The significance of M1/M2 macrophage-like monocytes in children with systemic lupus erythematosus

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Abstract
Monocytes/macrophages are important in the development of systemic lupus erythematosus. To research M1 and M2 macrophage-like monocytes in the peripheral blood of children with systemic lupus erythematosus and explore the clinical significance, M1 and M2 macrophage-like monocytes, tumor necrosis factor-α, interleukin-1, interleukin-6, interleukin-10, and interleukin-18 are tested in the peripheral blood of children with systemic lupus erythematosus by flow cytometry. A correlation analysis is made between M1 and M2 macrophage-like monocytes and erythrocyte sedimentation rate and C-reactive protein. As we found, the absolute number and percentage of M1 macrophage-like monocytes (CD163−CD14+), in macrophage-like monocytes (CD14+), in peripheral blood of the severe systemic lupus erythematosus group were higher than those of the control group and the mild–moderate systemic lupus erythematosus group (F = 28.4, 21.7, 122, 81.7; P < 0.05). But there was no obvious difference between these three groups in terms of the absolute number of M2 macrophage-like monocytes (CD163+CD14+). The absolute number and percentage of M1 macrophage-like monocytes in macrophage-like monocytes had positive correlation with C-reactive protein and erythrocyte sedimentation rate (r = 0.46, 0.44, 0.367, 0.47; P < 0.05); whereas, the absolute number and percentage of M2 macrophage-like monocytes in macrophage-like monocytes had negative correlation with CRP and erythrocyte sedimentation rate (r = −0.47, −0.45, −0.47, −0.32; P < 0.05). Thus, M1 macrophage-like monocytes have effective impact on inflammation in children with systemic lupus erythematosus. M2 macrophage-like monocytes, to a large extent, have the opposite functions compared to M1 macrophage-like monocytes. In children with systemic lupus erythematosus, macrophages play important role in the development of systemic lupus erythematosus and M1 macrophage-like monocytes have functions in active systemic lupus erythematosus and they can induce the inflammation and have correlation with severity of systemic lupus erythematosus.

Keywords
children, monocytes/macrophages, systemic lupus erythematosus

Introduction
Systemic lupus erythematosus (SLE), with its prevalence of 3.3–8.8/100,000, is one of the common immune diseases in pediatric rheumatology. Although the etiology and pathogenesis of SLE are still unknown, the recent studies show that innate immune cells, especially macrophages, participate in the development of SLE.
Macrophages are a highly plastic and heterogeneous cell type and can display functional differences in different micro-environments through a process referred to as polarization. Historically, activated macrophages have been considered to have two different types: M1 (classically activated) macrophages and M2 (alternatively activated) macrophages, which have opposite functions. M1 macrophages are induced by bacterial products such as lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, and interferon-γ (IFN-γ) secreted by Th1 cells and can phagocytose directly and kill pathogenic microorganisms and tumor cells. M2 macrophages are induced by steroids, interleukin-4 (IL-4), interleukin-13 (IL-13), interleukin-10 (IL-10), and transforming growth factor-β (TGF-β) secreted by Th2 cells. It limits immune response by secreting suppressive cytokines, reduces pro-inflammatory factors, and increases expression of scavenger receptor which participates in fibrosis. M2 macrophages mediate humoral immunity, tissue repair, vascularization, and tumor promotion/invasion.1 M1 or M2 macrophage polarization has been found in vivo, for instance, in the embryo and the placenta, during cancer and obesity, as well as SLE.2 MRL-Fas(lpr) mice fail to shift the macrophage phenotype from the “destroy” (M1) to the “heal” (M2) compared with the lupus-resistant mice.3 Lupus relieves magically after macrophages depletion and selectively injection of M2 macrophages.4 The SLE patients who received mesenchymal stem cell transplantation show M2 macrophages polarity recovery and increased phagocytosis.5 But the mechanism of macrophages is still unknown. Few reports are about the expression of M1 and M2 macrophages in Children with SLE.

Hence, the aim of this study is to detect the M1 and M2 macrophage-like monocytes and explore their clinical significance in children with SLE.

**Methods**

**Patients**

From January 2015 to June 2017, the patients under the age of 18 years diagnosed as SLE according to the classification of SLE revised in 2009 by Systemic Lupus International Collaborating Clinics (SLICC) were included.

Only patients diagnosed as orthostatic proteinuria were included in the control group. Patients with other rheumatologic diseases, autoimmune diseases, glomerulonephritis, nephritic syndrome, and infectious disease were excluded.

**Methods**

BD FACSCalibur flow cytometer was used to test M1 and M2 macrophage-like monocytes in peripheral blood (PB). M1 macrophage-like monocytes were marked by CD163–CD14+ and M2 macrophage-like monocytes were marked by CD163+CD14+. Monocytes were isolated from fresh blood of patients. And all the steps were taken according to the protocol of the flow cytometer. The reagents used were listed as follows: CD14-PE (A07764; Beckman Coulter Company); FITC Mouse IgG1 Control (554679; BD Pharmingen); and FITC Mouse Anti-human CD163 Clone: GH/61 (583897; BD Pharmingen).

**Statistical analysis**

Data were presented as mean and standard deviation. Comparisons between different groups were carried out using the Wilcoxon matched-pairs signed rank test. The correlation between different indices was studied using Spearman’s correlation analysis. \( P < 0.05 \) was considered statistically significant number.

**Ethics approval**

The research had been supported and got through by the Ethics Committee of the Children’s Hospital of Shanghai Jiaotong University. All the patients had signed informed consents for the research.

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**Results**

**General**

There were 38 patients diagnosed as SLE included in the experimental group: male (M)/female (F): 7/31 and the median age was 10.5 ± 3.73 years. There were 16 patients diagnosed as orthostatic
proteinuria included in control group: M/F: 12/4 and the median age was 12.4 ± 1.77 years. There were no significant difference between the two groups in age ($F=1.15; P > 0.05$). The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of patients with SLE was from 0 to 22 and the median was 10.1 ± 6.97. The SLEDAI of 11 patients was below 4, that of 6 patients was from 5 to 9, that of 14 patients was from 10 to 14, and that of 7 patients was more than 15.

**M1/M2 macrophage-like monocytes in the SLE and the control group**

The percentage of M1 macrophage-like monocytes (CD163–CD14+) in macrophage-like monocytes (CD14+) in the SLE group was more than that in the control group (33.6% ± 28.5% vs 25.1% ± 29.3%), and there was significant difference between them ($F=11.1; P < 0.05$), also the absolute number of M1 macrophage-like monocytes (CD163–CD14+) between the two groups ((148 ± 139) × 10⁶ L⁻¹ vs (66.3 ± 53.0) × 10⁶ L⁻¹, $F=5.15; P < 0.05$). The percentage of M2 macrophage-like monocytes (CD163+CD14+) in macrophage-like monocytes (CD14+) in the SLE group was lower than that in the control group (66.4% ± 28.5% vs 74.9% ± 29.3%), and there was significant difference between them ($F=11.2; P < 0.05$), but there was no difference in the absolute number of M2 macrophage-like monocytes (CD163+CD14+) between the two groups ((359 ± 255) × 10⁶ L⁻¹ vs (433 ± 117) × 10⁶ L⁻¹, $F=1.23; P > 0.05$). And there was no difference in the absolute number of macrophage-like monocytes (CD14+) between the two groups ((506 ± 256) × 10⁶ L⁻¹ vs (498 ± 116) × 10⁶ L⁻¹, $F=0.01; P > 0.05$). That indicated the M1 macrophage-like monocytes (CD163–CD14+) in SLE was higher than that in the control group. (The details could be seen Figure 1.)

According to SLEDAI, the patients with SLE were divided into the mild–moderate SLE group and severe SLE group. The percentage of M1 and M2 macrophage-like monocytes in macrophage-like monocytes (CD14+) of the severe SLE group was higher than that of the mild–moderate SLE group and the control group and that had significant difference ($F=21.7, 21.8, 28.4, 28.5; P < 0.05$). The percentage of M1 and M2 macrophage-like monocytes (CD163–CD14+) of the mild–moderate SLE group was higher than that of the control group, and they had the difference ($F=20.7, 21.8; P < 0.05$). The absolute number of M1 macrophage-like monocytes (CD163–CD14+) of the mild–moderate SLE group was higher than that of the control group, and they had the difference ($F=20.7, 21.8; P < 0.05$). The absolute number of M1 macrophage-like monocytes (CD163–CD14+) of the severe SLE group was higher than that of the mild–moderate SLE group and the control group and that had the significant difference ($F=122, 81.7; P < 0.05$). But the absolute number of M1 macrophage-like monocytes (CD163–CD14+) of the mild–moderate SLE group was higher than that of the control group and had the difference ($F=21.7, 21.8, 28.4, 28.5; P < 0.05$).
macrophage-like monocytes (CD163−CD14+) of the mild–moderate SLE group was not higher than that of the control group \((F=40.6; P > 0.05)\). There was no difference in the absolute number of M2 macrophage-like monocytes (CD163+CD14+) between the three groups \((F=29.8, 4.96, 34.7; P > 0.05)\). There was no difference in the absolute number of macrophage-like monocytes (CD14+) between the three groups \((F=16.0, 129, 113; P > 0.05)\). That indicated M1 macrophage-like monocytes increased in patients with active SLE in PB, while M2 macrophage-like monocytes had no change. (Details could be seen in Table 1.)

**Table 1.** The M1 and M2 macrophage-like monocytes in three groups.

|                   | Absolute number of monocytes | Percentage of M1-like monocytes | Absolute number of M1-like monocytes | Percentage of M2-like monocytes | Absolute number of M2-like monocytes |
|-------------------|------------------------------|---------------------------------|-------------------------------------|---------------------------------|-------------------------------------|
| Control group     | \((498 \pm 116) \times 10^6 L^{-1}\) | 25.1% ± 29.3%                   | \((66.3 \pm 53.0) \times 10^6 L^{-1}\) | 74.9% ± 29.3%                   | \((433 \pm 117) \times 10^6 L^{-1}\) |
| Mild–moderate SLE group | \((529 \pm 245) \times 10^6 L^{-1}\) | 27.0% ± 24.7%                   | \((107 \pm 92.6) \times 10^6 L^{-1}\) | 73.0% ± 24.8%                   | \((417 \pm 284) \times 10^6 L^{-1}\) |
| Severe SLE group  | \((494 \pm 269) \times 10^6 L^{-1}\) | 40.7% ± 26.0%                   | \((189 \pm 156) \times 10^6 L^{-1}\) | 59.2% ± 26.0%                   | \((303 \pm 232) \times 10^6 L^{-1}\) |

SLE: systemic lupus erythematosus.

Compared to control group: 
\*\(P < 0.01\), there was significant difference; 
\#\(P < 0.05\), there was significant difference.

Compared to mild–moderate SLE group: 
\#\(P < 0.01\), there was significant difference; 
\*\(P < 0.05\), there was significant difference.

The relationships between M1/M2 macrophage-like monocytes and clinical indices

In the SLE group, the percentage and absolute number of M1 macrophage-like monocytes (CD163−CD14+) had positive relationship with erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) \((r=0.46, 0.44, 0.367, 0.47; P < 0.05)\). The percentage and absolute number of M2 macrophage-like monocytes (CD163+CD14+) had negative relationship with CRP and ESR \((r=-0.47, -0.45, -0.47, -0.32; P < 0.05)\). That indicated the M1 macrophage-like monocytes could induce the autoimmune inflammation, while M2 macrophage-like monocytes had the opposite functions compared to M1 macrophage-like monocytes. The percentage and absolute number of M1 and M2 macrophage-like monocytes had no relationship with IL-1, IL-6, IL-10, IL-18, and TNF-\(\alpha\) \((P > 0.05)\).

Discussion

Different monocytes’ phenotypes and functions have been appreciated with a variety of autoimmune disorders including SLE.6 M1 and M2 macrophages were induced, respectively, by the cytokines secreted by Th1/Th2. M1 macrophages respond to Th1 and generate reactive oxygen species and nitric oxide to kill pathogens and cells. M2 macrophages respond to Th2 and mediate humoral immunity and tissue repair.3,7,8 Some researches had been taken in macrophages in SLE. Nakayama et al.9 had find that CD163 (the surface mark of M2 macrophages) increased in the skin and PB of SLE patients. Zizzo et al.10 found that M2 macrophages played an important role in the immunological pathomechanism in SLE. Mohammadi et al.11 found that the polarization of M1/M2 macrophages was important in the development of SLE; M1 macrophages were highly expressed in SLE, and peroxisome proliferator–activated receptor gamma (PPAR-\(\gamma\)) could improve the transfer of macrophages to M2 macrophages. Shao and Cohen12 found that M2 macrophages could increase cell apoptosis in SLE; M1 macrophages could improve the severity of SLE, but M2 macrophage had the opposite functions. But most of the researches were in animals or adult patients.

In our research, the percentage and absolute number of M1 macrophage-like monocytes were obviously higher in the SLE group and increased with the severity of SLE (SLEDAI increased), and it also had correlation with CRP and ESR, while the percentage and absolute number of M2 macrophage-like monocytes had negative relationship with CRP and ESR. It hinted that M1 macrophage-like monocytes had important functions in active SLE in children and M2 macrophage-like monocytes maybe played an important role in controlling the inflammation, which needs to be studied further. So, in children with SLE, M1 macrophage-like monocytes played an important role in inflammation in children with SLE and had correlation with the severity of disease. This was consistent to the researches by Mohammadi et al.11 and Li et al.4

Monocytes/macrophages are important in the development of SLE. M1 macrophage-like
monocytes have functions in children with active SLE, and they can induce inflammation and have correlation with severity of SLE. Further researches of the mechanism are still required.

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References
1. Sindrilaru A, Peters T, Wieschalka S et al. (2011) An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *Journal of Clinical Investigation* 121(3): 985–997.
2. Liu L, Allman WR, Coleman AS et al. (2018) Delayed onset of autoreactive antibody production and M2-skewed macrophages contribute to improved survival of TACI deficient MRLFas/Lpr mouse. *Scientific Reports* 8: 1308.
3. Iwata Y, Bostrom EA, Menke J et al. (2012) Aberrant macrophages mediate defective kidney repair that triggers nephritis in lupus-susceptible mice. *Journal of Immunology* 188: 4568–4580.
4. Li F, Yang YS, Zhu XH et al. (2015) Macrophage polarization modulates development of systemic lupus erythematosus. *Cellular Physiology and Biochemistry* 37: 1279–1288.
5. Deng W, Chen W, Zhang Z et al. (2015) Mesenchymal stem cells promote CD206 expression and phagocytic activity of macrophages through IL-6 in systemic lupus erythematosus. *Clinical Immunology* 161: 209–216.
6. Vilaiyuk S, Sirachainan N, Wanitkun S et al. (2013) Recurrent macrophage activation syndrome as the primary manifestation in systemic lupus erythematosus and the benefit of serial ferritin measurements: A case-based review. *Clinical Rheumatology* 32: 899–904.
7. Xiao X, Gaffer I, Guo P et al. (2014) M2 macrophages promote beta-cell proliferation by up-regulation of SMAD7. *Proceedings of the National Academy of Sciences of the United States of America* 111: E1211–E1220.
8. Mills CD (2012) M1 and M2 macrophages: Oracles of health and disease. *Critical Reviews in Immunology* 32: 463–488.
9. Nakayama W, Jinnin M, Makino K et al. (2012) CD163 expression is increased in the involved skin and sera of patients with systemic lupus erythematosus. *European Journal of Dermatology* 22(4): 512–517.
10. Zizzo G, Guerrieri J, Dittman LM et al. (2013) Circulating levels of soluble MER in lupus reflect M2c activation of monocytes/macrophages, autoantibody specificities and disease activity. *Arthritis Research & Therapy* 15: R212.
11. Mohammadi S, Saghaeian-Jazi M, Sedighi S et al. (2017) Immunomodulation in systemic lupus erythematosus: Induction of M2 population in monocyte-derived macrophages by pioglitazone. *Lupus* 26: 1318–1327.
12. Shao WH and Cohen PL (2011) Disturbances of apoptotic cell clearance in systemic lupus erythematosus. *Arthritis Research & Therapy* 13: 202.