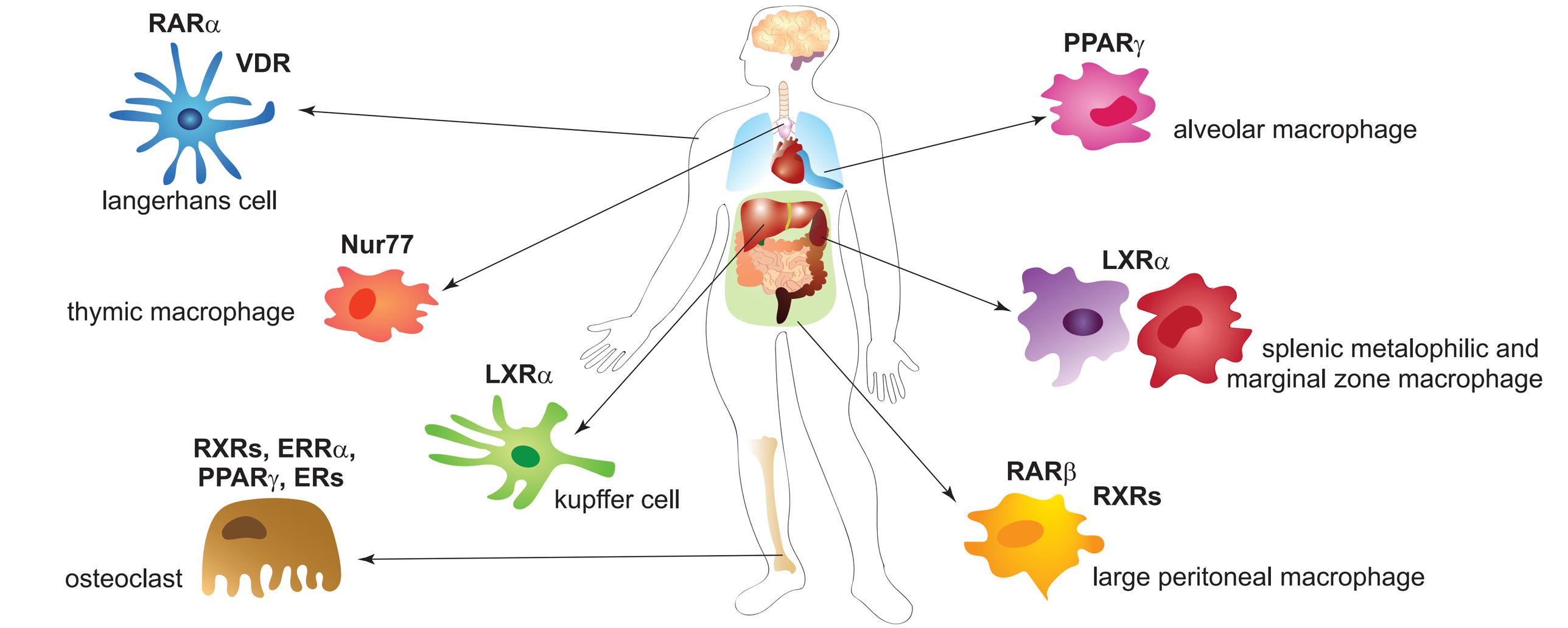
RARα
VDR
langerhans cell
Nur77
thymic macrophage
LXRα
kupffer cell
RXRs, ERRα,
PPARγ, ERs
osteoclast
PPARγ
alveolar macrophage
LXRα
splenic metalophilic and
marginal zone macrophage
RARβ
RXRs
large peritoneal macrophage

Molecular control of tissue-resident macrophage identity by nuclear receptors

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Abstract

Macrophages are key immune cells that reside in almost all tissues of the body, where they exert pleiotropic functions in homeostasis and disease. The development and identity of macrophages in each organ is governed by tissue-dependent signaling pathways and transcription factors that ultimately define specific tissue-resident macrophage phenotypes and functions. In recent years, nuclear receptors, a class of ligand-activated transcription factors, have been found to play important roles in macrophage specification in several tissues. Nuclear receptors are thus important targets for therapies aimed at controlling the numbers and functions of tissue-resident macrophages. This review outlines current knowledge about the critical roles of nuclear receptors in tissue-resident macrophage development, specification, and maintenance.

Introduction

Macrophages are key components of the innate immune system that mediate the clearance of pathogens, dead cells, and foreign particles by phagocytosis, a proccess first described by Ilya Metchnikoff [1]. These cells are present in all body tissues, where they play immunological roles and maintain tissue homeostasis. In addition to these features common to all macrophages, each tissue-resident macrophage (TRM) population has a unique phenotype, identity, and function. For instance, lung alveolar macrophages are involved in the clearance of surfactant proteins; liver-resident Kupffer cells (KCs) regulate iron metabolism and clearance of gut-derived microbial products; and microglia (brain-resident macrophages) regulate neuronal development and function, angiogenesis, and vascular anastomosis [2].

Recent studies have demonstrated that TRMs are mainly of embryonic origin and are maintained within tissues by self-renewal. However, some tissues, such as the intestine and dermis, contain bone-marrow derived macrophages under steady state; these macrophages are short-lived cells that are continously recruited to these tissues (for extensive reviews on macrophage ontogeny see [2-5]). Interestingly, the dependence of macrophage replenishmemt on blood monocytes is age and sex dependent [6]. Regardless of their origin, the functional specialization of TRMs is determined by the tissue in which they reside. Macrophages are exposed to local signals derived from their niche microenvironments. These signals play instructive

roles in establishing TRM identity and function by modulating the expression of distinct sets of transcription factors (TFs) and enhancer pattern in each TRM population [7,8].

Nuclear receptors in tissue macrophage development and specialization

During embryonic development, primitive macrophages initiate a core transcriptional program in the fetal liver that includes pattern recognition proteins, scavenger proteins, and cytokine receptors [9]. When macrophage precursors migrate to specific tissues and are exposed to niche signals, this core transcriptional program is diversified by other specific transcriptional programs, giving rise to differentiated TRMs [9]. The myeloid pioneer TF PU.1 is shared by all TRMs, binding throughout the macrophage nucleus to promoter and enhancer regions [7]. Other TFs such as CEBP, MAF, and MAFB work together with PU.1 to shape shared TRM functions. Within each destination tissue, these TFs combine with tissue-specific TFs to define the epigenetic and transcriptomic states of TRMs, with most of their transcriptional program being specific to the tissue of residence [10]. Several TFs that control macrophage tissue specialization are nuclear receptors (NRs) (for a concise review on NRs, see [11]). NRs form a superfamily of ligand-activated TFs that regulate a number of physiologic processes in humans, including metabolism, homeostasis, and reproduction. NRs are activated by steroid hormones and other lipid-soluble signals, including retinoic acid (RA), fatty acid metabolites, oxysterols, thyroid hormone, and vitamin D3 [11]. In addition, several synthetic compounds have been identified as NR ligands [11]. NRs are targets for the development of drugs to treat many diseases, including diabetes, cancer, inflammation, atherosclerosis, and endocrine and reproductive disorders. These receptors thus represent promising targets for new therapies aimed at modulating TRMs in disease pathogenesis and tissue repair. Progress toward this goal requires understanding of how NRs control TRM development, functional specialization, and maintenance in their host tissue. In this review, we summarize the role of different NRs and specific niche signals in the specification of TRMs (Figure 1).

Serous cavity macrophages

The serous cavities of mice (peritoneal, pleural, and pericardial cavities) contain two macrophage subsets—large peritoneal macrophages (LPMs) and small peritoneal macrophages—distinguished by their origin, size, cell surface markers, and gene

expression [12-14]. In steady state, the most abundant peritoneal cavity subset is formed by the LPMs. LPMs have high phagocytic activity against apoptotic and senescent cells [12,15], are implicated in the maintenance of intestinal microbial homeostasis by promoting the production of IgA by gut B1 cells, and participate in the resolution of liver injury [16,17].

A central role in LPM tissue specialization is played by the TF GATA-binding protein 6 (GATA-6) [13,16,18,19]. This was first demonstrated by studies showing that eliminating *Gata6* expression in macrophages interferes with LPM location, proliferation, and survival. Based on promoter studies and restriction of vitamin A availability, Okabe and colleges demonstrated that RA controls peritoneal LPM development, function, and identity through activation of RA receptor beta (RARβ) and the induction of *Gata6* [16]. Profiling of the dynamics of histone modifications across TRMs further showed that RARβ-induced GATA-6 acts in concert with a common set of primed enhancers established by PU.1 and other TFs to drive the selection of LPM-specific enhancers [7].

The lack of studies in RAR knockout mice has impeded the elucidation of specific roles of the three RAR isoforms or compensatory effects among them in the determination of LPM specification during development or its identity after birth. Another RA-induced NRs, the retinoic X receptors alpha and beta (RXRα and RXRβ), have recently been shown to control LPM identity in serous cavities [20]. Using mouse models lacking both RXR isoforms in macrophages, this study demonstrated that whereas RXRs are dispensable for LPM embryonic development, they are required for the expansion of LPMs during neonatal life and for LPM lipid metabolism and survival during adult homeostasis. Transcriptional and epigenomic profiling of LPMs revealed that RA signaling partially mediates LPM expansion and maintenance via RXRs. *Gata6* is downregulated in RXR-deficient LPMs; however, the expression profile of RXR- and GATA-6-deficient LPMs has limited overlap, suggesting that RXRs control peritoneal LPMs via GATA-6-dependent and GATA-6-independent mechanisms. Thus, other RXR heterodimers and/or RXR homodimers might regulate specific LPM transcriptional signature.

Recent reports have characterized the stromal niche that provides the RA that supports the LPM-specific transcriptional landscape [14,16]. LPMs are free-floating

cells that do not form direct contacts with stromals cell in steady state [21]. However, LPM *Gata6* expression is supported by the peritoneal microenvironment, as suggested by the loss of LPM *Gata6* expression when these cells are cultured *in vitro* or transferred outside the peritoneal cavity *in vivo* [7,8]. Initial studies demonstrated that the omentum expresses high levels of *Raldh2* (the rate-limiting enzyme catalyzing the final step in RA synthesis from retinol), suggesting a high local RA concentration at this location [16]. This conclusion is supported by recent evidence showing robust RA metabolism in fibroblasts and mesothelial cells present in the omentum and other mesothelial tissues in the pericardial and pleural cavities [14]. Mass spectrometry analysis identified all-trans retinoic acid (ATRA), a ligand for RARs, and 9/13cis RA, an activator of RXRs, as retinol-derived metabolites produced by the omentum [14]. These studies indicate that omental RA is necessary for GATA-6 expression in LPMs and therefore for their specific gene signature. However, a number of LPM hallmark genes, including *Rara* and *Rarg*, are GATA-6- and RA-independent [7,14]. Uncertainty remains about the nature of the RA-independent environmental signals that modulate LPM identity.

Osteoclasts

Osteoclasts are multinucleated bone-resident macrophages that maintain bone homeostasis through their bone resorption activity. Although they have been formally identified as cells of hematopoietic origin [22], a recent report demonstrated that osteoclasts required for normal bone development and tooth eruption originate from embryonic erythro-myeloid progenitors [23]. Osteoclast differentiation and function is highly controlled at the transcriptional level by changes in the expression of numerous regulatory genes, including several TFs (for a review, see [24]). NRs are known to regulate osteoclastogenesis and bone remodelling [25,26]. Here we focus on cell-autonomous effects of NRs in osteoclast differentiation. Several studies have linked the detrimental effects of thiazolidinediones on bone to the action of peroxisome proliferator-activated receptor gamma (PPARγ)-mediated stimulation of osteoclastogenesis and bone resorption in [27]. PPARγ promotes both osteoclast lineage commitment and osteoclast maturation by maintaining the levels of the key regulator of osteoclastogenesis c-fos in monocyte precursors and osteoclasts [28]. The pro-osteoclastogenic effects of thiazolidinediones are also mediated by PPARγ costimulator-1 (PGC-1β) [29] and the orphan NR estrogen-related receptor alpha

(ERRα), which show homology to estrogen receptors (ERs) [30]. ERs also play a role in bone formation and resorption, as evidenced by the postmenopause increase osteoporosis. Although most effects of ERs on osteoclastogenesis are mediated by decreasing the expression of osteoclastogenic cytokines in osteoblasts, ERs also suppresses RANKL-induced osteoclast differentiation by regulating c-Jun expression and activation in osteoclast progenitors [31]. RXRs have a cell-autonomous function in osteoclast proliferation, differentiation, and activation [32]. Loss of RXRs in osteoclast progenitors resulted in deficient osteoclastogenesis and osteopetrosis in adult male mice and protection from bone loss in an experimental model of postmenopausal osteoporosis. RXR-deficient adult mice developed abnormally large, multinucleated, non-resorbing osteoclasts. Our studies demonstrated that this phenotype was driven by decreased *Mafb* expression and an altered proliferative response of RXR-deficient osteoclast progenitors to macrophage colony-stimulating factor (M-CSF). RXR-deficient osteoclasts also showed reduced expression of the master regulator of osteoclast differentiation NFATc1. Further studies will be aimed at elucidating whether this reduction is due to direct regulation of *Nfact1* expression by RXRs or is simply the consequence of altered osteoclastogenesis in RXR-deficient mice.

Alveolar macrophages

Alveolar macrophages (AMs) are one of the two major macrophage populations in the lung, together with insterstitial macrophages. AMs are derived from fetal liver progenitors [33,34] and are maintained in lung tissue by self-renewal, with a minimal contribution from circulating monocytes [35]. In homeostasis, AMs engulf and clear lipoprotein-containing alveolar surfactants [36]. The correct development, differentiation, and gene signature of AMs requires PPARγ [37,38]. Adult mice with PPARγ-deficiency in myeloid cells have a reduced AM pool. AMs also require PPARγ perinatally for their final differentiation from AM precursors to mature AMs, and PPARγ has been found to be required for the differentiation of fetal monocytes to AMs during the final days of fetal development [37]. Transcriptomic studies reported that PPARγ is the key TF for AM identity, giving them their specific gene signature, including lipid uptake and catabolism genes. PPARγ-deficient AMs show enhanced cholesterol

esterification and a foam cell-like phenotype [37]. These findings indicate that PPARγ in AMs controls both survival and function. Furthermore, the niche signals required for AM PPARγ expression have been elucidated. During development, PPARγ expression is driven by granulocyte-macrophage colony-stimulating factor (GM-CSF) secreted by alveolar type II epithelial cells and fetal monocytes in the lung microenvironment, thus driving complete differentiation of fetal monocytes into AMs [33,37]. An additional signal governing AM PPARγ expression is transforming growth factor beta (TGF-β), which is required for AM development, differentiation from AM precursors, and the maintenance of mature AMs. TGF-β is produced by AMs themselves, thus generating an autocrine loop to allow PPARγ-dependent AM self-maintenance [39].

Kupffer cells

Liver-resident KCs are embryo-derived macrophages that are capable of self-maintenance [35]. Recent reports described the unique KC transcription program and epigenetic landscape controlled by liver X receptor alpha (LXRα) [40-42]. LXRα expression is upregulated in embryonic fetal macrophages, and this expression is maintained in mouse KCs throughout life, suggesting that LXR-specific gene expression is important for the early establishment of KC identity [9]. Although LXRα depletion does not reduce KC numbers, it impairs expression of the KC maturity markers *Clec4F* and *Timd4* [40]. Recent studies using an elegant approach in KC-depleted mice have deciphered the liver macrophage niche signals that govern KC identity [41,42]. These studies revealed hierarchical TF interactions, in which PU.1 and RBPJ are required for later LXRα and SMAD binding to primed *cis*-regulatory elements that will define the KC genetic landscape. In homeostasis, KCs are in close contact with others cells in the liver niche: hepatocytes, sinusoidal endothelial cells, and hepatic stellate cells. This niche architecture imprints KC identity at the transcriptional level. When KCs are depleted, various liver signals recruit monocytes to the newly empty niche, and these monocytes then undergo a phenocopying process until they become a mature KCs. These infiltrating monocytes have preexisting but poised regulatory elements, including chromatin-bound PU.1 and RBPJ, and express the cell membrane receptor Notch. Once the monocytes have been recruited, sinusoidal

epithelial cell-produced DLL4 activates the Notch signaling pathway, leading to the expression of the KC-lineage-dependent TFs LXRα and SpiC, selection of KC enhancers, and TGF-β receptor expression. The authors propose that at this stage these cells are no longer monocytes but rather repopulating liver macrophages, KC-like cells, with a transcriptional identity between that of a circulating monocyte and a mature KC. This repopulating liver macrophage population senses TGF-β pathway activation, leading to SMAD expression and subsequent SMAD binding to chromatin. Furthermore, mass spectrometry analysis showed that desmosterol is the most abundant oxysterol species in the liver. This hepatocyte-produced ligand activates LXRα, leading to the expression of KC identity genes. However, since KCs were depleted in these studies using diphtheria toxin receptor (DTR)-conditional models, it is important to recognise that they do not reflect homeostatic conditions but rather systemic inflammation. Future studies are also needed to address the role of LXRα during embryonic and neonatal KC development.

Langerhans cells

The definition of Langerhans cells (LCs) has been a matter of debate, and many authors now consider them to be embryo-derived epidermis resident macrophages with some dendritic-cell features [43,44]. LCs have a low self-renewal rate, but under inflammatory conditions they can be replaced by hair follicle-infiltrating bone marrow-derived monocytes that give rise to new LCs [43]. Many TFs that govern LC identity have been identified [43]; however, little is known about NRs in LCs. LC development and identity are controlled by RARα-RA signaling [45], and a lack of RARα leads to an almost complete absence of LCs. After birth, RAR-deficient mice have abnormally large and immature LCs, with low MHCII expression on postnatal day 1 that declines almost to zero from day 3 and remains absent into adulthood. Interestingly, *in vitro* studies in human blood monocytes and mouse bone marrow cells demonstrate that RA blocks LC differentiation [45]. The authors suggested that RARα maintains gene expression at the epigenetic level in a ligand-independent manner, as shown previously [46]. This would imply a complex regulatory mechanism in which activation of RARα impairs its own DNA-binding ability. Furthermore, transcriptomic studies show that RARα is required for the expression of LC identity genes, including the

canonical LC TF Runx3. The role of RAR in LC identity is further demonstrated by the finding that RAR-deficient LCs upregulate expression of the LC-suppressing TF C/EBPβ [43].

Another NR, the vitamin D receptor (VDR), is required for M-CSF-dependent local proliferation and wound healing by LCs after cutaneous injury [47]. These studies demonstrate a critical role for local LCs *versus* recruited monocytes in skin repair. In addition, they demonstrate that the local microenvironment modulates LC self-renewal after injury. Thus, induction of M-CSF (a master regulator of macrophage proliferation) by the active mebabolite of vitamin D, 1,25-dihydroxyvitamin D, is abolished in VDR-deficient cultured fibroblasts. It remains to be determined whether the action of VDR in skin repair depends on circulating 1,25-dihydroxyvitamin D or in autocrine activation of vitamin D by local LCs.

Metallophilic and marginal zone macrophages

During embryogenesis, the macrophage population of the naïve spleen is composed exclusively of red pulp macrophages. From around 1 month after birth, the spleen architecture is formed by red and white pulp. White pulp harbors bone marrow-derived splenic macrophages and, in the marginal zone between white and red pulp, metallophilic and marginal zone macrophages (both called MZ macrophages) [48]. During homeostasis, white pulp macrophages capture blood-borne antigens. Some TFs have been shown to control the development of specific splenic macrophages, Spic and IRF8 regulate transcription in red-pulp macrophages [49,50], whereas the NR LXRα regulates transcription in MZ macrophages [51]. Mice lacking LXRα lack MZ macrophages but show no changes in red-pulp splenic macrophages, possibly indicating that LXRα is dispensable for the development of embryo-derived splenic macrophages. However, the empty MZ niche allows adoptively transferred LXRα-sufficient monocytes to enter and differentiate into MZ macrophages, finally demonstrating that adult LXRα-dependent bone-marrow hematopoiesis is the source of these macrophages [51]. Moreover, constitutive genetic or pharmacological LXRα activation accelerates MZ macrophage differentiation but does not induce marginal zone markers in red pulp macrophages during macrophage renewal [51]. The authors suggested that additional signals at the border with the red pulp might govern marginal

zone macrophage identity. The endogenous LXRα ligands and niche signals remain unknown, although oxysterols are possible candidates [51], as in KCs. Further work is also needed to identify the mechanism by which LXRα controls the development of mature MZ macrophages from bone marrow progenitors and define the functional impact of LXRα in these splenic macrophages.

Thymic macrophages

Thymus-resident macrophages are critical for clearing the vast numbers of apoptotic thymocytes generated during lymphocyte selection in the thymus [52]. The orphan receptor Nur77, the master regulator of Ly6C^{NEG} patrolling monocytes [53], has recently been shown to control the development and function of a subset of resident macrophages in the thymus [52]. Mice lacking Nur77 expression in myeloid cells presented a drastic reduction of CD11b^{-}F4/80^{+} thymic macrophages, with no changes in other thymus-resident macrophages or in macrophages residing in the spleen, lung, brain, pancreas, peritoneum, or bone marrow [52]. The authors also observed a reduced apoptotic cell engulfment capacity in Nur77-deficient CD11b^{-}F4/80^{+} thymus macrophages [52]. Using parabiosis and bone marrow transplantation studies, as well as monocyte-tracking mouse models, the authors demonstrated that CD11b^{-}F4/80^{+} thymus macrophages derive from hematopoietic progenitors and not from short-lived circulating monocytes. Furthermore, M-CSF-depleted mice mimic the defects associated with Nur77 depletion, revealing M-CSF as a key niche signal regulating CD11b^{-}F4/80^{+} thymic macrophage maintenance [52]. These studies demonstrate the importance of Nur77-dependent CD11b^{-}F4/80^{+} macrophages in the maintenance of thymic homeostasis and self-tolerance, since the lack of this NR leads to accelerated thymic demise and pro-inflammatory cytokine production.

Conclusions and future perspectives

Macrophages are key components of tissue immunity, present in almost all tissues throughout the body. TRMs are not a homogeneous population, but are instead hugely diverse. This diversity is driven by microenvironment-derived signals that regulate the expression of unique TFs in each TRM population, leading to differences in epigenetic

profiles, transcriptional programmes, and functions. TFs of the NR family are important pharmacological targets for the control of gene transcription, with defined roles in macrophage immune functions and lipid metabolism. In the last decade, a growing body of evidence has demonstrated that NRs play important roles in TRM diversification and identity, specifically in macrophages residing in the serous cavities, bone, lung, liver, epidermis, spleen, and thymus. Whether any NRs play a role in macrophages residing in other tissues will require further investigation. In this regard, it is very important to consider the Cre-driver used to study each specific TRM population. LysM-cre transgenic mice have been extensively used to study the role of NRs in TRMs. However, this Cre system results in low recombination in some TRMs [54,55], and the use of this system therefore may have precluded the discovery of some phenotypes in specific tissues. Other important aspects of NR regulation of TRMs remain to be elucidated. For instance, little is known about the environmental signals that induce the expression of different NRs in each TRM population. Moreover, since NRs are ligand-induced TFs, the production of specific endogenous NR ligands by each tissue environment might represent an additional level of regulation explaining the diversity of NRs with roles in TRM specification. Natural ligands that bind to and activate specific NRs in TRMs include RA in peritoneal macrophages and desmosterol in KCs. However, more work is needed to identify other endogenous ligands and regulators mediating macrophage development, identity, and function in other tissues. A detailed study of how these microenvironmental signals and NR ligands fluctuate during homeostasis and disease would provide valuable information about TRM regulation. Further work is also needed to determine whether NRs with identified roles in mouse TRM development and identity play equivalent roles in human TRMs. Such studies will help to identify new drugs and mechanisms through which TRMs might be manipulated for therapeutic benefit.

Conflict of interest statement

Nothing declared.

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Figure 1. Regulation of tissue resident macrophages by nuclear receptors and niche signals. ERRα, estrogen receptor alpha; VDR, vitamin D receptor; LXRα, liver X receptor alpha; NR, nuclear receptor; RA, retinoic acid; RARα, retinoic acid receptor alpha; Raldh2, retinaldehyde dehydrogenase 2; RXR, retinoid X receptor; PPARγ, perioxisome proliferator-activated receptor gamma; DLL4, delta ligand 4; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; TGF-β, transforming growth factor beta.

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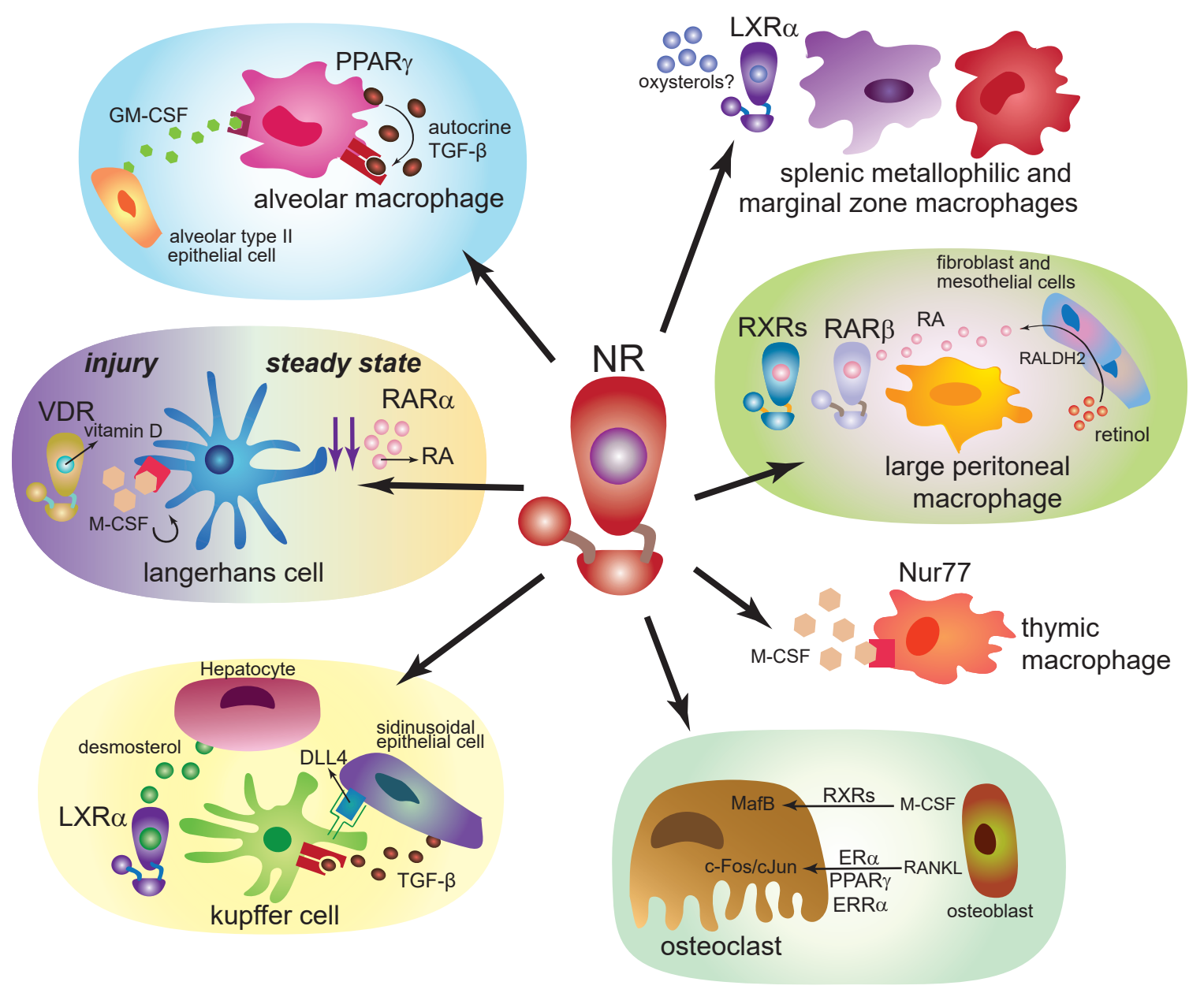
PPARγ
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autocrine
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alveolar macrophage
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epithelial cell
LXRα
oxysterols?
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marginal zone macrophages
fibroblast and
mesothelial cells
RXRs
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RA
RALDH2
retinol
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injury
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VDR
vitamin D
RARα
RA
M-CSF
langerhans cell
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Nur77
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