Cytokine Storm - A marker for uncontrolled innate immune responses

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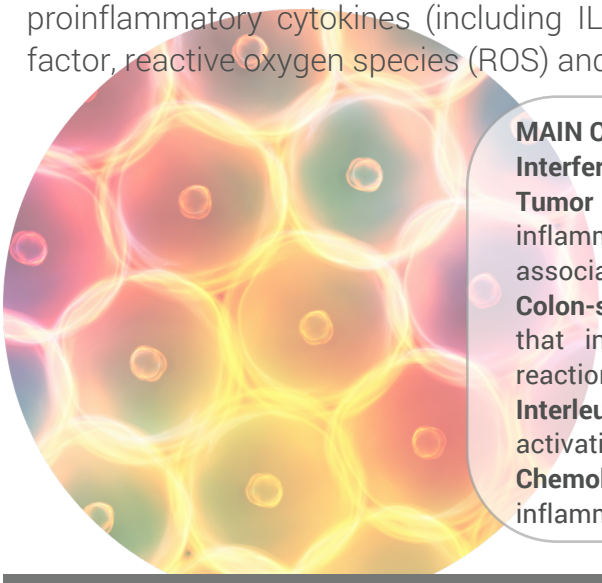
When well-controlled, the innate immune response is the first line of defence of infections. However, excessive responses cause damage to the body. The term “cytokine storm” portraits an immune response out of control. Cytokine storms are associated with various infectious diseases including COVID-19 and sepsis as well as graft-versus-host disease, inflammatory and autoimmune diseases and has also been a consequence of therapeutic interventions. Inflammation associated with a cytokine storm starts locally and then progresses into a systemic response. Even if the term goes way back, there is still a lack of understanding of the molecular mechansims underlying the concept and its contribution to pathogenesis.

Recently, much attention has been given the role of proinflammatory cytokines in pathology during viral infections and the link to disease severity in Acute Respiratory Distress Syndrome (ARDS), one of the major causes of death in COVID-19 patients. ARDS as a consequence of influenza (including avian influenza - H5N1) or corona viruses (Including SARS and MERS CoVs) is characterized by accumulaiton of inflammatory cells, edema formation and marked increase in cytokines. It has been shown that following a viral infection, low levels of antiviral interferons and high levels of proinflammatory cytokines (IL-1b, IL-6, TNFa and chemokines) are produced. This response is accompanied by increased levels of neutrophils and monocytes in blood and tissue. Several proinflammatory cytokines (including IL-6, IL-8, IL-1b), granulocyte-macrophage colony-stimulating factor, reactive oxygen species (ROS) and chemokines contribute to ARDS.



MAIN COMPONENTS OF CYTOKINE STORM

Interferons - a family of cytokines central in virus-directed innate immunity.

Tumor necrosis factor a (TNFa) - a pyrogen cytokine produced in acute inflammation and infection. It has a central role in viral diseases and is associated with inflammatory and autoimmune diseases.

Colon-stimulating factors (CSF) - components of an ampification cascade that increases cytokine production and perpetuates the inflammatory reaction.

Interleukins - a family of cytokines involved in immune cell differantiation and activation. Mediate traffic of immune cells to the site of infection.

Chemokines - a family of cytokines with a strong chemotactic effect. Recruits inflammatory cells from intravascular space into the inflammation site.

CYTOKINE STORM IN COVID-19 PATIENTS

Cytokine storm is considered to be one of the major causes of ARDS and multiple-organ failure in critically ill COVID-19 patients, with increased serum levels of cytokines and a positive correlation with mortality. In COVID-19 patients, high expression of Th1 cytokines such as IL-1b, IFN-g, IP-10 and MCP-1 has been detected but also Th2 cytokines are involved including IL-4 and IL-10. Serum levels of IL-6 are increased in COVID-19 patients and positively related to disease severity. Additionally, IL-6 is suggsted also to be responsible for the observed increased Th17 cell actvation in COVID-19 patients.

Read more:

The pathogenesis and treatment fo the “Cytokine Storm” in COVID-19. Ye et al. *J of Infection* (2020)

Identfication of Oxidative Stress and Toll-like Receptor 4 signaling as a Key Pathway of Acute Lung Injury. Imai et al. *Cell* (2008)

The cytokine storm in COVID-19: An overview of the involvement of the chemokine/kemokine-receptor system. Coperchini et al. *Cytokines adn Growth factor Revirews* (2020)

In vitro 3D models of cytokine storm

- Evaluation of effect of novel drugs in acute inflammatory responses

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Cytokine storm is associated with various infectious diseases including COVID-19 and sepsis as well as graft-versus-host disease, inflammatory and autoimmune diseases. Like most other inflammatory processes the cytokine release is complex and complicated to model experimentally. There is no single assay that can completely model all aspects of the process. However, combining different approaches and carefully designing the experimental setup our models are an excellent tool for evaluation of novel therapies.

In our 3D in vitro model using human whole blood a massive cytokine release is experimentally induced in by activating the same pathways activated by invading pathogens. By culturing the cells in a 3D structure, the cells and soluble inflammatory mediators are allowed to interact in a more physiologically relevant setting. The assay have a fast kinetic and model the acute phase of the cytokine storm.

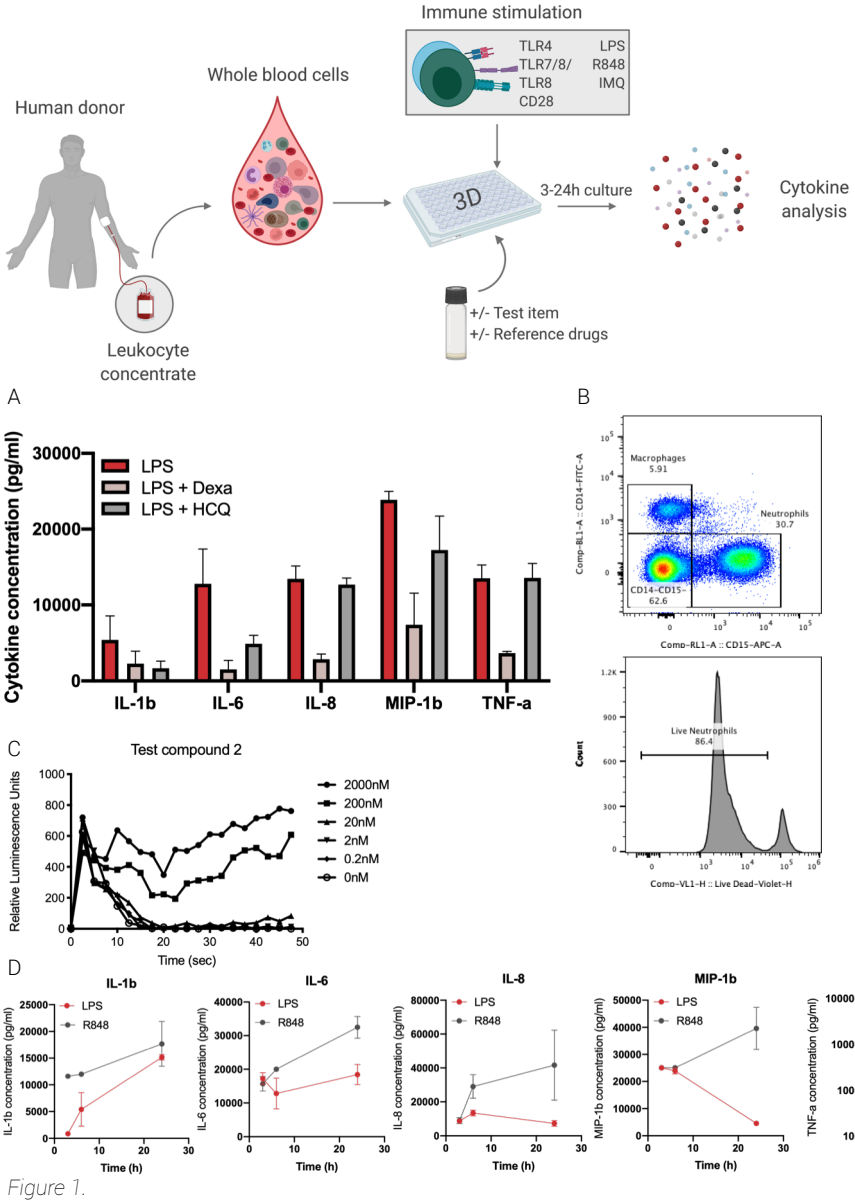


Figure 1.
A) Cytokine response after LPS activation of whole blood from 3 donors (3D plate) for 6 hours. Dexamethasone and Hydrochloroquinine were used as reference compounds. B) Representative staining of neutrophils and macrophages and analysis of viability following 6 hour incubation. C) Level and kinetics of ROS production induced by test item. D) Kinetics of cytokine production in response to different stimuli.

LPS - toxin routinely used to activate a systemic inflammatory response through Toll Like Receptor 4 (TLR4). Often used to model the effect of bacterial infection and sepsis.

Resiquimod (R-848) - agonist of TLR7/8. Mimics the cytokine response induced by single stranded RNA and viral infections. In light of the recent identification of cytokine storm as an underlying cause of **ARDS** in **COVID-19** infections, this model is attractive to evaluate effects on the acute inflammatory response. Validated reference compounds includes both Dexamethasone and Hydrochloroquinine.

Cytokine storm can also be induced by **superantigens** including anti-CD3 and anti-CD28 antibodies. Binding of superagonist antibodies stimulates T cells without antigen-receptor stimulation and the model can be adapted to investigate potential **drug induced cytokine storm**.

3D CELL CULTURE SYSTEMS by CELLEVATE

Cellevate develops the next generation cell culture systems based on nanomaterials. Culture of cells in a network of nanofibers allows them to proliferate and interact in conditions resembling the in vivo environment. Thus, more predictive, reliable and translatable data can be generated. The usage of 3D cultures in cell assays can provide more realistic in vitro models for data, with more clinical relevance and successful research in drug discovery.

Cellevate

Read more:

The pathogenesis and treatment fo the "Cytokine Storm" in COVID-19. Ye et al. *J of Infection* (2020)
Into the eye of the Cytokine storm. Tisoncik et al. *Microbiology and Molecular biology reviews* (2012)

Illustration created by Redoxis AB using BioRender.com (2020).

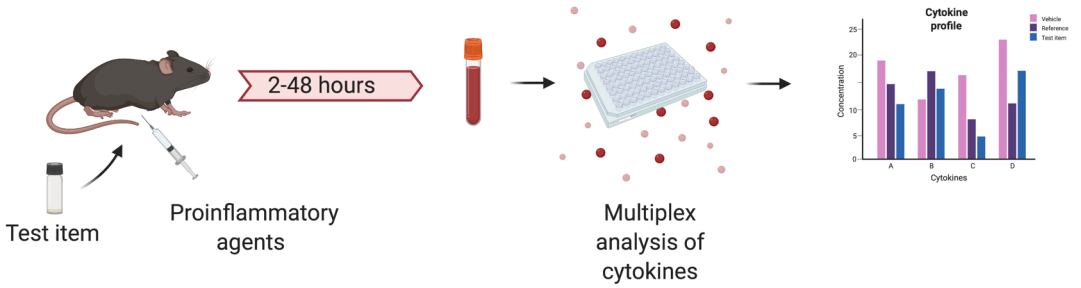
Experimental models of cytokine storm

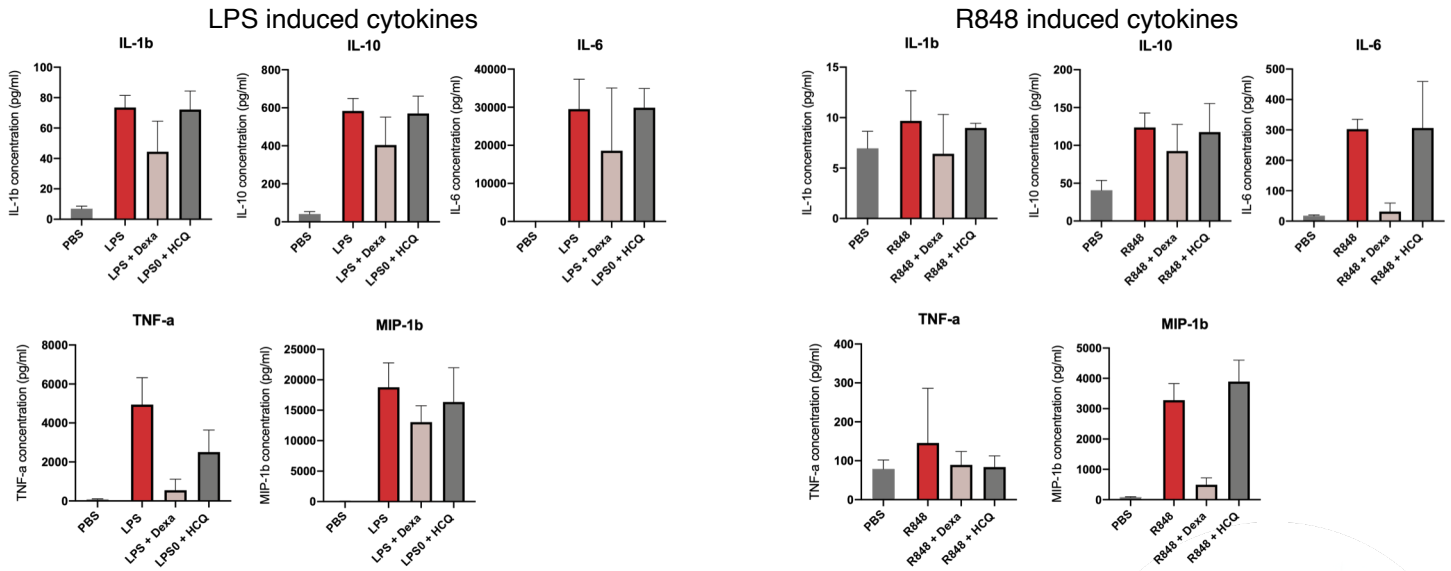
- In vivo models of cytokine release for evalution of acute innate immune responses

Administration of the endotoxin Lipopolysaccharide (LPS) or other TLR stimulating agents to mice induce acute inflammatory responses similar to the inflammatory response that occures during the early stages of septic shock and also seen in acute systemic responses in ARDS in COVID-19 patients. Shortly after administration of endotoxin, cytokines and chemokines including TNF-a and IL-6 are released. This model is a fast and cost-effective model for screening of anti-inflammatory properties of test items aimed for treatment of inflammatory and autoimmune conditions.

A massive cytokine release can be experimentally induced in serval ways. Toxic agents activating Pattern Recognition Receptors (PPRs) such as Toll Like Receptors (TLRs) are often used to model the effect of invading pathogens. LPS is one such toxin that is routinly used to activate a systemic inflammatory resonse. These models have fast kinetics and model the acute phase of the cytokine storm. LPS activates TLR4 and mimics aspects of a bacterial infection. This model is often used as an experimental model for sepsis.

Resiquimod (R-848) is an agonist to TLR7 and TLR8 and mimics the cytokine response induced by single stranded RNA and viral infections. Cytokine storm can also be induced by superantigens including anti-CD3 and anti-CD28 antibodies. Binding of superagonist antibodies stimulates T cells without antigen-receptor stimulation.





EXPERIMENTAL OUTLINE	
Disease induction protocol:	LPS/R8484 i.p hour 0
Strain:	C57BL/6, CD1
Suggested group size:	5 mice/group
Duration:	2 hours
Test item:	Pre/post induction
Read outs:	Cytokine profile
Positive controls:	Dexamethasone, HCQ, Cyclosporine, Anti-TNFa

Read more:

The pathogenesis and treatment fo the "Cytokine Storm" in COVID-19. Ye et al. *J of Infection* (2020)

Mouse models of Sepsis and Septic Shock. Korneev . *Molecular Biology* (2019)

Comprehensive comparison of three different animal models for systemic inflammation. *Seemann et al. J of Biomedical Sci.* (2017)

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