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Intestinal Macrophages in Mucosal Immunity and their role in Systemic Lupus Erythematosus Disease

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Abstract

Monocytes play an important role in inducing host systemic immunity against invading pathogens and inflammatory responses. After activation, monocytes migrate to tissue sites, where they initiate both innate and adaptive immune responses, and become macrophages. Although mucosal macrophages produce inflammatory cytokines in response to pathogens, the perturbations in innate immune signaling pathway have been implicated in autoimmune diseases such as systemic lupus erythematosus (SLE). In this review, we focus on the role of human macrophages in intestinal innate immune responses, homeostasis, and SLE disease. We further discuss gender difference in the intestinal macrophages and its role in the physiology and pathogenesis of SLE.

Introduction to Intestinal Macrophages

The epithelium and the lamina propria constitute the gut mucosal barrier, and tight junctions connect epithelial cells extracellularly and intracellularly ¹. The gut mucosal barrier protects the host from pathogens but allows very low levels of bacterial product translocation to the system to maintain systemic immune homeostasis, as mice raised in sterile conditions have severe immune deficiency ². Therefore, gut epithelial cells and other immune cells have to

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discriminate “good” or “bad” antigens through innate and adaptive immune responses at the mucosal site.

Intestinal macrophages have poor capacities to proliferate and are shorter-lived compared to those from other tissues ³. Previous studies have revealed that intestinal macrophages are derived from constant recruitment of peripheral monocytes, which may be different from macrophages of other tissue sites ⁴. In humans, peripheral monocytes are defined to two or three subsets based on CD14 and CD16 expression ⁵: CD14+CD16+ and CD14+CD16– subsets, or classic (CD14++CD16–), intermediate (CD14++CD16+), and non-classical (CD14+CD16++) subsets ⁶. Whether intermediate and non-classical monocytes are derived from classical monocytes is unknown. Upon activation by toll-like receptor (TLR) 4 ligand LPS, classical monocytes are activated and become macrophages or dendritic cells (DCs), produce cytokines and migrate to the tissue sites ³. The intermediate monocytes have been reported to be involved in cardiovascular diseases ⁶, whereas non-classical monocytes preferentially produce pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-6) in response to TLR agonists, which may be involved in autoimmune diseases ⁷. Due to their large numbers in the periphery and the processors of DCs, monocytes also play an important role in adaptive immune responses ⁸.

Macrophages play an important role in intestinal homeostasis and immunity through phagocytosis of antigens, as well as maintaining tolerance to self-antigens and innocuous antigens ⁸. Intestinal macrophages are neither M1 nor M2 macrophages based on their expression of high levels of MHC-II, CD163 and CD206 as well as their production of TNF- α and IL-10 ^{8,9}. Macrophages residing in the intestine function to take up and clear antigens, produce inflammatory cytokines, initiate adaptive immune responses and maintain homeostasis ⁴. Notably, human intestinal macrophages express surface CD14, HLA-DR, CD68, and some TLRs ¹⁰, which mediate recognition of pathogens, phagocytosis, and cytokine production. Moreover, intestinal macrophages express high levels of MHC-II and are professional antigen-presenting cells ¹⁰. They also play a role in modulating mucosal immune responses through cytokines (e.g., IL-10). For example, the intestinal macrophages suppress IL-17 production and induce of tolerance ¹¹.

Innate immune responses of intestinal macrophages

TLRs recognize pathogens through pathogen-associated molecular patterns (PAMPs), express in different cell types, especially highly express in antigen-presenting cells ¹². Both monocytes and macrophages express TLR2 and TLR4, recognition receptors for diverse bacterial products ¹³. TLRs express in human intestinal macrophages and play a key role in innate and adaptive immune responses ¹⁰. Upon a TLR recognition and activation by its ligand, TLR cell signaling pathway is triggered which results in pro-inflammatory cytokine production ¹⁴. Generally, intestinal macrophages do not produce large amount of pro-inflammatory cytokines in response to TLR stimulation ^{3,15}, which may be important for maintaining tolerance and preventing uncontrolled inflammation at the intestinal mucosal site. Moreover, intestinal macrophages secrete anti-inflammatory cytokines such as IL-10 but no other TLR-mediated pro-inflammatory cytokines in response to TLR stimulation ³. Compared to monocytes, intestinal macrophages express low level of surface CD14 to limit

Th17 cell development^{13, 15}; low level of MyD88, a key TLR signaling transcription factor; and high level of IRAK-M, a negative TLR signaling regulator to favor IL-10 production and to prevent responses to TLR agonists^{10, 16}. This evidence suggests a potential role of intestinal macrophages in autoimmunity. Nonetheless, human intestinal macrophages are fully phagocytic with bactericidal activity¹⁷.

Gender differences in intestinal macrophages, implication in SLE disease

SLE is an autoimmune disease characterized by pathological autoantibodies against cellular nuclear antigens and by the formation of immune complexes that eventually lead to organ damage¹⁸. Notably, the ratio of female to male is 9:1 in lupus disease prevalence, and the disease is triggered in most females after puberty¹⁹. This evidence suggests that female sex hormones, especially estrogen, may have inductive effects on SLE²⁰. For instance, it has been shown that anti-estrogen reduces the production of immune complex and prolongs survival of lupus-prone female mice (NZB × NZW) F1 and MRL-lpr/lpr mice²¹.

A good model for investigating female sex hormones in lupus disease is pregnancy. During pregnancy, peripheral monocytes in women with SLE express decreased levels of chemokine receptors CCR2, CCR5 and CXCR3, as well as alteration of the response to microbial stimuli²². Mice intestinal macrophages express both estrogen receptor (ER- α) and ER- β ²³. TLR8 has been shown as estrogen-responsive receptors in human PBMCs²⁴. Moreover, the ER- α expression was essentially negative while ER- β is the predominant ER subtype in the human colon by immunohistochemistry assays^{25, 26}. Human circulating monocytes express low to undetectable levels of both ER- α and ER- β ²⁷. It remains unclear how estrogen regulates monocyte function *in vivo*, although *in vitro* monocytes respond to estrogen stimulation^{28, 29}. In contrast, progesterone and testosterone receptors are not expressed in monocytes³⁰. During pregnancy, fifteen to sixty percent of lupus patients have reported exacerbation of lupus disease³¹. Moreover, maternal mortality rate in SLE patients is higher compared to healthy pregnant women^{32, 33}. Nonetheless, it is unclear whether this failure results from increased inflammation or autoantibodies in lupus patients during pregnancy.

Females have increased TLR7 responsiveness compared to males^{34, 35}. Previous studies from our group and others observed increased CD16-positive monocyte subsets and monocyte activation in women compared to men and in SLE patients compared to healthy controls *in vivo*³⁶. Notably, estradiol has been shown to regulate CD16 expression on monocytes *in vitro*, but results from these assays are controversial^{37, 38}. Moreover, increased CD16-positive monocytes are observed in blood during inflammatory conditions, suggesting that inflammation may drive monocytes' differentiation from classical to non-classical *in vivo*³⁹. Different monocyte subsets show differences in TLR responsiveness, and thus, there may be a sex difference of monocytes/macrophages in the clearance of microbial products and TLR signaling pathways during inflammation^{19, 20}. This effect may account for the high prevalence of SLE in women.

Gaudreau et al. found that the intestinal mucosa of female SNF1 mice not only expressed higher levels of type 1 interferons, TLR7 and TLR8, but also had a larger number of IL-17-, IL-22- and IL-9-producing cells in the lamina propria compared to their male counterparts,

even before puberty⁴⁰. In addition, our group observed increased proportions of non-classic monocytes, decreased proportions of classic monocytes, elevated levels of plasma sCD14 as well as reduced surface expression of CD14 on monocytes comparing women to men and lupus patients to controls³⁶. These data suggested a potential sex difference in macrophages activation through TLRs signaling pathway.

Recent studies showed that macrophages maintain interstitial homeostasis and induce inflammatory responses through inflammasome activation⁴¹. Yang et al. found that ATP-induced IL-1 β production was increased in macrophages of both male and female SLE patients³⁰. Furthermore, increased NLRP3 expression has been found in unstimulated macrophages from female SLE patients, whereas increased AIM2 expression in males and decreased AIM2 expression in females have been found in unstimulated macrophages in lupus disease³⁰. These results suggest that inflammasome activation in macrophages might contribute to gender difference in lupus disease.

Interestingly, a sex difference in gut microbiota may exist in SLE disease⁴². Hevia et al. found that a significantly lower gut *Firmicutes* to *Bacteroidetes* ratio was present in women with SLE even after disease remission⁴³. Previous studies have shown that *Firmicutes* are beneficial bacteria to the host⁴³. Taken together, there may be a direct or indirect link between pathogen-mediated macrophage activation, sex hormones and SLE pathogenesis.

Intestinal macrophages in SLE disease

Disruption of the balance between immunity and tolerance has been linked to autoimmune diseases, such as SLE. However, little is known on the role of intestinal macrophages in SLE. Human intestinal macrophages are resistant to respond to TLR stimulation due to reduced levels of key responding molecules^{16, 17}. However, altered expression of type-1 interferon-stimulated genes (ISGs), HLA-DR, and Fc γ receptors (Fc γ Rs) has been observed in monocytes/macrophages of SLE patients^{44, 45}. Moreover, altered inflammatory cytokine production from monocytes/macrophages also has been found in SLE patients⁴⁶.

Extensive studies have been conducted in TLR7, TLR8, and TLR9 signaling pathways in SLE disease⁴⁷. Anti-DNA and anti-RNA autoantibodies are involved in the formation of immune complex, which have the abilities to activate monocytes via TLR7/8/9 to produce inflammatory cytokines, and play a role in SLE pathogenesis⁴⁸. Furthermore, elevated monocyte counts, increased CD16 expression and IL-6 production on monocytes were found in SLE patients compared to controls⁴⁹. Plasma sCD14 and IL-6, cytokines released by monocytes in response to LPS, are increased in lupus patients relative to controls³⁶. These results imply that not only TLR7, TLR8, and TLR9, but also TLR4 signaling pathway may be involved in SLE disease pathogenesis. In addition, macrophages from SLE patients have impaired phagocytosis, which may result in insufficient removal of apoptotic cells and debris and result in excessive autoantigens in SLE disease⁴⁶. This impairment may be due to reduced C1q expression on macrophages because C1q is involved in clearance⁵⁰. However, the exact mechanism is unknown.

Conclusion

Macrophage-mediated maintenance of tolerance and prevention of pro-inflammatory responses to TLR ligands at the intestinal mucosal site are important for mucosal immunity. Altered TLR-mediated innate immune responses in intestinal macrophages may play a key role in SLE disease pathogenesis. Future directions might focus on the role of sex hormone in intestinal macrophage function, gut mucosal immunity, microbial translocation, and autoimmune diseases.

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