Lactate induces alternative polarization (M2) of macrophages under lipopolysaccharide stimulation in vitro through G-protein coupled receptor 81

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To the Editor: Sepsis is a life-threatening systemic inflammatory response syndrome caused by the host’s maladjusted immune response to infection. Hyperlactatemia is an important manifestation of severe sepsis. Initial hyperlactatemia is associated with increased mortality in sepsis. [1] Hyperlactatemia and immunosuppression often occur simultaneously in the late stage of sepsis. G-protein-coupled receptor 81 (GPR81) is a cell-surface G-protein coupled receptor, which is activated by lactate. It has been reported that lactate inhibits the function of macrophages through GPR81, and alleviates liver injury in immune hepatitis. [2] In sepsis, alternative polarization (M2) of macrophages reduces the production of pro-inflammatory factors, weakens the phagocytosis of macrophages, and inhibits the immune response. GPR81 pathway is closely related to M2 polarization of macrophages. Therefore, we designed this experiment to explore the relationship between lactate and M2 of macrophages and further explored the relationship between this effect and the GPR81 pathway in sepsis.

In the first part of this experiment, raw 264.7 mouse macrophages (Shanghai Zhong Qiao Xin Zhou Biotechnology Company, Shanghai, China) were plated in 24-well polystyrene dishes. In control 1 group, macrophages were not treated with lipopolysaccharide (LPS; Sigma Aldrich, St. Louis, MI, USA) and lactate. In LPS group, macrophages were treated with LPS. In LPS + lactate group, macrophages were treated with LPS and lactate. LPS was used at 100 ng/mL in macrophages. In vitro incubations were performed for 24 h. Then, 10 mmol/L lactate (Sigma Aldrich) was added to the cells for 15 min and subsequently washed out, and then the cells were incubated for an additional 4 h before collection of the supernatant for enzyme-linked immunosorbent assay (ELISA) or of the cell lysate for Western blotting. In the second part of this experiment, all macrophages were treated with LPS and lactate. In GPR81(-) group, macrophages were plated in 24-well dishes and treated with 10 µmol/L Silencer Select siRNA targeting GPR81 complexed with Lipofectamine (Santa Cruz Biotechnology Inc, Delaware Ave, CA, USA). In control 2 group, macrophages were treated with 10 µmol/L scrambled siRNA complexed with Lipofectamine. The siRNA treatment was repeated 24 h later. The cells were cultured for 24 h after the second siRNA treatment. Then the cells were treated with LPS and lactate. Primary antibodies for Western blotting were a goat polyclonal antibody to GPR81 (1:250) (Santa Cruz Biotechnology Inc) and a mouse monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase (1:2000; Abcam Company, Cambridge, MA, USA). The supernatant was assessed for tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-10, chemokine (C-C motif) ligand (CCL)-3, CCL-20, CCL-22, and CCL-24 levels using commercially available ELISA kits. The TNF-α, IL-6, IL-10, CCL-3, CCL-20, and CCL-22 ELISA kit was purchased from the MultiSciences Biotech Company (Zhejiang, China). The mouse CCL-24 ELISA kit was purchased from the Abcam Company.

In our study, the levels of TNF-α, IL-6, CCL-3, and CCL-20 were increased significantly under LPS stimulation. Lactate induced expression of GPR81 in macrophages under LPS stimulation. Lactate reduced the secretion of TNF-α and IL-6 by macrophages under LPS stimulation. Lactate decreased the expression levels of CCL-3 and CCL-20 in macrophages under LPS stimulation but increased the expression levels of CCL-22 and CCL-24. After intervention with siRNA against GPR81, all immunosuppressive effects caused by lactate were weakened [Figure 1].

Our results preliminarily confirmed that systemic hyper-inflammation exists in sepsis. Sepsis is characterized by an excessive inflammatory response in the early stage and...
immunosuppression in the late stage. Most sepsis patients die of immunosuppression. In patients with sepsis, the expression levels of human leukocyte antigen decreased significantly (<30%), the ability to present antigen decreases, and the ability to produce pro-inflammatory factors decreases significantly. Stimulated by LPS, monocytes, and macrophages release IL-10 and other immunosuppressive molecules in large quantities. These

Figure 1: Effects of lactate on macrophage M1 and M2 polarization and the role of GPR81. Part 1: Effects of lactate on macrophage M1 and M2 polarization. Part 2: The role of GPR81 in effects of lactate on macrophage M1 and M2 polarization. In control 1 group, macrophages were not treated with LPS and lactate. In LPS group, macrophages were treated with LPS. In LPS + Lactate group, macrophages were treated with LPS and lactate. In control 2 and GPR81(−/−) group, macrophages were treated with LPS and lactate. Results are presented as mean ± standard error (n=3). ∗P < 0.001, †P < 0.01, ‡P < 0.05. CCL: Chemokine (C-C motif) ligand; GPR: G-protein-coupled receptor; IL: Interleukin; LPS: Lipopolysaccharide; TNF: Tumor necrosis factor.
Many cells in the surrounding microenvironment have an immunosuppressive effect on the outcome. Increasing evidence shows that lactate produced by tumor cells has an immunosuppressive effect on anti-inflammatory treatment cannot change the immunosuppressive state and does not significantly improve the outcome. Increasing evidence shows that lactate produced by tumor cells has an immunosuppressive effect on many cells in the surrounding microenvironment. The role of lactate in immunosuppression and its mechanism are of great significance for the treatment of sepsis. M2 polarization of macrophages increased during infection, which was associated with LPS. When infection leads to changes in the body micro-environment, monocytes and macrophages undergo classical (M1) and alternative (M2) polarization. LPS often induces M1 polarization of mononuclear macrophages and promotes inflammation. LPS can also induce LPS tolerance. The production of LPS tolerance is closely related to M2 polarization of mononuclear macrophages. M2 polarized monocytes have reduced production of pro-inflammatory factors, have weakened phagocytosis, and induce immunosuppression.

Finally, we investigated the relationship between GRP81 and the effects of lactate on the immune status under LPS stimulation. In our study, lactate induced expression of GRP81 in macrophages under LPS stimulation. After intervention with siRNA against GRP81, the expression levels of CCL-3 and CCL-20 in macrophages were significantly increased, while the expression levels of CCL-22 and CCL-24 were significantly decreased under LPS + lactate intervention. All immunosuppressive effects caused by lactate were weakened. These results demonstrated that lactate might lead to M2 polarization of macrophages through GRP81. Intervention with GRP81 might be an effective immunomodulatory therapy in sepsis. Several GPRs have recently been identified to negatively regulate Toll-like receptor-mediated inflammation through interactions with the intracellular proteins. The most widely studied function of GRP81 is its ability to protect tissues from injury as observed in mouse models of hepatic, pancreatic, and brain injury. Studies have shown that lactate down-regulates Toll-like receptor 4-mediated nuclear factor kappa-B activation, IL-1 release, and caspase 1 cleavage through GRP81 in macrophages in vitro. Lactate alleviates inflammation and organ injury through GRP81 in immune hepatitis mice. M2 polarization of macrophages is closely related to inflammation. Our study demonstrated that lactate might lead to M2 polarization of macrophages through GRP81.

Plasma levels of TNF-α and IL-6 were significantly increased in septic shock with hyperlactatemia, while the levels of TNF-α and IL-6 in the supernatant were significantly decreased when stimulated with lactate in our study. The reason may be that septic shock is a complex pathophysiologic process, and the mechanism of TNF-α and IL-6 elevation in vivo has not played a role in vitro experiments.

In summary, after intervention with lactate, M2 polarization of macrophages increased, while M1 polarization of macrophages decreased under LPS stimulation. After knockdown of GRP81, immunosuppressive effects caused by lactate were weakened. These results demonstrate that lactate leads to M2 polarization of macrophages through GRP81 under LPS stimulation. More specific mechanisms require further research.

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Conflicts of interest
None.

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