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Abstract

More than 100 years have passed since Elie Metchnikoff discovered macrophage. Over the recent decade, attracting information about macrophage polarization have been reported. This is because many molecules have been identified as markers of macrophage polarization. Additionally, mechanistic insights have been demonstrated by experiments with various stimuli-induced macrophage polarization. Historically and simply, macrophages are divided into M1 (classically activated) and M2 (alternatively activated). However, some of them are not specific yet. Studies in the field of cardiology revealed the plasticity of macrophages and their subsets are divided into details: Mhem, MHb, Mox and M4 macrophages. M2 macrophages were further divided in M2a, M2b, M2c and M2d. There appears to be more phenotypes of macrophages. However, there still lack studies in dermatological field. This review summarizes the spectrum of macrophage activation and finding about various roles of macrophages in the dermatological field.

1. Introduction

In 1882, Elie Metchnikoff, a Russian zoologist, discovered the cells which have phagocytic ability and named the cells macrophage. After this discovery, the main function of macrophages is known to be a major host defense mechanism in innate immunity, and he was awarded the 1908 Nobel Prize in Physiology or Medicine. Macrophages were found in almost all tissues of living body and considered to participate in immune function. However, the main function of macrophages had been only focused on their powerful foreign matter reaction and phagocytic ability in all tissues, including Kupffer cells in the liver and alveolar phagocytes in lung. This is also possible to say in skin tissue. Macrophages in inflammatory skin lesion had been considered to ingest products of inflammation. Macrophages ingesting melanin are called melanophages, and macrophages ingesting hemosiderin are referred to as siderophages. Giant cells in granulomatous tissues have been also suggested as phagocytic macrophages. After decades of the discovery, recent studies revealed the diverse roles and plasticity of macrophages in various systemic diseases including cardiovascular diseases and cancer diseases [1]. Similarly, there is an increasing number of evidences concerning with the pathophysiological roles of macrophages in skin

diseases including atopic dermatitis (AD), psoriasis and skin cancers. Finding about various roles of macrophages in the dermatological field are introduced in this review.

2. Macrophage activation

2.1. Origin of macrophages

Macrophages were characterized as mononuclear phagocytic cells oriented from bone marrow progenitor, and it has been considered that tissue macrophages were derived only from circulating monocytes coming from bone marrow (Fig. 1). Additionally, macrophages are equally derived to all tissues during embryonic development by the yolk sac and fetal liver, and they stay there as resident macrophages [2]. After birth, they appear to be supplemented by circulating monocytes. However, more various unidentified origins of macrophages have been speculated, and the existence of them are still unclear in skin.

2.2. Differentiation of macrophages

Historically and simply, macrophages are divided into M1 (classically activated) and M2 (alternatively activated). Typical cell surface markers for M1 are HLA-DR and CD80, and those for M2 are CD163 andCD206. Apart from resident embryonic and

unknown originated macrophages, macrophages are differentiated from circulating peripheral blood mononuclear cells, monocytes, which can migrate into tissue in response to various stimuli (Fig. 1) [1]. In mouse, there are 2 types of monocyte: $Ly6C^+$ and $Ly6C^-$ monocytes. $Ly6C^+$ monocytes are recruited into inflammatory reaction in tissue via CC-chemokine receptor 2 (CCR2) pathway. $Ly6C^+$ monocytes are called classical monocytes that could differentiate into M1 macrophages. $Ly6C^+$ monocytes and/or M1 macrophages participate in phagocytosis and inflammatory process in tissue. $Ly6C^-$ monocytes are non-classical type, and they are recruited for tissue repair and likely to differentiate into M2 macrophages via CX_3C-chemokine receptor 1 pathway. In human, monocytes are classified based on the cell surface expression of CD14 and CD16. In general, $CD14^+CD16^+$ monocytes differentiate into M1 macrophages, and $CD14^+CD16^-$ monocytes differentiate into M2 macrophages. However, further types of macrophages can be identified by the ratio of CD14 to CD16. $CD14^{++}CD16^-$ monocytes are classical monocytes that express high level of CCR2. The $CD14^{++}CD16^+$ monocytes are intermediate monocytes which contribute to specific inflammatory process such as atherosclerosis. The $CD14^+$ $CD16^{++}$ monocytes are non-classical monocytes which perform a *in vivo* patrolling function. The differentiation of human monocytes into macrophages have distinct pathophysiological process from that of mouse.

2.3. Polarization of macrophage: M1, M2a, M2b, M2c, M2d, Mhem, MHb, Mox and M4

M1 macrophages are typically induced by toll-like receptor (TLR) ligands and/or interferon (IFN)-γ, and their cell surface marker. They are similarly induced by other cytokines including interleukin (IL)-1, tumor necrosis factor (TNF)-α, IL-12, IL-17 and IL-23 (Fig. 2). M1 macrophages express TLR-4, IL-1β and nuclear factor (NF)-κB and produce various inflammatory cytokines including tumor necrosis factor (TNF)-α, IL-6 and IL-12. CD80 and CD86 are utilized as cell surface markers for M1 macrophages. Inflammation by the production of reactive oxygen species and free radical is the attribute of M1 macrophages [3, 4]. M2 macrophages are induced by IL-4, IL-10, IL-13 and immune complexes. In general, M2 macrophages produce IL-4 and IL-10 to prevent inflammation. Studies in the field of cardiology revealed the plasticity of macrophages and their subsets are divided into details: Mhem, MHb, Mox and M4 macrophages (Fig. 2) [5]. These subsets of macrophages were originally found in the lesion of human atherosclerotic plaque, and their inducers and roles were identified in vitro experiments. Mhem macrophages are induced by blood haem. Mhem macrophages express CD163 and liver X receptor -α/β. Mhem macrohpages produce IL-10 and heme oxygenase-1 to reduce lipid accumulation and oxidative stress in the plaque of atherosclerosis. MHb

macrophages are induced by blood haemoglobin/haptoglobin. MHb macrophages express CD163 and CD206, and they produce IL-10 to prevent inflammation in the plaque of atherosclerosis. Mox macrophages are induced by oxidized lipids. Mox macrophages express TLR-2 and nuclear factor erythroid 2-related factor 2, and they have antioxidant capacity by producing IL-1β and cyclooxygenase (COX)-2 in the plaque of atherosclerosis. M4 macrophages are induced by chemokine (C-X-C motif) ligand (CXCL) 4. M4 macrophages express CD206, matrix metalloproteinase (MMP)7 and MRP8, and they produce IL-6, TNF-α and MMP7 to reduce inflammation in the plaque of atherosclerosis. M2 macrophages are further divided in M2a, M2b, M2c and M2d (Fig. 2) [6]. These macrophages were originally found by *in vitro* experiments. Studies have revealed that macrophages express characteristic cytokines, chemokines and growth factors via specific stimuli (Fig. 2). The existence and roles of each macrophage have been identified by *in vivo* experiments mainly with mouse disease models, and there is increasing evidence that these phenotypes of macrophages are confirmed in human diseases. These macrophages have some aspects of M1 phenotype and express M1-related markers or genes. M2a macrophages are tissue remodeling macrophages that are induced by IL-4 and IL-13. M2a macrophages express CD163, CD206 and CD369, and they produce IL-1 receptor, CC chemokine ligand (CCL) 17,

transforming growth factor-β and insulin-like growth factor. M2b macrophages are regulatory macrophages that are induced by combination of immune complex with TLR agonists or IL-1R agonists. M2b macrophages express CD86, CCL1 and TNF superfamily member 14, and they produce conspicuous amount of pro-inflammatory cytokine, IL-10. M2c macrophages are induced by IL-10. M2b macrophages are also called regulatory macrophages (Mregs). M2b macrophages can repolarize to M2a or M2c in response to some inducers, and they can inhibit the polarization of monocytes to M1 macrophages [6]. M2c macrophages express CD163, CD206, CCR2 and TLR-1. STAT3 is activated by IL-10, and high expression levels of IL-10 and TGF-β are observed in M2c macrophages. M2c macrophages can remove apoptotic cells. M2d macrophages are tumor associated macrophage (TAM) that are induced by the combination of TLR ligands with A2 adenosine receptor agonists or IL-6. M2d macrophages produce IL-10 and vascular endothelial growth factor (VEGF). No typical cell surface marker is reported for M2d macrophage. M3 macrophages and M17 macrophages are also implicated in some reports. However, these macrophages do not appear to be established now. Therefore, it is considered that macrophages are heterogeneous, and each macrophage can vary from others, indicating the necessity of further proper identification of macrophages in skin pathophysiology

3. From perspective of non-skin organs

3.1. Atherosclerosis

Diverse phenotypes of macrophage (M1, M(Hb), Mhem, M2, Mox and M4) and their different functions have been demonstrated in atherosclerosis [5].

Atherosclerosis-related macrophages possibly exist in the skin lesion of vascular plaque. Moreover, atherosclerosis is a well-known comorbidity of psoriasis. IL-17 is known to activate macrophages in the atherosclerotic plaque. Since IL-17 also activates macrophages in the skin lesion of psoriasis and atopic dermatitis [7], it is possible that atherosclerosis-related-like macrophages are included in the pathogenesis of psoriasis and atopic dermatitis as well as that of atherosclerosis.

3.2. Control of blood pressure

An astonishing report added skin as an organ of blood pressure regulation. The cutaneous accumulation of sodium is linked to the retention of water to maintain the isotonicity. Macrophages have tonicity-responsive enhancer binding protein that binds to the promoter of gene encoding VEGF-C. The secreted VEGF-C increases lymph capillary density and attenuates the blood pressure to high salt [8].

3.3. Obesity

Pro-inflammatory cytokines such as TNF-α and IL-6 are overexpressed in the adipose tissue macrophages during obesity to contributes to systemic inflammation, resulting in the development of non-skin and/or skin diseases including atherosclerosis and psoriasis. Macrophages are considered to play the central role in the onset of obesity [9]. In lean human, M2 macrophages are predominant, while obesity increases the number of M1 macrophages.

4. Macrophages in skin diseases (Fig. 3)

4.1. Wound healing

Skin wound healing is composed of three stages: acute inflammatory phase, regeneration phase and remodeling phase [10]. In acute inflammatory phase, M1 macrophages have strong phagocytic activity. In regeneration phase, M2 macrophages migrate to the wound bed and form granulation tissue. M2 macrophages remain in the scar at remodeling phage, and they are believed to produce some growth factors including fibroblast growth factors, keratinocyte growth factors and VEGF. Macrophages stimulate and recruit fibroblasts from wound edge or bone marrow. Some

fibroblasts differentiate into myofibroblasts, and transition of macrophages to myofibroblasts is suggested. Keratinocyte-macrophage crosstalk are also considered to be crucial for tissue repair. Macrophages and myofibroblasts undergo apoptosis or disappear from the wound during the last stage. In diabetic wounds, the number of M2 macrophage is decreased and that of M1 macrophage is increased, suggesting the prolonged inflammation phase of wound [11]. There are chemokine CX3C receptor^{high} embryonic macrophages around sensory nerve axon in the dermis after birth [12]. These embryonic macrophages contribute to nerve regeneration after injury.

4.2. Contact dermatitis

Contact dermatitis is a cutaneous immunological response to some chemical hapten. Clinically, metals, plants and drugs can cause allergic contact dermatitis. $CD4^+$ T cells and $CD8^+$ T cells play multiple roles in inflammatory responses in the elicitation phase of contact dermatitis. Proinflammatory cytokines such as IFN-γ were produced by these T cells accumulating in the skin lesion of contact dermatitis. The secreted IFN-γ can evoke the differentiation of M1 macrophages. In the lesion of human skin contact dermatitis, the observation of inducible nitric oxide synthase (iNOS)- and arginase-1-expressing $CD14^+$ dermal monocytes is reported [13]. These $CD14^+$ monocytes may

differentiate into both M1 and M2 macrophages. Since iNOS mostly contributes to the contact dermatitis-related inflammation, M1 macrophages are critical for contact dermatitis [14]. In general, M2 macrophages suppress Th1 responses, and skewed polarization towards M2 of macrophages in the lesion of contact dermatitis attenuates the inflammation [14]. CXCR1 deficiency reduces the expression of markers of M1 macrophage in the mouse models of contact dermatitis [15]. However, other mouse studies suggested the importance of M2 macrophages in the pathogenesis of contact dermatitis. It is reported that they can exacerbate 2.4-dinitrofluorobenzene-induced contact hypersensitivity by expressing MMP12 and recruit various inflammatory cells in mouse [16].

4.3. Urticaria

In addition to mast cells, $CD4^+$ Th2 cells are known to have particular roles in the pathogenesis of urticaria by producing IL-4 and IL-13. Therefore, a predominance of M2 macrophages is shown in the skin lesion of urticaria [17]. The M2 macrophages were shown to express $CD163^+$ in the previous report, but the real functions and roles of them are not elucidated yet. Factor XIIIA expressing dermal dendrocytes were reported to phagocyte mast cell granules in the skin lesion of drug-induced acute urticaria [18].

These dendrocytes may be $CD163^+$ macrophages, since the cells expressing factor XIIIA are identified as $CD11c^-$, $CD163^+$ macrophages in the dermis of human skin [19].

4.4. Atopic dermatitis

AD is a common inflammatory skin disease. There was an increase in macrophage numbers in the lesion of AD skin [20]. These macrophages express CD36 which is related with phagocytosis of apoptotic immune cells. The expression of CD36 by macrophages is increased in acutely and chronically inflamed lesions of AD skin, suggesting the removal of apoptotic immune cells during the inflammation of AD [20]. There are also some macrophages expressing CD163, a marker of M2 macrophage [21]. In general, AD exhibit Th2 responses, and the initiation of skin inflammation is thought to be mediated by infiltration of Th2 lymphocytes releasing IL-4, IL-5, IL-13, and IL-31. However, the accumulation of activated inflammatory immune cells causes a mixed Th1/Th2/Th17 pattern with expressing IFN-γ and IL17 in the chronic phase [22]. Therefore, heterogeneous and overlapping macrophage and dendritic cell populations are shown in the skin lesion of human AD [7, 20]. The studies utilizing mouse models of AD show the possible roles of macrophages. Macrophages are suggested to act as antigen-presenting cells [22] In the lesion of chronic inflammation, macrophages play

key roles in tissue remodeling, repair and resolution of inflammation. TLRs are first reported to protect invading pathogens in innate immunity, but recent studies revealed the phagocytotic ability and antigen processing ability of macrophages in adaptive immunity. Impaired TLR-2 expression and TLR-2-mediated cytokine secretion are reported in macrophages from AD patients [23], suggesting the subsequent susceptibility to skin infection with *Staphylococcus aureus* in AD. Dermal lymphatic hyperproliferation is a characteristic feature of AD lesion. It was reported that macrophages express lymphangiogenic factor, VEGF-C, in the skin lesion of IL-4-transgenic mouse model of AD [24].

4.5. Psoriasis

CD11c+ dendritic cells were reported as a major cell type in the skin lesion of psoriasis patients [25]. These dendritic cells express TNF-α and iNOS, and they are so called TIP-DCs. However, the existence of CD163+ macrophages was also identified after that in the skin of psoriasis patients [26]. These macrophages also express iNOS and TNF-α, indicating the strong contribution of macrophages to the pathogenesis of psoriasis as well as dendritic cells. They express IL-23 and play a pathogenic role in IL-23 mediated psoriasis-like skin inflammation [27]. It is well known that IL-17 is a major effector

cytokine in psoriasis. Considering that IL-17 is reported to sway the phenotype of macrophages [7], macrophages stimulated with IL-17 can be called M17. Macrophages express βKlotho in skin lesions of psoriatic patients, but the real roles of them need further investigation [28].

4.6. Hidradenitis Suppurativa

Histologically, macrophage infiltration is observed in both acute and chronic lesions of hidradenitis suppurativa [29]. The skin lesions of hidradenitis suppurativa have a unique inflammatory signature including the expression of TNF-α, IL-12, IL-23 and IL-1β, suggesting the potential roles of M1 macrophages [30]. Abundant expressions of IL-12 and IL-23 by macrophages were shown in the skin lesion of human hidradenitis suppurativa [30]. Although the expression of TLR2 and CD68 was reported in these M1-suggested macrophages, the real phenotype is not identified yet. Alternatively, predominant existence of $CD163^{+}$ macrophages was reported in the skin lesion of hidradenitis suppurativa [31]. These macrophages were categorized as M2 macrophages, which upregulate the expression of CCL18 to stimulate fibroblast-derived collagen production in skin lesion of hidradenitis suppurativa.

4.7. Alopecia areata

Serum CCL17 levels are associated with the disease activity of alopecia areata, and the existence of CCL17 producing $CD68^+$ macrophages was shown in the lesional hair follicles of human alopecia areata patients [32]. The phenotype of these macrophages is not elucidated yet. However, CCL17 is generally produced by M2 macrophages to promote tissue fibrosis, implying the probable roles of M2 macrophages in the pathogenesis of alopecia areata. On the contrary, it has been reported that M2 macrophages exist in the unaffected mouse skin and that M1 macrophages are observed in the skin of murine alopecia areata model [33]. Other phenotype of macrophage is implied in the report.

4.8. Autoimmune bullous diseases

Macrophages are critical for subepidermal blister formation in experimental bullous pemphigoid. The existence of CD163+ CD206+ macrophages were reported in the skin lesion of bullous pemphigoid (BP) and pemphigus vulgaris (PV) [34]. These macrophages were categorized as M2 macrophages. However, IL-17 and IL-23 induces IL-1β and subsequent metalloproteinase-9 production in the CD163+ macrophages of BP patients [35], and the overexpression of IL-17 and IL-23 was also reported in the

lesion of PV patients [36]. Since IL-17 increases M1 macrophages, macrophages observed in the skin of bullous diseases may not be simple M2, or they may be heterogenous. Speculatively, these macrophages may be M17. CD163+ macrophages express programmed death-ligand 1 in a BP patient treated with programmed death-1 inhibitor, suggesting the involvement of immune checkpoint regulation of macrophages in the pathogenesis of BP [37].

4.9. Collagen diseases

Studies suggest that M1 macrophages play an inflammatory role in systemic lupus erythematosus. Additionally, the reduced populations of M2a and M2c macrophages is suggested to contribute to the lack of anti-inflammatory activity in the disease [38]. Although the phenotypes of macrophage in the skin lesion of systemic lupus erythematosus, the existence of heterogeneous phenotype of macrophage is reported in the skin lesion of discoid lupus erythematosus. Reduced expression of miR-146a with subsequent increased expression of regenerating family member 3 α increases inflammatory macrophage migration in dermatomyositis [39]. Macrophages in the skin lesion of human systemic sclerosis express both markers associated with inflammatory and alternative activation, and they have profibrotic activities [40]. The phenotypes and

roles of macrophages in the skin lesion of collagen diseases are still unclear, and they may depend on the disease activity. However, further identification of them is desired.

4.10. Granulomatous skin diseases

Formation of giant cells in granuloma tissues was considered to induced by fusion of macrophages. Granuloma macrophages born via characteristic differentiation pathways. They include the pathways of so-called innate immunity such as Toll-like receptors and nucleotide-binding oligomerization domain-like receptors [41]. The innate immunity may induce autophagy in macrophages and giant cells, and autophagy-related protein expression is reported in some granulomatous skin diseases [42]. Various immune responses including Th1, Th2 and Th17 pathways are involved in the granuloma formation of skin diseases, and the identification of M1 and/or M2 macrophages has been reported. In tuberculosis, M1 macrophages are predominant in the lesion of granuloma tissue of early stage, but M2 macrophages are present in the peripheral area of granuloma tissue of chronic stage [43]. In sarcoidosis, M1 macrophages are predominant in the lesion of granuloma tissue of the skin. CD163-positive M2 macrophages are observed in the peripheral area of granuloma tissue of the skin, especially of the skin of patients with systemic sarcoidosis [44]. The

M2 macrophages also express programmed death-ligand 1 (PD-L1) in the skin of the patients with systemic sarcoidosis. In granuloma annulare (GA), the roles of macrophages are not reported yet. The reasons for this may be diverse variants of GA (localized, generalized, perforating and maculopapular) and histological varieties. The several studies showing IFN-γ and TNF-α in the lesion of GA may indicate the presence of M1 macrophages [45]. However, M2 macrophage markers are demonstrated in GA and necrobiosis lipoidica [46].

4.11. Leprosy

M1 and M2 macrophages have been well defined in the *Mycobaterium leprae* immune response. In tuberculoid leprosy, activation of the classical pathway by M1 macrophages induces the production of TNF-α, IFN-γ, and iNOS, which destroy the bacillus by free radical production [4, 47]. Lepromatous leprosy form shows a predominance of M2 macrophages that induce IL-10, TGF-β and FGF-β, which contribute to the immunosuppressive response with tissue repair [48] . The presence of M4 macrophages is shown in the lesion of leprosy [49]. The expression levels of M4 macrophage markers (CD68, MRP8, MMP7, IL-6 and TNF-α) are higher in patients with the lepromatous form than the patients with tuberculoid leprosy. Thus, lepromatous

form is suggested to be less effective in the elimination of the bacillus and is associated with the evolution to one of the multibacillary clinical forms of infection.

4.12. Skin Cancers

Although the roles of macrophages have been extensively documented in the literatures with respect to cancers in non-dermatologic fields, there are few reports regarding the roles of macrophages in skin cancers except for malignant melanoma. In general, various inflammatory cells infiltrate in the cancer tissues. Macrophages in the cancer tissue are called TAM, and they are polarized towards M1 or M2 phenotype. The M1 TAM has anti-tumor abilities, but M2 TAM promotes cancer growth by producing growth factors such as vascular endothelial growth factors [50]. The phenotype of TAM in non-melanoma skin cancers is unclear yet. However, studies have suggested the accumulation of CD163 TAM is relevant to poor prognosis of malignant melanoma [50]. In contrast, the poor prognosis has been reported in patients with reduced circulating M1 macrophages. The accumulation of CD163 TAM is also suggested to be relevant to poor prognosis of cutaneous T-cell lymphoma, diffuse large B-cell lymphoma and Hodgkin’s lymphoma.

5. Conclusion remarks

Over the recent decade, attracting information about macrophage polarization have been reported. This is because many molecules have been identified as markers of macrophage polarization. Additionally, mechanistic insights have been demonstrated by experiments with various stimuli-induced macrophage polarization as shown in this review. However, some of them are not specific yet, and there may be more phenotypes of macrophages. Moreover, there still lack studies of macrophage phenotyping in skin diseases including acne vulgaris, rosacea and drug eruption that are shown to have histological macrophage infiltration in the skin lesion. This review includes hypothetic aspects, but hopefully, future studies will clarify the details about phenotypes of macrophages and their attractive roles of skin diseases.

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Figure legends

Fig. 1. Differentiation of macrophages. In mouse, $Ly6C^+$ monocytes differentiate into M1 macrophages, and $Ly6C^-$ monocytes differentiate into M2 macrophages. In human, $CD14^+CD16^-$ monocytes differentiate into M1 macrophages, and $CD14^+CD16^-$ monocytes differentiate into M2 macrophages.

Fig. 2. Polarization of macrophage: M1, M2a, M2b, M2c, M2d, Mhem, MHb, Mox and M4. Abbreviations: AR, adenosine receptor; ATF, activating transcription factor; CCR2, CC-chemokine receptor 2; CXCL, chemokine (C-X-C) ligand; COX, cyclooxygenase; IL, interleukin; LXR, liver X receptor; MMP7, matrix metalloprotease 7; MRP8, myeloid-related protein 8; NFE2L2, nuclear factor erythroid 2 like 2; TGF, transforming growth factor; TLR, toll like receptor; TNF sf14, tumor necrosis factor superfamily member 14; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Fig. 3. Macrophages in skin diseases. Reported macrophage polarization in skin diseases is shown. Most reports demonstrate M1 and M2. Abbreviations: MMP7, matrix

metalloprotease 7; iNOS, inducible nitric oxide synthase; PD-L1, programmed death-ligand 1; TLR, toll like receptor.