Environmental Adaptation: the Macrophage Niche in Barrier Tissues

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Abstract

Macrophages are found throughout the body, where they play crucial roles in tissue development, homeostasis and remodelling, as well as being sentinels of the innate immune system that can contribute to protective immunity and inflammation. Barrier tissues such as the intestine, lung, skin and liver are exposed constantly to the outside world, placing special demands on resident cell populations such as macrophages. Here we review the mounting evidence that although macrophages in different barrier tissues may be derived from distinct progenitors, their highly specific properties are shaped by the local environment, allowing them to adapt precisely to the needs of their anatomical niche. We discuss the properties of macrophages in steady state barrier tissues, outline the factors that shape their differentiation and behaviour and describe how macrophages change during protective immunity and inflammation.

1. Introduction

Macrophages (mϕs) are one of the most abundant populations of leukocytes in the body. They play important roles in innate immunity and inflammation[1], but are also crucial for the development and homeostatic maintenance of normal tissues, as well as in the repair of damaged tissue[2]. Hence, there is considerable interest in exploring how mϕs are tailored to the physiological demands of their environment and how this changes when homeostasis is perturbed.

Until very recently, it was believed that mϕ behaviour could be explained by dividing them into discrete subsets ("M1-like" and "M2-like") based on their functional properties. However work over the past few years shows that this approach is over-simplistic[3] and that mϕs are highly heterogeneous, possessing specialised properties that are precisely adapted to individual tissues. In parallel it is now clear that local factors in the environment control how mϕs develop and function under both steady state and inflammatory conditions. Here we discuss how this new understanding of macrophage biology provides insights into the behaviour of these cells in the most immunologically active tissues in the body, the barrier surfaces of the skin, intestine, lung and liver. As these sites are exposed continuously to the external environment and are of crucial physiological importance, they require constant monitoring for pathogens, but also maintenance of tissue integrity. As a result, immune cells

in the barrier surfaces are subject to unique demands and the macrophage populations have many unusual properties.

2. Macrophages in Steady State Tissues

Mϕs are sessile mononuclear cells found in all tissues of the body, where they have the appearance of large vacuolated cells with abundant cytoplasm containing lysosomal granules. Classically, tissue mϕs have been identified on the basis of F4/80 and CD68 expression in mice and humans respectively, but recent multi-parameter flow cytometric analyses and genome-wide transcriptional profiling have revealed additional generic markers that identify mϕs across a range of tissues[4] (Table 1). Of particular note and of practical importance, the high affinity IgG receptor CD64 and Mer tyrosine kinase (MerTK) are common to most tissue mϕs in mice and are not expressed highly by other mononuclear phagocytes (MPs), such as dendritic cells (DCs)[5].

Layered on top of this common signature, mϕs in individual tissues are remarkably heterogeneous in terms of their surface phenotype, transcriptome and epigenome (Table 1 and see below). Although many of their functions are conserved across tissues, including key housekeeping functions such as clearance of apoptotic and senescent cells, individual populations of mϕs are highly adapted to the needs of their environment, fulfilling roles specific to the particular tissue, and even subcompartments within tissues[6]. This heterogeneity is not surprising given the significant differences between organs in terms of their physiological functions, exposure to microbiota, nutrients and metabolites, and the fact that mϕs develop synchronously with their organ of residence[7]. For a time, it was believed that these differences might reflect distinct developmental origins of mϕs, but it now seems more likely that local environmental imprinting is the major determinant in mϕ identity and function, irrespective of their origin[8]. As these general topics have recently been reviewed extensively (eg see [7-12]), here we restrict our discussion of those aspects of mϕ origin and development that are specific to the barrier surfaces.

A) Intestinal macrophages.

i) Mucosal Macrophages: Mϕs are abundant in all layers of the normal small and large intestines, including the lamina propria (LP) of the mucosa, the muscularis externa and the

serosa that separates the intestine from the peritoneal cavity. The largest population is in the LP, where they are often found immediately below the single layer of columnar epithelium. In mice, mature mϕs of the LP express high levels of MHCII, and most express CD11c to some extent, often resulting in their confusion with DCs[13 14-17] (Table 1). They are also rich in receptors associated with phagocytic activity and uptake of apoptotic cells, such as CD163, CD206, TIM4, $\alpha_v\beta_5$ integrin and CD36[14,18-21]. Importantly, human intestinal mϕs share many of these features[14,22] (Table 1).

Unlike many other tissue resident mϕs, those in the adult intestine are dependent on constant replenishment by bone marrow derived monocytes which then differentiate locally under control of factors in the environment including TGFβ[20,23-25] (Figure 1A). This continuous process allows flexible adaptation to the rapidly changing niche represented by the mucosa. During their differentiation, mucosal mϕs progressively acquire their characteristic phenotype, together with a number of functions that contribute to homeostasis in the steady state intestine (Figure 1A). They have avid phagocytic activity and are bactericidal without exogenous stimulation and together with their subepithelial location, they are ideally situated to deal with pathogenic microbes that invade across the intestinal epithelium, as well as contribute to the symbiotic relationship with the microbiota[19,25-27]. Local mϕs can also help preserve integrity of the mucosa in a number of ways. First their scavenger properties will enable them to deal with the large amount of cell death that occurs routinely in this highly dynamic tissue. Secondly they secrete mediators that drive epithelial cell renewal, including hepatocyte growth factor (HGF)[28], members of the Wnt signalling pathway[29-31] and PGE_2[32]. Finally they produce metalloproteinases that may promote tissue remodelling[20]. As a result, loss of mucosal mϕs in mice leads to dysregulated enterocyte differentiation and increased susceptibility to inflammatory damage([33,34] and see below).

A prominent feature of steady state intestinal mϕs is their constitutive production of IL10[14,21,35,36], together with some TNFα and IL1β[14,37,38]. Despite this, they fail to produce pro-inflammatory cytokines, nitric oxide or reactive oxygen species (ROS) when stimulated by agents such as TLR ligands[14]. This does not reflect a failure to express appropriate pattern recognition receptors (PRR), but rather may be due to active inhibitory mechanisms that block the relevant signalling pathways[22,39,40]. Signalling via the IL10 receptor plays a

crucial role in the functional "anergy" of intestinal mϕs, with defects in this pathway leading to mϕ hyperactivity and inflammatory bowel disease[21,41-43]. IL10 produced by mϕ themselves is not essential for this process and additional sources of this cytokine in the mucosa, such as $CD4^+$ T cells, seem to be more important[21].

Intestinal mϕs are also important sources of mediators that help maintain other immune cells in their vicinity. Mϕ-derived IL10 sustains the expansion and survival of inducible $FoxP3^+$ T_{reg} in the LP[35,44], a process that is important for tolerance to orally administered antigens[44]. In parallel, the numbers of endogenous $FoxP3^+$ T cells in different segments of the intestine correlate with mϕ numbers[45]. IL1β produced by mucosal mϕs may play a similar role in promoting the survival of local IL17 producing $CD4^+$ T cells[37] and in driving secretion of CSF2 from type 3 innate lymphoid cells (ILC3)[38]. Mature intestinal mϕs also produce a number of chemokines that can recruit T cells and other immune cells, including their own monocyte precursors[17,20] (Figure 1A).

The high expression of MHCII by steady state intestinal mϕs raises questions of whether they can behave as antigen presenting cells (APCs) *in vivo*. However mϕs are sessile in the mucosa and do not migrate to the draining mesenteric lymph node (MLN)[13]. Therefore, they are unlikely to be important for priming naïve T cells, which are found only in secondary lymphoid organs and not the mucosa. Whether mϕs might present antigen to T cells after their arrival in the mucosa remains to be resolved. Mϕs may also cooperate with mucosal DCs during the induction of local immune responses through antigen transfer to migratory DCs[46,47]. Additionally, human intestinal mϕs are capable of producing retinoic acid by metabolism of dietary vitamin A, a property that is restricted to intestinal DCs in mice and which could suggest that mϕ might assist the imprinting of gut homing in human T cells[48,49].

ii) Specialised macrophage microenvironments within the intestine?

Much of what we know about intestinal mϕs comes from studies using cells isolated from the LP of whole tissue or biopsies. Thus there is limited information on how mϕs might behave in the different anatomical compartments within the mucosa. There are relatively more mϕs in the LP of the colon than the small intestine[50], possibly reflecting differences in the functions and bacterial loads in these tissues.

149 Mϕs are found in a number of locations within the mucosa itself, ranging from
150 immediately next to the basement membrane underlying the epithelium to the central core
151 of the LP, and at different positions along the villus-crypt axis (Figure 2A). A specific
152 population expressing CD169 is found near the crypt base, close to the submucosa and
153 these may have distinct functions and developmental requirements[51,52]. Substantial
154 numbers of mϕs are also found in the external muscularis layer of the intestine (Figure 1A).
155 These are morphologically, phenotypically and transcriptionally distinct from those in the LP,
156 selectively expressing a number of genes associated with tissue repair[53]. Being located close
157 to neurons in submucosal ganglia, they also engage in two-way interactions with the enteric
158 nervous system and respond to luminal bacteria via signals from nor-adrenergic nerves[54,55].
159
160 ***B) Lung Macrophages.***
161 The lung harbours at least two different mϕ populations that occupy distinct
162 anatomical niches (Figure 1B). The largest of these inhabits the alveolar space (**alveolar**
163 **macrophages** - **AMs**), where they represent ~90-95% of leukocytes and reside in a precisely
164 defined niche on the luminal side of the lung alveoli. In both mice and humans, AMs can be
165 identified by their high auto-fluorescence and expression of CD64, as well as high levels of
166 CD11c and CD169 (Table 1)[4,56-58]. However, important phenotypic differences exist between
167 AMs in mice and men (Table 1). For instance, although SiglecF is a signature molecule for
168 murine AMs[4], its functional paralog, Siglec8, is absent from human AM[58] (Table 1). AMs
169 develop from foetal liver monocytes under the control of CSF2 (GM-CSF) in the first days of
170 life, paralleling the development of the alveoli[59] (Figure 1B) and then maintain themselves
171 by *in situ* self-renewal [57].
172 One of the principal homeostatic functions of AMs is regulating the levels of
173 pulmonary surfactant, the proteolipid complex synthesised and secreted by the respiratory
174 epithelium (Figure 1B). Indeed the transcriptional signature of AMs features several genes
175 implicated in lipid metabolism[4,60] and pulmonary alveolar proteinosis (PAP) develops due to
176 excessive surfactant accumulation when AM development is defective, for instance in mice
177 and humans in whom the CSF2-CSF2R axis has been disrupted[8]. There is continual bi-
178 directional crosstalk between AM and AEC, which is crucial for AM differentiation and
179 functions, including lipid catabolism[61] and cytokine production[62]. For instance, CSF2

produced by AEC induces the expression of PPARγ in AMs, which controls much of their unique phenotypic and functional identity. AMs also maintain the integrity of the alveolar space by removing senescent cells and inhaled particles, and by acting as a first line of defence against pathogens[63]. Like intestinal mϕs, AMs respond poorly to activation by TLR ligands and other stimuli[63,64], allowing them to scavenge and eliminate environmental antigens in a non-phlogistic manner. This hyporesponsive state is maintained by inhibitory receptors on AMs, including CD200R, IL10R and TGFβR which recognise their respective ligands on AECs[64]. Binding of epithelial-derived surfactant proteins, of which SP-A and SP-D are the most abundant, to receptors such as SIRPα can also modify phagocytosis, cytokine production and TLR responsiveness of AMs[65]. AMs are also critical in maintaining airway tolerance to innocuous antigens by supporting the differentiation of antigen-specific T_{reg}[66]. AMs may also directly regulate the reactivity of AECs to their environment through the release of exosomes and microvesicles containing suppressor of cytokine signalling (SOCS) proteins[67]. Continual interaction with the AEC is also proposed to account for AMs remaining relatively sessile under both steady state conditions and after challenge with bacterial stimuli[68].

Mϕs are also found in the lung parenchyma (interstitium) between the alveoli and capillary beds (Figure 1B). These **interstitial macrophages** (**IMs**) can be distinguished from AMs by their distinct surface phenotype[4,56,58,69] (Table 1) and they have a unique transcriptional signature[70]. Similar to intestinal mϕ, IMs are $MHCII^+$ and express variable levels of CD11c[4,58,69,71], which can lead to their misclassification as DCs. Because different investigators have used divergent criteria to define IMs, there is limited understanding of their role in lung homeostasis[72-74]. However a prominent feature of IMs is their constitutive production of the anti-inflammatory cytokine IL10[73,74], which is reported to control the immunogenicity of lung DCs[73]. Furthermore, they produce growth factors such as PDGFβ that are known to regulate fibroblast proliferation[75]. Although the developmental origin of IMs remains controversial[69,70,76,77], they have been shown to require replacement by monocytes, suggesting that different anatomical niches in the same tissue use distinct mechanisms to maintain their macrophage populations[69,70,76].

C) Skin macrophages.

The skin consists of two anatomically distinct regions, the dermis and the epidermis, each of which contains phenotypically, developmentally and functionally distinct mϕ populations (Figure 2A).

Langerhans cells (LCs) are found in the stratified squamous epithelium of the epidermis and express the langerin molecule (CD207; see Table 1) responsible for the formation the characteristic Birbeck granules found exclusively in LCs[78]. For many years, LCs were considered the archetypal non-lymphoid DCs, but it is increasingly clear that they display features of both mϕs and DCs[79] (Figure 3A). As well as fulfilling classical DC functions, such as migration to draining LNs and antigen presentation[79], mouse LCs express high levels of the DC-specific transcription factor Zbtb46[80] and lack expression of the macrophage markers CD64 and MerTK[79]. Despite this, they can be distinguished from cDC2s based on their expression of CD24 and lack of expression of CD26[81] (Table 1). Unlike DCs, LCs are derived from foetal liver monocytes[7], can exist autonomously from blood monocytes through *in situ* self-renewal and express the mϕ-restricted TF MafB[80]. However significant differences exist between murine and human LC (Table 1), with the latter sharing characteristics with murine cDC1s, including machinery for cross presentation of antigens[82]. Unlike most other tissue mϕs, LCs rely on the alternative CSF1R ligand, IL34, for their development, which is produced constitutively by keratinocytes[83,84]. Keratinocyte-derived TGFβ is also indispensable for LC development and maintenance[85] (Figure 2A).

Given their ability to migrate to the draining LNs and act as APCs, LCs have been implicated in initiating immune responses. As discussed below, this can involve priming of effector T cells in the context of infection. However in steady state conditions, they may have an intrinsically tolerogenic role[86], with increased contact hypersensitivity to haptens having been described in mice lacking LCs[87]. Thus LCs may be similar to other DC-like APCs in being able to adapt flexibly to the needs of their environment.

The mϕ compartment of the underlying dermis is heterogeneous[79] and they are phenotypically distinct from their epidermal neighbours in both mice and humans (Table 1)[88] (Figure 3A). Notably, mature **dermal mϕs** in mice exhibit bimodal expression of MHCII and although the relationship between the $MHCII^{+}$ and $MHCII^{-}$ subsets remains unclear, they display differences in transcriptome and turnover kinetics[76,88]. Dermal mϕs develop initially from embryonic progenitors, but as in the intestine, these are displaced

progressively by BM-derived monocytes[89]. Adult dermal MHCII^{+} mϕs then require continuous replenishment from circulating monocytes[88] (Figure 2A), in a process influenced, in part, by the microbiota[88]. Apart from a clear role of CSF1R signalling[90], to date, little is known regarding the factors involved in dermal mϕ differentiation. Whether MHCII-defined subsets exist in human skin remains unclear, because HLA-DR expression is often used as the starting point for identifying MPs in human skin[91,92]. Although dermal mϕs are poor APCs[88], as in the intestine they may help maintain the dermal T cell compartment[91], possibly through their constitutive production of IL10[88] (Figure 2A). They have also been proposed to act as sentinels of invasion, expressing a number of genes associated with killing of microorganisms and displaying avid phagocytic ability[88].

D) Liver macrophages.

Although not always thought of as a barrier tissue, the liver receives all blood draining the intestine via the portal vein and is thus continually exposed to products of both the diet and the microbiota. **Kupffer cells (KCs)** are the principal mϕs of the liver, where they reside in the sinusoids, in a perfect position to monitor materials emanating from the intestine (Figure 2B). KCs develop during embryogenesis from yolk sac precursors and fetal liver monocytes, which then self-renew throughout life[7]. However, circulating monocytes have also been shown to contribute (albeit at low levels) to the KC pool during liver growth in the first few weeks of life[93] and HSC-derived cells may also contribute to the KC pool with age[94] (Figure 2B). As well as generic mϕ markers, KCs express intermediate levels of CD11b and MHCII, distinguishing them from other CD11b^{hi} myeloid cells in the liver[93] (Table 1). In addition, murine KCs express high levels of the phagocytic receptor Tim4[93,95], the complement receptor VSIG4 (or CRIg)[96,97] and uniquely among tissue mϕs, the C-type lectin Clec4F[23,93,98] (Table 1). Human hepatic mϕs express CD68, CD64 and CD163[99,100], but whether these markers are restricted to KCs, or are also present on other liver MP is unclear. Notably, although Clec4F is not conserved in humans, both VSIG4 and Tim4 are expressed by human KCs[96] (Scott, Guilliams *Unpublished observations*) (Table 1).

KCs have been suggested to act as a firewall preventing systemic dissemination of microbes and their products from the intestine[101,102] (Figure 2B). The liver has been particularly associated with the induction of tolerance to orally administered proteins[103]

and administration of antigen into the portal vein has been reported to induce systemic tolerance, while portal vein shunting abrogates oral tolerance[104-107]. Given their phagocytic capacity and their expression of MHCII, KCs have been suggested to be key to this process, both as APCs[108-111] and by regulating the local immune environment via the production of immunosuppressive cytokines including IL10 and TGFβ[112,113]. The normally tolerogenic properties of KCs however, have been suggested to be overridden by stimuli such as TLR ligands, suggesting they could also play a role in active immunity against microbial infections[114-116], however, whether this represents true KC plasticity or the presence of non-KCs which respond to TLRs remains to be investigated.

KCs also play important homeostatic roles in iron metabolism and recycling[117], and they express a number of genes involved in these processes, including *Cd163*, *Slc40a1*, *Hmox1*, *Hpx* and *Scd1*[93] (Figure 2B). These properties are shared with splenic red pulp mϕs, which are also exposed constantly to blood[95,118]. Stimulation of iron metabolism in KCs by IL6 and IL1 can contribute to control of infection via deprivation of iron from pathogens[119]. Indeed, patients with a deficiency in hepicidin, which induces the expression of a number of proteins involved in iron scavenging and sequestration in KCs, are more susceptible to infection with iron-dependent microbes[120-122]. The KC transcriptome is also enriched for genes involved in lipid metabolism[93] and KCs have been implicated in the pathogenesis of diseases associated with excessive lipid consumption, including non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)[123-125] (see below).

3. Tissue macrophages under non-steady state conditions

One of the most important gaps in our current knowledge of tissue-resident mϕs is that the study of their behaviour under non-homeostatic conditions such as infection and inflammatory disease remains in its infancy (Box 1 and Figure 3). Below we review what is known about barrier tissue mϕs under these circumstances, focusing on work that is compatible with the recent advances in understanding their phenotype and biology.

A) Intestinal Macrophages

Intestinal disorders such as IBD and infection are accompanied by intense infiltration with mϕs and monocytes[126]. The success of anti-TNFα therapy in Crohn's

disease highlights the practical relevance of understanding the underlying processes. In both humans and animals, the majority of the infiltrate in inflamed mucosa is made up of monocytes and immature mϕs, while the numbers of mature mϕs are usually normal, or even reduced compared with steady state intestine[14,15,21,127]. Furthermore, the relatively immature cells account for most of the pro-inflammatory mediators such IL1, IL6, TNFα, IL23, NO and ROI[14-18,127,128]. As well as causing tissue damage and/or targeting microbes directly, these mediators can also activate other effector cells of the innate and adaptive immune system, such as monocytes, neutrophils, T_h17 cells and T_h1 cells. Pro-inflammatory monocytes/mϕs also produce chemokines such as CCL2, CCL3, CCL4, CCL5, CCL8 and CCL11 which recruit eosinophils and neutrophils, as well as more monocytes[51,129,130]. As in steady state intestine, the enhanced recruitment of monocytes into inflamed intestine is driven mostly by CCR2[126,131], although other chemokine-receptors may also contribute, such as CCL3 and its receptor CCR1[132].

The monocytes that infiltrate the inflamed mucosa of humans and mice are phenotypically indistinguishable from those which replenish the mature mϕ pool under steady state conditions. However their development appears to be arrested before they acquire significant anti-inflammatory properties such as production of IL10 or hypo-responsiveness to stimulation[14-16]. The reasons for this block in differentiation are unclear, although one possibility could be that the monocytes recruited to the inflamed intestine are already intrinsically different. A process of this kind has been found in murine *Toxoplasmosis*, where IL12 released from the inflamed mucosa alters monocyte differentiation in the BM via the production of IFNγ[133], but this has not yet been explored in other contexts.

It is controversial whether the original fully mature mϕs also contribute to intestinal inflammation (Box 1 and Figure 3). Although mature resident mϕs do not become pro-inflammatory during experimental colitis induced by chemicals or T cells[14,16,18], this can occur under conditions when inflammation occurs in the absence of IL10 mediated control of mϕ activity[21,128]. One population of resident mϕs that may contribute directly to inflammation is that expressing CD169, which recruits monocyte neutrophils via production of CCL8[51,52]. Furthermore, muscularis mϕs play important roles in postoperative paralytic

ileus by producing nitric oxide in response to local trauma, leading to activation of neighbouring neurons[54].

Mϕs also play a role in the protective immunity that expels large extracellular microorganisms such as intestinal helminths[134,135]. During such T_h2 responses, mϕs produce arginase and RELMα[135,136], together with chemokines that can recruit eosinophils and other effector cells[130]. This generates an environment detrimental to parasite survival, encouraging their expulsion. As in other forms of intestinal inflammation, newly recruited monocytes seem to be the most important source of activated, effector mϕs in these T_h2-dependent immune responses[135]. However as IL4 dependent local proliferation and activation of pre-existing resident mϕs has been described in parasite infection of the serous cavities[137], similar processes might be feasible in the intestine.

Mϕs are important for tissue repair and restoration of homeostasis after inflammation in the intestine. This may involve their ability to drive epithelial stem cell renewal[32,138], while IL1-mediated induction of IL22 from ILC3 helps restore epithelial barrier function and has anti-microbial effects[131,139]. Mϕs may also protect against intestinal inflammation induced by the chemical DSS by suppressing production of the alarmin IL33[140], while their ability to produce arginase during T_h2 mediated immune responses is a crucial component of tissue repair after helminth infection[141,142]. As a result of these properties, depletion of mϕ delays recovery from experimental colitis[138,140,143]. Whether these are functions of pre-existing resident mϕ or of the monocytes recruited during the initial pathology is again unclear, although recent studies have shown that apparently pro-inflammatory monocytes recruited during murine Toxoplasmosis may protect against immunopathology by producing PGE_2 supressing neutrophil activation[127]. Both GM-CSF and VEGF-C produced during inflammation have been shown to induce reparative properties in intestinal mϕs[144].

B) Lung Macrophages

Given their positioning in the airway, it is unsurprising that **AMs** are key effector cells in the protective response against bacterial, viral and fungal infections. By virtue of their expression of a range of PRRs and high phagolysosomal capacity, AMs excel at engulfing and destroying extracellular bacteria such as *Streptococcus pneumonia*[63]. AMs also orchestrate the recruitment of neutrophils and effector monocytes to the lung through release of IL1β,

364 which induces CXCL8 production by the respiratory epithelium[145]. They are also potent
365 producers of type 1 IFN in response to viral infections and orchestrate the recruitment of
366 anti-viral monocytes[146]. AMs enhance viral clearance during influenza infection and there is
367 increased lung pathology in systems in which AMs have been depleted[64,147]. Conversely, the
368 activation threshold of AM may be heightened following severe viral infection, leaving
369 individuals more susceptible to bacterial infections. This may involve changes in expression
370 of inhibitory ligands by AEC [64].

371 AMs have also been implicated in the development and progression of asthma,
372 although it remains uncertain whether they play a pathogenic or protective role[148]. On one
373 hand, depletion of AMs worsens allergen-induced airway inflammation[149] and adoptive
374 transfer of normal AMs can protect sensitised lungs from damage[149]. Moreover, AMs from
375 asthmatic patients produce more IL10 than their counterparts from healthy lungs[150].
376 However, AMs from allergen-sensitised mice are more able to stimulate T cell responses
377 and AMs from asthmatic patients express higher levels of costimulatory molecules such as
378 CD80[151], suggesting asthmatic AMs may be able to promote pathogenic T_h2 responses,
379 perhaps through their production of IL13[147].

380 The role of **IMs** in lung inflammation or infection is poorly understood, although
381 they have been shown to confer protection against allergic airway inflammation by
382 producing IL10[152]. Whether this is a property of resident IMs or whether elicited monocyte-
383 derived macrophages can also do this is unclear. Similarly, whether IM-derived IL10 plays an
384 important role in other models of disease remains to be determined. IM also release EGF
385 which has been suggested to promote alveolar fluid clearance through promotion of
386 epithelial sodium channels[153].

387 Pulmonary mϕs are important in driving the fibrogenesis, matrix remodelling and re-
388 epithelialisation of the alveolar wall that are all essential for the restoration of barrier
389 integrity and efficient gas exchange following lung injury. AMs produce multiple growth
390 factors that promote re-epithelialisation of the alveolar wall, including VEGF, PDGF, FGF,
391 TGFβ[154,155]. TNFα from AMs also upregulates CSF2 production from AECs, stimulating AEC
392 proliferation[156,157] and supporting AM maintenance. Efferocytosis of apoptotic cells also
393 promotes pro-reparative functions of AMs, including the production of PGE2, PAF and
394 TGFβ[158]. Somewhat paradoxically, lung mϕs have been implicated in the pathogenesis of
395 interstitial lung diseases, such as idiopathic pulmonary fibrosis (IPF) in which there is

uncontrolled fibrogenesis. The relative roles of tissue-resident AMs, IMs and monocyte-derived infiltrating mϕs in this condition remain poorly understood[8]. For instance, although AMs can promote resolution of experimental bleomycin-induced fibrosis[159], AMs from PF patients produce many pro-fibrotic mediators including TGFβ[160] and CCL18[161] and depletion of mϕs (AMs or infiltrating mϕs) reduces fibrogenesis in the same model[162]. However, this could be explained by recent work demonstrating that origin of AMs can influence their function. Experimental fibrosis disrupts the autonomous renewal of AMs, leading to the recruitment of BM-derived AMs that are more pro-fibrogenic than their resident counterparts[163]. How origin dictates function remains unclear, although one possibility is that resident and BM-derived AMs might occupy different micro-anatomical niches and that this controls their function. As discussed in Box 1, the roles of developmentally-distinct macrophages in other settings has not been examined, including during pulmonary emphysema where mϕs may also contribute to loss of alveolar architecture through their enhanced production of matrix metalloproteinases MMP1 and MMP12[64].

C) Skin Macrophages

Langerhans cells have been shown to induce active T_h17 responses during cutaneous *Candida albicans* infection[164] and can participate in effector $CD8^+$ T cell priming in LNs during Herpes simplex virus (HSV) infection, either by presenting antigen directly to T cells or after transfer to cDC1s[165,166]. Recently, it was shown that CD1a on LCs can amplify T_h17-driven models of dermatitis and psoriasis[167]. Importantly, blocking CD1a through administration of anti-CD1a antibodies significantly reduced skin inflammation[167], providing a putative therapeutic option for patients with T_h17 mediated skin diseases. Although phenotypically-distinct ($MHCII^{hi}$) monocyte-derived LCs have been shown to accumulate during models of injury/inflammation[168], it remains unclear if these or pre-existing resident cells are responsible for the pro-inflammatory functions of LC under these conditions.

Little is known regarding the roles of **dermal mϕs** under non-homeostatic conditions. Dermal monocyte-derived cells have been shown to accumulate in and drive development of psoriasis-like inflammation[79,168], but not in other forms of inflammation such as contact allergen induced dermatitis[88]. Dermal mϕs may play a role in the first line of defence against

pathogens, having been shown to induce neutrophil extravasation in responses to local infection with *Staphylococcus aureus*[169]. Dermal mϕs are also essential for wound healing and restoration of tissue integrity following mechanical skin injury. Again the relative contribution of fully mature resident mϕs versus elicited monocyte-derived cells is unclear, but macrophages are essential for neovascularisation, collagen fibril assembly and scab formation, in a process dependent on IL4R signalling[170]. IL4/IL13 polarised dermal macrophages are also implicated in driving tissue fibrosis through their production of RELMα which promotes pro-fibrotic collagen crosslinking by dermal fibroblasts[170]. A population of flt3L-dependent, migratory moDCs has been reported in the dermis under both homeostatic and inflammatory conditions, but the exact nature of these cells remains unclear, as does their relationship to dermal mϕs (Box 1).

D) Liver Macrophages

KCs have been implicated in several acute and chronic hepatic pathologies, including ischemia/reperfusion (I/R-) injury, acetaminophen hepatoxicity (AILI), liver fibrosis, alcoholic liver disease (ALD), viral hepatitis, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC). However contradictory findings have been reported on the exact roles of KCs in these conditions[171]. In I/R-injury, for example, KCs have been attributed both pathogenic and protective roles, driven by TNFα, IL1β and reactive oxygen species (ROS) and IL10 respectively[172-174]. Analogous findings have been reported in viral hepatitis, where KCs have been suggested to produce both anti-viral mediators and to suppress protective immunity[175]. These contrasting conclusions may reflect the fact that previous studies did not distinguish between *bona fide* KCs and infiltrating monocytes/mϕs, or used depletion strategies that targeted all myeloid cells in the liver.

More recent studies have attempted to explore the relative roles of these cell types in inflammatory liver pathology. Notably, KCs maintain their distinct transcriptional profile following acetaminophen-induced liver injury, remaining largely identical to their steady state counterparts[176]. However, as the damage following paracetamol overdose is restricted anatomically, it will be interesting to determine if specific KCs located in the damaged areas do respond to the injury and if perhaps this has been overlooked using bulk transcriptomic

techniques. Interestingly, the infiltrating monocytes/mϕs are thought to both aggravate the early stages of this disorder[177] and to be necessary for the subsequent resolution of the inflammation[176]. Notably, recruited monocyte-derived mϕs do not appear to develop into *bona fide* KCs under these conditions rather generating short-lived macrophages [176] (Scott, Guilliams *unpublished observations*). In contrast, monocyte-derived KCs can be found during *Listeria monocytogenes* infection[178]. In this infection, early uptake of bacteria triggers KC death by necroptosis and bacteria are subsequently eliminated by recruited monocyte derived macrophages which later develop into bona fide KCs[178]. However, it remains to be seen if KC death following bacterial uptake is required for the effective clearance of the bacteria and return to liver homeostasis, furthermore, the impact of mϕ origin on these functions requires further study (Box 1). Thus there is still much to learn regarding the specific functions of KCs and recruited monocyte-derived mϕs in the liver under non-homeostatic conditions. The use of the newly defined markers capable of discriminating between KCs and other recruited monocyte-derived mϕs, as well as investigating differences in micro-anatomical location, will be critical to truly assess mϕ function during these pathologies.

4. Summary and Future Perspectives

Many of the central dogmas about the origin and function of mϕs in barrier tissues have been completely revised in recent years, with an increasing awareness of their heterogeneity and diversity of physiological roles. Rather than depending on their origin, the properties of mϕs are highly tissue specific and appear to be imprinted locally, ensuring precise adaptation to the demands of their environment. These properties offer clear possibilities for targeted therapeutic intervention, with the aim of restoring homeostasis. However for this to be achieved, the factors driving the specification of different tissue-resident mϕ populations under homeostatic conditions would be crucial to identify. Determining the relative roles played by resident and infiltrating mϕs during infection or inflammation could create further options for preventing their recruitment or activation.

Box 1: Macrophage behaviour under non-homeostatic conditions

Disruption of local homeostasis due to inflammation or infection results in a drastically altered local environment, with damage to tissue cells and induction of an innate immune response. These lead to increased production of inflammatory cytokines and chemokines, together with recruitment of inflammatory cells including neutrophils and monocytes, the latter of which can differentiate into mϕs. An important unanswered question concerns the relative roles of newly recruited and pre-existing mϕs in inflammation (Figure 3). Although it is clear that recruited monocytes are sufficiently plastic to respond appropriately to the changing environment, there is less evidence that the tissue-resident mϕs can modify their homeostatic functions to become pro-inflammatory under such circumstances. Indeed the findings that the tissue conditioned properties of resident mϕs are determined at the level of the epigenome[23] would suggest it may be difficult for these cells to change in response to new triggers, although this too is likely to be tissue-specific. Importantly there is evidence that pre-existing tissue-resident and newly recruited inflammatory mϕs can respond differently to stimuli, at least in the peritoneal cavity, lung and liver[163,178,179]. This has clear implications for designing mϕ-targeted therapy in inflammation.

In many tissues, inflammation is associated with a reduction in the resident mϕ population, often referred to as the 'mϕ disappearance reaction'. The mechanisms responsible are likely to be specific to each inflammatory insult, but could include cell death, increased adherence to tissue stroma, or emigration from the tissue. A controversial topic is whether monocytes and/or mϕs can migrate from inflamed tissues to draining LNs and present antigen to T cells, thus behaving as "monocyte-derived DCs" (mo-DCs). This is a longstanding concept in myeloid cell biology and although mϕs do not migrate to LNs under steady state conditions, during inflammation in tissues such as the gut and lung, some monocytes may upregulate CCR7, migrate to draining LNs and present antigen to naïve T cells[180]. As these cells express MafB, but not Zbtb46, they appear to be mϕs rather than part of the genuine DC lineage[80]. Although this is likely a relatively rare process, it would clearly be an effective way of expanding the range of APC capable of driving effector T cell responses in protective immunity and underlines the plasticity of recruited monocytes.

During recovery from inflammation or infection, the mϕ population typically returns to steady state levels and can also contribute to the restoration of tissue homeostasis. Again it is unclear if these processes reflect differentiation of the recruited inflammatory monocytes/mϕs into mϕs with repair functions or via a second wave of monocyte recruitment and mϕ differentiation. Similarly, it is not known if the replenishment of mϕ numbers occurs through proliferation of the remaining resident mϕ population or additional recruitment of monocytes, or a combination of these processes (Figure 3).

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Figure Legends

Figure 1: Barrier tissue macrophage function under homeostatic conditions. A) Resident mϕs in the lamina propria of the intestine express high levels of many receptors for apoptotic cells, ideal for clearing the large amounts of cell death found in this rapidly turning over tissue[14,18-22]. Production of trophic factors for epithelial stem cells and tissue remodelling metalloproteinases helps maintain barrier integrity[20,28-34]. Being actively phagocytic and bactericidal, LP mϕs are crucial in shaping host-microbiota symbiosis and may send processes across the epithelial barrier to sample contents of the lumen. They acquire many antigens avidly and pass these on to neighbouring migratory DCs for presentation to T cells in the draining mesenteric lymph node[46,47]. Intestinal mϕs in adults are replenished continuously by circulating Ly6C^{hi} monocytes[14,16,25], which differentiate locally under the control of environmental signals such as TGFβ[20], a process associated with the expression of the TF RUNX3 and KLF10 (Ref. 23). The inability of mature intestinal mϕs to respond to pattern recognition receptor triggering is controlled by IL10 (Refs 21, 41-43). Constitutive production of IL10 and other cytokines sustains the survival of other immune cells in the vicinity, including FoxP3$^+$ T$_{reg}$[35,44] and ILC3 (Ref. 38), while release of chemokines such as CCL2 allows the mϕs to recruit their own monocyte precursors and other leukocytes[17,20]. Mϕs in the muscularis mucosa are involved in two-way interactions with sympathetic neurons of the enteric nervous system and express high levels of the β2 adrenergic receptor (β2AR)[53,54]. Signalling through the β2AR drives anti-inflammatory and pro-repair properties in the mϕs, including the production of IL10 and RELMα, while bone morphogenic protein 2 (BMP2) produced by muscularis mϕs in response to microbial signals regulates neuronal function[53,54]. B) Alveolar mϕs (AMs) in the lung are crucial for maintaining patency of the alveolar space, where they regulate surfactant levels and phagocytose inhaled microbes and other particulate materials[59,61]. They communicate intimately with alveolar epithelial cells, removing dead cells and controlling their renewal. AMs maintain an anti-inflammatory environment via expression of inhibitory cytokines and receptors that regulate T cell responses and local innate immune reactions. AMs are derived from foetal liver monocytes during the neonatal period that subsequently self-renew for much of adult life[57,59]. AM differentiation is driven by CSF2 acting by inducing the expression of the TF PPARγ[59,61,62], whose ligands may include lipid-rich materials such as surfactant present in the alveolar space. Other TFs involved in AM development include Bach2 and C/EBPβ[181,182]. The specific functions of steady state interstitial mϕs (IMs) are not yet known, but may include second line defence against microbes, promotion of anti-inflammatory T cell responses and shaping local DC functions[73]. The origin(s) of IMs remain controversial[69,70,76,77] and the signals and TFs involved in their specification are yet to be determined.

Figure 2: Barrier tissue macrophage function under homeostatic conditions. A) Langerhans cells (LCs) in the epidermis have transcriptional and functional properties of both mϕs and DCs[79,80]. They are highly phagocytic and proficient at acquiring antigen from the environment, but can also transport this to draining lymph nodes and present it to T cells, helping to maintain tolerance in the steady state. LCs are derived from yolk sac precursors and foetal liver monocytes[77, 89], and their differentiation is regulated by TGFβ[85] and the CSF1R ligand IL34 (Refs. 83), together with the TFs AhR and RUNX3. Dermal mϕs appear to

contain descendants of both embryonic precursors and Ly6C^{hi} monocytes, with the latter being dominant in adult life[88], but the factors involved in their differentiation and their tissue-specific roles remain to be determined. B) Kupffer cells are located at the intersection of the enteric and peripheral circulatory systems. Thus they are in an ideal position to act as a firewall against microbes and other factors arriving from the intestine in the portal veins[101,102], and they have been implicated in maintaining tolerance to these materials, either directly by presenting antigen to T cells, or by maintaining an immunosuppressive local environment[103-114]. Kupffer cells play a crucial role in recycling of iron from senescent red blood cells and are also involved in metabolism of lipids and transport of the resulting products into bile[95,117]. As they are closely associated with other parenchymal cells such as hepatocytes and liver sinusoidal endothelial cells (LSECs), it is likely that KCs may be important in the homeostasis of these cell types[8]. Kupffer cells develop from foetal liver monocyte precursors[89] and this is driven by heme derived from the recycling of effete red blood cells[95], together with the ID3 TF[183]. Additional, as yet unidentified, signals are likely to be involved in the specification of all these tissue-resident mϕ populations.

Figure 3: Macrophages in barrier tissues under non-homeostatic conditions. Disruption of homeostasis by infection or inflammation leads to the recruitment of Ly6C^{hi} monocytes and other inflammatory leukocytes such as neutrophils and eosinophils. The Ly6C^{hi} monocytes generate inflammatory mϕs and together, these are the main sources of mediators such as TNFα, IL1 and IL6 (Refs. 17, 176). It appears that most of these monocytes do not differentiate into fully mature mϕs as they would under homeostatic conditions, due to an arrest in this process and so these inflammatory cells may be short-lived[14]. An additional source of pro-inflammatory mϕs during inflammation may be monocytes whose properties have already been programmed differently before leaving the bone marrow in response to signals generated in the inflamed tissue[133]. The role of the original tissue resident mϕ population in inflammation remains unclear. They may act as early sentinels of tissue damage and recruit monocytes and granulocytes via production of CCL2, CCL8, CCL11, CXCL2 and other chemokines. However in many tissues, the numbers of resident mϕs are often reduced during the immediate response to tissue injury, the so-called macrophage disappearance reaction. Whether the remaining cells can alter their normal anti-inflammatory properties to contribute to pathology and protective immunity is not fully understood and may depend on the circumstances or tissue involved[14,17,137,146,163, 168,176]. Many questions also remain unanswered regarding the fate of the monocytes and mϕs upon return to homeostasis. For example, do activated tissue-resident mϕs return to steady state? Do the recruited monocyte-derived mϕs die, persist in the tissue as monocyte-derived mϕs or become monocyte-derived tissue resident mϕs? Finally, it is unclear if additional monocytes are recruited to help replenish the resident mϕ niche and how each of these cells might contribute to the tissue repair process.

Table 1: Surface Markers of Major Macrophage Subsets in Barrier Tissues.

Tissue	Macrophage Subset	Surface Phenotype		
		Mouse	Human	References
Intestine	Lamina Propria	$CD64^+$ $SIRP\alpha^+$ $MHCII^+$ $CD163^+$ $CD68^+$ $F4/80^+$ $MerTK^+$ $CD11b^+$ $CX3CR1^{hi}$ $CD11c^{+/-}$ $CD206^+$ $Tim4^{+/-}$ $\alpha v\beta 5^+$ $CD36^+$	$CD64^+$ $SIRP\alpha^+$ $HLA\text{-}DR^+$ $CD163^+$	14,16-20,22,81
Lung	Alveolar	$CD64^+$ $F4/80^+$ $MerTK^+$ $SIRP\alpha^+$ $CD11b^-$ $MHCII^-$ $CX3CR1^-$ $CD11c^{hi}$ $SiglecF^+$ $CD169^+$ $CD206^+$ $CD163^-$	$CD64^+$ $CD11b^+$ $HLA\text{-}DR^+$ $CD163^+$ $Siglec8^-$ $CD169^+$	56,57,58,81
	Interstitial	$CD64^+$ $CD14^+$ $MHCII^+$ $SIRP\alpha^+$ $F4/80^+$ $MerTK^+$ $CD11b^+$ $CX3CR1^{hi}$ $CD11c^{+/-}$	$CD64^+$ $CD14^+$ $HLA\text{-}DR^+$ $SIRP\alpha^+$ $CD36^+$ $CD169^-$ $CD11c^{+/-}$	56,58,69-71,81
Skin	Langerhans Cells	$CD64^-$ $F4/80^+$ $MerTK^-$ $CD11c^+$ $CD11b^+$ $EpCam^+$ $MHCII^+$ $SIRP\alpha^+$ $CD207^+CD24^+$ $CD26^-$	$CD1a^+$ $CD14^+$ $HLA\text{-}DR^+$ $CD207^+$	78,79,81,82
	Dermal	$CD64^+$ $F4/80^+$ $MerTK^+$ $EpCam^-$ $CD207^-$ $MHCII^{+/-}$ $CD11c^{+/-}$	$CD64^+$ $CD1a^-$ $CD14^+$ $FXIIIa^+$ $CD163^+$	81,88,91
Liver	Kupffer Cells	$CD64^+$ $F4/80^+$ $MerTK^+$ $MHCII^+$ $CD11b^{int}$ $CD11c^{lo}$ $Clec4F^+$ $VSIG4^+$ $Tim4^+$ $SIRP\alpha^+$ $CX3CR1^-$	$CD64^+$ $CD163^+$ $CD68^+$ $VSIG4^+$ $Tim4^+$	23,81,93,95-100,176

Figure 1

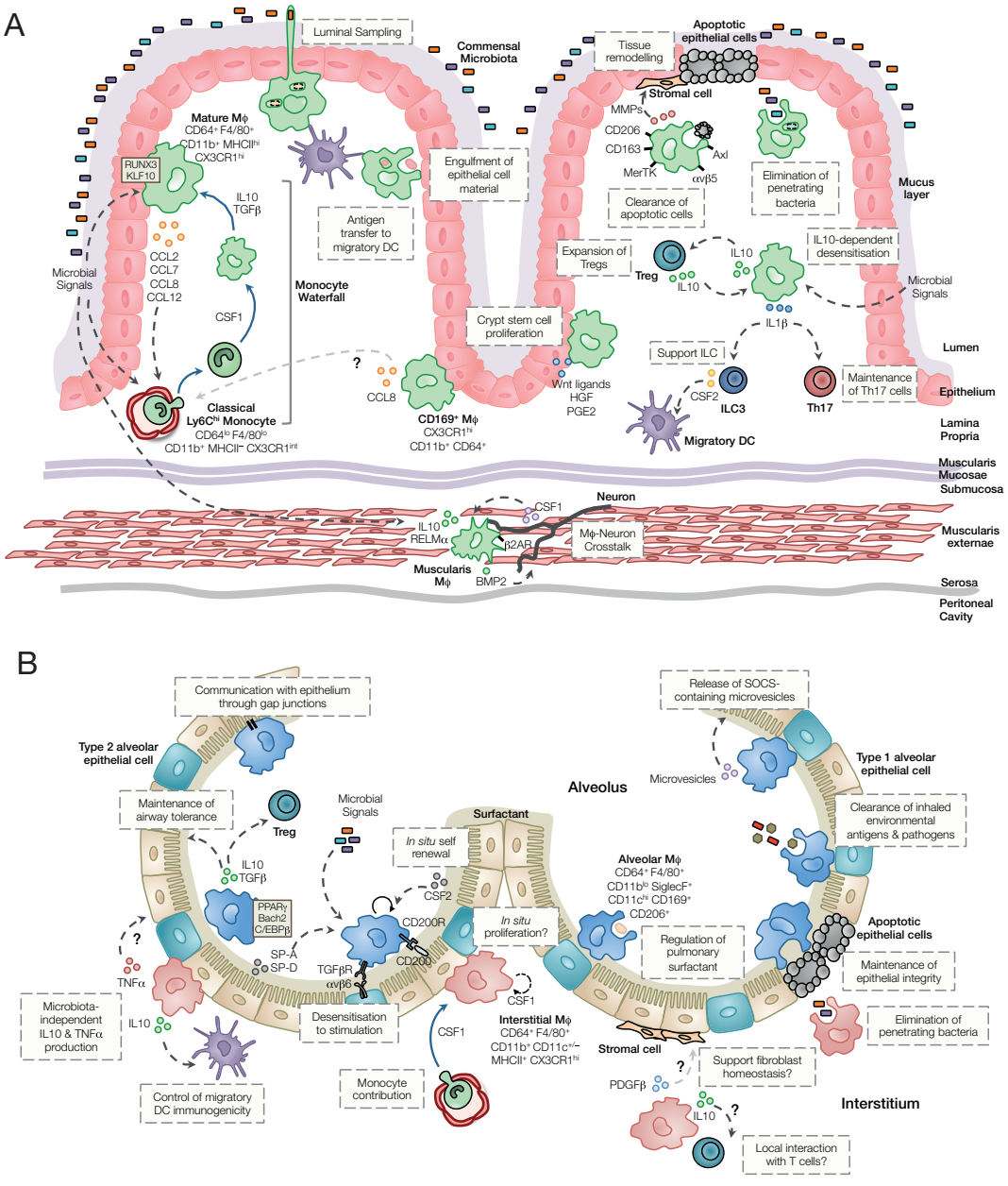

A
Luminal Sampling
Commensal Microbiota
Mature Mϕ
CD64+ F4/80+
CD11b+ MHCIIhi
CX3CR1hi
RUNX3
KLF10
IL10
TGFβ
CCL2
CCL7
CCL8
CCL12
Microbial Signals
CSF1
Monocyte Waterfall
Classical Ly6Chi Monocyte
CD64lo F4/80lo
CD11b+ MHCII- CX3CR1int
Antigen transfer to migratory DC
Engulfment of epithelial cell material
Crypt stem cell proliferation
CCL8
CD169+ Mϕ
CX3CR1hi
CD11b+ CD64+
Wnt ligands
HGF
PGE2
Tissue remodelling
Apoptotic epithelial cells
Stromal cell
MMPs
CD206
CD163
MerTK
Axl
αvβ5
Clearance of apoptotic cells
Elimination of penetrating bacteria
Mucus layer
Expansion of Tregs
Treg
IL10
IL10-dependent desensitisation
Microbial Signals
IL1β
Support ILC
CSF2
ILC3
Th17
Maintenance of Th17 cells
Migratory DC
Lumen
Epithelium
Lamina Propria
Muscularis Mucosae
Submucosa
Neuron
CSF1
IL10
RELMα
β2AR
Mϕ-Neuron Crosstalk
Muscularis Mϕ
BMP2
Muscularis externae
Serosa
Peritoneal Cavity
B
Communication with epithelium through gap junctions
Type 2 alveolar epithelial cell
Maintenance of airway tolerance
Treg
IL10
TGFβ
PPARγ
Bach2
C/EBPβ
TNFα
Microbiota-independent IL10 & TNFα production
Control of migratory DC immunogenicity
Microbial Signals
In situ self renewal
CSF2
CD200R
CD200
TGFβR
αvβ6
SP-A
SP-D
Desensitisation to stimulation
Monocyte contribution
CSF1
Surfactant
In situ proliferation?
Alveolus
Release of SOCS-containing microvesicles
Microvesicles
Type 1 alveolar epithelial cell
Clearance of inhaled environmental antigens & pathogens
Alveolar Mϕ
CD64+ F4/80+
CD11blo SiglecF+
CD11chi CD169+
CD206+
Regulation of pulmonary surfactant
Apoptotic epithelial cells
Maintenance of epithelial integrity
Elimination of penetrating bacteria
Interstitial Mϕ
CD64+ F4/80+
CD11b+ CD11c+/-
MHCII+ CX3CR1hi
Stromal cell
Support fibroblast homeostasis?
PDGFβ
IL10
Local interaction with T cells?
Interstitium

Figure 2

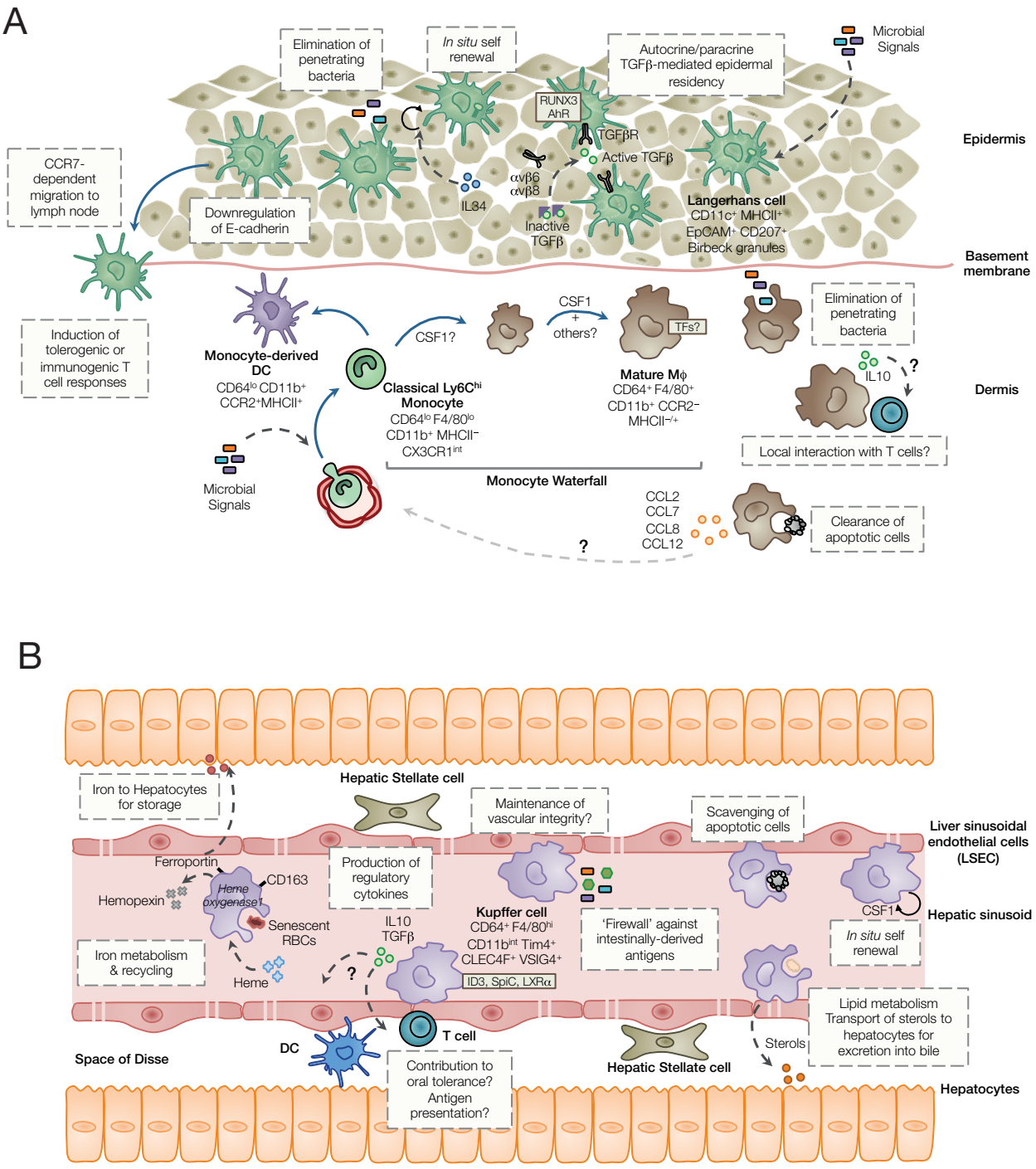
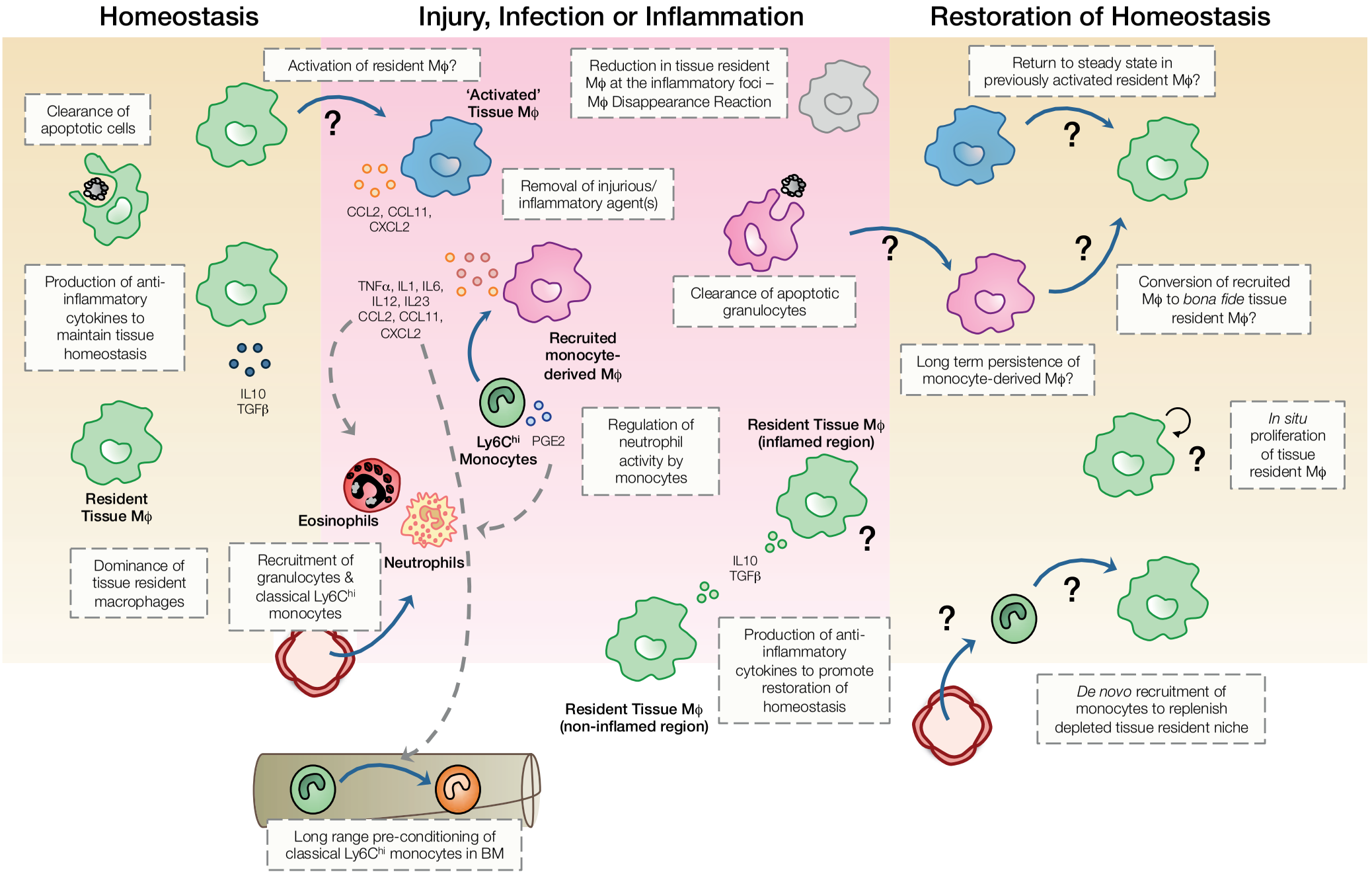

A
Elimination of penetrating bacteria
In situ self renewal
Autocrine/paracrine TGFβ-mediated epidermal residency
Microbial Signals
RUNX3
AhR
TGFβR
Active TGFβ
αvβ6
αvβ8
IL34
Inactive TGFβ
Epidermis
CCR7-dependent migration to lymph node
Downregulation of E-cadherin
Langerhans cell
CD11c+ MHCII+
EpCAM+ CD207+
Birbeck granules
Basement membrane
Induction of tolerogenic or immunogenic T cell responses
Monocyte-derived DC
CD64lo CD11b+
CCR2+MHCII+
CSF1?
Classical Ly6Chi Monocyte
CD64lo F4/80lo
CD11b+ MHCII−
CX3CR1int
CSF1
+
others?
TFs?
Mature Mϕ
CD64+ F4/80+
CD11b+ CCR2−
MHCII−/+
Elimination of penetrating bacteria
IL10
?
Dermis
Local interaction with T cells?
Microbial Signals
Monocyte Waterfall
CCL2
CCL7
CCL8
CCL12
?
Clearance of apoptotic cells
B
Hepatic Stellate cell
Iron to Hepatocytes for storage
Maintenance of vascular integrity?
Scavenging of apoptotic cells
Liver sinusoidal endothelial cells (LSEC)
Ferroportin
Production of regulatory cytokines
Hemopexin
Heme oxygenase1
CD163
Senescent RBCs
IL10
TGFβ
Kupffer cell
CD64+ F4/80hi
CD11bint Tim4+
CLEC4F+ VSIG4+
ID3, SpiC, LXRα
'Firewall' against intestinally-derived antigens
CSF1
Hepatic sinusoid
In situ self renewal
Iron metabolism & recycling
Heme
?
T cell
DC
Space of Disse
Lipid metabolism Transport of sterols to hepatocytes for excretion into bile
Sterols
Hepatic Stellate cell
Contribution to oral tolerance? Antigen presentation?
Hepatocytes

Figure 3


Homeostasis
Injury, Infection or Inflammation
Restoration of Homeostasis
Activation of resident Mϕ?
'Activated' Tissue Mϕ
Reduction in tissue resident Mϕ at the inflammatory foci – Mϕ Disappearance Reaction
Return to steady state in previously activated resident Mϕ?
Clearance of apoptotic cells
CCL2, CCL11, CXCL2
Removal of injurious/ inflammatory agent(s)
Production of anti-inflammatory cytokines to maintain tissue homeostasis
TNFα, IL1, IL6, IL12, IL23 CCL2, CCL11, CXCL2
Recruited monocyte-derived Mϕ
Clearance of apoptotic granulocytes
Conversion of recruited Mϕ to bona fide tissue resident Mϕ?
Long term persistence of monocyte-derived Mϕ?
IL10 TGFβ
Ly6C^hi Monocytes
PGE2
Regulation of neutrophil activity by monocytes
Resident Tissue Mϕ (inflamed region)
In situ proliferation of tissue resident Mϕ
Resident Tissue Mϕ
Eosinophils
Neutrophils
Dominance of tissue resident macrophages
Recruitment of granulocytes & classical Ly6C^hi monocytes
IL10 TGFβ
Production of anti-inflammatory cytokines to promote restoration of homeostasis
Resident Tissue Mϕ (non-inflamed region)
De novo recruitment of monocytes to replenish depleted tissue resident niche
Long range pre-conditioning of classical Ly6C^hi monocytes in BM