REVIEW

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Short histological kaleidoscope – recent findings in histology. Part IV

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Abstract

The paper provides an overview of the current understanding of different cells and structures' biology [*e.g.*, blood–retinal barrier, Bruch membrane, Clara (club) cells, brush cells and tuft cells, Merkel cells, Hofbauer cells, cytokeratins], including their origin, structure, function, and role in disease pathogenesis, and of the latest findings in the medical literature concerning the paracortex of the lymph nodes.

Keywords: Bruch membrane, club cells, brush cells, Merkel cells, Hofbauer cells.

Introduction

New modern investigation methods, molecular and microscopical examination techniques, highlight information about histological structures and their functions are published each year in various medical literature papers. As seen in parts one, two and three, this short kaleidoscope emphasizes the review of less common histological aspects regarding the biology and function of certain cells and structures [blood–retinal barrier (BRB), Bruch membrane (BM), Clara (club) cells, brush cells (BrCs) and tuft cells, Merkel cells, Hofbauer cells, cytokeratins (CKs)], and the tissular elements such the paracortex of the lymph nodes (LNs).

Blood–retinal barrier

BRB is a complex selective structure that monitors the transport of ions, macromolecules, and water between systemic circulation and the retina [1]. BRB plays an essential role in maintaining the physiological composition of the unique retinal microenvironment and promoting proper visual function.

Localization and structural elements of the BRB

According to the localization, two components can be described: the outer BRB (oBRB) interposed between the choroid and retina, and the inner BRB (iBRB), mostly represented by the inner retinal microvasculature [2, 3].

The oBRB contains the retinal pigment epithelium (RPE), BM, and the choroid [2]. The outer layer of the choroid is vascularized by larger suprachoroidal blood vessels and the inner layer is supplied by fenestrated choroid capillaries, which function in the delivery of nutrients to the retina and the removal of by-products of tissue metabolism. Choriocapillaris in the proximity of the retina participate in the formation of the BM. The basal lamina of these capillaries is fused with the basement membrane of the RPE; thus, BM is composed mainly of collagen and elastic fibers, organized into three planes: a central elastic layer, flanked by two collagenous layers [1].

The inner retinal microvasculature is represented by three vascular plexuses that supply the fibrillary layers: the outer plexiform layer, the inner plexiform layer, and the optic nerve fibers layer [4]. iBRB is formed by the endothelial tight junctions of the retinal capillaries, the basement membrane, and the pericytes; glial cells such as astrocytes, Müller cells, and microglial cells are also associated with the retinal capillaries [2, 4].

Retinal pericytes are more numerous in the retinal microvasculature than other tissues and are crucial for stabilizing the iBRB. Their main function is to control the inner retinal blood flow and vascular wall remodeling. Structurally, pericytes participate in angiogenesis due to their multipotential differentiation and maintain the appropriate niche microenvironment by the synthesis of stromal fibronectin. Degeneration of pericytes in diabetic retinopathy leads to increased permeability of iBRB and alteration of barrier function [5].

The distribution of retinal astrocytes varies according to the density of blood vessels and nerve fibers. The presence of astrocytes in the proximity of retinal capillaries suggests that resident astrocytes immigrate from the bloodstream [6]. Furthermore, astrocytes are mainly associated with the optic nerve fibers that they ensheath, and with retinal neurons in the inner nuclear layer. Their dual role is to release trophic substances, antioxidants, pro- and anti-inflammatory mediators, and cytokines in the retinal microenvironment, and to reinforce the iBRB by wrapping their processes around the tight junctions of the micro-vascular endothelium [7].

Müller cells are the most numerous retinal glial cells with an essential supportive role due to the radial arrangement of their processes extending throughout almost the entire thickness of the retina [6]. Developmental studies demonstrated that Müller cells and retinal neurons arise from a common stem cell; these findings led to the hypothesis of cooperating functional units formed by one Müller cell and a group of neurons that share a common progenitor. Thus, Müller cells seem to occupy a central position within each columnar array of neurons and mediate synaptic transmission by releasing "gliotransmitters" from the processes extending toward synapse sites [6, 8]. Moreover, Müller cells send processes ensheathing the retinal capillaries and thus regulate the permeability of the iBRB and control the molecule exchanges to provide metabolic support for the adjacent neurons [9]. By the processes terminating on both synapses and blood capillaries, Müller cells are interposed between neurons and the bloodstream, to mediate neuro-vascular coupling and to ensure the optimal microenvironment in the retina [6, 8].

Retinal microglia are in close apposition to neurons and pericytes. As specialized macrophages, retinal microglia phagocytose degenerated cells and play important roles in synaptic pruning. Synaptic pruning is a remodeling process, consisting of the elimination of excessive neuronal contacts for promoting robust synaptic networks. Upon pathogens' invasion, microglia represent the first line of defense, mediating local inflammatory and immune reactions [6, 10].

Functional characteristics of the BRB

Retinal homeostasis is maintained by the selective control of the flux of molecules into and from the retina. BRB allows the delivery of oxygen and nutritive substances, while preventing the passage of blood-borne toxins [3].

BM functions as a size-selective filter by enabling the passive diffusion of small molecules, while restricting the passage of larger molecules [1]. Moreover, due to the BM's continuous structure, inflammatory cells' infiltration towards the neural retina is limited, and the physical properties of the BM ensure the stability of the RPE and the shock-absorbent function [11]. Fenestrated endothelium of choriocapillaris displays pores of 6 to 12 nm and is permeable to larger molecules [1]. Choroid is impermeable to hydrophilic substances and large molecules but allows the passage of lipophilic compounds with a positive charge [1].

The structure of iBRB has common features to the neuro-vascular unit of the blood–brain barrier. The passage of molecules from systemic circulation is controlled based on the molecular size, the electrical charge, and hydrophilic/lipophilic properties. The intercellular junctions of iBRB are permeable for small hydrophilic molecules, whereas the lipophilic molecules are transported by transcellular route [4]. The increased transendothelial resistance of the iBRB is due to three mechanisms: (*i*) the complex junctions interconnecting the endothelial cells, represented by *zonula occludens*, *macula adherens*, and gap junctions; (*ii*) the continuous endothelium, without pores or fenestrations; and (*iii*) the absence of pinocytotic vesicles.

The molecular profile of the intercellular junctions is characterized by the presence of specific integral membrane proteins: occludin and claudins, as determinants of endothelial barrier function [12, 13]. Claudins are a family of proteins in the structure of tight junctions with essential roles in the regulation of endothelial permeability (by creating para-cellular pores that allow the passive diffusion of molecules) [14, 15]. In the iBRB tight junctions, the most prominent component is Claudin-5, which functions according to the circadian rhythm and allows an increased influx of nutritive substances during the day [14]. In addition to being a tight junction structural protein, occludin plays complex roles in mediating cell signaling and cell proliferation, as well as controlling paracellular molecule passage [16].

Pathophysiology of the BRB and clinical applications

Alterations in the restrictive function of BRB lead to the onset of various retinal vascular pathologies and vision alteration. Diabetic retinopathy is the consequence of iBRB breakdown, whereas age-related macular degeneration (AMD) is caused by the impairment of the oBRB [2].

Both the oBRB and iBRB have influx transporters for substances entering the retina and efflux pumps for the molecules exiting the retina. Based on these functional characteristics, various drug delivery systems have been developed. To ensure the efficient intra-retinal dose, therapeutic substances should be available to the influx transporters, but unavailable to the efflux pumps. Another possibility would be to use inhibitors of the efflux pumps, to prevent drug elimination and to maintain the therapeutic agents at the desired site [12].

Exploring the Bruch membrane

BM, situated between RPE and choriocapillaris, serves as a barrier and a supportive matrix for retinal homeostasis. It is an intricate structure, and its dynamic functions are crucial for maintaining the health and the integrity of the retina [17].

Histological structure and function

BM consists of five layers: the basement membrane of the RPE, the inner collagenous layer, the elastic layer, the outer collagenous layer, and the basement membrane of the choriocapillaris [18]. This meticulously layered arranged composition of various macromolecules, including collagen, elastin, and proteoglycans [18] enables the membrane to perform diverse functions: *structural support* (BM serves as a scaffold, anchoring the RPE and maintaining the structural integrity of the retina [19]), *selective barrier* (BM acts as a selective filter, crucial for maintaining the RPE's physiological environment, by regulating the passage of nutrients, oxygen, and waste products between RPE

and choroid [17]), *nutrients exchange* (BM facilitates the transport of essential nutrients from the choroid to RPE, a transport that is vital for the RPE's proper function in supporting photoreceptor health [20]) all of these contributing to retinal health and function.

Pathological implications

Alterations in the BM's composition or integrity are associated with various ocular pathologies, including AMD, diabetic retinopathy, and retinal dystrophies [21].

Dysregulation of extracellular matrix (ECM) turnover, accumulation of lipids and debris, and inflammation within the BM contribute to the pathogenesis of these diseases. These pathological modifications are strongly linked to AMD development [22]. Changes in BM's composition are considered a hallmark of AMD pathogenesis. With age, BM thickens due to the accumulation of deposits including drusen, a mixture of cellular waste products, lipids, and proteins [23]. This thickening and altered composition compromise BM's normal functions: disruption of the BRB, impaired support for the RPE, and abnormal complement activation [23].

Molecular mechanisms

At the molecular level, interactions between components of the ECM, RPE cells, and immune mediators regulate BM homeostasis [24]. Dysregulation of matrix metalloproteinases, lysosomal enzymes, and inflammatory cytokines disrupts the delicate balance between matrix turnover and deposition, contributing to pathological changes in this membrane [23].

In AMD, studies suggest that specific micro-ribonucleic acids (miRNAs) (*e.g.*, miRNA-29a) might regulate the deposition of drusen, by inhibiting its formation, and the function of RPE cells [25]. Moreover, dysfunctional autophagy may play a role in the accumulation of waste products within BM in AMD; one study has explored the link between Beclin-1, a key protein in autophagy, and its association with AMD [26].

Recent advancement

Exciting research avenues are emerging to combat AMD by targeting the BM; researchers are exploring the potential of drugs or gene therapies that modulate BM composition or function to prevent AMD progression [1]. However, the latest research regarding nano-based ocular drug delivery systems includes nanomicelles, nanoparticles, nanosuspensions, nanoemulsions, and liposomes for precise delivery of drugs to the eye. This offers advantages such as prolonged drug release, targeted delivery, and reduced side effects [1]. For example, in AMD, Bevacizumab retained its antiangiogenic activity when loaded into the biodegradable core–shell electrospun nanofibers, these having the potential to be an alternative treatment option to frequent intravitreal injections of antiangiogenic agents [1]. The development of BM-mimetic scaffolds as potential implants for RPE regeneration holds promise for future therapeutic strategies.

Conclusions

Understanding the histology, pathology implications, recent advancements, and molecular mechanisms of the BM is crucial for unraveling the pathogenesis of retinal diseases and developing targeted therapies. Further research into its complex biology promises to yield novel insights into ocular health and disease.

New insights in Clara cells

Generalities

Clara cells, initially analyzed by the anatomist Rudolph Albert von Kölliker in 1888, were defined over the decades, as particular cells of the bronchial epithelium (Max Clara in 1937), and today, mainly as bronchiolar exocrine cells or club cells (CCs) [27, 28]. They display many key roles in both homeostasis and lung injury, including the synthesis of proteases, antimicrobial peptides, surfactant apoproteins A, B, and D, cytokines and chemokines, immune response modulation, xenobiotic metabolism, bronchiolar epithelium regeneration, mucociliary clearance of exogen factors regulation, fibrosis, and pulmonary neoplasia promotion [27, 28].

In healthy lungs, CCs are mainly distributed within terminal bronchioles, representing about 15–44% of all regenerating cells of this region and 9% of all epithelial cells covering the respiratory tract, being sparse in the trachea and the primary, lobar, and segmental bronchi. In addition, CCs were found in the kidneys, prostate, and gravid uterus [28–31].

Morphological features

CCs are also called bronchiolar cells or non-ciliated non-mucous secretory cells of the bronchiolar epithelium because they don't present cilia or produce mucin. They represent cuboidal cells with a large apical pole protruding in the bronchiolar lumen [28, 32]. Ultrastructurally, they display many mitochondria, a well-developed Golgi apparatus, and a smooth and rough endoplasmic reticulum (ER) within the apical cytoplasm, highlighting their intense metabolic potential. Furthermore, they present apical secretory granules enveloped in a dense membrane. The granules contain proteins, glycoproteins, and lipids. The nucleus has a central position with numerous nucleoli [27, 28]. Moreover, CCs present high quantities of cytochrome P450 (CYP) and have increased apocrine and merocrine secretion [32, 33]. It seems like adrenergic fibers promote CC synthesis activity [27, 28].

Functions of CCs in normal lung

CCs sustain lung hemostasis through many pathways: detoxification of xenobiotic compounds and oxidant elements with CYP, synthesis of the main elements of the extracellular material that covers the respiratory bronchioles, modulation of the fluid content in the terminal parts of the respiratory system [27, 28, 34]. Moreover, they have stem cell abilities, ensuring the regeneration of ciliated cells and their own [27, 34].

CCs are involved in the purification of a high amount of toxic gases from the inhaled air such as furan, aromatic hydrocarbons, ozone, compounds of tobacco smoke, coumarins, nitrogen oxides, and other toxic derivates [28]. The main triggers involved in the detoxification process are represented by monooxygenases, flavin-containing monooxygenases, and CYP [35]. During this process, CCs undergo several transformations: they become swollen, with

many cytoplasmic vacuoles, nuclear chromatin irregularities and clunking, and mitochondrial and ER enlargement; these morphological findings suggest the protective role of CCs from the toxic exogen agents of the polluted air [28, 35]. It was shown that chronic tobacco smokers presented fewer CCs in their respiratory tract epithelium than nonsmokers [36].

CCs have an increased secretory activity producing numerous biologically active mediators, like anti-inflammatory proteins, antibacterial peptides, Clara cell secretory protein [also called uteroglobin, CC protein 16 (CC16), CC protein 10 (CC10), secretoglobin, human protein 1, etc.], Krebs von den Lungen-6 (KL-6) protein, lipids, glycoproteins, and surfactant apoproteins A, B, and D. All these factors maintain, defend, and repair the respiratory environment [27, 28].

Several researches have shown that CCs have regenerating properties, acting like stem cells, replacing ciliated cells, and type 1 and 2 pneumocytes *via* β-catenin. β-Catenin is a member of the cadherin family which assures the morphogenesis and strength of intercellular junctions [27, 37]. Additionally, the transformation of CCs into other types of epithelial cells which occurs during the renewal or alteration of the bronchiolar epithelium is promoted by the soluble form of E-cadherin [37–39].

Wide evidence revealed that the morphology and role of CCs depend on the modifications of the respiratory system environment [27, 28]. In a homeostatic state, CCs are non-proliferative cells, protecting the respiratory tract. Alteration of the normal respiratory epithelium stimulates dormant stem cells causing self-regeneration and production of elective precursor cells (Clara) [27, 40]. Moreover, the respiratory insult could stimulate the transformation of Clara cells into an intermediate type A cell. This type of cell proliferates, creating ciliated cells, two daughter cell types, and mature cells (named type B). Type B cells have also the potential to transdifferentiate into CCs [27, 40–42]. In response to various respiratory lesions CCs secrete mucus, becoming mucous cells. This mucous metaplasia represents a defense mechanism that eventually is lost in chronic pulmonary pathology [40–42]. Yet, the process of stem CCs transdifferentiating remains an enigma [27, 40–42].

Impact of CCs in pulmonary diseases

Many data have shown that CCs are the main triggers in different respiratory pathologies, promoting inflammation and fibroblast proliferation *via* Clara cell secretory protein, leading to fibrosis [28, 42]. Thus, CCs are involved in several chronic respiratory diseases such as tracheomalacia, pulmonary emphysema, pulmonary fibrosis, pulmonary hypertension, chronic obstructive pulmonary disease, acute respiratory distress syndrome, and cystic fibrosis [28, 42, 43].

Furthermore, it was reported that several types of pulmonary carcinomas, especially adenocarcinoma (ADK) may arise from CCs [27, 28]. This finding is supported by recent data, which revealed that Kirsten rat sarcoma virus (*Kras*) gene mutation stimulates CCs inducing pulmonary tumorigenesis, followed by ADK development [44]. Also, CCs promote carcinogenesis through the alteration of the catenin pathway, and amplification of chronic inflammation and fibrosis [27, 45].

CCs as clinical markers

It was reported that CCs produce many proteins in the respiratory tract, urine, and bloodstream, with clinical utility in the diagnosis and prognostic of different diseases [28].

Therefore, several reports highlighted that the patients with lung fibrosis, sarcoidosis, and artificial ventilation with high positive end-expiratory pressure values presented high levels of CC proteins within the blood serum and pulmonary transudate [46, 47]. They concluded that the level of CC16 represents a good marker of pulmonary hemostasis [46, 47].

Furthermore, research conducted by Wutzler *et al.* observed an increased concentration of CC16 in subjects with numerous lesions, suggesting that this protein represents a useful marker of secondary respiratory insufficiency in patients with multiple lesions [48].

Increased levels of Clara cell secretory protein in bronchiolar secretion are believed to be a valuable indicator of high capillary–alveolar barrier permeability [46]. Other reports suggested that the serum and urinary levels of Clara cell secretory protein and surfactant represent good diagnostic markers of incipient silicosis and fibrotic alterations in pneumoconiosis [49, 50].

Furthermore, studies have shown a correlation between low levels of CC16 and enhanced predisposition of pulmonary viral infections and oxidative stress. The concentration of this protein could represent a useful indicator of pulmonary alteration in different acute and chronic pathologies [46].

Additionally, it has been revealed that, in preterm newborns, high serum levels beyond 79 μg/mL of KL-6 protein represent a good prognostic marker of progressive bronchopulmonary dysplasia [50].

Moreover, research conducted on a large cohort with evolved pulmonary carcinoma revealed an association between the serum levels of CC16 and the mortality prevalence in these patients [51].

Conclusions

The findings support the pivotal role of CCs in hemostasis and respiratory diseases, with clinical utility in many pulmonary pathologies. Still, their vast potential has not been fully investigated, and further researches are needed.

Brush and Tuft cells – the complex chemosensory cells

Definition and morphology

BrCs or tuft cells are a minor chemosensory cell population, scattered as solitary cells, throughout all parts of the airways (from the nasopharynx to alveolar epithelium) [52], in the thymus, urogenital mucosa (testicular efferent ductules, urethra) [53, 54], and the digestive tract (*e.g.*, submandibular gland, taste buds, small intestine, stomach [55], gallbladder, pancreatic epithelium [54, 56], and recently, in pancreatic precancerous lesions [55]). In the respiratory mucosa, they are distinct from other epithelial and resident immune cells [57]; in the trachea, tuft cells are commonly referred to as *cholinergic BrCs* [58]; outside the respiratory tract, the terms *tuft*, *caveolated*, *multivesicular*, *and fibrillo-vesicular cells* have also been used [54, 56].

The morphological variety of these cells in different

locations causes diversity in nomination. The cells can have a triangular cell body, an elongated one, or a classical spindle shape with microvilli. BrCs have often a long basolateral process that goes over a few other cell bodies and protrudes to the basal membrane far from its origin [59].

BrCs are non-ciliated, with long, stiff apical microvilli (approximately 120–140/cell) [54], and they are basally in contact with afferent nerve fibers. Alveolar BrCs (0.5% to 10% of the alveolar epithelial cells) [54] exhibit: a spherical appearance, stiff, straight microvilli with flattened tips and with parallel alignment, perinuclear filaments, and junctional complexes with alveolar type I cells. In the trachea (1% to 7% of the alveolar epithelial cells), the BrCs have triangular cell bodies and reach the tracheal lumen only with a very tinny process that is commonly not observed in tissue sections; there are straight microvilli, filaments extending deep into the apical part of the cell, an apical vesicular compartment (numerous small membrane vesicles between the filament bundles), and junctional complex with the neighboring cell. Nose chemosensory cells possess a thin apical process and rigid microvilli [59].

In the small intestine, the tuft cells represent 0.4–2% of all epithelial cells under homeostatic conditions [55]. They present tuft-like apical protrusions, extending above neighboring epithelial cells, and a large filamentous network stretching down from the apical surface to the perinuclear region, which is interdigitating with an extensive tubulo-vesicular network [55].

Markers

BrCs have a unique development in close association with nerve fibers from neuronal sensory system, and they are present in the airways at the early postnatal stages [60]. These nerve fibers form local swellings ("varicosities") along their ascent between the epithelial cells, containing the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP) [59].

The microvilli contain F-actin, intermediate filaments, villin, fimbrin, girdin, advillin, and espin; the deeper cytoplasmic filament bundles are immunoreactive for CK18. Also, ankyrin can be found, linking the plasma membrane to the underlying cytoskeleton [55].

BrCs have a widespread expression of Hoxa family transcriptional regulators, with critical roles in spatiotemporal embryonic patterning and shaping of the neural synaptic circuits [57].

In the mouse trachea, the tuft cells express several canonical genes [such as arachidonate 5-lipoxygenase activating protein (*Alox5ap*), *Pou2f3*, and growth factor independent 1B transcriptional repressor (*Gfi1b*)] [61], other genes required for the synthesis of leukotrienes (LTs) [*e.g.*, phospholipase A2 group IVA (*Pla2g4a*), *Alox5*, and leukotriene C4 synthase (*Ltc4s*)] and the synthesis of cyclooxygenase [prostaglandin (PG)-endoperoxide synthase 1 (*Ptgs1*) and hematopoietic PG D synthase (*Hpgds*)] [58, 62]. It was observed that the tuft cells from at least five tissues share a transcriptional signature core including G protein subunit alpha transducin 3 (GNAT3), phospholipase C beta 2 (PLCB2), and transient receptor potential cation channel subfamily M member 5 (TRPM5; Ca^{2+} triggered cation channel) [53]. Other tuft cell markers include the epithelial interleukin (IL)-25 cytokine, and the choline acetyltransferase (ChAT) enzyme [58]. Nearly all tuft cells also express ChAT, the enzyme that synthesizes the neurotransmitter acetylcholine (ACh) [53] and SRY-box transcription factor 9 (SOX9) [60].

Immunostaining for intestinal tuft cell markers is expressing succinate receptor 1 (SUCNR1; most widely studied intestinal tuft cell receptor), free fatty acid receptor 3 (FFAR3), dopamine receptor D3 (DRD3), taste 1 receptor member 3 (TAS1R3; abundant in the ileum), vomeronasal type 2 receptor 26 (VMN2R26; olfactory receptor). Other markers of tuft cells are expressed in physiological conditions (F-actin (phalloidin), doublecortin-like kinase 1 (DCLK1), and acetylated tubulin as seen in lingual taste receptor cells, for nutrient sensors function [63]) and pathological conditions (regulator of G protein signaling 13 (*Rgs13*) gene, in asthma [61]).

The context-dependent tuft cells transcriptional signature from specific ligands (helminths or microbes), across multiple tissues, suggests G-protein coupled receptor (GPCR)-induced intracellular signal transduction as a common function [55].

p63-expressing lineage-negative epithelial stem or progenitor cells are considered a source of tuft cells [64]. Studies of lineage tracing identified *Dclk1*+ tuft cells from *p63*+/keratin 5 (*Krt5*)+ basal progenitors [64, 65].

Function and involvement in immune reactions

In the airways, tuft cells sense bacteria, allergens, and noxious stimuli [53] and utilize ACh for paracrine signaling [56]. BrCs are "chemosensory" and express elements of the bitter taste transduction system (including Gα-gustducin and TRPM5) and directly activate cholinergic neural sensory circuits [57]. The bitter-tasting substances and free amino acids present in the liquid lining the airways activate chemosensory cells through binding to GPCR coupled to the G-protein subunit α-gustducin. Next, PLCB2 mediates the release of Ca^{2+} and activates the TRPM5. This leads to Na^{+} influx and opening of voltage-gated Ca^{2+} channels ending in ACh neurotransmitter release and excitation of sensory nerve fibers [the vesicular acetylcholine transporter (VAChT) is present in airway BrCs] [59].

BrCs respond to airborne irritants by activating a neural reflex and reducing the respiratory rate [57].

BrCs also relay immune signals. Tuft cells are the dominant source of IL-25, and cysteinyl LTs (CysLTs), lipid mediators of vascular permeability and chemotaxis [58].

In the airway mucosa but also the intestine, the tuft cells serve as immune sentinels during parasitic infection; they release IL-25 through the secretion of CysLTs and activation of their CysLT3R receptors, leading to allergen type 2 inflammation [57, 62]. LTC4 and IL-25 are the two principal inflammatory mediators of tuft cells that initiate type 2 inflammation [58]. Tuft cells express a cell type-specific marker *Rgs13*, a known risk gene for asthma [61]. The viruses that exacerbate asthma generate LTE4, sufficient to induce eosinophilia in asthmatics, independent of CysLTR blockade [57]. In conclusion, the potent synergy of cytokine and lipid mediator signaling, IL-25 and CysLTs, in the aeroallergen-trigged activation, design the airway tuft cells as being powerful modulators of type 2 immunity in the lungs [58].

The BrCs are involved in several allergen-triggering diseases. In mouse and human models, the highest increase in tuft cell numbers was found in the recovery phase of

influenza and other viral infections, in the alveolar region of the lungs [66], as bronchiolitis, coronavirus disease 2019 (COVID-19), influenza A viruses [67], but also after viral exacerbations of asthma [57].

As they possess a similar sensing equipment as the tongue taste receptors (GPCRs), newly emerged research highlights the nutrient sensors function of the tuft cells [63]. Also, as seen in lingual taste receptor cells, F-actin (phalloidin), DCLK1, and acetylated tubulin are widely used tuft cell markers, that can evidentiate the microvillus structure, whole-cell shape, and dense microtubules [63].

Regeneration and pathological involvement

The tuft cells react differently during the immune reactions and regeneration process in the lung or intestine. The lung has a significant regenerative capacity [65]. However, the role of tuft cells in lung regeneration following viral infection remains unclarified [60]. More recently, tuft cells that emerge in the alveolar region of the lung upon viral infections were reported [66]. Ectopic tuft cells (ChAT+ or POU2F3+) increased threefold in the upper airways of individuals with COVID-19 [68], with an expansion independently occurring from type I or type III interferon signaling. TRPM5 is known to regulate tuft cell development [60]. Unlike in the intestine, lung tuft cells do not require IL-25, or IL-4Ra, but do require *Pou2f3* transcription factor gene [65]. Lineage studies showed that post-influenza infection, ectopic tuft cells are generated from *Krt5*+ *Trp63*+ cells. However, there is no consensus about *Krt5*; there are authors that sustain that in the case of influenza, a tuft cell population that transiently expressed p63 but not Krt5 was labeled [60]. Other regulators of tuft cell number are Wnt inhibitors (increasing the number), influenza infection downregulating Wnt signaling, and Notch signaling (decreasing the number) [60]. A severe lung injury stimulates tuft cells' development [65]. In total, the observation sustains that lung regeneration is yet, independent of the presence of ectopic tuft cells in the parenchyma [60].

In the small intestine, tuft cell proportions change dramatically in response to commensal microbes, pathogens, inflammation, and damage conditions, suggesting a fundamental role for this cell type in barrier function [55]. Like this, the tuft cells detect helminth infection, protist colonization, and bacterial dysbiosis [53, 69]. Generally, tuft cells modulate bacterial homeostasis and microbial metabolites to initiate type 2 immunity, remodeling like this the commensal of the small intestine antimicrobial landscape [69, 70]. The tuft cells are dependent on T-helper (Th) 2 cytokines for their amplification and promote the growth and differentiation of surrounding epithelial cells [65]. Also in the intestine, signal transducer and activator of transcription 6 (STAT6) and type 2 innate lymphoid cells (ILC2s) are required for the expansion of the tuft cell compartment [57]. Therefore, in the small intestine, tuft cells will infection-induce an increase of ILC2s [67]. In the small intestine, tuft cells also activated ILC2s in a model of food allergy, where sensitization occurs through tape stripping of the skin [53].

Involvement in carcinogenesis

Accumulating evidence based on specific markers of the tuft cells has shown that the tuft cells specific genes (*Pou2f3*, *Dclk1*) are involved in carcinogenesis in pancreas, lung, stomach, colon, liver [52].

DCLK1+ tuft cell population is important for intestinal homeostasis and regeneration and is increased in inflammation-induced carcinogenesis [71]. The macrophage-derived IL-1β, tumor necrosis factor-alpha (TNF-α), and IL-6 cytokines are significantly increased in dextran sodium sulfate (DSS) colitis and promoted stemness of *Dclk1*+ tuft cells, that serve as the cellular origin of cancer [72]. Also, it was observed the up-regulation of *Pou2f3*, a master regulator of tuft cell differentiation, and a tuft cell-like cancer cell differentiation in the human colorectal cancers [73].

The clustering of DCLK1+ cells is an early event in Kras-induced pancreatic tumorigenesis and may contribute to intraductal papillary mucinous neoplasm initiation [74]. Experiments in mice showed that GNAT3 ablation, a gustatory pathway G-protein expressed by metaplastic tuft cells promoted the progression of metastatic pancreatic ductal ADK [75]. Pancreas-specific deletion of *Pou2f3* in KC mice (KPouC mice) resulted in a loss of tuft cells and accelerated tumorigenesis [76].

Notch activation in *Dclk1*+ tuft cells in the gastric cardia can contribute to Barrett's esophagus (BE), which is a precursor to esophageal ADK [77]. In human gastric cancers, tuft cells were rarely observed but showed positive associations with well-differentiated lesions in mouse models, suggesting that, while tuft cells are associated with precancerous pathologies, their loss is most associated with the progression to invasive cancer [78].

There is hope for future research in validating tuft cells as novel strategies for cancer therapies [52].

Conclusions

The tuft cells are involved in immunological allergen defense, promoting allergic inflammation, therefore stimulation and proliferation of these cells could represent significant pathobiological and therapeutic targets.

The preexisting intestinal conditions can be settled by changes in tuft cell and ILC populations; these could represent unexplored mechanisms contributing to severe disease in humans or preventing systemic infection.

A new emerging role is the involvement of tuft cells in tissue regeneration and carcinogenesis.

The differentiations of distinct signals required for tuft cell development in various locations may provide important clues to the function of these enigmatic cells.

Merkel cells

Described for the first time in 1875 by Friedrich Sigmund Merkel as "Tastzellen" or "touch cells", Merkel cells are a unique specialized population of epidermal-derived cells, scattered among the keratinocytes and melanocytes of the basal layer in the epidermis [79]. The disposition along the dermo–epidermal junction enables its synaptic connections with type 1 amyloid beta (Aβ)-afferent somatosensory nerve endings, thus forming the Merkel cell–neurite complex, a slowly adapting light touch receptor of the skin [80, 81].

There has been a long debate about their origin lineage. Whether these cells are derived from a neural progenitor during embryogenesis or from an epidermal ancestry line,

was a constant dispute over the years. Two successive transgenic mouse research, eventually showed in 2009 that Merkel cells had an epithelial origin in mammals [82, 83]. It was demonstrated in both assays that the complete lack of Merkel cells was caused by the loss of atonal BHLH transcription factor 1 (*Atoh1*) in epidermal progenitors. Further evidence that the Merkel cell population was unaffected by the *Atoh1* deletion in the neural crest lineage was provided by Morrison *et al.* [83]. Further research using mouse models showed that the acquisition of the Merkel cell phenotype with *Atoh1* expression appears to be limited to a particular subset of keratinocyte progenitors that have an active Sonic Hedgehog pathway [84, 85].

Tilling *et al.* investigated the human Merkel cell lineage by co-immunodetection of the Merkel cell marker protein, CK20, with other proteins known to be produced in either, the skin's neural stem cells, or epidermal cells. There was no cell co-labeling of CK20 with any of the well-known neural crest lineage markers, SRY-box transcription factor 10 (SOX10) or paired box 3 (PAX3). On the other hand, approximately 71% of epidermal interfollicular and 25% of follicular Merkel cells contained β1 integrin, which is recognized to be abundant in epidermal stem cells. Furthermore, there was notable co-immunolabeling of CK20 with leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1), which was likewise concentrated in epidermal stem cells (about 20% in the interfollicular epidermis and 7% in the hair follicle, respectively). In isolated Merkel cells, more epidermal markers were found. Cells that co-express epidermal markers and CK20 could indicate a transitional state between differentiated ones and stem cells [86].

Initially, it was thought that Merkel cells have a limited lifespan, and there is also a controversy over their replacement frequency, and the identities of Merkel cell progenitor cells, that maintain the cell population. More mice recent research, using 5-Ethynyl-2'-deoxyuridine (EdU) birth dating experiments regarding the longevity of Merkel cells, reveals that that these cells (produced during embryogenesis) remain viable even in late adulthood. This comprehensive research aimed at examining the dynamics of Merkel cell generation, survival rate, and potential of replacement, and discovered that during adult skin homeostasis, new Merkel cells are rarely produced and that the number of touch dome Merkel cells remains constant throughout the hair cycle [87]. Furthermore, after birth, there is a rare production of new Merkel cells, except for after an injury. On the other hand, Merkel cell formation was markedly increased by modest mechanical injury, caused by shaving, as demonstrated by live imaging and EdU tests. Through genetic ablation of cells and fate-mapping studies, authors were able to verify that touch dome keratinocytes give rise to new touch dome Merkel cells in adult mice [87]. The first reported genetic study that provisioned support in this regard pointed out that keratin 17 (*Krt17*)+ stem cells situated in the rete ridges of glabrous skin and the touch dome areas of the epidermis of hairy skin retain squamous differentiation, and that every 7–8 weeks, the whole supply of mature Merkel cells is replaced. Lastly, when selective genetic ablation was performed on the *Krt17*+ keratinocytes located in the touch domes, it was observed that the Merkel cell–neurite complex's innervation is mostly maintained by these cells, not by mature Merkel cells [88].

When Friedrich Merkel identified these cells, he described them using the term "helle Zellen" (clear cells). He was the first that aimed to characterize these cells in mammals, as transparent cells, at the epidermal foundation in specific regions of hairy skin [79]. Later on, Merkel cells were reported to be present in several locations: within the basal layer of human oral [89] and esophageal [90] mucosa epithelial tissue, in the hair follicles, forming belt-like clusters [91], near the opening of the eccrine ducts (at the base of epidermal ridges) in the glabrous skin of palms and soles [92], and, according to Felix Pinkus, in the "Haarscheiben" of hairy skin (due to their close disposition near the hair follicles, arrays of Merkel cells named "hair discs" form disk-like structures, associated with dermal myelinated nerve fibers) [93] or, as currently mentioned, in touch domes [94].

Scarce evidence exists in up-to-date studies focusing on investigating the presence, number, and distribution of Merkel cells in normal human tissue specimens, and much more, only a few refer to these aspects in patients with skin-associated diseases. Previous studies reported an increased number and significant density of these cells in the masticatory mucosa covering the gingiva and anterior hard palate, in the lips [95], human eyelid [96], male prepuce [97], labia minora [98], human vagina [99], hand finger pads and plantar surface of the toes [100]. A study searching the correlation between the number and distribution of Merkel cells with the expression of cluster of differentiation (CD)200 in scalp biopsies, taken from patients with cicatricial alopecia secondary to discoid lupus erythematosus and lichen planopilaris, has reported that the total number and follicular number of Merkel cells were lower by comparison with healthy control group, and also had a significant lower occurrence in CD200-negative cases. Down-regulation of Merkel cells in correlation with the low presence of CD200+ cells may imply a loss in the physiological immune privilege in these patients [101].

Ultrastructural studies revealed the morphological features of these specialized post-mitotic cells: measuring 10–15 μm in length, Merkel cells tend to arrange into clusters, close to the nerve endings, in the proximity of the basement membrane, to which they are anchored using hemidesmosomes. The cell surface exposes up to 50 lobulations or spine-like projections (sometimes named microvilli), with a length varying up to 2.5 μm, that display few and small desmosomes, enabling their attachment to the surrounding keratinocytes. The nucleus appears pale stained, large, and lobulated, and possesses few nucleoli. A loosely organized cytoskeleton with perinuclear and peri-neurofilaments, low-density identifiable intermediate filaments, occasionally forming aggregates that resemble tonofibrils, can be characteristic. The most definitory feature is yet the presence of high electron-dense core granules in the cytoplasm, enveloped by a simple membrane, surrounded by a clear, electron-lucent halo space. These cytoplasmatic secretory granules are concentrated with high density in areas where the cells establish contact with the primary afferent nerve endings. The immunohistochemical characterization of the dense-core granule content and their pattern of distribution are a hallmark of these particular cells. For

example, CK20 is roughly dispersed throughout the cytoplasm, differentiating them from Merkel cell carcinoma cells; in carcinoma, there is also a display of CK20 positivity, but in a dot-like arrangement, with a distinctive spiral or layered disposition. Positive staining of other neuroendocrine markers such as synaptophysin, chromogranin A, and neuron-specific enolase, in the dense-core granules, are helpful to attest to the presence of Merkel cells. In addition to those, several other markers namely, serotonin, SP, or vasoactive intestinal polypeptide, were reported to show inconstant positivity [102–105].

The human skin Merkel's cell dense-core granules contain a wide range of neuropeptides, some of which may function as neurotransmitters; they enable Merkel cells and the contact nerve endings to carry out their typical function as mechanoreceptors for light touch stimuli. Merkel's cell ability to convert mechanic tactile sensations into electric signals is dependent on the presence of the ion channel piezo-type mechanosensitive ion channel component 2 (PIEZO2) protein, which was found to be highly expressed. This PIEZO2 protein facilitates the passage of electric signals Ca^{2+} mediated and the release of serotonin, thus enhancing the stimulation of type I afferent nerve endings [106–108]. The interaction between Merkel cells and sensory nerve endings is under a continuous remodeling process during skin homeostasis, showing a highly malleable interaction with a strong correlation degree regarding their appearance, revival, development, progress, and repositioning in the course of epithelium turnover [109].

There are several potential roles for the Merkel cells that have been suggested. Owing to their close physical connection to the sensory nerve, and the presence of different neuropeptides and neuron-specific proteins, their function in somatosensation and mechanical transduction was attested. The endocrine secretory performance of these cells was assumed by the fact that Merkel cell cytoplasm is home to a variety of small amine or polypeptide hormones stored in membrane-bound, small granules. Recent studies that explored the interaction between Merkel cells and Aβ fibers in experimental mechanical-induced itch, revealed the potential of this complex to modulate skin impairment by diminishing the degree of the pruritus [110–113]. These findings can be of great importance for future studies in patients with chronic pruritus such as senile pruritus, psoriasis, or atopic dermatitis, in developing new therapeutic agents targeting alloknesis (pathological mechanically evoked itch) pathways.

Hofbauer cells

The placenta, a unique transient organ that develops during pregnancy, has an essential role in supporting the growth and development of a fetus. The placenta serves as a connector between the mother and the fetus, enabling the transfer of vital nutrients, oxygen, and waste materials [114]. The placenta provides several functions. First, it facilitates the transfer of essential nutrients, such as glucose, amino acids, and vitamins, from the maternal bloodstream to the developing fetus. Additionally, it aids in the transportation of oxygen from the mother and eliminates waste products such as carbon dioxide from the fetus [115]. Also, the placenta synthesizes several types of hormones essential to pregnancy, such as human chorionic gonadotropin (hCG), progesterone, estrogen, and human placental lactogen (hPL). These hormones control the mother's physiological processes to facilitate the growth of the fetus, prevent menstruation, and prepare the breasts for the production of milk. There is also a barrier function of the placenta, defending the fetus against harmful substances such as pathogens and toxins found in the mother's bloodstream [116].

Hofbauer cells are specialized cells located within the complex structure of the placenta. They were initially described over 150 years ago, but their actual functions remained a mystery for a considerable period. In the past, Hofbauer cells were mainly acknowledged for their function in the immune defense of the placenta. Nevertheless, recent research has provided insight into the diverse ways in which they contribute to a healthy pregnancy [117].

The value of the latest studies on Hofbauer cells resides in their extensive impact on fetal development and the final results of pregnancy. Recent findings highlight the role of Hofbauer cells in the process of angiogenesis, which is crucial for ensuring the adequate supply of nutrients and oxygen to the developing fetus. Also, researchers indicate that Hofbauer cells may influence the development and functioning of trophoblast cells, which could potentially affect the exchange of nutrients and the overall health of the placenta. Understanding the histological structure and functioning of Hofbauer cells could provide valuable insights into the potential etiology of pregnancy complications [118].

Although there have been no significant advances in understanding the detailed histological structure of Hofbauer cells in recent years, current research is redirecting its focus toward exploring their functional diversity rather than only examining their morphological characteristics. Recent study evidence indicates that Hofbauer cells may possess a wider range of diversity within their population than previously believed. Their size, shape, and internal structures may vary depending on their location within the placenta and their specific functions [119]. The field is changing its emphasis from exclusively assessing histological characteristics to comprehending the practical relevance of these structures. Researchers are investigating the potential connection between the distinct configurations of Hofbauer cells and their various roles in the placenta, including immune defense, nutrient transport, and signaling.

Even though recent discoveries regarding their specific histological structure are lacking, Hofbauer cells exhibit certain clearly defined classical features. Hofbauer cells are characterized by their relatively large size and round or oval shape, distinguishing them from other types of placental cells. They contain significant amounts of cytoplasm, which is usually pale or lightly stained when observed using standard histological techniques. The nucleus is usually solitary, circular, or oval in shape, and located eccentric. Hofbauer cells exhibit a limited amount of visible organelles in typical histological preparations. The potential cause for this phenomenon could be attributed to the staining techniques used, since certain investigations suggest the presence of specialized structures. Also, Hofbauer cells can contain small lipid droplets in their cytoplasm, suggesting a potential involvement in fat storage or metabolism. Classically, Hofbauer cells exhibit a smooth cell surface

devoid of any visible protrusions such as microvilli or cilia. Recent research suggests the existence of potential cilia-like structures in certain cells, requiring further investigation. Hofbauer cells are mainly located in the connective tissue stroma of the placental villi. These villi are finger-like projections that increase the surface area for the exchange of molecules between the maternal and fetal blood [120, 121].

Hofbauer cells have been characterized for over a century as large, round cells with few visible organelles based on classical histological examinations. However, recent research provides a more complex representation, showing a diversity in their structure that suggests different functional roles within the placenta [121]. The conventional characterization of Hofbauer cells as mainly uniform may be an oversimplification. Research indicates that there are differences in size, shape and cytoplasmic content. Hofbauer cells within the placenta may display variations in both size and shape. Some cells may have a larger and more circular shape, while others may have a more elongated or even star-shaped morphology. The apparently empty cytoplasm observed in traditional histology can provide more valuable information when analyzed using advanced techniques. Electron microscopy studies have suggested the existence of specialized structures within some Hofbauer cells. As we mentioned, tube-like structures were observed extending from the nucleus of cells, possibly resembling cilia. These structures may play a role in distinct functions such as intracellular transport or communication [122].

The increasing acknowledgment of the heterogeneity of Hofbauer cells is intriguing as it suggests a connection between their structure and their function. With regard to region specific differences, Hofbauer cells located in proximity to the fetal blood vessels may possess anatomical adaptations to facilitate nutrient exchange, whereas those in close proximity to the maternal blood may display characteristics specifically designed for immune surveillance. Also, in terms of functional diversity, the conventional perspective is focused on Hofbauer cells as immune sentinels. Nevertheless, new evidence indicates that they have a more widespread function in maintaining the well-being of the placenta, including angiogenesis, placental remodeling, or trophoblast maturation. Hofbauer cells potentially play a role in the development of blood vessels in the placenta, which is essential for maintaining adequate blood flow. Structural variations may be related to their function in shaping the placenta during pregnancy. The maturation of trophoblasts can have an impact on the development and functioning of these cells, which in turn affects the exchange of nutrients [123].

Advanced microscopy techniques, such as electron microscopy combined with immunohistochemistry (IHC), can offer a more comprehensive understanding of the proteins and structures present in Hofbauer cells. This approach allows for a detailed examination of their location, which can provide valuable insights into their function. Functional assays will be developed to evaluate the activity of Hofbauer cells in various placental regions. This will help establish a correlation between specific structures and their functional roles. The future of Hofbauer cell research will likely focus on understanding the relationship between their intricate structure and the various functions they carry out in the complex placental environment. Acquiring this knowledge could have an essential impact on comprehending placental dysfunction and enhancing pregnancy results.

Cytokeratins

Epithelial tissues form the vital interface between our bodies and the world [124]. They line our organs, provide barriers against external threats, and regulate the exchange of substances [125]. Within these tissues, a network of resilient water-insoluble protein filaments, known as CKs, or simply keratins, plays a pivotal role in maintaining structural integrity and facilitating specialized cellular functions [126]. In the realm of histology, the microscopic study of tissues, CKs serve as invaluable molecular signposts, illuminating the complex landscape of cell types and aiding in the diagnosis of diseases, particularly cancers [127].

CKs belong to the superfamily of intermediate filaments, the rigid, rope-like structures that crisscross the cytoplasm of cells, extending from the nucleus towards the cell membrane. These filaments allow the cells to overpass mechanical and non-mechanical stress forms [128]. This family boasts over 50 individual CK proteins, classified as either acidic (type I) or basic (type II) and numbered CK1–CK50 (where, for example, CK1–CK8 are basic and CK9–CK20 are acidic) [129].

Epithelial cells express distinct pairs of type I and type II CKs, to polymerize as a filament, creating a unique fingerprint that reflects their origin or lineage and degree of differentiation (*e.g.*, K1–K10, K2–K11, K8–K18). The expression of these different pairs of CKs varies according to the position of cells in the epithelium or the type of epithelium. For example, simple epithelia often express CKs 8 and 18, while the stratified squamous epithelia of our skin predominantly contain CKs 5 and 14 [130].

Another classification uses their molecular weight, which belongs to the interval of 40–70 kDa. Thus, there are low molecular weight (LMW) keratins, like CK5, and CK6, and high molecular weight (HMW) keratins, like CK7, CK8, CK19, and CK20. The initial nomenclature of CKs followed a descending order, from HMW – CK1 towards LMW keratins [131].

In pathology, CK IHC has revolutionized how we visualize and study tissues. IHC employs precisely engineered antibodies that bind with exquisite specificity to different CK proteins [132]. When coupled with a detection system, these antibodies generate a visible signal at the site of the targeted CK. This allows pathologists to observe the distribution and pattern of CK expression within cells under the microscope, offering crucial diagnostic clues [133].

A classical application of CK IHC is in the identification of tumors originating from epithelial cells. Carcinomas often retain some of the CK expression patterns of the tissues they arose from, while undifferentiated tumors may still express basic epithelial CKs [134]. This allows pathologists to confirm the epithelial nature of a tumor and often suggest a likely source. Furthermore, specific CKs are associated with distinct tumor types [135]. For instance, the presence of CK20, along with the absence of CK7, points toward a colorectal carcinoma, while the

reverse pattern suggests carcinomas of specific other origins [136].

Beyond cancer diagnosis, CK IHC helps in unraveling a vast range of pathologies. Identification of rare epithelial cell types within tissues aids in diagnoses within dermatopathology and assists in understanding inflammatory and autoimmune conditions involving epithelial tissues [137].

The intricate relationship between CKs and cellular identity extends beyond disease diagnosis. In developmental biology, investigating the changing patterns of CK expression helps map the complex processes of tissue formation in the embryo [138].

Type I CKs show remarkable diversity in their expression patterns throughout the body's epithelial tissues. Specific CKs like CK9 and CK10 dominate the keratinizing layers of the skin [139], while CK12 is unique to the eye's cornea [140]. In the multi-layered non-keratinizing epithelia lining the oral cavity and the esophagus, CK13 takes prominence [141]. The more broadly expressed CK14, CK15, or CK17 hold key roles in epithelia's basal and stem cell compartments [142], whereas CK16 marks areas of active cell proliferation [143]. Simple epithelia, a single-layered sheet lining our organs and glands, depend heavily on CK18 and the versatile CK19 [144]. Finally, CK20 stands out with its specific expression in the digestive and urinary tracts, as well as Merkel cells in the skin [145].

Type II CKs complement their type I counterparts, ensuring a robust cytoskeleton. CK1 and CK2 reinforce the structure of keratinizing stratified epithelia. CK3 joins CK12 in the cornea but can also be found in other stratified tissues. CK4 supports the structure of non-keratinizing epithelia [146]. The key players CK5 and CK6 are found in the basal layers of stratified epithelia, alongside myoepithelial cells that aid in gland function [147]. CK7 is particularly important in simple epithelia, especially those forming ducts, as well as the mesothelium [148]. Finally, CK8 is widely expressed in simple epithelia and is commonly retained even in tumor cells, making it valuable for diagnostic purposes [149].

CKs typically function as heterodimers, with one type I and one type II forming a pair. Pan-CK cocktails are powerful tools in IHC, designed to provide broad-spectrum detection of epithelial cells [150]. One of the most widely used is AE1/AE3, which combines antibodies targeting a wide range of both type I and type II CKs. This makes it especially valuable for confirming the epithelial origin of tumors, particularly poorly differentiated carcinomas where cellular features may be less distinct [151]. Other pan-CK cocktails also exist, often with CKs 8, 18, and 19 as core components and variations to fine-tune their detection. For more specific diagnostic questions, we can use cocktails like CK5/6 to identify squamous cell carcinomas and mesothelioma [152] or CK7/CK20 which is crucial in pinpointing the origin of carcinomas or even precancerous lesions of the esophagus [153]. CAM 5.2 targets CK8/18 and is used with other markers to support carcinoma diagnoses and epithelial differentiation of sarcomatoid and glial tumors [154]. Additionally, specialized cocktails target HMW CKs such as CK34βE12, specific carcinoma types, or highlight features like glandular differentiation, giving pathologists the tools to tackle various complex diagnostic challenges [155] (Figure 1).

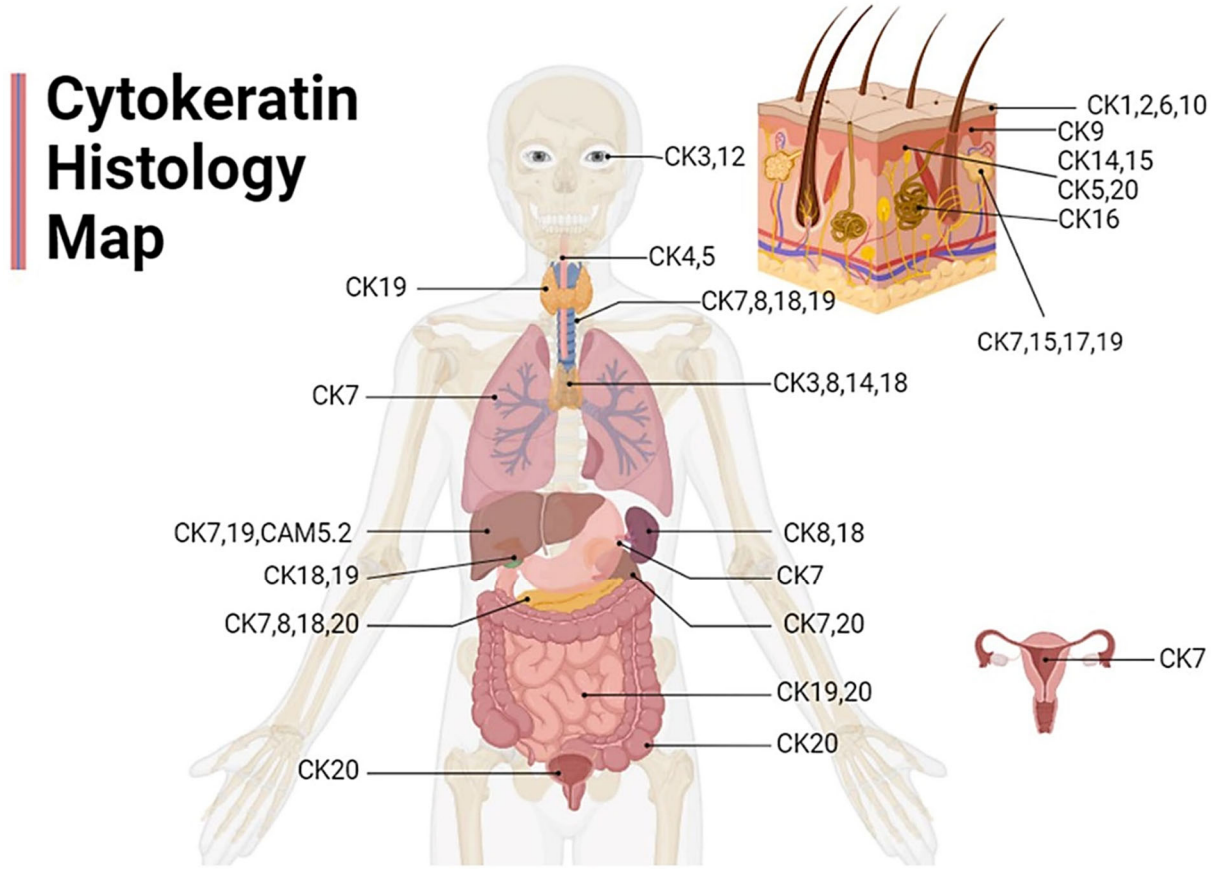

Figure 1 – ***Cytokeratin (CK) immunohistochemical positivity map in various epithelia.***

In conclusion, CKs represent far more than mere structural components of epithelial cells. They are intricate markers of cellular lineage, differentiation, and disease. Harnessing their power through IHC empowers histological investigation in both clinical and research settings. As research continues to unveil novel CKs and their roles in cellular function, their applications in tissue analysis will undoubtedly expand, contributing to our ever-evolving understanding of the human body in health and disease.

The paracortex of lymph nodes

LN analysis falls into routine examination for general pathologists. As main stations in lymph passage, LNs offer comprehensive information about the structures or organs they drain and about preserved or impaired immune function. Moreover, lymphatic dissemination of tumoral cells is crucial in oncological cases, in tumoral staging, and in assessing prognosis.

The parenchyma of the LN is mainly divided into cortex and medulla. The cortex however presents two different compartments: the superficial area – the B-cell zone and the deep area, regarded as the paracortex – the T-cell zone [156]. Although these structural compartments are well established, the microscopic aspect is highly diverse, according to various antigen stimulation. Thus, the concept of histology is, to an extent, controversial in the LN, since the majority, if not all human LNs show adaptative morphological changes.

From a structural point of view, the LN consists of several lobules (also labeled as units or compartments), arranged side-by-side, converging towards the hilum. Each lobule includes the opening of an afferent lymphatic into the marginal sinus, the corresponding superficial cortex (with two to six lymph follicles), a single deep cortical unit, and cords and sinuses that continue this unit. Deep cortical units of adjacent lobules fuse at the level of their lateral, peripheral region. Since each afferent lymphatic vessel gathers the lymph from distinct drainage areas, every lobule might encounter a unique array of antigens [157].

This chapter will focus on the deep unit of the cortex, the paracortex. It will address the cell population within this compartment, as well as alterations encountered in reactive and tumoral processes.

Cell population

The majority of the cells belonging to the paracortex are small, nomadic, permanently circulating T-cells. There is a 3:1 ratio between helper (CD4+) and cytotoxic (CD8+) T-cells [158]. Rare B-cells can also be identified scattered within the thickness of the paracortex. Recently, researchers identified a subset of memory T-cells, that have the potential to reconstruct the complete range of memory and effector T-cells. These were called T-memory stem cells (TSCM) and are key players in ongoing studies, in the battle against infectious diseases, including the design of vaccines, chronic infections, autoimmune diseases, and malignancies [159, 160]. As one of the aforementioned changes, research showed that aging leads to diffuse degeneration of lymphocytes belonging to the cortex and paracortex [161].

T-cells cooperate with interdigitating dendritic cells (DCs), antigen-presenting cells (APCs) for T-cells, which correspond with the dendritic reticulum cells found in the cortex. Interdigitating cells have an abundant, clear, or pale cytoplasm that confers a mottled aspect to the dark basophilic background assured by the small T-cells. They have a central nucleus and cytoplasmic extensions that interdigitate with processes of adjacent DCs [157]. Antigenic stimulation increases the influx of these interdigitating DCs. Towards differentiation to memory cells, CD8+ T-cells initially communicate with type 2 DCs found in the interfollicular region and the cortical ridge, and only afterward do they encounter type 1 DCs, in the paracortex [162].

The deep cortex is also the headquarters of the post-capillary venules (PCVs). These are lined by peculiar, plump, cubo-cylindrical endothelial cells, with a round nucleus and evident cytoplasm. These characteristics, along with the narrow lumen of these vessels contrast with the general aspect encountered in venules from other tissues. PCVs can also be found in the interfollicular unit of the superficial cortex, but towards the cortico-medullary junction, the lining cells revert to the characteristic flattened endothelial cells [157]. The main role of PCVs is in assuring lymphocytic influx in the LN, thus enabling immune cell recirculation. Lymphocytes afterward exit through the paracortical sinuses [163].

Morphological changes in reactive conditions

Reactive changes are site-dependent and to an extent, considered physiological. Marked alterations are encountered in the main stations of drainage, such as LNs belonging to the pulmonary hilum, mesenteric, cervical, axillar, and inguinal areas [158].

The major response pattern within the paracortex is the cellular reaction, with the development of antigen-specific T-cells and memory T-cells. Reactive hyperplasia is a relatively frequent process, occurring most often in youngsters. It is seldom found alone, but more commonly associated with follicular or sinus hyperplasia [164]. The most striking alteration is the expansion of the paracortex, as the number of T-cells increases [165]. Moreover, since the size of the deep cortical units within different lobules can vary, the paracortex of a reactive LN might have an uneven, heterogeneous appearance [157]. There are three major patterns of paracortical hyperplasia: an increase in the number of lymphocytes, blasts, or the interdigitating cells. However, the cell population is polymorphic, encompassing both small and large lymphocytes, without significant cytological atypia, macrophages, plasma cells, and eosinophils. Epstein–Barr virus, cytomegalovirus, and herpes virus elicit a predominant paracortical pattern of reactive lymphadenopathy [166].

The paracortex, among other compartments of the LN, is the preferred site for the emergence of granulomas [158].

Morphological changes in tumoral processes

Primary malignancies

The most frequent primary malignancies centered in the paracortex are nodal T-follicular helper cell lymphomas, a category that encompasses several individual entities of lymphomas. In such cases, the proliferation of malignant cells invades the cortical compartment, which becomes thinner. Malignant cells demonstrate atypia: pleomorphic

nuclei with vesicular chromatin, and prominent nucleoli, as well as mitotic figures. IHC can aid in the positive diagnosis of malignancy, demonstrating the loss or gain of specific markers, such as CD10, B-cell lymphoma 6 (BCL6), programmed cell death 1 (PD1), inducible co-stimulator (ICOS), C–X–C motif chemokine ligand 13 (CXCL13) [167]. Molecular tests can validate the diagnosis, by detection of clonal T-cell receptor rearrangements.

Secondary tumoral involvement of lymph nodes (lymphatic spread of tumors)

Interestingly, it has been demonstrated that tumors elicit immune suppression of regional LNs, decreasing the activity of the paracortex. Moreover, the level of suppression directly corresponds with the proximity of the LN and the tumoral site [168]. One mechanism responsible for this is the downregulation of interdigitating DCs, with a reduction of both the density of interdigitating cells and the length of their cytoplasmic processes [169]. Morphological and immunohistochemical studies demonstrated that LNs with metastasis show a decrease in the total number of T-cells [170]. Recently, a fascinating discovery came to our attention: the profound acidic pH of the paracortex and the implication of T-cells in the acidifying process [171]. Although the low pH does not block T-cell activation, it considerably reduces cytokine activity and affects cellular communication and immune regulation. The migration dynamics of T-cells within the LN are also altered by the use of immunotherapy that targets immune checkpoint molecules [172].

Conclusions

The paracortex of the LNs plays a crucial role in cellular immunity, through antigen recognition and activation of T-cells. Comprehending the intricate interplay between immune cells, the structural reticular stroma, cytokines, chemokines, and adhesion molecules is essential for elucidating the mechanisms governing adaptive immune responses, offering potential avenues for advancing immunotherapeutic interventions.

Conflict of interests

The authors declare that they have no conflict of interests.

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