REVIEW ARTICLE

Of skin and bone: did Langerhans cells and osteoclasts evolve from a common ancestor?

Christopher G. Mueller and Benjamin Voisin*

CNRS UPR 3572, Laboratory of Immunopathology and Therapeutic Chemistry/Laboratory of Excellence MEDALIS, Institut de Biologie Moléculaire et Cellulaire, University of Strasbourg, 67000 Strasbourg, France

Abstract

Skin Langerhans cells are antigen-presenting cells of the interfollicular epidermis and the upper part of the hair follicle, whereas osteoclasts are specialized bone-resorbing macrophages. Although at first view these two cell types appear to have little in common, a closer analysis reveals shared features, and when taking into account their surrounding environment, a hypothesis can be developed that Langerhans cells and osteoclasts have evolved from a common ancestral cell type. In this mini-review, we have compared the ontogenetic features of Langerhans cells and osteoclasts from a genetic and a functional point of view, an issue that so far has been overlooked. The gene programs that control cell differentiation, and the body parts where they reside, present surprising similarities. Whereas the function of osteoclasts in bone degradation has been established since the first vertebrates, Langerhans cells may have undergone a stepwise adaptation from aquatic to terrestrial life. Their cell function co-evolved with the imperatives of the skin to protect against physical impact, heat, water loss and pathogens, which implied the capacity of Langerhans cells to associate with skin appendages and to develop immunostimulatory functions. For the highly versatile and efficient immune system of modern vertebrates, Langerhans cells may be a memory of the past.

Key words: Langerhans cell; osteoclast; macrophage; dendritic cell; evolution; hair follicle.

Shared ontogenic features of Langerhans cells and osteoclasts

Langerhans cells (LCs) were identified by Paul Langerhans in 1868 when he initially described the cells as cutaneous epithelial nervous cells. A century later they were characterized as antigen-presenting cells and classified as members of the dendritic cell family. Like dendritic cells, macrophages and B cells, LCs present antigenic peptides associated with the major histocompatibility complex (MHC) I or II to T cells, but – a feature shared only with dendritic cells – they can also migrate from the skin to the lymph nodes to activate native T cells (Maurer & Stingl, 2001). LCs and dendritic cells undergo migration, antigen processing and T-cell activation in response to innate immune stimuli, such as those triggering Toll-like receptors, a process called maturation. LCs and dendritic cells therefore associate the innate with the adaptive immune response. LCs are also equipped with CD1 molecules that, like MHC-I molecules, are composed of an α and a β chain and present lipids to special T cells. Other than antigen presentation, from a functional perspective, LCs seem to have little in common with macrophages, at least what is considered the classical macrophage. However, the definition of macrophage is loose. In fact, macrophages comprise a large family of myeloid cells adapted to their microenvironment. As every tissue contains its own pool of specialized macrophages -of embryonic origin or continuously supplied by monocytes – the diversity of these cells is large. It comprises the Kupffer cells of the liver, microglia of the brain, alveolar macrophages of the lung, osteoclasts of the bones, peritoneal macrophages and a heterogeneous population in lymphoid organs. In addition, tissues can also be infiltrated with inflammatory macrophages of monocytic origin and polarized into M1 or M2 types according to the inflammatory program (Gordon & Taylor, 2005). Osteoclasts (OCLs) are

Correspondence

Christopher G. Mueller, CNRS UPR 3572, Laboratory of Immunopathology and Therapeutic Chemistry/Laboratory of Excellence MEDALIS, Institut de Biologie Moléculaire et Cellulaire, University of Strasbourg, 15 rue René Descartes, 67000 Strasbourg, France. T: + 33 (0)3 88417114; F: + 33 (0)3 88610680; E: c.mueller@cnrs-ibmc.unistra.fr

*Current address: Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

macrophages specialized in the degradation of bone. They are characterized by the production of a series of degradative enzymes, acidification of the extracellular space and a directional cell secretory system to channel the localized degradation to bone surface (Cappariello et al. 2014). OCLs function in close association with osteoblasts, cells of the mesenchymal lineage that deposit bone matrix. Active OCLs release factors that promote osteoblast function, leading to a balanced equilibrium between bone resorption and deposition. Animals that lack functional OCLs display osteopetrosis and thus severe growth and developmental defects. Reciprocally, their overactivity leads to osteoporosis. On the other hand, mice deficient in LCs under steady-state conditions do not show a prominent phenotype (Bennett et al. 2005; Kissenpfennig et al. 2005). This may be attributable to the plasticity of the myeloid lineage and the capacity of monocytes or dermal mononuclear phagocytes to replace LCs under inflammatory conditions.

At first view, LCs and OCLs appear thus very different in form and function, but shared features can be uncovered. OCLs are very large, multinucleated cells formed by the fusion of mononuclear precursor cells. Soft tissue macrophages can also fuse to giant cells such as giant foreign body cells, and dendritic cells can undergo differentiation into OCL-like cells in response to osteoclastogenic factors (Rivollier et al. 2004; Ibanez et al. 2016). However, whether bona fide LCs fuse into multinucleated cells with OCL function is still debated, although in LC histiocytosis, cells with morphological and antigenic features typical of OCLs have been observed (Egeler et al. 2010). Both LCs and OCLs are part of the myeloid lineage (Gordon, 2003; Satpathy et al. 2012). During embryogenesis, LCs arise from the yolk sac and the fetal liver to colonize the epidermis, where they autorenew throughout life (Hoeffel et al. 2012; Schulz et al. 2012). Under inflammatory conditions, monocytic precursors can differentiate into LCs (Ginhoux et al. 2006). *In vitro*, like LCs, OCLs can be obtained from primitive macrophages of the yolk sac and the fetal liver (Thesingh, 1986; Kanazawa & Kudo, 2005); however, whether they seed autorenewing OCLs or OCL precursors is not yet known. Surprisingly, OCLs can also process and present antigen to T cells and their T cell-activating potential differs according to the precursor. Bone marrow monocyte-derived OCLs induce regulatory T cells, whereas OCLs obtained from dendritic cells promote activation and polarization of T cells into Th1 cells (Ibanez et al. 2016). These findings underline the importance of the type of OCL precursor, which can be monocytes, macrophages or dendritic cells (Jacome-Galarza et al. 2013).

Mice lacking the transcription factor PU.1 lack all myeloid cells including OCLs and LCs (Tondravi et al. 1997; Chopin et al. 2013). Transforming growth factor (TGF)-β is required for LC formation and plays a critical role in bone homeostasis by affecting OCL differentiation (Borkowski et al. 1996; Fuller et al. 2000). Both cell types show dependence on signaling by the colony-stimulating factor 1 receptor (CSF-1R),

as mice deficient in this receptor lack LCs and OCLs (Dai et al. 2002; Ginhoux et al. 2006). In addition, CSF-1R-deficient mice are devoid of microglia (Erblich et al. 2011) and show a reduction in the number of monocytes and small intestine Paneth cells (Huynh et al. 2009). The CSF-1R is activated by CSF-1 produced by fibroblasts and endothelial cells, and mice with a mutation in the gene coding for CSF-1 (op/op), rendering it functionally defective, are likewise characterized by reduced OCLs and osteopetrosis (Yoshida et al. 1990). Although LCs depend on CSF-1R, op/op mice have almost normal numbers of LCs (Witmer-Pack et al. 1993), and osteopetrosis was more severe in CSF-1R$^{-/-}$ mice than in op/op mice. Recently, interleukin (IL)-34 was identified as the alternative ligand for CSF-1R and was found to support myeloid cell viability and the formation of macrophages from progenitors (Lin et al. 2008). Mice deficient in IL-34 lack LCs (Wang et al. 2012), demonstrating that LCs require the activation of CSF-1R by IL-34. As might be expected, IL-34 also activates osteoclastogenesis (Nakamichi et al. 2012).

IL-34 is produced by keratinocytes (Wang et al. 2012), whereas CSF-1 is principally synthesized by mesenchymal cells and endothelial cells (Ryan et al. 2001). It appears therefore that the association with a neighboring cell type is important in determining cell fate. Indeed, LCs arise only in the epithelium of skin, eye, vagina and the oral surfaces (Young et al. 1985; Sato & Hirano, 1997; Hamrah & Dana, 2007). OCLs on the other hand form in tight association with the osteoblasts, cells of mesenchymal origin and sources of CSF-1. RANKL (TNFSF11/TRANCE), produced by osteoblasts, is another key cytokine for osteoclastogenesis. Conjointly identified by research groups working on osteoclasts and dendritic cells, RANKL was found to promote the differentiation of myeloid cells into OCLs in the presence of CSF-1 (Suda et al. 1999). RANKL activates the differentiation into OCLs via the receptor RANK, which signals through different pathways including necrosis factor (NF)-κB (hence the name receptor activator of NF-κB) (Anderson et al. 1997). RANKL–RANK interaction is antagonized by a non-signaling decoy receptor, OPG (osteoprotegerin) (Simonet et al. 1997). Mice deficient in or overexpressing either of the three proteins display alterations in bone density (Walsh & Choi, 2014). This has been confirmed in humans as diseases characterized by osteoclast-poor forms of osteopetrosis involve loss-of-function mutations in RANK or RANKL (OMIM #612301 and OMIM #259710, respectively); inversely, decreased bone density results from defective OPG (OMIM #239000) or hyperactive RANK (OMIM #174810). RANKL and OPG are produced by the osteoblasts, therefore an equilibrium is established whereby under normal conditions bone synthesis by osteoblasts is concomitant with bone matrix resorption by OCLs. Alterations in this equilibrium lead to bone matrix deposition or, conversely, to bone loss. These alterations are triggered by a number of different factors that respond to changes in physical impact (body size, weight, movement), the requirement of calcium

and phosphate (hormonal control) or inflammation. However, RANKL not only plays a key role in the regulation of bone mass; it also has an important impact on the immune system. Mice deficient in RANKL lack lymph nodes (Kong et al. 1999), and RANKL stimulates dendritic cell maturation (Anderson et al. 1997). Strikingly, mice deficient in RANKL show a reduction in LC numbers (Barbaroux et al. 2008). LCs express RANK, and RANKL is produced by keratinocytes in the embryo and the newborn mouse (Duheron et al. 2011). Inflammatory conditions can upregulate RANKL expression in the adult (Loser et al. 2006).

In view of these similarities in ontogeny, a valid question is why OCLs are found in the bone and LCs reside in the epidermis. In fact, in the proliferative disorder of Langerhans cell histiocytosis (LCH), cells with Langerhans and osteoclast phenotypic and functional features, occur in osseous sites (calvarian bones, but also femur, ribs and vertebrae) and extraosseous sites (facial and scalp skin, soft tissue of the mouth, lymph nodes, lungs and other internal organs) (Egeler et al. 2010). Also, under *in vitro* culture conditions and in osteopetrotic mice, dendritic cells can convert to OCLs (Akagawa et al. 1996; Wakkach et al. 2008). Normally, however, cell specialization is confined to distinct body sites, and it is probable that the answer to this question lies in the complex regulatory mechanism imposed by the microenvironment. For instance, GM-CSF, which is a key cytokine for dendritic cell differentiation but inhibits osteoclastogenesis, is produced by keratinocytes (Miyamoto et al. 2001).

Functional specialization from a common ancestor cell

From an evolutionary standpoint, the current vision is that bone appeared as mineralization of body parts giving rise to teeth and to protective measures, such as shells or spines. This integumentary skeleton (or dermal skeleton, exoskeleton), consists of the dermatocranium, skeletal components of the pectoral apparatus, teeth and tooth-like elements of the pharyngeal cavities. It provided the animal with advantage of locomotion and protection but made it heavier, limited its size and the development of sensory organs. The delocalization of mineralized material from the outside to the inside of the body facilitated agile locomotion with attachment of muscular systems and the development of the skin as a sensory organ. The skin has therefore undergone a series of adaptations to replace the heavy collagenous – mineralized – materials of the dermal skeleton with the lighter but equally protective keratin proteins of the epidermis, such as reptilian scales. The alternative strategy was to develop skin appendages, fish scales, feathers and hair. These served as physical protection but also could be adapted to allow a lightweight resistance to air for flight. With the adaptation to terrestrial life, the regulation of water loss and the resistance to temperature changes became a major element of survival. Hence, the mucosal

surface of fish skin underwent important changes, including keratins, and phospholipids with bound calcium to render the epidermis of terrestrians resistant to water loss. The development of sweat glands and hair was necessary to regulate heat exchange. In addition, temperature changes, reproductive seasons and growth phases imposed on the skin the ability to continuously adjust to these forces. As part of its protective program, the epidermis constantly renews and either continuously sheds epidermal layers or undergoes a seasonal shedding process (molting). The skull bones likewise undergo a remodeling program, such as in salmon when migrating from salt water to fresh water or in deer when antlers follow changes of the reproductive seasons. Also the dermal appendages follow phases of renewal. Fish scales are remodeled to fulfill special inorganic material needs (Witten & Huysseune, 2009) and hair can function as a social and sexual character and can adapt to temperatures changes (Randall, 2007).

Genetic programs that oversee endoskeleton formation are active in the skin and in the development of dermal appendages. The Runx, hedgehog or Sox gene families, to name a few, are key gene families that regulate osteoblastogenesis and play a major role in dermal appendage development or renewal (Ross & Christiano, 2006; Wagner & Aspenberg, 2011). RANKL is required for tooth eruption, mammary gland development and hair cycling. Although mammary glands and hair develop normally in the embryo of knock-out mice, pregnancy-induced mammary gland growth and the juvenile and adult hair cycling are RANK- and RANKL-dependent (Fata et al. 2000; Duheron et al. 2011). It is intriguing that IL-34 is required for microglial cells, specialized macrophages of the brain. Fish also contain such microglial cells and there is evidence suggesting that these cells extend into the skin (Herbomel et al. 2001). Myelomonocytic cells of dendritic morphology could also be seen in fish skin using a chemokine receptor reporter transgenic construct expressed in LCs of higher vertebrates (Aghaallaei et al. 2010). This suggests that fish contain LC-like cells. In chicken and probably also in reptiles (turtle), epidermal cells with LC phenotypic and functional features have been described (Perez-Torres et al. 1995; Igyarto et al. 2006). This raises the possibility that bona fide LCs, as characterized in mammals, derive from an evolutionary early ancestor; however, in the absence of a sophisticated immune system of higher vertebrates, LCs of these animals would have fulfilled a different functional need. In view of the role of RANKL in controlling the hair follicle cycle (Duheron et al. 2011), its action on LCs and the requirement of CSF-1R signaling for LC development, it is tempting to speculate that LCs would play a more important role in the homeostasis of skin than is presently thought. Like OCLs that together with osteoblasts regulate bone mass, LCs may be required together with keratinocytes to confer on the skin a capacity to change. Indeed, it has been proposed that LC may coordinate epidermal renewal by affecting cell
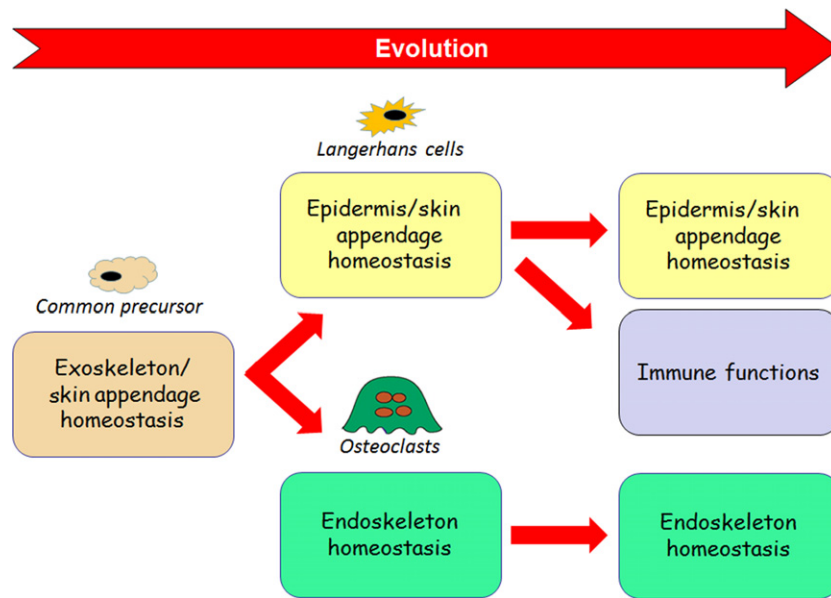
Fig. 1 Proposed evolutionary relationship between Langerhans cells and osteoclasts. In early vertebrates a common precursor cell unites the skin-specialized homeostatic functions of Langerhans cells and the endoskeleton-specialized homeostatic functions of osteoclasts. Whereas osteoclast functions remain unchanged, Langerhans cells acquire immuno-regulatory activities.

division of keratinocytes that are organized in proliferation units comprising at their center a LC (Potten & Allen, 1976). Alternatively, LCs in evolutionary older vertebrates may have exerted more macrophage-type activity to degrade material to promote epidermal shedding. Interestingly, fish scales are resorbed by mononucleated cells, whereas the endoskeleton is largely resorbed by multinucleated giant OCL-like cells (Witten & Huysseune, 2009). The hair cycle comprises a catabolic process, called catagen, during which keratinocyte-comprising hair follicles undergo apoptosis. Also in this process, LCs could play a role in the degradation of cellular material. LCs may be a better choice to eliminate apoptotic cell debris than surrounding macrophages, since LCs are already closely associated with the epithelial cells and do not need to be recruited through chemokines or inflammatory mediators, unlike macrophages dispersed throughout the dermis. Indeed, during catagen, LCs were observed to phagocytose hair follicle melanin (Tobin, 1998).

With the evolution of the immune system from bony fish onwards, cell types became specialized to mount an immune response against pathogens and had to learn to distinguish between self and non-self. For this, cells were needed to continuously educate potentially autoreactive T cells to ignore self-material. Among the body surfaces most exposed to pathogens is the skin. In mammals LCs have the capacity to migrate from the skin to secondary lymphoid organs, where the T cells reside, to present to them self-antigens and maintain self-tolerance. It is therefore possible that the function of LCs in skin homeostasis was co-opted by the immune system to reuse degraded cellular material for the education of T cells. LCs developed a migratory

ability and acquired the capacity to partially degrade material and to present derived peptides on the major histocompatibility complex to T cells (Fig. 1). Possibly a sign of this acquired function are the CD1 molecules, which present lipids derived from bacteria or from cellular membranes in a similar manner to that of peptides on MHC-I. The T cells that recognize CD1-lipid complexes already present in jawed vertebrates and some are resident in the epithelium (Castro et al. 2015).

Taken together, the features shared by LCs and OCLs raise the possibility that an ancestral cell regulating exoskeleton/skin hemostasis of early vertebrates gave rise to skin-specialized LCs and bone-specialized OCLs of higher vertebrates. Beyond its general interest in biology, investigations into this proposed model may provide unexpected answers for medically relevant issues such as osteoimmunology, autoimmunity and cancer.

Acknowledgements

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