cells are preincubated with GM-CSF [63]. Preincubation of unprimed LC with IL-1α, TNFα, IFNγ and TGFβ does not affect their ability to induce a response and, indeed, some combinations of cytokines cause a reduction in the immune response [63]. If murine LC are cultured in TNFα alone, the viability of the cells is maintained but they do not mature functionally and are poor stimulators of MLR [64]. However, TNF does induce LC differentiation, causing the downregulation of macropinocytosis [65]. Culture of hu-

man DC progenitors, $CD34^+$ cells isolated from peripheral blood, in GM-CSF and TNFα causes their differentiation into cells with DC activity [66].

Other immunomodulatory cell populations in the skin

LC are the principle APC in the skin, but there is evidence that other cells can present antigen and affect cutaneous immune responses. Their contribution to functional antigen presentation may be masked by LC under normal circumstances. However, when the efficiency of antigen presentation by LC is impaired by UVB radiation for example, then the alternative APC may become important.

One cell population of mice and rats, the Thy-1^+ dendritic epidermal T cells (DETC), may induce suppressive immune responses [67] and the ratio of DETC to LC in the epidermis influences sensitization [9]. Thy-1^+ DETC do not express Ia on the cell surface [17] making MHC class II-restricted antigen presentation unlikely, and there is evidence that Thy-1^+ DETC do not migrate to the DLN [41, 68]. Intravenous administration of haptenated Thy-1^+ cells into mice suppresses the induction of CH responses [67, 69]. While in a normal cutaneous response, these suppressor circuits may act to limit an inflammatory cascade, in LC-depleted skin the major signal from the skin may be to suppress the response. It is therefore possible that UVB may be acting via populations other than the LC to induce immunosuppression of cutaneous immunity.

Secondary antigen-presentation pathways have also been proposed as an explanation for the UVB resistance found in certain strains of mice. It has been suggested that UV-resistant mice possess a second antigen-presentation pathway, separate from epidermal LC, possibly mediated by DC in the dermis [70]. Tape-stripped skin which is selectively depleted of epidermal cells, is able to support CH in UVB-resistant, but not UVB-sensitive, mice. However surgical excision of hapten-painted skin within 1 h of application, which removes both the dermis and epidermis, prevents the induction of CH. Recently, it has been shown that dermal cells, haptenated in vitro, from UVB-exposed UV-resistant mice can transfer CH responses to naive mice. In contrast haptenated dermal cells from UVB-exposed UV-susceptible strains are unable to transfer CH and instead induce tolerance [71]. Around 2% of cells in dermal cell suspensions normally express MHC class II; after UVB exposure this drops to around 1.4% in both resistant and susceptible strains [71]. However, the results suggest that in resistant strains the dermal population is able to provide a secondary antigen-presenting pathway, while in susceptible strains these cells induce tolerance. MHC class II^+ LC-like interstitial dendritic antigen-presenting cells have been differentiated from dermal macrophages [12]. Morphology and phenotype were used to distinguish these subsets, with the $CD11b^+$, Ly6c (monocyte/endothelial antigen) subset, thought to be dermal macrophages, having no APC function in syngeneic MLR. The LC-like cells are found in the perivascular and interstitial dermis of resistant and susceptible strains [12].

Other cell populations, not necessarily dendritic, may be involved in presentation of antigen from the skin. One type which appears in the human epidermis during the elicitation phase of the CH response, consists of $CD1^-$ $OKM5^+$ (CD36 monocyte/platelet marker) MHC class II^+ cells [72]. They have been found not to suppress the CH response and have been shown to be responsible for up to 50% of antigen-presentation capacity during hypersensitivity responses [72]. However, a cell population with the same phenotype, which is found in the epidermis after irradiation by certain wavelengths of UV (UVB and UVC) [73], activates a suppressor T-cell population [74]. In another study, UVB induced a loss of cells with LC markers and the appearance of MHC class II^+, $CD1a^-$, $CD36^+$ (on 60% of $CD1a^-$ MHC class II^+ cells), $CD11b^+$ macrophages which may have antigen-presenting activity [75]. Neutrophils (MHC class II^-, $CD11b^+$, GR-1^+), macrophage-like APC (MHC class II^{high}, $CD11b^+$) and macrophages (MHC class II^{low}, $CD11b^-$) infiltrate into the murine epidermis after exposure to UVB [76].

Veiled cells

After an appropriate signal, LC migrate into the afferent lymphatics where they are described as veiled cells (due to their long actively moving processes which resemble veils). In one study the afferent lymph ducts of calves were cannulated, enabling the ALVC to be studied [54]. The ability of the ALVC draining from the site of intradermal antigen challenge [variable surface glycoprotein (VSG) from *Trypanasoma brucei*] to stimulate peripheral blood mononuclear cell (PBMC) proliferation, was measured in monozygotic bovine twins. It was found that they induce proliferation in PBMC from VSG-immunized calves, as rapidly as 30 min after intradermal application of antigens. There was no proliferation of PBMC from VSG-naive calves. Similar work has been carried out in sheep, using the protein antigens OVA and purified protein derivative (PPD) from *bacillus Calmette Guerin* [53]. There was a marked proliferation of OVA- and PPD-specific T-cell lines when incubated with afferent lymph cells from OVA- and PPD-challenged animals. Afferent lymph cells collected prior to challenge did not induce significant proliferation in the antigen-specific T-cell lines. The stimulation was antigen specific as the afferent lymph cells of OVA-challenged sheep did not induce proliferation of PPD-specific T cells, and vice versa. In addition, the ability of ALVC to cluster with primary resting T cells has been described, a property not shared by nonprofessional APC and one of the reasons why antigen presentation by DC is essential for the initiation of primary responses.

The draining lymph node

The veiled cells drain into the paracortical area of lymph nodes where they are called IDC as their dendritic projec-

tions show extensive contact with surrounding cells. The IDC present antigen in a MHC-restricted manner to T cells in the local lymph nodes. T cells which are specific for the antigen/MHC complex and which receive the correct signals from the APC (soluble signals and cell-cell interaction) are induced to proliferative and differentiate.

The changes in the phenotype and function of DC as they migrate to the DLN are thought to be similar to the changes seen in vitro when freshly isolated epidermal LC are cultured. Evidence has been presented in an earlier section showing that LC lose their ability to process native peptide after being cultured for 72 h [21]. However, cultured LC are significantly better at stimulating autologous and allogeneic T-cell responses than are freshly isolated LC. These changes in function are likely to be due to alterations in the phenotype of LC during their migration from the skin and their differentiation into DC. The phenotype of IDC after migration in vivo is similar to the phenotype of LC after differentiation in culture. MHC class II is upregulated on cultured LC [77] and DC isolated from lymph nodes show increased expression compared with epidermal LC [78]. Similarly, ICAM-1 is upregulated on cultured epidermal LC [58] and DC isolated from lymph nodes have higher levels of ICAM-1 than LC isolated from the epidermis (Table 2) [79]. There are also changes in morphology during culture with the majority of LC losing Birbeck granules [80].

Antigen presentation

Antigen presentation is a complex interaction requiring recognition by the T cell receptor (TCR) of peptide antigen bound in the groove of an MHC molecule. Other signals are also required and are provided by soluble cytokines and adhesive associations between the APC and the T cells. During a T-cell/APC interaction, binding of the TCR to the peptide/MHC complex occurs and there are interactions between a variety of adhesion molecules on the T cell and the APC and their ligands [81].

The ability of DC to initiate primary immune responses can be explained, in part, by their capacity to form stable clusters with resting antigen-specific T cells. Splenic DC from mice form clusters with T cells and B cells in vitro in the absence of exogenous antigen [82], while other APC can only cluster with sensitized T cells [83]. Antigen-independent adhesion between T cells and the APC precedes antigen-dependent clustering, and may allow DC to 'sample' different T cells [84]. In vitro assays have shown that clustering occurs prior to, and is essential for, T-cell proliferation [85]. The interaction between LFA-1 and its ligands ICAM-1,2,3 and CD2 and LFA-3 may be important in this first stage of antigen presentation. However, antibodies to LFA-1 fail to block antigen-independent clustering of murine spleen DC although they do block the function of clusters by causing a decrease in cell proliferation and cluster stability [86].

The importance of ICAM-1 expression in antigen presentation has been shown by examining the function of mutant APC from mice which had an 80–95% reduction in ICAM-1 expression [87]. The ICAM-1^{low} APC had a greatly impaired ability to present antigen to T cells. Reconstitution of ICAM-1 by transfection of the gene into these cells restored normal antigen presentation. ICAM-1 induced important costimulatory signals through the LFA-1 molecule on T cells [88]. The ICAM-1/LFA-1 interaction is also necessary in the clustering of T cells with other cells [89], and some groups have suggested that antigen-independent adhesion is the first step toward recognition of the antigen/MHC complex by T cells [81].

There has been much interest recently in the costimulatory functions of members of the B7 family which are ligands for CD28 and CTLA-4 on T cells. B7-2 (CD86) expression is found within 24 h of activation of human B cells, while B7-1 (CD80) expression peaks several days later [90]. B7-2 is found on peripheral blood DC [91], resting human monocytes and on activated T cells, B cells and NK cells [92]. CD28 is expressed widely on both human and mouse resting T cells, while CTLA-4 expression seems to be limited to activated T cells [90]. While both B7-1 and B7-2 are ligands for CD28 and CTLA-4, in vivo interactions may be influenced by the availability of the ligands. B7-2 is found constitutively at low levels on murine epidermal LC, and after 24 h in culture there is a dramatic upregulation in its expression (Table 2) [93]. B7-1 is not found on epidermal LC normally, but it is induced (to lower levels than B7-2) during culture [93–95], and is present on splenic DC [94].

The upregulation of B7-1 and B7-2 during LC culture and their expression on lymphoid DC suggest the involvement of keratinocyte-derived cytokines. B7-2 upregulation can be partially decreased during culture in the presence of an anti-GM-CSF antibody [93], which may reflect a minor role for GM-CSF in the induction of B7-2. Alternatively, the isolated LC may have received a signal to upregulate B7-2 expression during isolation which then may be difficult to reverse [93]. Lipopolysaccharide does not seem to upregulate B7-2 on DC although it does increase the expression of B7-2 on macrophages and B cells [96].

Therefore, both B7-1 and B7-2 are expressed on DC in lymphoid tissue with upregulation during in vitro culture of LC, and a connection with APC activity. Freshly isolated LC are less able to stimulate alloresponses than cultured LC [94, 95, 97] and cultured LC induce alloresponses similar to freshly isolated LC when B7-1 and B7-2 interactions are blocked using CTLA4-Ig [95]. CTLA4-Ig is a fusion protein with the extracellular portion of CTLA-4 spliced to the constant region of the human IgG1 molecule, which acts as a soluble ligand for B7-1 and B7-2 [98]. Interestingly, the alloresponse induced by fresh or cultured LC can be abrogated using CTLA4-Ig and anti-ICAM-1 [95]. The available evidence suggests that CD86 may be the more important costimulatory molecule in alloresponses. The ability of human DC, obtained by culturing $CD34^+$ peripheral blood progenitor cells with GM-CSF and TNFα, to induce alloresponses is inhibited by monoclonal antibodies against CD28 [99]. However,

though monoclonal antibodies against CD80 have little effect on the alloresponse, monoclonal antibodies against CD86 suppress the alloresponse by 70% [99]. When used together, monoclonal antibodies to CD80 and CD86 cause a 90% reduction in alloresponse [99]. CTLA4-Ig binding is completely inhibited in the presence of monoclonals to CD80 and CD86, suggesting that there is not a third ligand for CTLA-4 on DC.

Due to these and other changes in phenotype summarized in Table 2, IDC and cultured LC become specialized at presenting antigen to T cells, and are efficient at activating unprimed lymph node cells and in the induction of alloresponses in vitro [21]. It has been stated that DC are important in the induction of primary immune responses, and good evidence for the role of DC in primary immune responses has been provided using a transgenic mouse model [100]. Transgenic mice were bred that differed in the amounts of I-E MHC expressed on the surface of the major APC populations (DC, B cells and macrophages) [100]. These mice were then immunized with an I-E-restricted peptide antigen. After 8 days the $CD4^+$ T cells were removed from lymph nodes draining the site of immunization and cultured with the immunizing antigen. There was a correlation between the in vitro proliferative response of the $CD4^+$ T cells and the percentage of I-E-expressing cells in the mouse. However, there was no correlation between I-E expression on B cells or macrophages, and the size of the CD4 T-cell recall response.

The fate of the cutaneous DC

DC are not found in the efferent lymph [101] and there are few FITC-bearing DC left in the DLN 6 days after skin painting [102]. It seems likely that some system in the lymph nodes allows the DC to be destroyed without causing damage to the surrounding cells. The clearance of large numbers of inflammatory cells during the resolution of acute inflammation may involve apoptosis of the effector cells followed by ingestion by macrophages [103]. It is possible that DC in the DLN, starved of keratinocyte-derived growth factors which would maintain their viability, undergo apoptosis and are then removed by macrophages without damaging lymph node tissue. Other mechanisms may also act to clear DC from lymph nodes such as activated NK-like cells, or hapten/peptide specific antibody.

Production of a cutaneous immune response

T cells that recognize, and bind to, the specific antigen/MHC complex on the IDC and receive the necessary costimulatory signals, are induced to proliferate. Activation signals also induce differentiation of the T cells which undergo changes in both morphology and phenotype. These T cells exit the lymph node in the efferent lymph and enter the bloodstream via the thoracic duct. Recently, activated $CD45RO^+$ T cells, the so called memory subset, have been shown to exhibit specialized recirculation patterns. In the sheep the loss of L-selectin on these cells means that they are less likely to recirculate to peripheral lymph nodes and instead they circulate preferentially through tissue sites including the skin [104].

Commonly, cutaneous immune responses are studied using CH and DH responses as models. The inflammatory response generated during challenge with antigen in both these systems is associated with infiltration of mononuclear cells into the skin. Two subsets of CD4 Th cells have been identified in the mouse: Th1 and Th2 cells [105]. Th1 responses mediate cell-mediated immunity, including CH and DH responses, which are dependent on IFNγ [106, 107]. The cytokines produced by Th2 cells are important in B-cell activation and differentiation and therefore these cells preferentially stimulate humoral immunity. Interestingly, the ability of UVB to suppress sensitization to haptens corresponds to a loss of production of Th1-type cytokines by lymph node cells taken from lymph nodes draining irradiated skin [108]. Although a corresponding increase in Th2-type cytokines has not been detected, it is possible that UVB induces a functional inactivation of Th1-type cells. In support of this it has been shown that the ability of splenic T cells from UV-treated animals to transfer immunosuppression of DH responses to normal recipients, can be blocked by neutralizing antibodies to IL-4 or IL-10 given 4 and 24 h after transfer [109]. Therefore the suppressive action of these T cells on cell-mediated DH responses requires the production of Th2-type cytokines by the T cells.

Secondary cutaneous responses

The antigen-presenting pathway that has been described, LC processing antigen in the periphery and presentation to specific T cells in the DLN, is relevant to all cutaneous immune responses. However, this mechanism may be augmented by alternative antigen-presentation pathways in a secondary response, where there will be a pool of activated or semi-activated T cells specific for the antigen. Earlier, it was noted that subpopulations of these cells show preferential recirculation to tissue sites including the skin. Therefore in a secondary response, T cells could encounter antigen in the periphery bound to LC or other antigen-presenting cell populations such as B cells, macrophages and keratinocytes. Memory/activated T cells expressing CD45RO can be activated by a wide range of APC, while activation of naive T cells requires DC [110]. Therefore skin-homing populations of T cells, after being presented with antigen in the periphery, could release inflammatory factors that initiate a cascade response leading to the migration of effector cells through the endothelium into the skin.

There is some evidence that endothelial cells could present antigen to circulating T cells in the bloodstream. This mechanism would allow for antigen-specific migration of T cells into inflammed tissues. Endothelial cells do not express MHC class II constitutively but this molecule is highly upregulated during infection and allogeneic

transplant rejection [111]. Although cultured cells from virtually any organ can be induced to express MHC class II, endothelial cells are interesting because of the high degree to which class II can be upregulated by cytokines such as IFNγ and IL-1 [112]. As well as adhesive interactions, soluble mediators may be important in antigen presentation. However, cytokines in the blood stream would be rapidly washed away from the inflammatory site. It has been suggested that proteoglycans expressed on endothelial cells might act to bind and present soluble cytokines to circulating leucocytes [113]. Cytokines bind proteoglycans at low-intermediate affinity and some cytokines can specifically bind the glycosaminoglycan sidechains of proteoglycan [113]. Therefore, it is possible that antigen could be presented by endothelial cells in the postcapillary venules to recirculating 'memory' T cells.

Conclusion

The DC of the skin are very important in the production of skin immune responses. The migration of antigen-bearing LC from the epidermis to the DLN and their differentiation into efficient antigen-presenting cells are vital for the induction of primary immune responses. Other antigen-bearing cells, including dermal dendritic cells, may migrate in a similar fashion, and may influence immune responses. The faster kinetics of secondary responses suggest that antigen presentation in the DLN may not be as important as in primary responses. In this case it is likely that DC in the skin, together with other APC, initiate and influence the effector response.

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