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# Role of oxidized LDL-induced “trained macrophages” in the pathogenesis of COVID-19 and benefits of pioglitazone: A hypothesis

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## ABSTRACT

**Background and aims:** Older adults and people who have cardiovascular disorders (their common pathogenetic mechanism is progressive atherosclerosis) are at higher risk for severe illness from COVID-19 (coronavirus disease 2019). Their common pathogenetic mechanism is progressive atherosclerosis in which oxLDL (oxidized LDL) plays major role. Receptor-mediated uptake of oxLDL by the monocyte-derived macrophages activates the long-term epigenetic reprogramming of innate immunity, which is termed “trained immunity.” The aim of this work is to investigate the mechanisms and treatment possibilities that can control the activities of these specific macrophages.

**Methods:** Search in Medline and PubMed relevant articles on the trained immunity and cytokine storm of COVID-19.

**Results and Conclusions:** When oxLDL-trained macrophages encounter SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) in the lung, it causes unregulated cytokine secretion, leading to the alveolar damage. Therefore, blocking macrophage training by pioglitazone, a thiazolidinedione, could control the hyperactivation that the virus would trigger.

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Older patients with hypertension or diabetes, which are critical disorders for the development of atherosclerotic plaque, a leading cause of cardiovascular diseases, are more likely to develop severe COVID-19 [1]. After all, they have weaker immune systems. But here is the puzzling thing: it is often not the SARS-CoV-2 per se that is so lethal, but the reaction of immune system to it. In some people, the immune system goes into overdrive and creates what is known as a “cytokine storm”, which is often deadly. Therefore, delineating the mechanisms behind these chronic diseases may provide clues for the clinical management of the severe COVID-19.

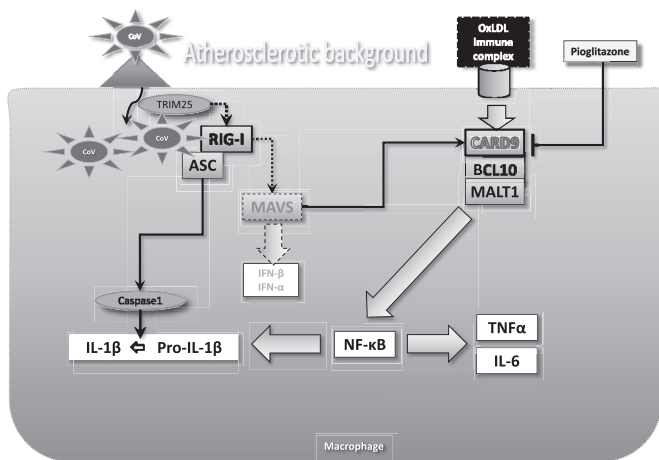
In atherosclerosis, macrophages respond with the production of a large variety of pro-atherogenic cytokines and chemokines upon stimulation with oxidized low density lipoprotein (oxLDL). Brief encounters of monocytes with pro-atherogenic stimuli can induce a long-lasting inflammatory monocyte/macrophage phenotype in the circulation. This innate immune memory has been termed “trained innate immunity” that is present in patients with atherosclerosis or associated risk factors [2]. Training with oxLDL stimulates the epigenetic reprogramming at the level of histone modifications at the promoters of TNF $\alpha$  (tumor necrosis factor alpha), IL-6 (interleukin 6), IL-8, and CD36 (cluster of differentiation

36). Importantly, CD36, one of the major scavenger receptors, is responsible for the recognition and internalization of oxLDL [3].

Acute lung injury can result from the local generation of reactive oxygen species (ROS) and the subsequent formation of oxidized phospholipids. Lung has a large surface area that is exposed to the aerobic environment and thus is a highly susceptible site to oxidative events for such lipid modifications. In SARS-CoV-infected human cases, marked production of oxidized lipids are observed in the inflammatory exudates lining the injured air spaces, pneumocytes, as well as macrophages [4]. Consequently, oxLDL-trained macrophages encounter with the huge amount of oxidized lipids in the virus-infected areas and exhibit greater oxidized lipid uptake, leading to transient lipid depletion [5]. Intriguingly, Hu et al. demonstrated that the serum lipid levels, especially total cholesterol, HDL-cholesterol and LDL-cholesterol in patients with COVID-19 infection were significantly lower [6] (Fig. 1).

In fact, up to 90% of circulating oxLDL can be found in oxLDL immune complexes (oxLICs). oxLIC-mediated inflammasome priming in macrophages is CARD9 (caspase recruitment domain-containing protein 9)-dependent. CARD9 is critical adaptor protein and a central integrator in innate immune cell activation that

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**Fig. 1.** After receptor-mediated uptake, oxLDL-immune complex stimulates CBM signalosome, leading to the NF- $\kappa$ B activation. NF- $\kappa$ B transcription factor increases the expression of cytokines, including TNF $\alpha$ , IL-6, and Pro-IL-1 $\beta$ . Cytosolic SARS-CoV binds to TRIM25, deactivating its RIG-I ubiquitinating function. While SARS-CoV inhibits type I IFN production, it could stimulate major inflammasome adaptor ASC to activate caspase-1. As an IL-1 $\beta$ -converting enzyme, caspase-1 cleaves pro-IL-1 $\beta$  to mature IL-1 $\beta$ , an important mediator of inflammation. Pioglitazone may suppress the activity of the major constituent of CBM complex, CARD9; thus, may inhibit the NF- $\kappa$ B activation.

triggers the inflammatory signaling pathway in response to viral infection. CARD9 signalosome, which is a tripartite protein complex formed from CARD9, BCL10 (B-cell lymphoma/leukemia 10), and MALT1 (mucosa-associated lymphoid tissue lymphoma translocation protein 1) (CBM complex), leads to the activation of NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and mitogen-activated protein kinase (MAPK) signaling, resulting upregulation of several cytokines and chemokines [7].

In alveolar epithelial cells, the cytoplasmic virus receptor RIG-I (retinoic acid-inducible gene I) directly recognizes and binds SARS-CoV RNA. Viral infection-induced conformational change in RIG-I makes it susceptible to the polyubiquitination and activation by the E3 ubiquitin ligase, TRIM25 (tripartite motif-containing protein 25) [8]. However, SARS-CoV binds TRIM25 and suppresses RIG-I activation, and subsequently type I interferon production as well [9]. SARS-CoV-induced delayed type I interferon response promotes high initial virus titers and further aberrant inflammatory monocyte-macrophages recruitment [10].

In SARS-CoV-infected macrophages, although RIG-I cannot activate immunity, it engages the CARD9-BCL10 module for NF- $\kappa$ B activation and triggers the major inflammasome adaptor protein, ASC (apoptosis-associated speck-like protein containing a CARD) [11]. Consequently, while SARS-CoV-infection in oxLDL-trained macrophages cannot produce protective type I interferons, they readily potentiates existing low-grade inflammation through ASC-mediated caspase-1 activation [10,11]. Consequently, this dysregulated cytokine response can lead to a hyper-inflammatory condition called “cytokine storm”, which may lead to the lung and other organ injuries [10 (and references therein)].

Accumulating evidence indicate that peroxisome proliferator-activated receptor-gamma (PPAR- $\gamma$ ) is expressed in different immune cells, such as macrophages, dendritic cells, and T cells. It has been shown that PPAR $\gamma$  activation by its high-affinity synthetic

ligand, troglitazone, impairs nuclear localization of NF- $\kappa$ B through inhibition of CARD9 expression [12]. However, troglitazone was withdrawn from the market in 2000 due to concerns over increasing reports of serious hepatic adverse events. Pioglitazone is another available thiazolidinedione that also inhibits the activation of NF- $\kappa$ B and MAPK pathways by reducing the expression of CARD9 [10].

Although the trained innate immunity is undoubtedly beneficial in the context of recurrent infections and vaccination, it might be detrimental in the context of chronic inflammatory disorders, such as oxLDL- and lipoprotein (a)-induced atherogenic pathologies. When these trained macrophages encounter a severe viral threat, such as SARS-CoV-2, the inflammatory response is increased uncontrollably. All COVID-19 patients who develop severe respiratory failure display hyper-inflammatory responses with features of either immune dysregulation or macrophage activation syndrome. Pioglitazone can control immune-inflammatory flood by blocking the protein (CARD9) at the centre of the immune activation mechanism in macrophages [10].

The anti-inflammatory and insulin-sensitizing activities of pioglitazone are two distinctly separable effects. A sub-therapeutic dose of pioglitazone produces anti-inflammatory effects by suppressing the CARD9-stimulated TNF $\alpha$ , IL-1 $\beta$ , and IL-6 production without altering metabolic parameters. The role of low dose pioglitazone needs to be studied in COVID-19 [10].

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## References

- [1] Cao X. COVID-19: immunopathology and its implications for therapy [published online ahead of print, 2020 Apr 9] *Nat Rev Immunol* 2020. <https://doi.org/10.1038/s41577-020-0308-3>.
- [2] Netea MG, Joosten LA, Latz E, et al. Trained immunity: a program of innate immune memory in health and disease. *Science* 2016;352:aaf1098. <https://doi.org/10.1126/science.aaf1098>.
- [3] Groh L, Keating ST, Joosten LAB, Netea MG, Riksen NP. Monocyte and macrophage immunometabolism in atherosclerosis. *Semin Immunopathol* 2018;40:203–14. <https://doi.org/10.1007/s00281-017-0656-7>.
- [4] Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell* 2008;133:235–49. <https://doi.org/10.1016/j.cell.2008.02.043>.
- [5] Levitan I, Shentu TP. Impact of oxLDL on cholesterol-rich membrane rafts. *J Lipids* 2011;2011:730209. <https://doi.org/10.1155/2011/730209>.
- [6] Hu, X, Chen D, Wu L, He G, Ye W. Low Serum Cholesterol level among patients with COVID-19 infection in Wenzhou, China (February 21, 2020). Available at: SSRN: <https://ssrn.com/abstract=3544826>.
- [7] Zhong X, Chen B, Yang L, Yang Z. Molecular and physiological roles of the adaptor protein CARD9 in immunity. *Cell Death Dis* 2018;9:52. <https://doi.org/10.1038/s41419-017-0084-6>.
- [8] Gack MU, Shin YC, Joo CH, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature* 2007;446:916–20.
- [9] Hu Y, Li W, Gao T, et al. The severe acute respiratory syndrome coronavirus nucleocapsid inhibits type I interferon production by interfering with TRIM25-mediated RIG-I ubiquitination. *J Virol* 2017;91. e02143-16.
- [10] Erol A. Pioglitazone treatment for the COVID-19-associated cytokine storm. *OSF Preprints* doi:10.31219/osf.io/avfpp.
- [11] Poeck H, Bscheider M, Gross O, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol* 2010;11:63–9. <https://doi.org/10.1038/ni.1824>.
- [12] Kock G, Bringmann A, Held SA, Daecke S, Heine A, Brossart P. Regulation of dendritic cell activation by peroxisome proliferator-activated receptor-gamma ligand troglitazone. *Blood* 2011;117:3569–74. <https://doi.org/10.1182/blood-2010-08-302224>.