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Langerhans cell origin and regulation

Matthew Collin and Paul Milne

Human Dendritic Cell Laboratory, Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

Abstract

Purpose—To summarize recent research on the ontogeny of Langerhans cells and regulation of their homeostasis in quiescent and inflamed conditions

Recent findings—Langerhans cells (LCs) originate pre-natally and may endure throughout life, independently of bone marrow derived precursors. Fate mapping experiments have recently resolved the relative contribution of primitive yolk sac and fetal liver hematopoiesis to the initial formation of LCs. In post-natal life, local self-renewal restores LC numbers following chronic or low grade inflammatory insults. However, severe inflammation recruits *de novo* bone marrow derived precursors in two waves; a transient population of classical monocytes followed by uncharacterized myeloid precursors that form a stable self-renewing LC network as inflammation subsides. Human CD1c+ dendritic cells have LC potential *in vitro*, raising the possibility that DC progenitors provide the second wave. LC development depends upon TGFβ receptor signaling with distinct pathways active during differentiation and homeostasis. LC survival is mediated by multiple pathways including mTOR and ERK signaling, mechanisms that become highly relevant in LC neoplasia.

Summary—The study of LCs continues to provide novel and unexpected insights into the origin and regulation of myeloid cell populations. The melding of macrophage and DC biology, shaped by a unique habitat, is a special feature of LCs.

Keywords

Langerhans cell; Dendritic cell; TGFβ

Introduction

Langerhans cells (LCs) are myeloid cells resident in the epidermis and stratified epithelia of the corneal, buccal, gingival and genital mucosae [1]. They were first described by Paul Langerhans as neurons [2] but the advent of monoclonal antibodies revealed expression of MHC class II and macrophage antigens [3, 4] and a potential bone marrow origin [5]. LCs soon became the paradigm of migratory dendritic cells (DCs); tissue resident sentinels that could capture antigen, migrate to lymph nodes and develop potent immunostimulatory

Tel +44 (0)191 222 7785, matthew.collin@ncl.ac.uk

Conflicts of Interest

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capacity [6, 7]. In recent years, however, the story of LCs has become more complex. LCs, microglia and many populations of tissue macrophages have their earliest origins from primitive yolk sac hematopoiesis and may persist by local self-renewal for the entire life of an organism. These exciting developments have revised the concept of a mononuclear phagocyte system in which LCs and macrophages are continually replenished by monocytes [8].

LCs have now become an exemplar of this class of tissue-resident myeloid cell, leading some authors to re-classify them as macrophages [9], although uniquely, they retain genuine DC function. Critically, the demonstration of a primitive origin does not exclude a secondary origin from bone marrow and the LC network is renewed by bone marrow derived cells following severe inflammation [10, 11]. New work has revealed that this occurs in two waves, involving classical monocytes and an uncharacterized myeloid progenitor.

Recent studies have also examined the molecular regulation of LC development. The requirement of LCs to integrate into an epithelial layer and then to disengage from this layer demands special properties that may be instructive to compare with mesenchymal to epithelial transitions [12]. A key conceptual advance has been to draw a distinction between mechanisms involved in the development of LCs and those required for the maintenance of a stable LC network.

The pre-natal origin of Langerhans cells

Mammalian hematopoiesis has multiple overlapping origins. The first myeloid cells, so-called yolk sac macrophages, are generated from primitive erythro-myeloid progenitors (EMP) in the yolk sac between days 7 and 9 of mouse embryogenesis and at about 16-18 days of human gestation. EMPs have restricted potential and do not produce definitive long term multipotent hematopoietic stem cells (HSC). The majority of definitive HSCs arise in the aorta-gonad-mesonephros (AGM) region after day 9 in the mouse (day 32 in humans) and migrate first to the fetal liver and then to the bone marrow. Primitive waves of restricted hematopoiesis may contribute to fetal liver hematopoiesis overlapping with but not contributing to the adult HSC pool.

A primitive origin of LCs from yolk sac macrophages

It has been known for many years that yolk sac macrophages migrate throughout the embryonic tissues, entering the rudimentary skin and brain. However, fate-mapping experiments have only recently revealed that these primitive macrophages proliferate *in situ* giving rise to long-lived populations of LCs, microglia and macrophages that persist into post-natal and adult life (reviewed in [13]). The development of LCs and microglia is critically dependent upon the local production of IL-34 acting through the M-CSF receptor [14, 15].

The contribution of fetal liver monocytes to LC development

In the brain, microglia arise exclusively from yolk sac macrophages, as demonstrated by Ginhoux and colleagues using a Runx1 inducible fate-mapping system [16]. The same

technique applied to LCs yielded a more complex result in which LCs were initially labeled to the same level as microglia when the marker was activated at day 7.5, but between days 13.5 and birth, the level of marker fell by at least two-thirds. The authors interpreted this as evidence for a secondary origin of LCs from the unlabeled fetal liver, via a monocyte-like precursor potentially derived from definitive hematopoiesis [17]. However, this result was challenged by Geissmann and colleagues who adopted a powerful genetic strategy to demonstrate that LCs could develop in the absence of *c-myb* and *flt3*, obligate factors for the formation of definitive HSCs. This led them to propose that LCs were mainly derived from EMPs via a yolk sac macrophage intermediate, although they could not completely exclude an additional contribution of definitive hematopoiesis to the LC pool with these experiments [18]. In a subsequent study, fate-mapping with CSF-1 and *flt-3* inducible markers that tag primitive and definitive hematopoiesis, respectively, showed that primitive EMPs made substantial contributions to fetal liver myelopoiesis until day 16.5 [19]. This important finding suggested that the fetal liver monocyte proposed by Ginhoux to be a significant precursor of the LC pool, was ultimately derived from primitive hematopoiesis (Figure 1).

A distinct population of yolk sac progenitors gives rise to LCs via fetal liver monocytes

A further study in which fetal liver monocytes were specifically tagged, found evidence that they were indeed derived from primitive EMPs generated in the yolk sac, in broad agreement with the results of Geissmann and colleagues. However, crucially, LCs and tissue macrophages were found to originate from a distinct subset of late *c-myb*⁺ EMPs that did not form yolk sac macrophages but which gave rise to fetal liver monocytes [20]. Broadly speaking, this result resolves the controversy by showing that LCs can arise from primitive EMPs via the fetal liver. However, there is still disagreement about the relative importance of two distinct routes: the *c-myb*-independent yolk sac macrophage described by Geissmann and the *c-myb*⁺ fetal liver monocyte that takes over once the circulation is established. Both groups show that a third component of *flt-3* dependent definitive hematopoiesis makes a minor contribution (Figure 1). It has been postulated that resident cells laid down by primitive waves of myelopoiesis have intrinsic differences from those recruited from the progeny of definitive hematopoiesis in post-natal life [21]. Much more work is required to expound this concept; while some evidence is described in cardiac tissue macrophages [22], studies are currently lacking in LCs.

Bone marrow independence of human LCs

Human hematopoiesis also begins in yolk sac blood islands and transitions through the fetal liver to the bone marrow in a similar sequence but is fully established in the adult pattern by birth. Human LCs are fully formed at the practical limit of studies on human fetuses at around weeks 18-24 [23, 24].

A number of lines of evidence corroborate the view that LCs are self-renewing and independent of BM precursors in humans: LCs in skin grafts onto xenogeneic hosts incorporate BrdU and proliferate [25]; human limb transplants maintain donor-type LCs [26]; and, humans with *GATA2* or *IRF8* mutation have persisting LCs and tissue

macrophages, despite complete absence of circulating monocytes and DCs [27, 28]. GATA2 is another factor that is required for definitive hematopoiesis while IRF8 is required for the terminal differentiation of monocytes and DCs. A systematic study of the role of IRF8, IRF4, PU.1 and Id2 in LC development shows concordant results with human IRF8 mutation [29].

Following human bone marrow transplantation, rapid replenishment of LCs by bone marrow-derived cells is reported, even after non-myeloablative conditioning in the absence of overt graft versus host disease [30-32], although one recent study does not concur with these results [33]. This contrasts with the finding in mice where recipient LCs show robust survival after bone marrow transplantation [34]. Multiple factors may combine to limit the capacity of human LCs to self-renew following bone marrow transplantation: human recipients are many decades older than neonatal laboratory mice; human skin is continually exposed to UV light; and, patients have usually been treated with multiple cytoreductive drugs.

Self-renewal of LCs in the absence of inflammation

Approximately 2-5% of LCs express Ki-67 and are in cell cycle in the steady state. Staining of epidermal sheets reveals pairs of daughter cells scattered across the interfollicular regions [35, 36]. A beautiful random-color fate mapping tool has been used to show that equal-sized patches of epidermis are colonized by the progeny of multiple individual LCs that enter the skin during development [37]. Attempts to define a subset of LCs that act as local 'stem cells' have been unsuccessful suggesting that that mature LCs have an intrinsic ability to enter cell cycle when required. The regulation of LC density is incompletely understood but ablation of mTORC1 [38] or disruption of ERK/mTOR signaling via targeted ablation of the adaptor protein p14, leads to failure of LC homeostasis soon after birth [39]. The loss of p14 has recently been shown to interfere with TGF β signaling [40]. Mice with these mutations are also unable to form long term resident LC populations after inflammation.

The importance of TGF β signaling in human and mouse LC development has been known for many years [41-43]. The failure of LC development in Runx3 and Id2 knockout mice is also linked to the observation that Runx3 is required for transcription of Id2, a key target gene of TGF β . A permissive role for Pu.1 in activating Runx expression has also recently been highlighted [29].

Recent data recognize a distinction between TGF β signaling pathways that affect the initial formation of LCs and those required to promote stability of the LC network (Figure 2). Surprisingly, although adult TGF β knockout mice lack LCs, deletion of the classical TGF β receptor chain TGF β RI/ALK5 did not prevent the formation of LCs but caused early attrition of the network through enhanced migration of LCs in early post-natal life [44, 45].

This conundrum has recently been addressed by the elegant work of Strobl and colleagues showing that alternative TGF β signaling involving bone morphogenetic protein 7 (BMP7), the ALK3 receptor and phosphorylation of smad 1/5/8, is prominent in LC development [46]. In contrast, signals transduced through TGF β RI/ALK5 and smad2/3 appear to be more

important in maintaining homeostasis of the LC network. Numerous mechanisms are cited for this effect including the expression of adhesion molecules EpCAM/TROP1, TROP2 and E-Cadherin and beta-catenin [47]. Another critical pathway maintaining LCs in a quiescent state in response to TGF β , is the expression of Axl, a member of the TAM family of receptors for apoptotic cells (Tyro3, Axl, Mer). Axl ligand Gas 6 is abundant in the suprabasal epidermis and Axl expression maintains TLR hyporesponsiveness, contributing to the stability of the LC network [48].

Replenishment of Langerhans cells after inflammatory insults

Inflammation of the epidermis results in increased turnover and trafficking of LCs to draining lymph nodes [49]. In atopic conditions, proliferation increases to meet this demand [35] but denudation of the epidermis recruits de novo bone marrow derived progenitors. Several studies have demonstrated a two-wave reconstitution of the LC compartment involving a transient population of classical monocytes followed by a myeloid precursor that restores stable self-renewing LCs (Figure 3). The initial wave of monocyte-derived LCs retain markers Gr-1 and lysozyme and develop low langerin and EpCAM expression. [50-52]. A microscopic dissection of this process showed that CCL2 and CCL20 (CCR2 and CCR6 ligands, respectively) are expressed by discrete portions of the hair follicle that function as portals for monocyte recruitment [51]. Four to six weeks later, after inflammation has subsided, Id2-dependent LCs with high langerin and EpCAM are observed, originating by an uncertain pathway from the bone marrow [52]. Previous studies in mice implicating flt-3 dependent pathways in the long term reconstitution of LCs suggest that a second wave of precursors may be derived from classical DC progenitors [10, 53]. Further experiments in mice are warranted to test the potential of blood DCs and pre-DCs to provide long term LC reconstitution in the absence of monocytes. The existence of a second wave of LC precursors is not universally accepted and long term survival of the LC network (up to six weeks) was recently reported in a lineage-restricted Id2 knockout mouse model [29]. Strain and preparative differences may explain some of this discrepancy and further confirmation of the two-wave model is required.

Two human LC precursors?

In humans, CD14⁺ monocytes can be induced to express langerin in response to GM-CSF, IL-4 and TGF β [54, 55]. In addition two recent papers highlight the potential of CD1c⁺ blood DCs to acquire high levels of langerin and CD1a, forming LC-like cells in vitro in response to TSLP and TGF β [56] or GM-CSF and BMP7 [57]. Monocytes express lower levels of langerin and do not form Birbeck granules as readily as CD1c⁺ DCs [57] although it is possible that this difference is effaced when monocytes are activated with Notch ligands as might occur in vivo [55, 58]. A unifying hypothesis that accounts for the expression of langerin by both monocytes and CD1c⁺ DCs is that they represent the two waves of repopulation reported in mouse models. A number of observations are accommodated by this model. First, the puzzling report of CD1a⁺ 'LC precursors' in human blood [59] is reconciled by the knowledge that the antibody clone used to detect CD1a in this study is actually specific for CD1c, thus correctly identifying the CD1c⁺ DC as a potential LC precursor [60]. Second, the claim that CD14⁺ dermal cells are LC precursors [61] is

consistent with recent studies showing that they are related to monocytes and therefore might be induced to express langerin at a low level [62]. Finally, the search for langerin+ DCs in humans revealed small populations of langerin+ CD1c+ DCs in the dermis, lung and lymph node [63]. These cells are distinct from and independent of LCs in the steady state, but the expression of langerin may reflect a latent potential of DC precursors to differentiate into LCs under inflammatory conditions. There are many differences between classical monocytes and CD1c+ DCs at the molecular level and it will be very interesting to unravel the critical factors, possibly involving Runx3 and Id2, that might permit LC differentiation from a DC lineage.

Langerhans cell neoplasia

Understanding the regulation of LC development and homeostasis has important implications for a human disease, Langerhans Cell Histiocytosis (LCH). LCH has the hallmarks of a 'functional neoplasia' i.e. a fundamentally neoplastic disease that retains functions of a normal cellular counterpart. LCH is caused by activating mutations of the RAF/MEK/ERK pathway that result in accumulation of Langerhans-like cells with an immature phenotype in ectopic sites [64-66]. The study of normal LC development may identify survival signaling circuits that can be interrupted or differentiation pathways through which maturation and senescence may be induced, in order to treat this disease. The subtle dysregulation of ERK that causes LCH also illustrates how critically poised the development of LCs is in healthy conditions.

Conclusion

The study of LCs continues to provide conceptually challenging results. LCs are the exemplar of a resident myeloid cell population arising from primitive hematopoiesis and able to self-renew throughout life. Future studies will be aimed at uncovering functional differences between long term resident myeloid cells and those recruited by an inflammatory insult. In ontogeny and after inflammation, LC precursors come in waves. At first sight, there are many differences between the precursor populations involved in these two processes. However, further studies are required to understand exactly which aspects of the developmental program that generated LCs in pre-natal life is recapitulated in the replenishment of a stable LC network after inflammation. LC development is complex and signals that control the development and differentiation of LCs are distinct from those that maintain a stable LC network; more work is required to unravel these mechanisms. It seems likely that the LC has yet more secrets to reveal that will have wide ranging impacts in the field of myeloid cell biology.

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Key points

- 1) LCs have arise from primitive EMPs generated in the yolk sac via two routes: c-myb independent yolk sac macrophages and c-myb+ fetal liver monocytes
- 2) LCs form a stable self-renewing network that does not require bone marrow-derived precursors in post-natal life
- 3) Distinct TGFb signaling pathways are involved in the development of LCs and maintenance of their homeostasis
- 4) Inflammation recruits two waves of LC precursors: a transient population of classical monocytes followed by a second wave of uncharacterized myeloid cells that creates a stable LC network
- 5) In vitro experiments in humans suggest that myeloid DCs have LC potential but whether this lineage provides the stable LC population after inflammation, remains speculative

Figure 1. Initial generation of LCs during prenatal life

The LC is formed from primitive erythro-myeloid progenitors (EMPs) that first arise in the yolk sac in a Pu.-1 dependent fashion and migrate to the epidermis as yolk sac macrophages. This is followed by a second wave of fetal liver monocytes derived from late EMPs that acquire c-myb expression and a small third component of hematopoietic stem cell (HSC)-derived LCs originating in the aorta-gonad-mesonephros (AGM). The relative contribution of each wave to the LC network at birth is indicated by the respective colors: yellow for yolk sac, brown for fetal liver monocyte and red for HSC. Approximate comparison of mouse and human gestation is shown below. YS: yolk sac; FL fetal liver; BM bone marrow.

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Figure 3. Two-wave model of LC replenishment after inflammation

Transient recruitment of classical monocytes in a CCR2 and CCR6 dependent fashion dependent upon M-CSF but not Id2 is followed by long term Id2-dependent repopulation of the LC network from an uncharacterized precursor. Previous work suggests that flt3 and β-catenin signaling are involved in the long term repopulation of LCs. The role of notch signaling and the local production of GM-CSF, TGFβ, TSLP and BMP7 have not been tested in *in vivo* models.