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MINI-REVIEW

Monocyte subsets and their differentiation tendency after burn injury

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Abstract **Monocytes are critical effectors and regulators of immune response. Studying the nomenclature of monocyte subsets may be beneficial for understanding the complex function of monocytes in steady and inflammatory states. A monocyte has the potential to differentiate into dendritic cells or macrophages, and this behavior significantly changes in severely burned patients and mice. The findings in the present study may help enhance understanding on the perturbation of the immune system after severe burn injury.**

Keywords monocyte; differentiation; burn

Introduction

Suppression of the immune system contributes to the occurrence of sepsis and organ dysfunction, which are profound problems in the treatment of severe burns. The reasons for immune depression in burn injury include loss of barrier functions, tissue ischemia, foreign body introduction, and invasion of microorganisms. These factors stimulate the response of the immune system, resulting in abnormality in the complement system, dysfunction of immune cells, and production of soluble immunosuppressive substances [1]. Monocytes circulating in the blood stream participate in the immune system and are important in connecting innate immunity with adaptive immunity. Monocytes are a heterogeneous cell population that can be divided into numerous subsets based on the molecules expressed on the cell membrane or the cytokines secreted from cells. These monocyte subsets can differentiate into dendritic cells and macrophages. However, the change in the behavior of monocytes may have an important role in immune system disturbance after burn injury.

Monocyte subsets

Using flow cytometry, Passlick *et al.* identified a subset of cells co-expressing CD14 and CD16 (a receptor for IgG and C-reactive protein) antigens in human peripheral blood. They found that the $CD14^{+}CD16^{+}$ monocytes can express higher quantity of major histocompatibility complex (MHC) class II antigens and can decrease the ability to perform Fc receptor-mediated phagocytosis of erythrocytes [2]. Ancuta *et al.* [3] further divided $CD14^{+}CD16^{+}$ monocytes into two groups, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{+}$, according to cell size, granularity, and CD14 expression. Based on the expression of CD14 and CD16 on human monocytes, a group of scientists proposed some standards to classify subsets of blood monocytes: classical monocytes $CD14^{++}CD16^{-}$, intermediate monocytes $CD14^{++}CD16^{+}$, and non-classical monocytes $CD14^{+}CD16^{++}$. Subdivisions of mouse monocytes similar to humans were defined based on the expression of Ly6C and CD43: classical monocytes $Ly6C^{++}CD43^{+}$, intermediate monocytes $Ly6C^{++}CD43^{++}$, and non-classical monocytes $Ly6C^{+}CD43^{++}$ [4]. The + denotes an expression level that is ~10-fold above the isotype control, whereas ++ represents a level that is ~100-fold above the isotype control.

The population of classical monocytes accounts for about 85%, whereas intermediate and non-classical monocytes account for approximately 5.4% and 9.2%, respectively [5]. Thus, identifying the intermediate and non-classical monocytes correctly can be difficult. Aside from properly using isotype controls, several researchers have attempted to utilize additional surface markers. Wong *et al.* detected the gene expression of three human monocyte subsets by microarray, and found that several genes can be significantly expressed in the different subsets. Considering the average fold change of

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mean fluorescence intensity (MFI) on intermediate or non-classical monocytes detected by flow cytometry, the expressions of CLEC10A, GFRA2, CD163, CD11b, SLAN, CD1d, CCR6, HLA-DR, CD294, and Siglec10 genes showed more than two-fold change, which may be used as additional surface markers for separating intermediate and non-classical monocytes. Zawada *et al.* [6] analyzed the transcriptomes of three monocyte subsets using SuperSAGE combined with high-throughout sequencing. They found that the potential surface markers for discriminating intermediate and non-classical monocytes are HLA-DR, CD74, endoglin, and CD202B. After segregating human monocytes into three subsets based on CD14 and CD16, the mechanism underlying diseases may be identified and the effect on patients may be predicted. The expansion of the CD16^{+} monocytes has been well documented in infection or inflammatory conditions. Poehlmann *et al.* [7] noted that the expanded CD16^{+} monocytes mainly comprise monocytes of the intermediate phenotype in sepsis. One question raised from this result is where the CD16^{+} monocytes come from under this condition. An *in vitro* study showed that the percentage of non-classical monocyte population expanded in 20 h through post-stimulation, and an increase in tumor necrosis factor (TNF)-α and interleukin (IL)-6 was observed after 2 h [8]. Besides TNF-α, other cytokines can also induce the expression of CD16 in monocytes. West *et al.* observed the expansion of intermediate monocytes in trauma patients, and further studies demonstrated that the percentage of intermediate monocytes after trauma was strongly correlated with plasma C-reactive protein, transforming growth factor-β (TGF-β), and macrophage colony-stimulating factor (M-CSF) levels. TGF-β and M-CSF can induce the expression of CD16 in monocytes [9,10]. In addition, the evidence from gene expression profiling supports the theory that classical monocytes are highly versatile, are capable of responding to a variety of external cues, and are ready to develop into intermediate and non-classical monocytes [5,6].

Differentiation of monocytes into dendritic cells or macrophages

Monocytes can differentiate into dendritic cells (DCs) or macrophages in appropriate situations. Do specific monocyte subsets tend to differentiate into DCs or macrophages? A macrophage is a type of white blood cell that ingests foreign materials, whereas DC is a special type of cell that acts as a professional antigen-presenting cell (APC) that is capable of activating naive T cells and stimulating the growth and differentiation of B cells [11]. Two kinds of molecules, dendritic cell-specific ICAM grabbing non-integrin (DC-SIGN) and CD1, are specifically expressed in DCs. DC-SIGN contains a carbohydrate-recognition domain enabling cytokine-derived DCs to recognize and mediate phagocytosis/uptake of a broad range of pathogens, whereas CD1 molecules (CD1a and CD1b) facilitate the presentation of nonpeptide antigens into T cells. A monocyte can differentiate into DCs under inflammatory conditions. In the process of differentiation, the activation of toll-like receptors (TLRs) triggers the release of cytokines and stimulates the differentiation of immature to mature dendritic cells. Ligands for TLR2/1, TLR2/6, TLR4, and TLR5 can significantly induce the expression of both DC-SIGN and CD1b for two distinct populations of cells identified as DC-SIGN^{+}CD1b^{-} (a macrophage-like phenotype) and DC-SIGN^{-}CD1b^{+} (an immature dendritic cell phenotype) [12]. To define the differentiation tendency of monocyte subsets, Aguilar-Ruiz *et al.* [13] transferred Mo subpopulations into the peritoneum of immunocompromised mice, and found that differentiating CD16^{-} Mo (24 h after transfer) could acquire greater APC capacity in LPS-induced peritonitis. By contrast, CD16^{+} had more phagocytic cells and produced higher amounts of TNF and IL-6 than CD16^{-} Mo early after the transfer of zymosan. These results were further verified by Balboa *et al.* [14] when they found that CD16^{-} Mos that differentiated into CD1a^{+} DC-SIGN^{high} cells achieved efficient recall response, whereas CD16^{+} Mos that differentiated into CD1a^{-}DC-SIGN^{low} population were characterized by poor Ag-presenting capacity. An increasing number of evidence indicates that classical monocytes have a tendency to differentiate into dendritic cells, whereas non-classical monocytes are prone to differentiating into macrophages. However, some paradoxical results exist. In a model of transendothelial trafficking, the subpopulation of monocytes showed that CD16 (Fcγ receptor III) may become predisposed to migratory DCs that can induce allogeneic T cell proliferation [15]. Whether monocytes differentiate into dendritic cells or macrophages depends on the kind of transcription factors that are activated. High PU.1 activity favors DCs at the expense of macrophage fate by inhibiting expression and activity of the macrophage factor MafB, which belongs to the family of Maf proteins, a subgroup of AP-1-type bZip transcription factors.

Disturbance of monocyte function following burn injury

Burn injury is often associated with reduction in DCs. D'Arpa *et al.* [16] reported that the levels of circulating DCs significantly decreased on the first day after burn injury compared with healthy controls. Mice receiving 15% scald burn on their dorsum in 100 °C water bath for 8 s experienced significant reduction in circulating DCs derived from monocytes (moDCs) for up to 14 d post-burn. One reason for this finding is that the differentiation potential of moDC precursors is inhibited. Burn injury augments MafB expression in monocytes and impairs the differentiation of monocytes into moDCs. Silencing of MafB in *ex vivo* culture prior to DC differentiation by using small interfering RNA technique results in the increase in the differentiation

potential of monocytes into moDCs [17].

Burn injury not only reduces the number of moDCs, but also changes their functions. The expression of human leukocyte antigen (HLA) class II molecules on classic monocytes is significantly reduced in burn patients [18]. Depressed HLA-DR expression on all monocytes and DC subset was observed in septic patients in a clinical observation [19], which is in agreement with findings on the reduction of major histocompatibility complex II^+ ($MHC\text{-}II^+$) expression on moDCs. However, whether or not the dysfunction of moDCs is associated with the inherent alteration of monocytes after burn injury should be further tested. Monocytes stimulated with specific cytokines can differentiate into different kinds of macrophages. M-CSF is a key cytokine for the generation of unpolarized macrophages from monocytes *in vitro*. Unpolarized macrophages can be driven away by polarized macrophage subsets based on environmental cues. Classically activated macrophages (M1) were formed with the stimulation of interferon-γ (IFNγ) and tumor necrosis factor-α (TNF-α) *in vitro*. M1 macrophages can produce pro-inflammatory cytokines and possess enhanced microbicidal activity. Thus, they are also called pro-inflammatory macrophages. Originally, alternatively activated macrophages (M2) were derived from unpolarized macrophages with M-CSF and IL-4. However, recent studies further separate M2 macrophages into three groups, M2a, M2b, and M2c, which are induced by IL-4 or IL-13, immune complexes and IL-1β or LPS, and IL-10 or TGF-β or glucocorticoids, respectively. M2 macrophages are $IL\text{-}12^-IL\text{-}10^+$ and have an anti-inflammatory function. These subsets of M2 macrophages can be distinguished from each other by chemokine profiles and gene expression. M2a macrophages are characterized by CCL-17 production and DC-SIGN gene traits; M2b macrophages are characterized by CCL-1 production and DC-SIGN gene-lacking characteristics; and M2c macrophages are characterized by CXCL-13-producing ability [20,21]. In addition, an increasing number of cytokines have been identified as inducers of M2 monocytes. Bone morphogenetic protein 7 (BMP7) significantly enhances monocyte polarization into M2 macrophages and increases the levels of anti-inflammatory cytokines [22]. Foucher *et al.* [23] also reported that IL-34 drives the differentiation of monocytes into immunosuppressive M2 in a manner similar to M-CSF, and that IFNγ and GM-CSF can prevent this effect.

Burn injury results in the production of many kinds of cytokines that may change the phenotype of monocytes. Kobayashi *et al.* [20,21] demonstrated that most of the monocytes isolated from blood of severely burned patients were M2b monocytes ($IL\text{-}17^-CCL1^+CXCL13^-$). The increase of CCL2 in the sera of burned patients contributes to the expression of CCL1, and stimulates monocyte conversion from resident monocytes to M2b monocytes. Fortunately, this pathological process can be intervened by propranolol, a competitive blocker of catecholamine binding to β-adrenergic receptors. The effects of propranolol on monocytes have also been observed in mice. Based on the chemokine expression and migration properties of monocytes isolated from mice, monocytes can be classified into two different groups: inflammatory subset $F4/80^+Gr1^+$ and resident subset $F4/80^+Gr1^-$[24]. Muthu [25] reported that the administration of propranolol enhanced the level of inflammatory monocyte subsets in circulation following burn sepsis treatment.

Conclusions

The function of monocytes has been extensively studied for many decades, but numerous aspects remain unknown. The nomination of monocyte subsets is based on cells isolated from steady-state conditions. Whether the cells isolated from inflammatory conditions can exhibit the same phenotype and function requires further study. Another challenge is applying the knowledge obtained from experiments into clinical application. For severe burn patients, finding septic biomarkers and alternative treatments for the weakened immune system is possible.

Compliance with ethics guidelines

Guangqing Wang and Zhaofan Xia declare that they have no conflict of interest. This manuscript does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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