

REVIEW

Metabolic adaptation of mucosal macrophages: Is metabolism a driver of persistence across tissues?

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Macrophages play essential roles in tissue homeostasis, defense, and repair. Their functions are highly tissue-specific, and when damage and inflammation stimulate repopulation by circulating monocytes, the incoming monocytes rapidly acquire the same, tissue-specific functions as the previous, resident macrophages. Several environmental factors are thought to guide the functional differentiation of recruited monocytes, including metabolic pressures imposed by the fuel sources available in each tissue. Here we discuss whether such a model of metabolic determinism can be applied to macrophage differentiation across barrier sites, from the lung to the skin. We suggest an alternative model, in which metabolic phenotype is a consequence of macrophage longevity rather than an early driver of tissue-specific adaptation.

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Macrophages are cells with multiple functions: they are phagocytes, engulfing and destroying microbial pathogens, clearing immune debris and apoptotic bodies^{1,2}. They are positioned at barrier sites throughout the body, offering first-line defense against infection. They also provide highly specialized, site-specific functions, from the mopping up of excess surfactant in the alveoli of the lungs, to iron metabolism in the spleen and synaptic pruning in the brain³. Tissue-specific macrophages are seeded by embryonic progenitors before birth, providing differentiated cells that are long-lived, capable of self-renewal, and educated by the local environment. Depending on the tissue anatomy, these resident macrophages can be replaced gradually by monocyte-derived cells that differentiate in the steady state to maintain local populations. More extreme replacement occurs in inflammation, infection, and/or immune pathology, which significantly deplete resident macrophage populations leading to an influx of monocyte-derived replacements^{4,5}.

Data suggest that the incoming cells acquire the phenotype and function of the resident cells that they replace once the inflammation has resolved, becoming almost (but not entirely) transcriptionally indistinguishable from the original tissue-dwelling cells^{6–10}. This differentiation process suggests that a program of tissue-specific education instructs the incoming cells, determining their final function. A current hypothesis is that these tissue-specific signals are largely metabolic, with access to specific energy sources determining the possible routes of differentiation^{11–13}. This model is well accepted in the lung, where the lipid-rich environment is associated with fatty acid oxidation and pro-repair or type 2-polarized macrophage function¹⁴. Here we discuss how this paradigm applies to other

barrier tissues by focusing on the skin and gut, debating where and whether metabolism leads the tissue-specific differentiation process, or tissue adaptation leads and metabolism follows.

Our review began as series of discussions between two colleagues, Clare Bennett, a Langerhans cell (LC) and macrophage expert working primarily in the skin, and Georgia Perona-Wright, a T cell and immunometabolism specialist focused on the gut and lung. Our discussion led us to consider the hypothesis that lipid metabolism does not confer macrophage specificity per se but is an adaptation for persistence and longevity that is shared across mucosal and other barrier sites. We have summarized our conversations here, exploring current thinking in the metabolic control of macrophage function.

TISSUE SPECIFICATION OF MACROPHAGE FUNCTION

Tissue-resident macrophages are seeded by a relatively homogeneous population of embryonic progenitors that circulate before birth to populate nascent tissue niches^{15–18}. Here the core macrophage transcriptional program is overlaid by environmental signals present in each tissue leading to transcriptional and epigenetic changes that enforce differentiation of cells uniquely adapted to the needs of that tissue^{18–20}, and equipped with the machinery needed for survival within that particular tissue environment. In some tissues such as the lung airway, the extrinsic and intrinsic signals that instruct this differentiation have been clearly delineated. For example, signaling by pulmonary transforming growth factor beta (TGFβ) and granulocyte-macrophage colony stimulating factor (GM-CSF) activates the transcription factors peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT enhancer binding protein beta

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(CEBP β), which are required with early growth response 2 (EGR2) for alveolar macrophage (AM) differentiation and survival²¹⁻²⁴. Likewise, in serous cavities retinoic acid activates expression of GATA-6 and CEBP β which together define tissue-specific macrophages at this site²⁵⁻²⁷. But while the core cytokines required for tissue macrophage specification have been identified in many tissues, there is considerable overlap and we still do not fully understand how niche factors combine to specify macrophage fate.

Fate-mapping studies in the mouse have revealed that over time, entry of bone marrow-derived monocytes leads to a certain ontogenic heterogeneity within tissue-resident macrophage populations^{28,29}. The extent to which embryo-derived macrophages persist in healthy tissues can be model-dependent, but it is clear that a spectrum exists across tissues: At one end, epidermal Langerhans Cells (LCs) and microglia in the brain become sealed in their compartments after birth³⁰⁻³² and are maintained as quiescent, self-renewing, embryo-derived macrophage populations throughout life, without contribution from circulating cells. At the other extreme, the skin dermis and lamina propria of the gut are highly accessible to the bloodstream, and embryonic macrophages are rapidly replaced and maintained by a constitutive influx of blood monocytes throughout life^{33,34}. Ontogeny of tissue-specific macrophages may be critical in determining function, as has been discussed in detail elsewhere (e.g. 5,35). The ratio of different ontogenies in each tissue can also shift suddenly. Inflammation and destruction of resident macrophages due to viral infection or immune pathology, for example, initiates an influx of monocytes into inflamed sites to refill the empty niche^{10,36}. Monocyte-derived resident macrophages retain a bias toward a pro-inflammatory state and do not fully replicate the embryo-derived cells they replace until the local tissue environment has returned to steady-state homeostasis^{10,37,38}. An interesting exception to this may be monocyte-derived LCs which we have shown rapidly differentiate within the inflamed epidermis³⁹.

This transition of inflammatory monocytes into quiescent tissue macrophages involves a shift in energy requirement, supporting persistent, homeostatic function rather than the acute activity of an inflammatory response. So, to what extent do metabolic needs shape monocyte differentiation? Monocyte-derived AMs that differentiate in the lung after influenza-dependent destruction of resident embryo-derived macrophages do not initially exhibit lipid-dependent metabolism, but over time adopt this profile as they become more like the resident AMs they replace¹⁰. Does this transition illustrate that access to different metabolic fuels in their new location contributes to the functional programming of the incoming monocytes? What is known about the interplay between tissue specification of macrophage differentiation and metabolic restriction in the environment?

METABOLIC REGULATION OF MACROPHAGE ACTIVATION

Immune cells that are actively participating in an immune response are radically different from cells at rest: they proliferate, alter surface proteins, and secrete cytokines and effector molecules. The biosynthesis involved in these changes imposes an acute energy demand on the cell, and early understanding of metabolic regulation argued that this demand was typically met by glycolysis^{40,41}. Glycolysis provides rapid ATP generation, and the increase in glucose uptake also drives the pentose phosphate pathway and the synthesis of lipid and amino acid precur-

sors, needed for rapid cytokine and effector molecule production⁴². The increased glycolytic flux maintains essential redox balance (NAD⁺/NADH)^{43,44}, and the increased glutaminolysis associated with glycolysis drives the hexosamine biosynthetic pathway that produces essential substrates for the glycosylation of recently synthesized proteins⁴⁵, an essential step for functional cytokine production. In contrast, resting cells use a slower but more efficient metabolism reliant on lipid consumption and oxidative catabolism, a decision thought to promote longevity and homeostatic quiescence^{46,47}. Resident memory T cells at barrier sites such as the skin are perhaps the poster child of quiescent immune cells, using lipids to fuel their persistence⁴⁸.

Macrophages have also been described as existing in one of these two binary states: resting cells that are associated with slow and sustained fatty acid oxidation, and acutely activated cells characterized by intense glycolysis⁴⁹. This glycolytic rush fuels the respiratory burst and the rapid production of reactive oxygen species (ROS), which is an essential part of macrophage pathogen clearance. Indeed, this metabolic switch is directly targeted by some pathogens as a means of escape (for example *Mycobacterium tuberculosis*^{50,51}). Activated macrophages are now known to exist in a wide variety of phenotypes, however, and many of these profiles have been associated with distinctive metabolic signatures (described in detail in recent reviews^{11,12}). *In vitro*, while toll-like receptor- and interferon- γ -stimulated macrophages are actively glycolytic, those with repair or resolution profiles such as macrophages activated in the presence of interleukin (IL)-4 or during helminth infection, are instead associated with mitochondrial metabolism and oxidative phosphorylation^{52,53}. *In vivo*, some elements of this dichotomy hold true: inflammatory macrophages show active glycolysis, and some helminth infections and other type II immune contexts have been associated with high oxidative phosphorylation in myeloid cells¹².

Recent reports have shown, however, that *in vivo* tissue location and fuel availability are significant influences on the metabolic characteristics and functional potential of local macrophages. The lung is the best-studied example to date, perhaps in part due to the relative ease of isolating AMs; understanding of immune metabolism has often leaped forward with technological advances that capture improved in-depth, *in vivo*, and spatial information⁵⁴⁻⁵⁶ (Table 1). The airways are depleted of glucose, to deter microbial outgrowth, but are abundant in lipids such as surfactants. Location within this lipid-rich environment is thought to impose a lipid-dependent, highly oxidative metabolism on local AMs, restricting their inflammatory potential and promoting a resolution function¹⁴ (Fig. 1). Svedberg et al.¹⁴ directly compared the metabolic requirements of differentiated AMs to those of peritoneal macrophages, and demonstrated that residency within the alveolar space is associated both with the quiescent, resolving, lipid-metabolizing profile of PPAR γ + AMs, and coincident downregulation of glucose consumption. The link between PPAR γ as the defining transcription factor of AMs and as a key regulator of lipid metabolism suggests a model in which the lipid-rich environment of the lung imposes a fatty acid dependency that activates PPAR γ and initiates downstream signaling that enforces an airway macrophage phenotype. Consistent with this model, resident interstitial lung macrophages, which regulate a homeostatic environment within a more nutrient-diverse lung space, do not require PPAR γ and remain more glycolytically active^{51,57}.

Table 1. Advances in methodology in immunometabolism. Understanding immune cell metabolism across models and in different tissue sites has progressed with significant developments in available methodologies. Classical biochemical approaches such as measuring metabolic flux with early Seahorse analysers relied on large numbers of cells, a factor which led to an initial dependence on in vitro cultures. Resident macrophages rapidly lose expression of genes that confer their tissue identity upon extraction from tissues, potentially confounding ex vivo analyses depending on the ease and speed with which the cells can be accessed and processed. The range of assays available in immunometabolism is rapidly expanding, particularly in single cell technologies based in flow cytometry and in systems-based approaches. New innovations in spatial transcriptomics and spatial metabolomics are beginning to provide detailed maps of metabolic environments, and future challenges include accessibility of these technologies and the addition of temporal information. Example publications are listed.

Methodology	Tissue/Cells used	Pros	Cons	
In vitro cultures				
<i>Extracellular flux analysis (eg. Seahorse analysis)</i>	Largely in vitro-generated macrophages (mouse bone marrow, human monocytes) Sorted primary cells	Direct functional measurement of metabolic activity	Requires abundant cells (100k+) that are stable in culture Impacted by culture conditions/medium used	14,59,109,110
<i>Biochemical assays with addition of (labelled) substrates including stable isotope tracers</i>	Largely in vitro-generated macrophages (mouse bone marrow, human monocytes) Sorted primary cells	Direct functional measurement of metabolic activity	Requires abundant cells that are stable in culture Impacted by culture conditions/medium used Stable isotope labelling uses “heavy” elements	111–113
Ex vivo				
Flow cytometry				
<i>Uptake of labelled or unlabelled substrates and dyes (e.g. BODIPY, Filipin III, mitotracker, glucose, amino acid metabolites)</i>	Tissue single cell suspensions Cell cultures	Measure of functional cellular activity at a specific point in time Can also be used for microscopy	Limited reagents to key pathways Autofluorescence and toxicity can affect readouts	114,115
<i>Met-flow, scMEP (single-cell metabolic regulome profiling) – detection of e.g. receptors, transporters and rate-limiting enzymes</i>	Tissue single cell suspensions Human peripheral blood mononuclear cells	In depth metabolic phenotyping of tissue macrophages Doesn't require sorting of macrophages Practical for profiling multiple populations of tissue-resident macrophages and/or longitudinal analyses	Phenotypic rather than functional readout May require extensive optimisation depending on the size of the flow cytometry panels	116,117
<i>SCENITH (Single Cell ENergetic metabolism by profiling Translation inHibition)</i>	Tissue single cell suspensions Human peripheral blood mononuclear cells	Captures the functional capacities of cells at the single cell level Doesn't require sorting of macrophages Amenable to low cell numbers (1000s)	Dependent on inhibition of pathways and measurement of protein synthesis as a readout for ATP production and metabolic activity; indirect measures Not suitable for quiescent cells with low levels of protein synthesis	118,119
“-omics”				
<i>Proteomics (mass spectrometry)</i>	Sorted macrophage populations	Unbiased overview of proteins expressed by the cell Can be performed on low (100s) numbers of primary macrophages	Capture of metabolism-associated proteins in small sample sizes may be obscured by abundant proteins such as cytoskeletal proteins	120

(continued on next page)

Table 1 (continued)

Methodology	Tissue/Cells used	Pros	Cons	
<i>Metabolomics (analysis of metabolites by mass-spectrometry)</i>	Whole tissues	Highly sensitive to small changes in metabolic pathways	Typically uses large numbers (millions) of cells, difficult to separate low metabolite levels from background when small numbers of cell are used. But single cell metabolomics technologies are being rapidly optimised	121-123
	Cultured cells	Untargeted approach allows discovery of global changes while targeted approaches are more sensitive to specific pathways of interest	Metabolite coverage strongly influenced by extraction techniques	
	Body fluids and cell supernatants	Can be performed at the single cell level	Large numbers of metabolites can make identifying and validating biologically meaningful differences challenging	
<i>Lipidomics (analysis of specific lipid species by mass-spectrometry or NMR)</i>	Whole tissues	Untargeted approach allows discovery of global changes while targeted approaches are more sensitive to specific pathways of interest	Vast diversity in lipid species makes analysis complex and/or requires targeted analysis	124
	Cultured cells Body fluids and cell supernatants	Compatible with tracer studies	Highly sensitive, so data can be noisy	
<i>Single cell RNAseq with metabolic pathway analysis (eg. Compass, a flux balance algorithm that integrates metabolic states with single cell data)</i>	Sorted tissue-resident macrophages or total tissues for single cell analyses	Single cell analysis captures heterogeneity in cell states and gene expression at the point of cell isolation	May not give real-time information on key metabolic proteins – missing post-transcriptional regulation, and temporal differences between mRNA, protein abundance and metabolic flux Analysis depends on quality and caveats associated with single cell RNA sequencing, e.g. depth of sequencing	55,125
In vivo				
<i>Tracing labelled substrates ("fluxomics") (e.g. using 18F-fluorodeoxyglucose with scanning techniques such as Positron Emission Tomography (PET))</i>	Whole mouse	In situ, real-time measurement of substrate usage by cells	PET requires limited substrates – glucose, glutamine metabolism May be limited by resolution of PET scans	126,127
	Human volunteers			
<i>Genetically modified mice</i>	Whole mouse	Defines a functional requirement for the gene/protein	Specificity depends on the Cre promoter; use of intersectional genetic approaches may improve specificity	59,115,128
<i>Spatial metabolomics with imaging mass spectrometry</i>	Tissue sections	Localisation of metabolites to defined tissue areas or potentially within cell organelles	Complicated sample preparation	129,130
			Limitations in how material is separated compared to e.g. liquid chromatography-mass spectrometry (LC-MS) for conventional metabolomics; this increases overlap of signals, noise and variability	

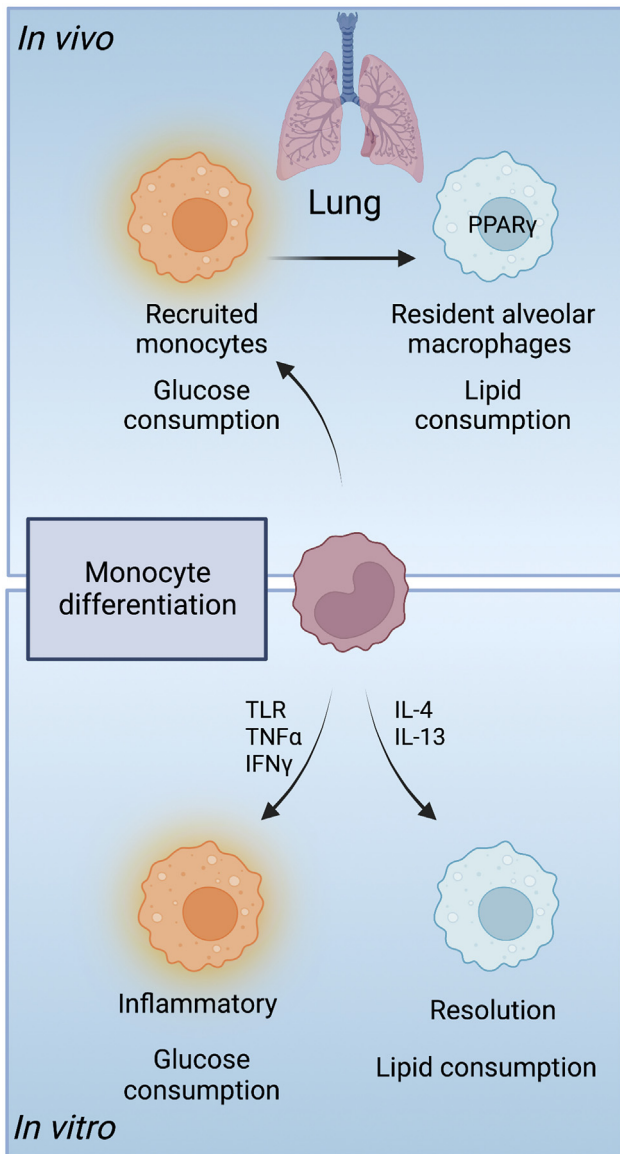


Fig. 1 Current paradigms of macrophage metabolic adaptation. Schematic showing current models of metabolic influence on monocyte differentiation. (Bottom) *in vitro* studies have shown that inflammatory monocyte-derived macrophages use glucose as a dominant fuel source, while resolution-phenotype macrophages preferentially consume lipids. (Top) *in vivo*, in the lung, recruited monocyte-derived alveolar macrophages are initially glycolytic and adopt an inflammatory state. Differentiation to resident, quiescent, alveolar macrophages is linked to expression of the transcription factor PPAR γ and a switch to lipid metabolism. IFN = interferon; IL = interleukin; PPAR γ = peroxisome proliferator-activated receptor gamma; TLR = *toll*-like receptors; TNF = tumor necrosis factor.

MODELS OF METABOLIC INSTRUCTION

The idea that local fuel availability directs the metabolic and functional profile of tissue macrophages is often discussed in the context of the lung but may also hold true elsewhere. In the liver, for example, a high-fat diet results in differentiation of distinct lipid-associated macrophages due to activation of Trem2-dependent signaling⁵⁸. These examples have established

a concept of metabolism directing differentiation and function: access to specific fuels determines activation potential and cell identity. However, the current data would also be consistent with a model in which macrophage persistence and residency are associated with a switch to quiescent metabolism, irrespective of tissue-specific adaptation (Fig. 2). Many mucosal and barrier sites are rich in lipids, and yet macrophage function is different and specific to each site. Sancho and colleagues have recently reported that the metabolic factor that most distinguishes macrophages at different sites is oxidative phosphorylation, a process that in T cells is associated with metabolic efficiency and long life⁵⁹. Is it possible that the distinctive metabolic profiles seen in macrophages at different tissue sites reflect their life span and persistence, rather than being imposed by the local tissue environment?

LC IN THE SKIN EPIDERMIS: LIPID METABOLISM AND CELL LONGEVITY

In the lung, airway macrophages are active in lipid-dependent, oxidative metabolism; are embedded in a lipid-rich environment; and are persistent, long-lived cells. Some insight into cause and effect in these associations can be gained from analysis of other lipid-rich sites. In the skin, for example, the epidermis is composed of layers of keratinocytes, constantly differentiating and moving away from the basal layer of the epidermis toward the outer edge of the skin. During this journey, the keratinocytes complete their lifespan, die, and are replaced⁶⁰ (Fig. 3). These cells form the constantly replenished stratum corneum that protects our body from dehydration, infection, and physical insults⁶¹. Like the alveolar space, the epidermis is therefore very lipid-rich. Keratinocytes secrete several lipids, the composition of which changes as they migrate and differentiate away from the epidermal basement membrane; these include glycolipids and phospholipids that are broken down to produce free fatty acids, cholesterol that are progressively catabolized, and glucosylceramides that are converted into ceramides⁶². Production of lipids is fundamental to tissue function; the layers of lipid lamellae are said to provide the “mortar” sealing the keratinocyte building blocks together to maintain an impermeable physiological barrier, and to provide an immunological barrier containing anti-microbial fatty acids and a lipid-rich niche for commensal bacteria⁶². Skin microbiota provide essential amino acids and other metabolites that play important roles in regulating skin immunity, for example via the aryl hydrocarbon receptor. The epidermis also lacks blood vessels, making epidermal cells dependent on exposure to the air for oxygen, resulting in a relatively hypoxic environment⁶³ and limiting nutrient concentrations including glucose at this site.

LCs in the skin, therefore, share several characteristics with AMs in the airways. They sit at the epithelial interface with the outside world. They persist as a self-renewing, remarkably long-lived population of epidermal macrophages, self-maintained throughout life without contribution from circulating monocytes. Evidence for this in people comes from recipients of hand allografts in which LCs from the donor are maintained in the skin of the transplanted limb for decades post-surgery⁶⁴. LCs are seeded before birth from embryonic CX3CR1+ macrophage precursors and, driven by undefined signals, the population undergoes a burst of proliferation approximately 2 days after birth to fill the epidermal niche⁶⁵. During the steady state LCs constitutively migrate at low levels to draining lymph nodes⁶⁶, where they are thought to help maintain

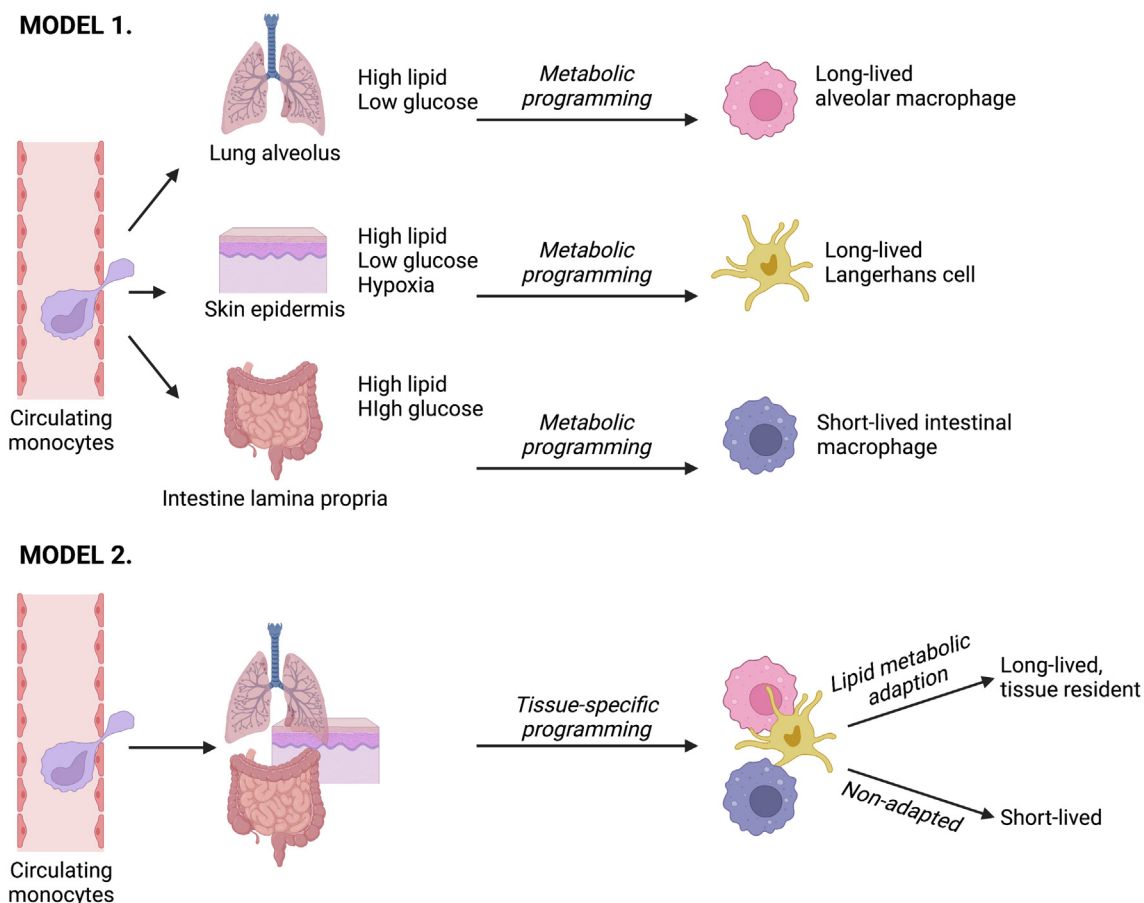


Fig. 2 Alternative models of metabolic adaption in tissue macrophages. (Top) the idea of tissue-specific metabolic adaption suggests that the unique metabolic environments of different tissues impose distinct patterns of differentiation and function on incoming monocytes and resultant macrophages. (Bottom) an alternative model is that monocytes entering a tissue receive signals such as cytokines and growth factors that specify cell fate and function. When that cell fate is long-lived, the macrophages adapt for quiescence and persistence by adopting an efficient, often lipid-based metabolic profile. When the cell is instead destined for rapid function, short life and early death, the metabolic profile remains dominated by rapid, inefficient metabolic pathways such as glycolysis.

tolerance to innocuous skin antigens⁶⁷. This migration is enhanced during infection or inflammatory disease, and, in this case, local proliferation of resident LCs is sufficient to refill gaps in the LC network [65,68].

Like AMs, LC identity at birth is partly conferred by TGF β ^{69,70}, but in the epidermis, LC cell fate is also dependent on IL-34^{71,72} and bone morphogenetic protein 7 (BMP7)^{73,74} which activate Id2 and Runx3^{75,76}. Once the LC network is established, IL-34 appears to be sufficient for long-term survival [71] while TGF β is required for retention of LCs within the epidermis^{77,78}. The transcription factor dependence of LCs is specific to their location and different from that of AMs, despite the lipid-rich environment surrounding both populations. Immune cells in the skin do show a distinctive metabolic profile; differentiating T cells that enter the skin epidermis metabolize exogenous lipids to survive as long-lived resident memory cells, characterized by the upregulation of fatty acid binding proteins and the fatty acid translocase CD36^{48,79}. As observed for AMs, expression of PPAR γ in epidermal T cells promotes uptake of fatty acids⁴⁸. Transcriptomic sequencing of human LCs that have migrated out of epidermal sheets, or which have been released by trypsinization, suggests that lipid metabolism pathways domi-

nate gene activity, including biosynthesis of unsaturated fatty acids, fatty acid metabolism, and amino acid metabolism⁸⁰. Moreover, recent data suggest that LCs may cluster with AMs as high lipid- and cholesterol-processing macrophages that are dependent on oxidative phosphorylation⁵⁹. Together these data suggest that, like AMs, LCs require lipid metabolism for maintenance as tissue-resident, quiescent cells, but the link between fuel use and differentiation is still uncertain. In addition, LC destruction by ultraviolet irradiation or pathogenic T cells activates recruitment of dermal monocytes across the basement membrane into the inflamed epidermis, wherein they differentiate into replacement LCs [36,39]. This differentiation takes place despite the inflammation, keratinocyte damage, and likely dyslipidemia in this context, suggesting intrinsic programming of LC differentiation rather than a cell fate imposed by the metabolic environment. Interestingly, the accumulation of neutral lipids in LCs isolated from lesions in psoriatic patients has been linked to a downregulation in autophagy, a cellular mechanism used to break down intracellular debris and regulate lipid metabolism⁸¹. Loss of lipid catabolism in psoriatic LCs was associated with a switch to a more inflammatory phenotype that contributed to disease pathogenesis⁸¹, consistent with a model

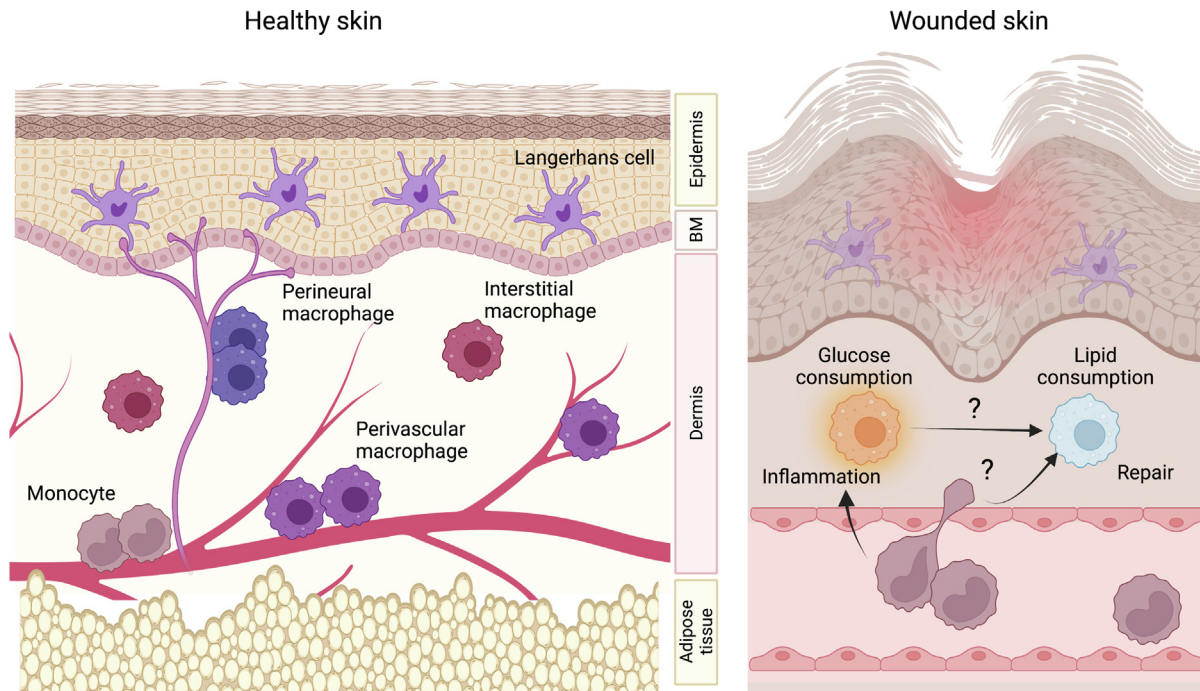


Fig. 3 Skin macrophage populations in health and during wounding. (Left) healthy skin is populated by many resident macrophage populations. Langerhans cells reside in the lipid-rich epidermis, where in the steady state they are maintained without contribution from circulating monocytes. In the dermis, most but not all macrophages are maintained by recruited monocytes. Dermal macrophages reside in sub-tissular niches as interstitial cells or located around dermal blood vessels (shown in red) or peripheral nerves (shown in pale purple). The dermis is underlain by a layer of adipose tissue. (Right) after wounding, monocytes are recruited into the site of injury where they differentiate into macrophages. Early after wounding, monocyte-derived macrophages resemble inflammatory macrophages found at other sites, relying on glucose to fuel proliferation and anti-microbial functions. As the wound heals, gene expression in local macrophages becomes dominated by lipid-consumption and repair processes. Question marks indicate open questions about how metabolic and environmental signals intersect to co-ordinate the macrophage response to tissue repair, and whether inflammatory and reparative macrophages are seeded from the same monocytic precursors.

in which lipid metabolism defines long-lived, quiescent macrophages, and an inability to use this metabolic adaption imposes an acute, inflammatory, activated phenotype rather than controlling cell identity.

SHORT-LIVED MACROPHAGES IN THE INTESTINE AND THE SKIN

The intestine is a site in which many fuels are readily abundant: it is rich in lipids associated with mucus and dietary fats, abundant in amino acids derived from food, and also well vascularized and hence glucose-rich too⁸². The presence of the microbiome also provides microbial metabolites that regulate intestinal immunity, including macrophage function^{82,83}. Most, but not all^{84,85}, macrophages in the healthy gut are constitutively replenished by circulating monocytes that differentiate over 5–6 days to become mature cells characterized by loss of proteins such as Ly6C, and instead high expression of the chemokine receptor CX3CR1^{34,86–88}. Mature lamina propria macrophages secrete chemokines that sustain monocyte recruitment⁸⁹ and their life span is short, thought to be limited by a tonic state of low-grade inflammation stimulated by the combination of microflora and dietary challenge⁹⁰.

The metabolism of intestinal macrophages is not yet completely described. Lamina propria macrophages in the large intestine are active in both glycolysis and oxidative phosphorylation⁸³ and their metabolism increases further under germ-free or antibiotic conditions⁹¹, suggesting that microbial products

such as butyrate are metabolically influential⁹². Small intestinal macrophages show lower rates of glycolysis, oxidative phosphorylation, and mTOR activity than those in the colon⁸³. Interestingly, fatty acid uptake and lipid storage in intestinal macrophages has been associated only with a rare population of CD4+Tim4+ tissue residents in the colon^{83,85}. These cells are long-lived and self-renewing⁸⁵, again associating lipid acquisition with macrophage persistence and longevity.

Another barrier location that is characterized by short macrophage life span and constitutive replenishment is the dermal layer of the skin³³. As in the intestine, neurons and blood vessels provide some local niches in the dermis that instruct specialized macrophage differentiation and may protect longer-lived resident specialist macrophage sub-populations^{93–95}, but the majority of macrophages in the dermal layer are continually replaced by circulating monocytes [29,33]. The dermal layer is less lipid-rich than the epidermis and is intricately connected to the circulation, so it shares environmental and cellular similarities to the gut and oral mucosa. Contrary to human and murine LCs, the dermal monocyte/macrophage transcriptional program is dominated by genes associated with immune function rather than metabolic pathways^{33,80,96}. Differentiation of MerTK+CD64+ dermal macrophages was associated with upregulation of the fatty acid transporter gene *cd36* in the study by Tamoutounour et al.³³, and dermal Trem2+ macrophages are enriched for pathways encoding cholesterol metabolisms and PPAR signaling. These

cells have been linked to pathology in the context of dysregulated cutaneous lipids in acne development⁹⁷. However, the metabolic changes associated with differentiation of dermal macrophage populations have not been investigated in detail. Given the constitutive turn-over of most dermal macrophages, one possibility is that switching to lipid metabolism pathways to provide long-term efficient fuel usage is not required. This would be consistent with the concept of the metabolism of tissue-specific macrophages as a late-stage adaptation enabling tissue persistence, rather than an early driver of differentiation.

The idea of tissue-specific adoption of slow burn metabolism as a feature of long-lived, resident macrophages, in contrast to the active metabolism of cells recently derived from infiltrating monocytes, is given extra complexity at barrier sites: the gut, skin, and lungs are all sites of multiple, frequent infections, and macrophage plasticity is an essential response to varied parasitic, viral, and bacterial challenges. Perhaps metabolic flexibility is needed in short-lived intestinal macrophage populations to preserve responsiveness to the rapidly changing environment? How are metabolic changes linked to cellular fates within changing tissue environments?

PERTURBATION IN THE SKIN: METABOLIC RESPONSES TO WOUND HEALING

Clues to answering this question may come from our understanding of wound healing in the skin; here the adoption of different metabolic pathways by recruited immune cells is entwined with repair of tissue damage^{41,98} (Fig. 3). Recent transcriptomic and functional analyses of macrophages at different time points post-wounding demonstrated that glycolysis/gluconeogenesis, Hif-1 α , and pentose phosphate pathways dominate early macrophage gene programmes⁹⁹, facilitating the macrophage microbicidal effector functions required to sterilize the wound. At subsequent time points, the expression of genes associated with fatty acid oxidation, lipid catabolism, and enhanced mitochondrial processes⁹⁹ is associated with a switch to a pro-fibrotic resolution phenotype that orchestrates epidermal re-growth, stromal repair, and restoration of tissue homeostasis⁹⁸. These studies highlight the coordinated acquisition of distinct metabolic pathways by recruited macrophages, with lipid uptake and catabolism associated with resolution. As in the intestine, where microbial metabolites can influence host cell behavior, in skin inflammation it has been suggested that metabolites produced during the initial inflammatory phase of the response activate differentiation of immune-suppressive macrophages⁹⁸. For example, production of itaconate from citrate/aconitate is associated with suppression of inflammation^{100,101}. On the other hand, binding of IL-4R α by IL-4 and IL-13 has a dominant immune-suppressive impact on macrophages during tissue repair¹⁰², and directly alters mitochondrial metabolism due to a switch from mitochondrial ROS production to oxidative phosphorylation⁹⁹, suggesting that metabolic adaptation may be a consequence, not a driver, of the switch from inflammatory to reparative macrophages. Similarly, activation of lipid metabolic pathways *per se* does not appear to be sufficient to regulate this switch: while excessive TNF α delays skin wound healing by suppressing differentiation of pro-resolution macrophages, this is due to the transcription factor activity of sterol response element binding protein 2, which switches on an inflammatory gene program in macrophages at the wound site, rather than due to suppression of lipid metabolism in these cells¹⁰³.

Current models of skin wound healing infer homogeneity within recruited monocytes, such that all cells transition through both inflammatory and reparative states during the wound healing process; pseudo-time analysis of gene expression programs in adoptively transferred macrophages supports this differentiation trajectory¹⁰⁴, perhaps as part of an evolutionarily conserved response to protect the tissue⁴¹. But significant heterogeneity exists within macrophages isolated from the skin wound site⁹⁹, and an alternative hypothesis is that highly activated glycolytic monocyte-derived macrophages die and are replaced by intrinsically different cells that enter the wound later in the healing process to become the resident (lipid-dependent) macrophage population¹⁰⁵. In the lung, inflammatory AMs activated by influenza virus infection that express high levels of Hif-1 α are more likely to die, while the Hif-1 α ^{low} less inflammatory cells proliferate and preferentially expand to repair the damaged lung¹⁰⁶. Rodero et al.¹⁰⁷ observed two waves of monocytes entering wounded skin by intravital imaging; Ly6C^{high} inflammatory monocytes were rapidly recruited into the wound, while Ly6C^{low}CX3CR1^{high} monocytes arrived later during the repair process. While the fate of these cells has not been determined, it is tempting to speculate that they may represent independent precursors of glycolytic pro-inflammatory and lipid metabolizing resolution macrophages required during wound healing. More detailed barcoding and fate-mapping studies are needed to resolve this question and to determine how findings in the skin apply to other tissue contexts.

CURRENT QUESTIONS

The adaptations of monocyte-derived cells during wound healing in the skin clearly show that metabolism and function are closely linked, but cause and effect are hard to distinguish. It may even be semantic to separate the two. Wculek et al.⁵⁹ have recently identified lipid metabolism and oxidative phosphorylation as defining metabolic features that distinguish different tissue macrophage populations. Their work, together with that of Gao et al.¹⁰⁸, demonstrates that macrophages in tissues with high lipid content, such as the lung alveolar space, are more dependent on oxidative phosphorylation to generate energy and thereby more sensitive to interference with this pathway⁵⁹ than macrophages in sites with lower lipid content. This is consistent with a model in which, under homeostatic conditions, local availability of lipids drives dependence on metabolic pathways that in turn enable tissue macrophage survival across tissues. As we begin to understand more about how metabolism and differentiation interact during homeostasis, we can begin to address the extent to which metabolic plasticity is retained within differentiated macrophages during infection and disease. We also currently understand little about how energetic requirements drive macrophage differentiation in the gut and the extent to which it is similar or different to the dermis. An interesting point is that the use of lipid catabolism pathways by resolving macrophages in the skin mirrors tissue repair mechanisms in the gut after helminth infection⁹⁸. This raises the question of how gut macrophages, or those at other sites, adapt to competition for fuels or the changing nutrient environment imposed by the presence of pathogens or shifts in microbiota imposed by chronic infections. Barrier sites frequently encounter concurrent and successive infections so the programming of tissue-resident macrophages, both functional and metabolic, requires ongoing flexibility.

SUMMARY

Metabolism is a key part of tissue differentiation. Metabolism and function are inherently linked, reflecting local signals, fuel availability, and environmental changes during and after inflammation. We argue that the current AM paradigm represents a simplified but useful model for understanding how energy usage intersects with macrophage differentiation and function, and our conversation has suggested that rather than instructing cell fate, macrophage metabolism might instead be an outcome of functional adaptation. The interaction between transcription factor expression and metabolic phenotype may be different in different tissues, and the skin offers new insight into metabolic adaptation of tissue-specific macrophages.

AUTHOR CONTRIBUTIONS

CB and GPW drafted and edited the manuscript and designed all figures. Both authors approved the final manuscript.

DECLARATIONS OF COMPETING INTEREST

The authors declare no competing interests. GPW supervises a PhD student funded in part by Sitryx Therapeutics.

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