Ontogeny and homeostasis of Langerhans cells

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Langerhans cells (LCs) refer to the dendritic cells (DCs) that populate the epidermis. Strategically located at one of the body’s largest interfaces with the external environment, they form the first line of defense against pathogens that breach the skin. Although LCs share several phenotypical and functional features with lymphoid and non-lymphoid organ DCs, they also have unique properties that distinguish them from most DC populations. In this review, we will discuss the key mechanisms that regulate LC homeostasis in quiescent and inflamed skin. We will also discuss recent evidence that suggests that LCs arise from dedicated precursors during early embryonic development.

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Langerhans cells (LCs) are hematopoietic cells that belong to the myeloid lineage. They form the only myeloid cell population of the epidermis accounting for up to 5% of total nucleated epidermal cells in mice and humans.

LCs are part of the dendritic cell (DC) lineage, a heterogeneous group of cells that control the induction of adaptive immune responses. DCs are usually classified into two groups that include classical and plasmacytoid DCs. Similar to classical DCs, LCs constitutively express major histocompatibility complex (MHC) class II molecules on the cell surface, lack lineage markers and express the CD11c integrin. They migrate from the epidermis to the skin draining lymph nodes through the lymphatic vessels, and present processed antigens to T lymphocytes (for review see Merad et al.3). However, LCs have unique homeostatic properties that affect their role in skin immunity. This review discusses the key features that distinguish LCs from classical DCs.

HISTORICAL PERSPECTIVE
In 1868, Paul Langerhans first described the presence of dendritic-shaped cells in the human epidermis. He hypothesized that these cells were intraepidermal nerve endings, owing to their impregnation with gold chloride that was thought to be specific for neurons.4 The cells remained an enigma to scientists for decades, and were successively considered to be Schwann cells,5 ‘effete’ or exhausted non-pigmentary melanocytes or cells of ectodermal origin6 (for review see Prunieras7). However, for many years, LCs were thought to be related to melanocytes and to share the same neural crest origin. As LCs share morphological features with melanocytes and localize closer to the skin’s surface, they were believed to be the progeny of exhausted melanocytes, moving towards the surface to be shed (for review see Prunieras7). However, several subsequent studies refuted the hypothesis that LCs were related to melanocytes. Ultrastructural studies using electron microscopy identified important differences between LCs and melanocytes, showing that LCs lacked melanin granules but possessed specific cytoplasmic granules that were later defined as Birbeck granules.8–10 Adoptive transfer studies were also against the melanocyte hypothesis.10,11 In these studies, mouse embryonic limb buds collected either before (E10.5) or after (E11.5) neural crest invasion were grafted into the spleen of adult mice. This time point was specifically chosen as it is at E11 of the 20/21 days of mouse embryonic development period that newly formed limb buds are colonized by melanoblasts, the precursors of melanocytes, originating from the neural crest. In these studies, LC-like cells were visualized in both sets of limb buds 3 weeks after transplantation, suggesting that LCs were unrelated to melanocytes. However, these results were not conclusive, as they could not exclude the possibility that the observed LCs were not derived from the host adult mouse.

The most definite proof against the melanocyte origin of the epidermal LCs came from a study by Reams and Tompkins. In this study, embryonic limb buds were grafted into chick embryo hosts to distinguish recipient from host-derived LCs.6 Similar to earlier studies,10,11 the limb buds were isolated either prior or after the seeding of neural crest cells and LC-like cells were identified in both set of limb bud grafts at the time of recovery from the chick embryo. However, these cells lacked Birbeck granules and were therefore named indeterminate cells. It is only when the skin derived from the grafts was put in in vitro culture for maturation that LCs with Birbeck granules appeared at later time points (8 days). Importantly, using the same experimental model, this study also established that purified layers of ectodermal cells gave rise to LCs upon transplantation to chick
Embryos. On the basis of these data, LCs were thought to develop independently of melanocytes and to be of ectodermal origin. The importance of these findings will be discussed later in the review.

During the same time, important information was obtained from electron microscopy studies revealing the presence of phagocyte-like cells containing Birbeck granules in the human dermis.12,13 While the debate persisted about the origins of LCs, Hashimoto was the first to speculate that LCs were a self-perpetuating ‘intraepithelial phagocytic system.’13 This view was supported by Shelley, Stingl and others who believed that LCs performed macrophage-like functions, and confirmed by Stingl et al. who showed that LCs express the receptors for antibody constant regions and for complement components, both characteristics of macrophages.14,15 However, it was only in 1979 that Katz and Frelinger’s studies unequivocally established the hematopoietic nature of LCs.16,17 In these studies, lethally irradiated mice were reconstituted with donor allogeneic or semiallogeneic hematopoietic bone marrow (BM) progenitor cells. A few weeks after transplant, the identification of LCs of donor origin in the skin of recipient mice established for the first time the BM origin of these cells. Although this revealed the capacity for LCs to be replenished from the BM under inflammatory conditions, it also led to the general assumption that the same mechanisms operated in the steady state throughout adult life.

The identification of MHC class II expression on both human and mouse epidermal LCs strongly suggested that these cells were antigen-presenting cells.18,19 Finally, their role as antigen-antigen-presenting cells or DCs was definitively established in a series of studies in the early 1980s.20–23

**HOMEOSTASIS OF LC UNDER STEADY STATE AND INFLAMMATORY CONDITIONS**

**Steady state: general concepts**

The homeostasis of classical DCs results from a balance between local proliferation, cell death and replenishment by blood-derived precursors.24,25 Although the results from Katz and Frelinger described above led to the concept that similar to most DCs, LCs were also maintained by circulating precursors, the development of congenic BM transplant mouse models allowed us to revisit this concept.26 Congenic BM transplantation is a useful model to study the turnover of hematopoietic cells in lethally irradiated animals in the absence of graft-versus-host disease (GVHD). In this model, recipient LCs that were partially eliminated by the radiation regimen, repopulated locally independently of donor circulating precursor cells and remained of host origin throughout life. By contrast, the majority of leukocytes, including lymphoid organ DCs, was replaced by donor hematopoietic precursor cells by 3 to 4 weeks post-transplantation.26 DCs in the airways and in the vaginal mucosa were also eliminated following lethal irradiation and replaced by blood-borne-derived cells.27,28 Parabiotic mice provide a physiological model to study the turnover of hematopoietic cells in the steady state. It is in this model that the LC ability to maintain and renew locally throughout life, independently of adult circulating precursors was unequivocally established.26 Parabiotic mice consist of two surgically attached congenic mice called parabionts, which share a common blood circulatory system but distinct organs for prolonged periods of time. Few weeks after the initiation of parabiosis, the mixing of DC populations can reach up to 30% in the spleen and lymph nodes in both parabionts.24,25 In contrast, there was no mixing of LCs in the epidermis, even after 1 year of parabiosis.26 Although equivalent studies cannot be carried out in humans, several reports suggest that human LCs share similar properties. Proliferating LCs were identified in human skin.29,30 A study in which human skin is grafted onto nude mice revealed the persistence of human LCs > 4 months after transplant.31 Strikingly, graft-resident LCs were also found to remain for a year into a transplanted human limb graft.32 Human LCs also seem to resist radiation-based hematopoietic stem cell transplantation as a small number of recipient LCs can be detected for at least 1 year after transplantation.33,34 These results establish that, like their murine counterparts, human LCs are both radiosensitive and locally self-renewing.

**Steady state: molecular control of LC homeostasis**

In contrast to classical DC homeostasis, steady state LC homeostasis seems to result from a balance between local proliferation, death and emigration of LCs to the draining lymph nodes, as LCs maintain themselves locally independently of circulating precursors. In adult mouse and human epidermis, a minor fraction of LCs is dividing at any single time, whereas the exact rate of LC death is not known. The exact half-life of LCs has yet also to be clearly established, but seems to be far longer than the 7–8 days half-life of most DC subtypes.24,35 In vivo imaging of LCs expressing the enhanced green fluorescent protein (EGFP) indicates that the steady state turnover of epidermal LCs is slow, with an estimated half-life of 53–78 days,36 which is consistent with earlier in vivo labeling studies.37 In transgenic mice expressing the diphtheria toxin receptor (DTR) under the langerin promoter, DT administration leads to conditional depletion of the entire LC population. In this model, LC reconstitution takes several months,38,39 whereas lymphoid organ DCs repopulate in few days upon DT administration in CD11c-DTR mice.40 The local proliferative capacity of LCs has long been identified.13,19,20,41,42 Recent studies in our laboratory established that 1–2% epidermal LCs actively proliferate at any given time both in mice and humans (Ginhoux, unpublished data), whereas lymphoid organ DCs divide at the rate of 5%.24,35 DC proliferation in peripheral lymphoid organs is dependent on lymphotixin-β (LTβ) and the Fms-like-tyrosine-kinase 3 ligand (Flt3L) receptor.44 In contrast, LCs are not affected in mice that lack Flt3L or its receptor Flt3.35 The mechanisms that control local LC proliferation remain to be established and one possibility is that LC proliferation in the epidermis is triggered by LC emigration to the draining lymph nodes. LC differentiation is also regulated differently than classical DCs. Flt3L and Flt3 control the differentiation of classical DCs in both mice and humans.45 Flt3-deficient mice have severe defects in DC development in lymphoid44 and non-lymphoid organs.35 Granulocyte/macrophage colony-stimulating factor (GM-CSF) is also an important cytokine for the differentiation of DCs in vitro and recent work from our group revealed the role of this cytokine in the differentiation of one subset of DCs in the intestine.46 Surprisingly, as mentioned above, mice lacking Flt3, Flt3L or GM-CSF have normal numbers of LCs in vivo.35,47 In contrast, mice deficient in transforming growth factor β-1 (TGFβ-1),48 inhibitor of DNA binding (ID2),49 Runx-related transcription factor 3 (RUNX3)50 or the macrophage CSF receptor (MCFS-R)51 lack LCs. However, the mechanisms that affect LC development in these mice remain unclear and may include defects in LC differentiation, proliferation and/or survival or in LC precursors as discussed later. These results further underline the separation between LCs and the classical DC lineage.

**Repopulation of LCs during inflammation**

LC homeostasis in inflamed skin depends on the type and strength of inflammation. In severe inflammatory injuries that lead to major LC loss, epidermal–dermal barrier damages, and the release of mononuclear chemokines, LCs are repopulated by circulating blood precursors.
Examples of severe skin injuries include skin exposure to ultraviolet (UV) light and cutaneous GVHD that occurs after allogeneic hematopoietic cell transplantation.\textsuperscript{56,51,52} Upon skin exposure to UV, LCs are repopulated by circulating Gr\textsuperscript{1} blood monocytes.\textsuperscript{51} Similarly in humans who have undergone allogeneic hematopoietic stem cell transplantation, higher numbers of donor-derived LCs are found in the skin of patients affected with cutaneous GVHD compared with patients without skin inflammatory injuries.\textsuperscript{53} In UV or GVHD-affected skin, LC repopulation is dependent on the expression of CCR2 and CCR6 by circulating cells.\textsuperscript{50,52} CCR2 most likely controls the recruitment of monocytes to the dermis, whereas CCR6 likely control their migration to the epidermis.\textsuperscript{23,52} Similar observations have been made using human cells in vitro. CD34\textsuperscript{+} hematopoietic progenitor cells give rise to LCs through a monocytic differentiation pathway accompanied by the sequential expression of CCR2 and CCR6.\textsuperscript{54} In this model, monocytes were shown to respond to CCR2 ligands expressed in the perivascular region of the dermis, and subsequently differentiate into LCs in response to CCR6 ligands expressed by keratinocytes at the epidermal–dermal junction.\textsuperscript{54} Consistent with these data, circulating monocytes\textsuperscript{55} and dermal CD14\textsuperscript{+} cells\textsuperscript{56} that have been isolated from human skin can differentiate into CCR6\textsuperscript{langerin}\textsuperscript{+} cells in vitro in response to TGF\textsubscript{beta}1.\textsuperscript{57} Subsequent studies using skin-equivalent models, in which skin is reconstituted in vitro from skin stem cells, confirmed that monocytes can differentiate into LCs in the epithelium and suggested a role for CXC chemokine ligand 14 (CXCL14), a chemokine that is expressed in the skin during the steady state but is upregulated after injury.\textsuperscript{58}

In contrast, in less severe injuries that lead to moderate LC loss, preserve the epidermal–dermal barrier integrity and are not accompanied by the release of inflammatory chemokines, the remaining LCs have the potential to repopulate themselves locally. Local LC repopulation have first been identified in two types of skin injuries including exposure to skin sensitizers and to x-ray irradiation.\textsuperscript{56} Similar results were recently obtained in a mouse model of atopic dermatitis, in which LC proliferation is controlled by keratinocyte-derived signals.\textsuperscript{59} Interestingly, LC loss in the absence of inflammation, as in the case of DT-mediated ablation in langerin-DTR mice, leads to much slower LC repopulation, which may suggest that inflammatory signals control the dynamics of LC proliferation.

These examples represent extreme scenarios and it is likely that during common skin injuries, LC repopulation occurs from both local and blood-derived precursors, as shown in a mouse model of Herpes Simplex virus skin infection.\textsuperscript{60}

THE ORIGIN OF LC

An apparent conundrum in understanding LC ontogeny is that while they are unequivocally of hematopoietic origin, they derive from precursors that are present in the skin before birth and before BM has developed (Figure 1). Indeed, early studies in rodents noted the presence of ATPase\textsuperscript{+} LC-like cells in the epidermis in later stages of embryonic development, around E16 and E17.\textsuperscript{10,61,62} A study from Romani et al.\textsuperscript{63} revealed that ATPase\textsuperscript{+} cells present in the E18 murine epidermis expressed adult LC markers including F4/80 and CD11b but acquired MHC class II and Birbeck granules only after birth.

Figure 1 Ontogeny of murine Langerhans cells. We propose that Langerhans cells (LCs) might derive from yolk sac (YS) primitive macrophages that migrate to the skin via the blood circulation during mid-embryogenesis. The YS is the first extraembryonic hematopoietic tissue during embryogenesis, starting from E7.5 to E12.5. YS myeloid precursors give rise to primitive macrophages (Gr\textsuperscript{1},CD11b\textsuperscript{+},M-CSF-R\textsuperscript{+},CX3CR1\textsuperscript{−},EGFP\textsuperscript{+}) that emerge around E8. YS-derived primitive macrophages will then join the intraembryonic circulation after the circulatory system has been fully established from E8.5 to E10. From the blood, they migrate to various tissues to become fetal macrophages. On the basis of all the evidence cited in the review, fetal macrophages are present in the developing skin as soon as E10.5. It is unlikely that the fetal macrophages are already in the future epidermis, which consists only at this stage of only a single layer of ectodermal cells. Fetal macrophages are rather in the subepidermal mesenchyme, or developing dermis. Importantly, fetal macrophages are already present in the skin before the onset of monocyte production by the fetal liver. YS primitive macrophages derived fetal macrophages might be sufficient to populate the LC compartment of embryonic skin, but at the present time, another later contribution of fetal liver monocytes, migrating from the blood to the epidermis between E12.5 and E18.5 cannot be excluded. From E16.5/E17, putative LC precursors with the same phenotype than fetal macrophages can be detected in the developing epidermis, which have lost its periderm and consist at this stage of multiple layers of keratinocytes. Epidermal LC precursors in E16.5 and E18.5 epidermis have a high proliferative index (up to 20\% of proliferating cells) and differentiate into LCs after birth.
Subsequent studies suggested that these cells were present in the epidermis as early as E16 and lacked langerin and CD24 expression, two markers of mature adult LCs. These putative LC precursors proliferate actively the first week after birth, while acquiring MHC class II and langerin expression. More recently, we and others found that these embryonic LC precursors express high level of the fractalkine receptor CX3CR1 (Ginhoux, unpublished data). CX3CR1 is a useful marker to specifically track LC precursors as mature LCs lose expression of this molecule during differentiation. Interestingly, we could not detect CX3CR1+CD11b+F4/80+IA+ or IA–LC precursors in adult skin, suggesting that most LC precursors that are present at birth have differentiated into mature LCs.

Similar to rodents, human LCs can be detected in the developing epidermis before birth. Human HLA-DR-ATPase can be detected as early as 6–7 weeks of estimated gestational age (EGA). Consistent with these results, HLA-DRCD1c leukocytes were recently found scattered in embryonic human skin starting from 9 weeks EGA and increased continuously as the skin develops. Langerin was found to be absent on these CD45CD1c cells between 9 and 10 weeks EGA, but exclusively appears on CD45CD1c cells in the epidermis after week 11. Expression of the proliferation marker Ki67 was also detected in over 17.0% of CD45+ cells in embryonic skin, but only after week 11. Expression of the proliferation marker Ki67 was also detected in over 17.0% of CD45 cells in embryonic skin, but only 3.7% in fetal skin and 0.9% in adult skin. Although it is difficult to compare the kinetics of murine and human embryonic development, these studies suggest that langerin+ LC precursors are recruited to the epidermis in both species at a comparable development stage of the epidermis (E16/E17 in mice and 9 weeks EGA for humans), when the epidermis loses its periderm and starts to acquire its stratum corneum (Figure 1) (E16/E17 in mice and between 8 to 12 weeks EGA for humans).

Identifying the LC precursor

Although it is now clear that LCs derive from embryonic hematopoietic precursors that seed the skin before birth, the origin of the precursors that migrate to the epidermis to give rise to the 'first' endogenous wave of LCs remains unknown and difficult to establish. Here, we discuss studies in the literature as well as recent findings from our group regarding the origin of these precursors.

During late embryogenesis, from E12 on, the fetal liver is the primary site of hematopoiesis, whereas the BM takes over right after birth (Figure 1). As blood monocytes were known to replenish LCs following inflammation, fetal liver-derived monocytes could represent potential LC precursors. We recently identified monocyte-like cells in the embryonic skin expressing CD11b, F4/80, CD115 (MCSF-R) and CX3CR1, with the difference that they lacked the Gr-1 marker, in contrast to the majority of circulating embryonic monocytes (Ginhoux, unpublished data). These cells correspond to the LC precursors described earlier by Romani et al. However, unlike in adult inflamed skin, the recruitment of LC precursors to the fetal epidermis is independent of CCR2 and CCR6, as CCR2 and CCR6 ligands are not expressed in fetal skin (Ginhoux, unpublished data), which is consistent with earlier data showing that mice that lack CCR2 and CCR6 have normal numbers of epidermal LCs.

Earlier studies have shown that LC-like cells are present in epidermal sheets derived from grafted limb buds isolated at E10.5, before the development of fetal liver-derived hematopoiesis. These results imply that at early time points of development, as soon as E10.5, the developing skin already contains progenitors with LC potential (Figure 1). Interestingly, in the rat, macrophage-like cells can be found in the dermis around E12. Highly proliferative macrophage-like cells were also detected in E16/17 rat epidermis and shown to acquire MHC class II around E18. A more recent study, using a CD115-EGFP reporter mouse model, identified the presence of EGFP+ cells in E10.5 limb buds. In addition, human embryonic macrophages were also detected in the developing dermis around 6 to 14 weeks EGA. Altogether, these observations suggest that fetal macrophages might represent the LC precursor. Interestingly, Breathnach hypothesized that these embryonic macrophages were of yolk sac (YS) origin, based on their close resemblance to the phagocytic macrophages described in the YS of 4 to 5 weeks old human fetuses.

The yolk sac hypothesis

The YS is the first extraembryonic hematopoietic tissue in murine and human embryogenesis (Figure 1). In mouse, the first hematopoietic cells appear in the YS blood islands around E7.5, shortly after the onset of gastrulation, and belong to the erythroid and myeloid lineages. Human hematopoiesis is also initiated in the YS during the third week of development and YS-derived stem cells are limited to myelo-erythroid development. YS myeloid precursors give rise to primitive macrophages, described to emerge around E8 in the YS blood islands. YS-derived primitive macrophages will then migrate to various tissues either directly at early time points, or through the blood after the circulatory system has been fully established from E8.5 to E10. Once in the tissues, they differentiate into several fetal macrophage populations even before the onset of monocye production by the fetal liver. These fetal macrophages have high proliferative potential. Interestingly, the MHC class II–LC precursors in E18.5 epidermis also present a high proliferative index (up to 20% of proliferating cells) (Ginhoux, unpublished data).

Phenotypically, YS primitive macrophages are Gr1+CD11b+MCSF-R+F4/80+CX3CR1-EGFP+ with the same phenotype (Gr1+CD11b+MCSF-R+F4/80+CX3CR1-EGFP+) as fetal liver monocytes (Ginhoux, unpublished data). In addition, YS primitive-derived macrophages or fetal macrophages with a similar phenotype (Gr1+CD11b+MCSF-R+F4/80+CX3CR1-EGFP+) can be found in the blood circulation as soon as E12.5 as well as in E12.5 limb buds (Ginhoux, unpublished data) and could correspond to the MCSF-R+ cells described by Rae et al. Altogether, these data constitute circumstantial evidence supporting the hypothesis that LCs are derived from YS primitive macrophages that migrate into the developing skin from E10.5 to E16.5. At the present time, a second contribution of fetal liver monocytes, migrating from the blood to the epidermis between E12.5 and E18.5 cannot be excluded, although this would seem redundant. Adoptive transfer of fetal liver monocytes in utero, as well as a genetic tagging model of the YS macrophages progeny should help to resolve this issue.

MOLECULAR CONTROL OF LC ONTOGENY AND DIFFERENTIATION

Until now, the reasons for an absence of LCs in certain KO mouse models was thought to be due to defects in LC proliferation, differentiation or survival based on the assumption that these cells were continually recruited during adult life. However, as embryogenesis is a critical stage of LC development, it raised the question whether these models actually lacked LCs also due to defects in LC precursors, in their generation or their recruitment to the embryonic skin.

TGFβ-1 is a key molecule for LC development and mice that lack TGFβ-1 lack LCs. Although both keratinocytes and LCs express TGFβ-1, it is the LC autocrine source of TGFβ-1 that is required for LC development, although the exact mechanism by which TGFβ-1 control LC homeostasis remains to be clarified. In humans, TGFβ-1 is needed for the in vitro differentiation of LC-like cells from CD34+...
hematopoietic progenitor cells, or purified monocytes. Recent data also showed that TGFβ-1 production, which is first detectable in the primitive epidermis at 9 weeks EGA, precedes langerin and CD1a expression on CD45+CD1c-LC precursors in human skin. This correlation between the production of this cytokine in the epidermis and the acquisition of the LC phenotype suggests that TGFβ-1 might have a key role in the differentiation of epidermal precursors into mature LCs. Similarly to the TGFβ-1 KO model, ID2 KO and RUNX3 KO mice lack mature LCs. Both ID2 and RUNX3 are involved in TGFβ signaling. ID2 acts downstream of TGFβ-1 and inhibits helix-loop-helix transcription factors. RUNX3 is a member of the RUNX family of transcription factors that mediate TGFβ responses and lack of appropriate TGFβ-induced RUNX3 signaling leads to a defect in LC development. It remains to be demonstrated whether LC precursors are present or absent in late embryogenesis of all these KO models.

The receptor for MCSF (also known as CSF-1) is also required for the development of LCs. However, Gsflop/op mice, which carry a mutation in the gene encoding MCSF, have normal numbers of LCs. This implies the existence of another ligand for the MCSF receptor (MCSF-R) that could compensate for the absence of MCSF. Consistent with this possibility, interleukin-34 (IL-34) has recently been identified as a ligand for MCSF-R in mice and humans. IL-34 binds MCSF-R with a higher affinity than MCSF. Although nothing is known about IL-34 expression during embryogenesis, MCSF expression has been followed in mouse fetal and extraembryonic tissues at various stages of development. Expression was predominantly reported in extraembryonic tissues, such as the YS, as early as E10. However, the exact role for MCSF-R ligands in LC development has yet to be determined. Interestingly, the zebrafish mutant model pum, which lacks a functional MCSF-R gene, exhibits macrophage defects. In these animals, early YS macrophages differentiate but fail to migrate out of the YS and to invade embryonic tissues. Therefore, we can hypothesize that the absence of LCs in MCSF-R KO mice is similarly due to a defect in YS macrophages, further underlining the affiliation between YS macrophages and LCs.

CONCLUSION
LCs have served as the prototype DC population, through which many key features have been elucidated, from the collection of antigens in the periphery to the migration, maturation and trafficking of cells to the T-cell areas of the lymph node. It is now clear that LCs represent a specific DC population that has a unique origin and specific differentiation and homeostatic requirements. How these differences determine LC functional attributes and control their role in skin immunity is an important challenge to resolve in the years to come.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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