PEER-REVIEWED MINI REVIEW

GM-CSF-PPARγ Axis Plays Critical Roles in the Development, Surfactant Homeostasis and Anti-inflammatory Activity of Alveolar Macrophages

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Abstract

AMΦs have an organ-specific function to maintain surfactant homeostasis that is critical to alveolar stability and lung function. AMΦs poses unique features such as aerobic respiration, high anti-oxidant capacity, and AMΦs alone among the all tissue MΦs are adapted to aerobic environment. Inflammation of the lungs causes respiratory failure and interferes with life sustaining. Therefore, treatment of foreign antigens with AMΦs should be done without inflammatory response as much as possible. AMΦs originate from fetal monocytes, are long-lived cells and their maintenance depend on self-renewing. GM-CSF is essential for the development of AMΦs but not other tissue MΦs. Characteristics of GM-CSF-induced human monocyte-derived MΦs (GM-MΦs) is the same as that of human AMΦs, indicating that the GM-MΦs become a model of human AMΦs. Expression of PPARγ in nucleus of AMΦs depends on GM-CSF, and is essential for the development of AMΦs. In this article, recent findings on the origin and the essential role of GM-CSF-PPARγ axis in the development, surfactant homeostasis and anti-inflammatory activity of AMΦs are reviewed.

Introduction

Macrophages (MΦs) exist various tissues in the body, and have roles in development, tissue homeostasis, inflammation, tissue regeneration, lipid metabolism and host defense against microorganisms and tumors. Tissue-resident MΦs constitute heterogeneous populations with unique functions and distinct gene-expression signatures, and are known by different names (e.g., alveolar MΦs (AMΦs) in the lung, peritoneal MΦs (PMΦs) in the peritoneum, Kupffer cells in the liver, microglias in the brain and osteoclasts in the bone).

AMΦs comprise up to 90-95% of the leukocytes present in mouse or human bronchoalveolar lavage (BAL) under steady state. AMΦs have an organ-specific function to maintain surfactant homeostasis in the lung which is critical to alveolar stability and lung function. AMΦs are known to possess high anti-oxidant capacity and the energy metabolism of AMΦs relies primarily on aerobic respiration, whereas that of other tissue MΦs depends primarily on glycolysis. These unique features of AMΦs may result from their location in the lung in which AMΦs are exposed to high oxygen tension and are also bathed in high concentrations of surfactant. AMΦs alone among the all tissue MΦs are adapted to aerobic environment.

Inflammation of the lungs causes respiratory failure and interferes with life sustaining. Therefore, treatment of foreign antigens with AMΦs should be done without inflammatory response as much as possible. Thus precise mechanisms control the high anti-oxidant activity and the balance of pro- and anti-inflammatory responses are necessary for AMΦs to ensure an appropriate response to environmental agents. Here, recent findings on the origin and an essential role of granulocyte-macrophage colony-stimulating factor (GM-CSF)-peroxisome proliferator-activated receptor γ (PPARγ) axis in the development, surfactant homeostasis and anti-inflammatory activity of AMΦs are reviewed.

Origin and self-renewal of AMΦs

In 1924, MΦs were defined by Aeschoff as cells of the reticulo-endothelial system (RES). This implied that macrophages originate from the tissue, and reside and renew within the tissue. In 1960, Ralph Van Furth proposed the concept of "the mononuclear phagocyte system (MPS)". According to the MPS, all MΦs, including all tissue-resident and inflammatory MΦs are derived from the bone marrow through circulating blood monocytes, and they are terminally differentiated cells. We previously showed the differences in the expression of asialo GM1, Mac-1 and the binding to FITC-LPS between AMΦs and PMΦs in the mouse[1)-3)]. AMΦs express asialo GM1^{+}, Mac-1^{low} and do not bind to FITC-LPS, whereas PMΦs are asialo GM1^{-}, Mac-1^{+} and bind to FITC-LPS. In agreement with the difference in FITC-LPS binding, PMΦs but not AMΦs activate tumor cytotoxicity by LPS stimulation[2)]. In 1981, we found that MΦs exist in fetal lung and fetal liver at E14, and the fetal lung MΦs are asialo GM1 positive as well as AMΦs obtained from adult mice. In 1988, we also found that AMΦs from adult mouse can proliferate and make colonies by stimulation with GM-CSF or M-CSF[3)]. These our studies strongly indicate that asialo GM1 positive lung MΦs already exist in fetus and maintenance of AMΦs in adulthood depend not only blood monocytes but also on the self-renewal.

Recent studies using the new method such as Fate-mapping elegantly showed that mouse tissue-resident MΦs in multiple organs, originate from embryonic progenitors such as primitive macrophages (CX_3CR1^{hi} $F4/80^{hi}CD11b^{lo}$) in yolk sac (microglia) or fetal monocytes (CX_3CR1^{-} $F4/80^{low}CD11b^{high}$) in fetal liver (MΦs in lung, spleen, liver, skin, kidney, gut), and can persist into adulthood and self-maintain by local proliferation[4)]. In 2013, Guilliams et al. reported that AMΦs originate from fetal monocytes within the first week of life,

mature by 3 days after birth and are long-lived cells[5]. The maintenance of them depends on their self-renewing capacity and circulating blood monocytes contribute minimally to the steady-state AMΦs pool[5]. Self-renewing capacity in human AMΦs was also detected by us[6]. In 2016, Nayak et al demonstrated that almost 100% of human AMΦs detected in BAL from the transplanted lungs are donor derived with a capacity to self-renewal[7]. However, monocytes can contribute to the AMΦs pool when AMΦs niche in lung is empty under extreme conditions of depletion or radiation injury[8]. In some tissues, embryo-derived MΦs show declining self-renewal activity and are replaced by monocyte-derived MΦs soon after birth (MΦs in intestine)[9] or with age (MΦs in the heart)[10].

GM-CSF is a critical factor for the differentiation of AMΦs

In 1988, we demonstrated that one of the mechanisms that control MΦs heterogeneity is the difference in colony-stimulating factor (CSF) by which MΦs differentiation is induced, and GM-CSF determine the phenotype of murine AMΦs, and M-CSF determine that of PMΦs[3]. Our results indicate that factor in the environment in which AMΦs locate determine the phenotype of AMΦs, because lung tissue is known to be rich in GM-CSF[11]. In 1994 and 1995, the essential role of GM-CSF in the functional differentiation of AMΦs was demonstrated from the studies of gene targeting of GM-CSF (GM-/-) and the common β-chain of the GM-CSF receptor (GMRβc-/-) in mice[12,13,14]. These gene-targeted mice develop pulmonary surfactant excess, which histologically resembles to the human pulmonary alveolar proteinosis (PAP). PAP is associated with a marked accumulation of foamy, lipid-filled MΦs, surfactant protein and lipids in the alveolar spaces of the lungs. Human PAP develops by the disruption of GM-CSF signaling caused by high levels of neutralizing anti GM-CSF antibodies in autoimmune PAP[15] or by mutations in *GM-CSFR* in congenital PAP[16]. Surfactant accumulation in these gene targeted mice and PAP patients is due to reduced clearance caused by reduced surfactant catabolism in AMΦs[17]. AMΦs from GM-/- mice and PAP patients also reduce the cell adherence, phagocytosis, bacterial killing, expression of cell surface receptors (TLR4, TLR2, CD14, mannose receptor, Fcγ receptor, M-CSF receptor, integrins) and LPS-mediated cytokine production, indicating that not only surfactant catabolism but also innate immune function in AMΦs are impaired[17]. In fact, these gene targeted mice and PAP patients are susceptible to respiratory infections[17]. In GM-/- mice, abnormality is observed only in AMΦs but not in other tissue MΦs, and the development of AMΦs stop at early AMΦs commitment from fetal monocytes[5].

In contrast to GM-CSF, M-CSF which is present at biologically active concentrations in the circulation and in most tissues is necessary for the development of many tissue MΦs including PMΦs, Kupffer cells, spleen MΦs, osteoclast in addition to blood monocytes. IL-34 produced from keratinocytes and neurons specifically promotes the development of Langerhans cells and microglias[18]. IL-34 and M-CSF share the receptor, Fms, but they bind different Fms domain, which cause different signal activation and bioactivities. These results taken together indicate that development of unique phenotype and functional properties in each tissue MΦs are largely specified by factors in the local environment in which the tissue MΦs exist.

GM-CSF-induced human monocyte-derived MΦs become a model of human AMΦs

Previously we demonstrated that human monocytes differentiate into 2 phenotypically and functionally distinct types of MΦs, dendritic cells and osteoclast-like multi-nucleated giant cells under the influence of M-CSF, GM-CSF and IL-4[19-25]. GM-CSF-induced human monocyte-derived MΦs (GM-MΦs) are the same as that of human AMΦs in morphology (fried egg-like shape) and the expression of cell surface antigens (c-fms^{low}, CD14^{low}, HLA-DR^{++}, HLA-DQ^{+} CD71^{+}, 710F^{+}). In contrast, morphology (spindle-like and some cells remain small and round) and the expression of cell surface antigens (c-fms^{high}, CD14^{high}, HLA-DR^{+}, HLA-DQ^{-}, CD71^{-}, 710F^{-}) of M-CSF-induced human monocyte-derived MΦs (M-MΦs) resemble those of human PMΦs and inflammatory MΦs. As shown in table 1, other characteristics of GM-MΦs are the same as those of human AMΦs. These results indicate that GM-CSF is a critical factor for the differentiation and function of human AMΦs, and the environment that stimulate the development of AMΦs but not origin of AMΦs (fetal monocytes or adult blood monocytes) is important to decide the phenotype and function of AMΦs, and human monocyte-derived GM-MΦs become a model of human AMΦs. In agreement with our studies, recent study in mice show that transcriptome analysis of embryonic host-derived and postnatal donor bone marrow-derived AMΦs coexisting within the same

Table 1. Characteristics of human monocyte-derived GM-MΦs and M-MΦs and human AMΦs

	Human AMΦs	GM-MΦs	M-MΦs
Expression			
C/EBPβ isoform	Small-isoform main	Small-isoform main	Large-isoform main
Hck	Not expressed	Not expressed	High
Bcl-2 family genes	Bcl-xL	Bcl-xL	Bcl-2
Functions			
T cell proliferation	Suppress	Suppress	Suppress
IFNγ production	Not suppress	Not suppress	Suppress
IL-10 production	Low	Low	High
H_2O_2 production	Low	Low	High
H_2O_2 sensitivity	Resistant	Resistant	Sensitive
Catalase production	CSF-independent	CSF-independent	CSF-dependent
In vitro survival	CSF-independent	CSF-independent	CSF-dependent
HIV-1 infection	Resistant	Resistant	Susceptible
M. tuberculosis infection	Susceptible	Susceptible	Resistant

mouse demonstrated >98% correlation, and overall functional analyses are similar[8].

GM-MΦs and human AMΦs, in contrast to M-MΦs, are highly resistant to H_2O_2 via the high basal level of catalase activity and a marked ability to express catalase in response to H_2O_2, indicating that GM-CSF but not M-CSF plays a critical role in the development of H_2O_2 scavenging ability via unique catalase producing activity in human MΦs[23]. A strong anti-oxidant mechanism of human AMΦs and GM-MΦs supported by high catalase activity may help them to be long survivors in an oxidant rich lung environment and contribute to lung homeostasis. In fact, human AMΦs and GM-MΦs are long-lived cells even in the absence of GM-CSF, and the survival depends on catalase produced in a CSF-independent manner[25]. Extracellular catalase has a novel role in the prevention of apoptosis through the dominant expression of BCL-X_L in human AMΦs and GM-MΦs, and BCL-2 in M-MΦs[25].

Critical roles of PPARγ in the development, surfactant homeostasis and anti-inflammatory function in AMΦs

AMΦs from healthy mice and human but not from PAP patients and GM-/- mice express both PU.1 and PPARγ in nucleus, and the expression depends on GM-CSF. PU.1 has general roles in myelomonocytic development, and is expressed in many tissue MΦs. PU.1 in AMΦs is critical for the differentiation and the innate immune functions of AMΦs, whereas the role of PU.1 on the surfactant lipid catabolism in AMΦs is not yet clear[17]. In contrast to PU.1, PPARγ is indispensable for the development of AMΦs, and PPARγ-/- mice stop the AMΦs development at E17.5 as well as in GM-/- mice[26]. PPARγ was dispensable for the development of MΦs located in the peritoneum, liver, brain, heart, kidneys, intestine and fat. In these tissue MΦs, other transcription factors such as Spi-C in red pulp MΦs[27], GATA6 in PMΦs[28] NFATc1 in osteoclasts[29] and IRF8 in microglias[30] are known as tissue specific developmental factors.

PPARγ is a ligand-activated, nuclear transcription factor that regulates genes involved in lipid and glucose metabolism, inflammation, and other pathways. Reduced expression of both PPARγ and ABCG1 but not ABCA1 was observed in AMΦs from GM-/-mice and PAP patients (Table 2)[31]. ABCG1 and ABCA1 are ATP-binding cassette (ABC) lipid transporter that mediate cholesterol efflux from MΦs. ABCA1 mediate cholesterol efflux to ApoA-I and ABCG1 mediate cholesterol efflux to HDL. Instillation of lenivirus (lenti)-PPARγ to AMΦs in GM-/- mice restored the ABCG1 expression and reduced lipid accumulation in AMΦs and in BAL[32]. Instillation of lenti-ABCG1 to AMΦs in GM-/- mice reduced cholesterol accumulation in AMΦs and BAL, and improved the lung function[33]. In contrast, ABCG1-/- mice exhibited PAP-like pulmonary lipidosis with massive deposition of cholesterol in both AMΦs and BAL[34,35]. Thus the cholesterol efflux mediated by ABCG1 which is induced by GM-CSF via PPARγ is necessary for the surfactant homeostasis in AMΦs.

As well as GM-/- mice or PAP patients, mice that lack PPARγ in AMΦs cause lung inflammation and increase the Th1 type cytokines/chemokines production by BAL cells[35]. Interestingly, ABCG1-/- mice also induce lung inflammation with a marked recruitment of leukocytes and increased proinflammatory cytokine production by AMΦs[36,37]. In the atopic asthma model, ABCG1-/- mice display IL-17-mediated enhancement of neutrophilia in the airway following allergen sensitization and challenge[38]. The neutrophilic, high IL-17 asthma phenotype observed in ABCG1-/- mice resemble to that described in severe asthma in human subjects[39]. Thus GM-CSF-PPARγ-ABCG1 axis –mediated cholesterol homeostasis in AMΦs serves as an important negative regulator of pulmonary inflammatory responses, and the breakdown of this axis induces pulmonary inflammation. As shown in Fig.1, functions regulated by PPARγ in AMΦs are related to anti-inflammation or resolution of inflammation that are expressed in M2MΦs. As well as AMΦs, differentiation to M2MΦs depends on oxidative phosphorylation by expression and activation of PPARγ, and is inhibited in PPARγ deficient MΦs[40].

Pulmonary surfactant consists of ~10% neutral lipids including free fatty acids, which are precursors of PPARγ ligands. Thus surfactant-rich lung environment constantly stimulates AMΦs by endogenous PPARγ ligands, and probably maintains lung homeostasis by suppressing inflammation and controlling respiratory function necessary for maintenance of life. Lipoprotein lipase (LPL) hydrolyze neutral lipids to generate free cholesterol and free fatty acids, which are precursors of PPARγ ligands. Interestingly, expression of LPL is decreased in GM-/- mice as shown in Table 2. Important role of LPL was reported from gene targeting of lysosomal acid

Table 2. Molecules expressed in alveolar macrophages from PAP patient, GM-/- mice, PPARγ-/- mice and ABCG1-/- mice

	GM-/- mice	PAP patient	PPARγ-/- mice	ABCG1-/- mice
PPARγ	↓	↓	None	→
PU.1	↓	↓	→	→
ABCG1	↓	↓	↓	↓
ABCA1	↑	↑	↑	↑
LXRα	↑	↑	↓	n.d.
LXRβ	n.d.	n.d.	↑	n.d.
LPLA2	↓	↓	→	n.d.
LPL	↓	n.d.	n.d.	n.d.
CD36	↓	↓	↓	n.d.
GM-CSF	None	↓	↑	n.d.
M-CSF	↑	↑	↑	n.d.

LXR: Liver X receptor.
LPLA2: Lysosomal phospholipase A2 specifically expressed in MΦs with predominance in AMΦs.
LPL: lipoprotein lipase.
n.d.: not determined.

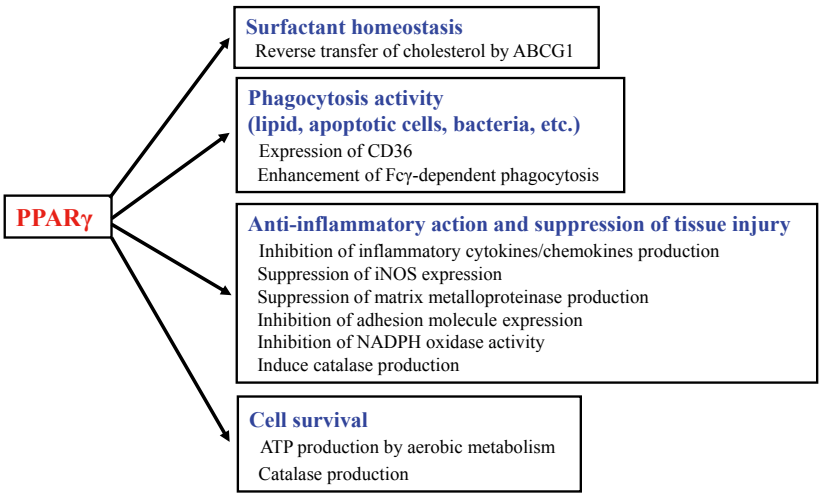

Fig. 1. PPARγ regulates the functions of alveolar macrophages

lipase (LAL) in mice. The LAL-/- mice results in respiratory inflammation, destruction in the lung with an increase in the number of foamy AMΦs and neutrophil infiltration, increase in proinflammatory cytokines/chemokines and matrix metalloproteinases in lung, and shows severe pathology of multiple organ failure[41]. MΦ-specific expression of human LAL or stimulation with PPARγ ligands corrects inflammation and pathogenic phenotypes in LAL-/- mice[41].

Conclusions

The lungs are important organ responsible for the breathing functions essential to making the energy necessary for maintaining life. Inflammation of the lungs causes respiratory failure and interferes with maintaining life. AMΦs expresse PPARγ2, which is the same subtype as expressed in adipocytes, suggesting that the role of lipid metabolism of AMΦs is very important and necessary, since the AMΦs exist in the unique lung environment with lipid-rich surfactant ocean. Expression of both PPARγ and PU.1 in the nucleus of AMΦs by GM-CSF is an essential condition for AMΦs to maintain lung homeostasis. Well-balanced activity of PPARγ and PU.1 make it possible to maintain surfactant homeostasis and treat the pathogen and foreign matter entering the lung as much as possible without inflammatory reaction and terminate efficiently even if inflammation is induced. Abnormalities in surfactant lipid metabolism of AMΦs caused by breakdown of GM-CSF-PPAR γ-ABCG1 axis induce accumulation of cholesterol rich foamy AMΦs and inflammation of the lungs, and are involved in the development of many pulmonary inflammatory diseases such as PAP, asthma and COPD. New drug discovery that target the GM-CSF-PPARγ-ABCG1 axis is expected.

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