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Trabajo Fin de Grado

IDENTIFICATION OF PATHWAYS FOR CYTOKINE STORM CONTROL

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RESUMEN

La tormenta de citoquinas es una reacción exagerada y descontrolada del sistema inmunológico que consiste en una retroalimentación positiva entre las citoquinas y las células del sistema inmune. Enfermedades como el HLH, Ébola y, actualmente, Covid-19, desarrollan la tormenta de citoquinas en los casos más graves.

Desarrollar vías para su control es esencial para evitar que cause fallos multiórganicos y, en última instancia, la muerte en los pacientes que la desarrollan.

Mediante el estudio de los tratamientos utilizados en enfermedades previas (HLH y Ébola) se pueden desarrollar vías efectivas del control de la tormenta de citoquinas en el Covid-19.

ABSTRACT

Cytokine storm is an uncontrolled overreaction of the immune system consisting of positive feedback between cytokines and immune system cells. Diseases such as HLH, Ebola and, currently, Covid-19, develop cytokine storm in severe cases.

Developing ways to control it is essential to prevent it from causing multi-organ failure and ultimately death in patients who develop it.

By studying treatments used in previous diseases (HLH and Ebola), effective ways of controlling the cytokine storm in Covid-19 can be developed.

RESUMEN INTRODUCCIÓN Y CONCLUSIONES.

La tormenta de citoquinas es un síndrome inflamatorio sistémico potencialmente mortal que implica niveles elevados de citoquinas circulantes e hiperactivación de las células inmunitarias. Puede ser desencadenado por diversas terapias, agentes patógenos, cánceres, enfermedades autoinmunes y trastornos monogénicos.

Una de las primeras terapias dirigidas para la abolición de la tormenta de citoquinas fue el antireceptor de interleucina-6, un anticuerpo monoclonal denominado tocilizumab, que se desarrolló para el tratamiento de la enfermedad de Castleman idiopática multicéntrica en la década de 1990. Se han descrito otros trastornos que causan tormenta de citoquinas y se han tratado con terapias inmunodirigidas, como la sepsis, la linfohistiocitosis hemofagocítica primaria y secundaria (HLH), los trastornos autoinflamatorios y la enfermedad por coronavirus 2019 (Covid-19).

Aunque se ha avanzado en la comprensión de las bases mecánicas de la iniciación y propagación de la tormenta de citoquinas, sigue habiendo una gran necesidad de terapias eficaces, una mejor comprensión de la etiopatogenia y la identificación de biomarcadores para predecir la respuesta al tratamiento y el pronóstico, a fin de permitir un enfoque de tratamiento estratificado y, en última instancia, de medicina de precisión.

A lo largo de este trabajo se ha llegado a la conclusión de que la tormenta de citoquinas podría ser una de las complicaciones más importantes y mortales en pacientes graves con HLH, Ébola y Covid-19.

Al dilucidar el papel clave de las citoquinas en el desarrollo de la fisiopatología de la enfermedad, se han puesto a disposición nuevas vías de control de la tormenta de citoquinas. Por un lado, se desarrollan terapias dirigidas directamente a las propias citoquinas. Alternativamente, la acción de las citoquinas podría inhibirse bloqueando las vías de señalización con inhibidores de JAK.

La supresión de la liberación de citoquinas en una fase temprana de la enfermedad sigue siendo controvertida. Sin embargo, el conocimiento de la fisiopatología de enfermedades como el HLH y el Ébola ha llevado al desarrollo de buenos enfoques para el tratamiento de los casos graves de SARS-CoV-2.

1. INTRODUCTION.

Cytokine storm is a life-threatening systemic inflammatory syndrome involving elevated levels of circulating cytokines and immune-cell hyperactivation that can be triggered by various therapies, pathogens, cancers, autoimmune conditions, and monogenic disorders (Fajgenbaum and June, 2020). Cytokine storm syndromes can lead to constitutional symptoms, systemic inflammation, and multiorgan dysfunction that can rapidly produce multiorgan failure and death if they are not promptly and adequately treated.

One of the earliest targeted therapies for abrogation of a cytokine storm was the anti–interleukin-6 receptor monoclonal antibody tocilizumab, which was developed for the treatment of idiopathic multicentric Castleman's disease in the 1990s. Other disorders have been described as causes of cytokine storm and targeted with immune-directed therapies, such as sepsis, primary and secondary hemophagocytic lymphohistiocytosis (HLH), autoinflammatory disorders, and coronavirus disease 2019 (Covid-19) (Fajgenbaum and June, 2020).

Although there has been an advancement in understanding the mechanistic basis for the initiation and propagation of cytokine storm syndromes, it remains a considerable unmet need for effective therapies, better understanding of the aetiopathogenesis, and identification of biomarkers to predict treatment response and prognosis to enable a stratified and ultimately precision medicine treatment approach (Puja Mehta et al., 2020).

2. HYPOTHESIS.

SARS-CoV-2 and the generated severe acute respiratory syndrome have reminded the importance of cytokine storm role, a form of uncontrolled systemic inflammatory reaction that can be triggered by a variety of factors. Studies have been carried out with the aim of controlling this unrestrained reaction that is triggered by a variety of diseases.

Currently, SARS-CoV-2 pandemic has forced further development of treatments for cytokine storm, due to its development in severe SARS-CoV-2 cases.

This project aims to find ways to control the development of cytokine storm in SARS-CoV-2 by studying it in previous diseases such as haemophagocytic lymphohistiocytosis (HLH) and Ebola.

3. OBJECTIVES.

- General objective.

Study the control of cytokine storm in SARS-CoV-2 by analysing this syndrome in previous diseases.

- Specific objectives.

1) Understand the molecular biology of cytokines and the development of the cytokine storm.
2) Describe the pathophysiology and control of the cytokine storm in HLH and Ebola.
3) Establish a possible control of cytokine storm based on previous studies of the three diseases mentioned (HLH and Ebola).

4. METHODOLOGY.

The bibliographic search for this systematic review followed the PRISMA (Preferred Reporting Items for Systematic reviews and MetaAnalyses) statement.

The bibliographic search began in February 2021 (15-02-2021), and the process was completed in April (30-04-2021) of the same year. The databases used to search for articles were PubMed and Web of Science.

The method followed for the selection of articles was the same in all databases. In addition, two books (Introduction to Human Immunology; and Cellular and Molecular Immunology) were selected for the development of section 5.

For the bibliographic review, the criteria on which the search was based were the interest in addressing the issues mentioned in the objectives. To this end, inclusion and exclusion criteria were established:

Articles published from 2015 onwards, preferably selecting those published in recent years. The language selected for the articles was English. In addition, only "Full text"

articles were selected. Exclusion criteria included: Articles prior to 2015 (with the exception of some articles used for the description in section 5, selected due to lack of information in recent articles), articles in other languages and those that did not have information of "cytokine storm control/treatment".

Search strategy:

The keywords used were: 'cytokine storm', 'control of cytokine storm', 'cytokines', 'signaling pathways', 'MAPK', 'JAK/STAT', 'Haemophagocytic lymphohistiocytosis', 'Ebola', 'SARS-CoV-2' and 'severe acute respiratory syndrome', using booleans 'AND' and 'OR' to relate terms.

Following the inclusion and exclusion criteria mentioned above, the selection of articles was divided into three phases:

- Pre-selection of articles based on their title.
- Reading the abstract, eliminating those that did not cover the necessary information.
- In-depth reading of the articles, eliminating those that were less relevant or far from the main objective of the study.

The route followed is detailed in the figure below:

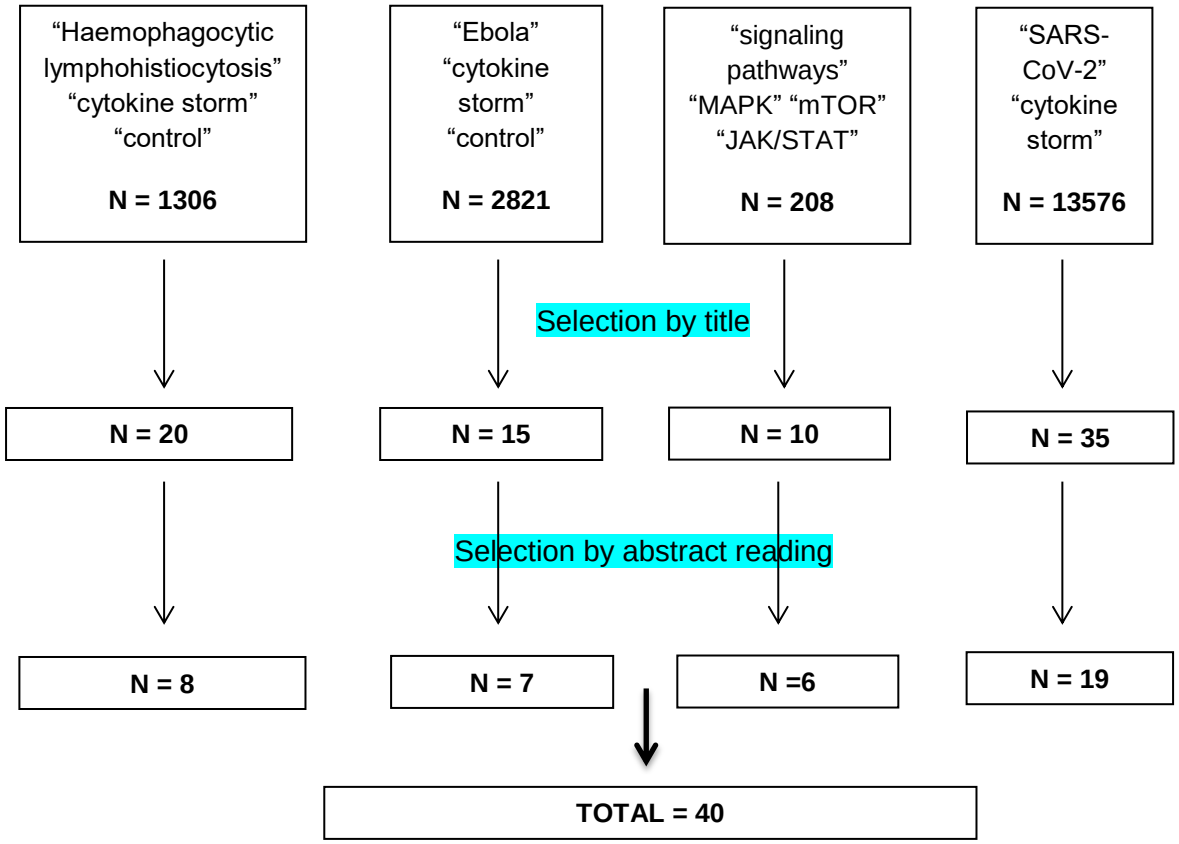

Figure 3.1. Selection process flowchart.

5. CYTOKINES.

Cytokines comprise a heterogeneous group of low molecular weight molecules that modulate the activity of the immune system. They are produced and secreted by various cellular types, some of them belong to the immune system. Cytokines mediate their activity through interaction with high-affinity specific cellular receptors expressed by target cells. This interaction leads to signal transduction that ultimately results in the expression of an altered gene pattern. Depending on the cytokine and the properties of the target cell, suppression or exacerbation of the genetic expression of one or more genes can be observed (Fainboim and Geffner, 2005).

In the activation phase of adaptive immune responses, cytokines are responsible for stimulating the growth and differentiation of lymphocytes, while in the effector phases of innate and adaptive immunity they activate different effector cells to eliminate microorganisms and other antigens.

Cytokines often act in an autocrine mode, binding to receptors expressed on the secreting cell, or in a paracrine mode, binding to receptors expressed on cells in their immediate environment. Although is not common, they can also act in an endocrine form, modulating the activity of cells at distant sites.

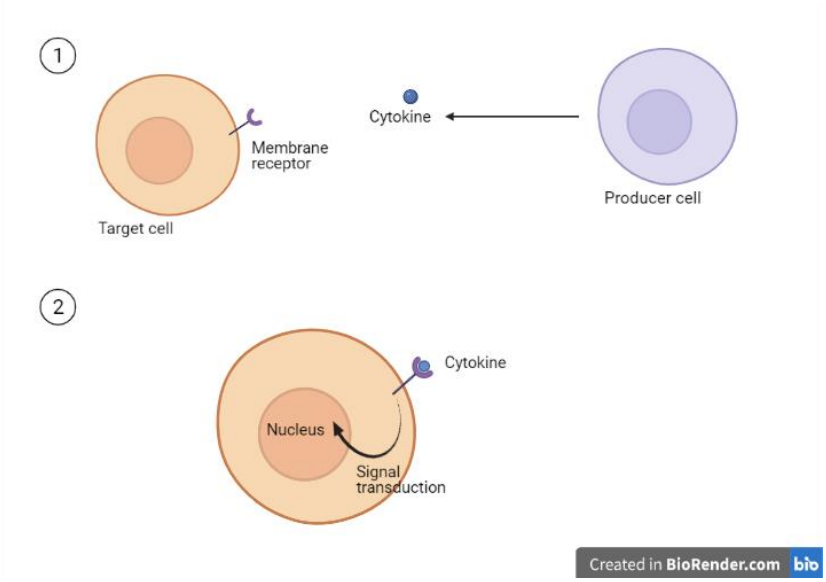

Figure 5.1. Biological activity of cytokines via membrane receptors and subsequent signal transduction. Created with BioRender.com

5.1. Cytokine properties.

Although cytokines are diverse in structure, they share a number of properties:

- Cytokine secretion is a brief, self-limiting event. Cytokines are not stored as pre-formed molecules and their synthesis is initiated by the transcription of new genes due to cellular activation (Abbas and Lichtman, 2004). This transcriptional activation is temporary and the messenger RNA that encodes the most of cytokines is unstable, so cytokine synthesis is also transient. In addition, the production of some cytokines may be is controlled by RNA processing and post-transcriptional mechanisms, such as proteolytic release of an active product from an inactive precursor.
- The actions of cytokines are often pleiotropic and redundant. Pleiotropism refers to the ability of a cytokine to mediate different biological actions, acting on different target cells. Redundancy refers to the ability of different cytokines to mediate the same biological action. Because of this redundancy, antagonists of a particular cytokine or mutation of a cytokine gene may have no functional consequences, as other cytokines compensate for it.
- Cytokines often influence the synthesis and actions of other cytokines. The ability of one cytokine to stimulate the synthesis of others produces a cascade. In some cases two cytokines may antagonise each other and this may produce a greater effect than expected (synergy) (Abbas and Lichtman, 2004).
- The actions of cytokines can be local and systemic.
- Cytokines initiate their actions when they bind to specific membrane receptors on target cells.
- External signals regulate the expression of cytokine receptors and, therefore, the response of cells to cytokines. For example, stimulation of T or B lymphocytes by antigens results in increased expression of cytokine receptors. For this reason, during an immune response, it is the antigen-specific lymphocytes that preferentially respond to the secreted cytokines. This mechanism maintains the specificity of immune responses, even if the cytokines themselves are not antigen-specific (Abbas and Lichtman, 2004).

Cellular responses to most cytokines consist of changes in target cell differentiation and proliferation. Many of the changes in gene expression induced by cytokines

result in differentiation of T and B lymphocytes and activation of effector cells, such as macrophages (Abbas and Lichtman, 2004). For example, cytokines stimulate antibody isotype change in B cells, differentiation of T-helper cells into TH1 and TH2 subpopulations and activation of phagocyte microbicidal mechanisms. Chemokines and tumour necrosis factor (TNF) do not act at the genetic level, they induce apoptosis by activation of cellular enzymes.

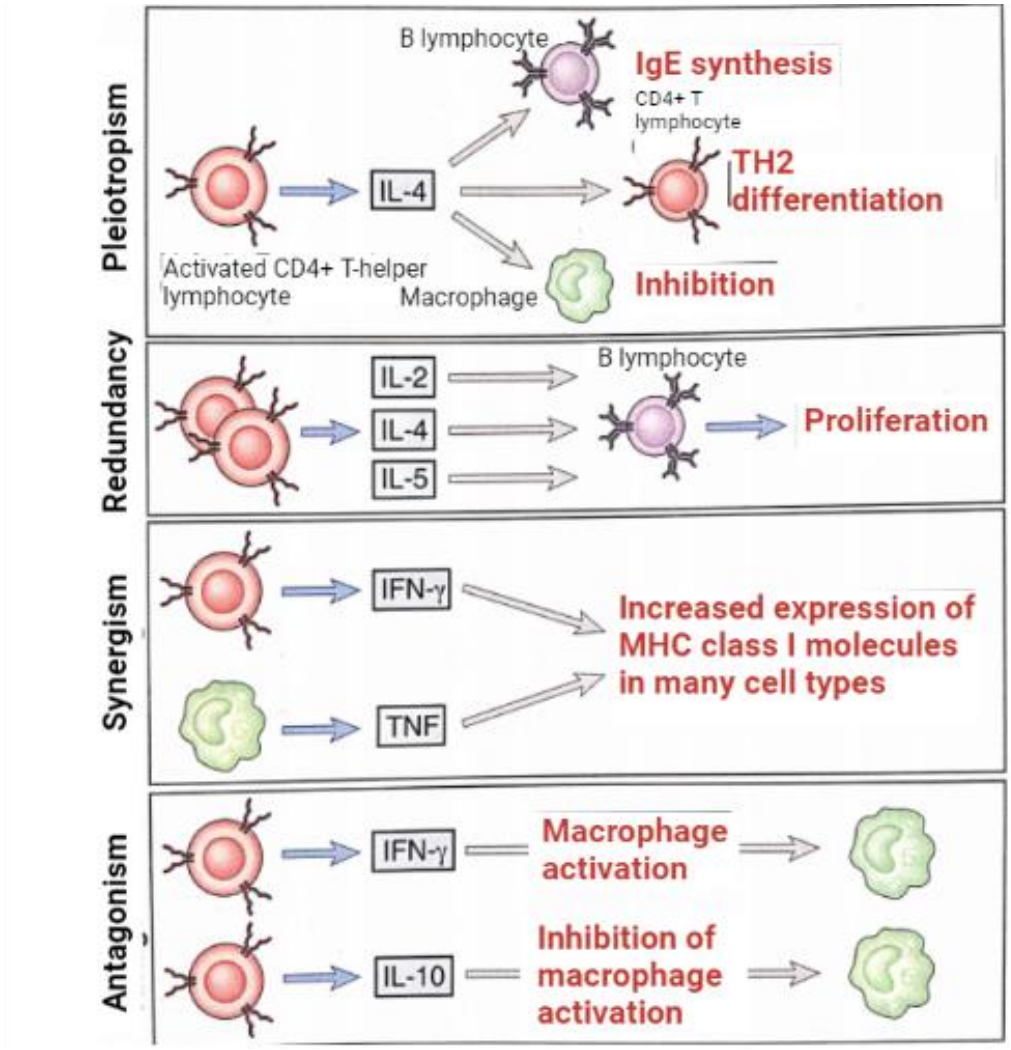

Figure 5.2 Properties of cytokines. Selected examples are shown illustrating the following properties of cytokines: Pleiotropism, redundancy, synergism, antagonism. (Abbas and Lichtman, 2004).

5.2. Cytokines functionality.

Cytokines are involved in the different effector and immunoregulatory mechanisms of both innate immunity and the adaptive response. It is not easy to define a comprehensive classification criterion for the different cytokines. Many of them are remarkably redundant in their activities and are involved in both innate and adaptive responses. Others determine, from innate immunity, the subsequent course of the adaptive immune response.

In order to provide guidance, as cytokines is a very complex subject, these categories are used to try to cover the explanation of all cytokines:

- Mediators and regulators of innate immunity.
- Mediators and regulators of adaptive immunity.
- Stimulators of haematopoiesis.

CYTOKINES THAT MEDIATE AND REGULATE INNATE IMMUNITY.

An important component of the initial innate immune response to viruses and bacteria is the secretion of cytokines, which mediate many of the effector functions of innate immunity. The cytokines of innate immunity attract and activate leukocytes and cause systemic alterations, such as increased synthesis of effector cells and proteins that enhance antimicrobial responses. In innate immunity, the main sources of cytokines are macrophages, neutrophils and NK lymphocytes, but endothelial cells and some epithelial cells produce many of these proteins (Abbas and Lichtman, 2004).

Table 5.1. Cytokines of innate immunity.

Cytokines	Cellular source	Biological effects
Tumour necrosis factor (TNF)	Macrophages T lymphocytes	- Adherent endothelial surface for leukocytes. - Stimulates endothelial cells and macrophages to secrete chemokines. - Stimulates IL-1 secretion. - Involved in inflammation and apoptosis.
Interleukin-1 (IL-1)	Macrophages	- It acts together with

	Endothelial cells Some epithelial cells	TNF in innate immunity and inflammation. - In low concentrations → mediator of local inflammation. - At high concentrations → Endocrine effects like TNF (fever, acute phase plasma protein synthesis, cachexia).
Chemokines	Macrophages Endothelial cells T lymphocytes Fibroblasts Platelets	- Chemotactic activity of leukocytes. - Regulate the circulation of lymphocytes and other leukocytes through peripheral lymphoid tissues.
Interleukin-12 (IL-12)	Macrophages Dendritic cells	- Stimulates IFN-γ synthesis by NK and T lymphocytes. - Stimulates the differentiation of $CD4^+$ T-helper into TH1 lymphocytes that produce IFN-γ. - Enhances the cytolytic functions of activated NK-lymphocytes and $CD8^+$ cytotoxic T-lymphocytes (CTL).
Type I Interferons (IFNs)	IFN-α : Macrophages IFN-β : Fibroblasts	- Inhibits viral replication. - Increases the expression of MHC (major histocompatibility complex) class I molecules. - Stimulates the development of TH1 lymphocytes.
Interleukin-10 (IL-10)	Macrophages T-lymphocytes (mainly TH2)	- Inhibits IL-12 synthesis by activated macrophages and dendritic cells. - Inhibits T-lymphocytes activation.
Interleukin-6 (IL-6)	Macrophages Endothelial cells	- Stimulates acute-phase protein

	T lymphocytes	synthesis. - Contributes to the systemic effects of inflammation. - Stimulates the growth of B-lymphocytes.
Interleukin-15 (IL-15)	Macrophages	- Stimulates proliferation of NK lymphocytes. - Growth factor for $CD8^+$ T-lymphocytes.
Interleukin-18 (IL-18)	Macrophages	- Stimulates IFN-γ synthesis by NK and T lymphocytes and exerts a synergistic action with IL-12 in this response.

CYTOKINES THAT MEDIATE AND REGULATE ADAPTIVE IMMUNITY.

Cytokines mediate lymphocyte proliferation and differentiation following antigen recognition in the activation phase of adaptive immune responses (Abbas and Lichtman, 2004). In addition, cytokines mediate the activation of specialised effector cells in the effector phase of adaptive immunity.

Microorganisms and antigens can induce the differentiation of T cells into effector populations, such as TH1 and TH2 subpopulations, which synthesise different types of cytokines and exert various functions.

Table 5.2. Cytokines of adaptive immunity.

Cytokines	**Cellular source**	**Biological effects**
Interleukin-2 (IL-2)	T lymphocytes	- Stimulates T lymphocytes growth. - Increases synthesis of other cytokines in T lymphocytes (IFN-γ and IL-4). - Proliferation and differentiation of other immune cells. - Apoptosis of antigen-activated T lymphocytes.
Interleukin-4 (IL-4)	$CD4^+$ T lymphocytes (TH2) Mast cells	- Inhibition of macrophage activation. - Differentiation of T lymphocytes into TH2

		lymphocytes. - Stimulates B lymphocyte isotype change to certain classes of immunoglobulins (IgE).
Interleukin-5 (IL-5)	$CD4^+$ T lymphocytes (TH2)	- Activates mature eosinophils. - Stimulates B lymphocyte proliferation and IgA antibody synthesis.
Interferon gamma (IFN-γ)	T lymphocytes (TH1, CD8+) NK lymphocytes	- Major macrophage-activating cytokine. - Stimulates expression of MHC class I and II molecules. - Promotes differentiation of $CD4^+$ T cells into TH1 and inhibits TH2 proliferation. - Activates neutrophils and stimulates cytolytic activity of NK lymphocytes. - Net effect: ↑ macrophage-rich inflammatory reactions and ↓ IgE-dependent eosinophil-rich reactions.
Transforming growth factor beta (TGF-β)	T lymphocytes Macrophages	- Inhibits proliferation and activation of lymphocytes and other leukocytes. - Inhibits immune and inflammatory responses, counteracting the effects of pro-inflammatory cytokines.
Lymphotoxin (LT)	T lymphocytes	- Activates endothelial cells and neutrophils. - Mediator of the acute inflammatory response. - Local action cytokine.
Interleukin-13 (IL-13)	$CD4^+$ T lymphocytes	- Inhibits macrophage

	(TH2)	activation. - Antagonises IFN- γ.

CYTOKINES THAT STIMULATE HEMATOPOIESIS.

Cytokines are necessary for the stimulation of haematopoiesis in the bone marrow. Some cytokines produced during innate and adaptive immune responses stimulate the growth and differentiation of bone marrow progenitor cells (Abbas and Lichtman, 2004).

In this way, inflammatory immune reactions also stimulate the synthesis of new leukocytes.

Mature leukocytes arise from pluripotent progenitor cells that are committed to a particular lineage (differentiation) (Abbas and Lichtman, 2004). This differentiation is stimulated by cytokines called colony stimulating factors (CSF).

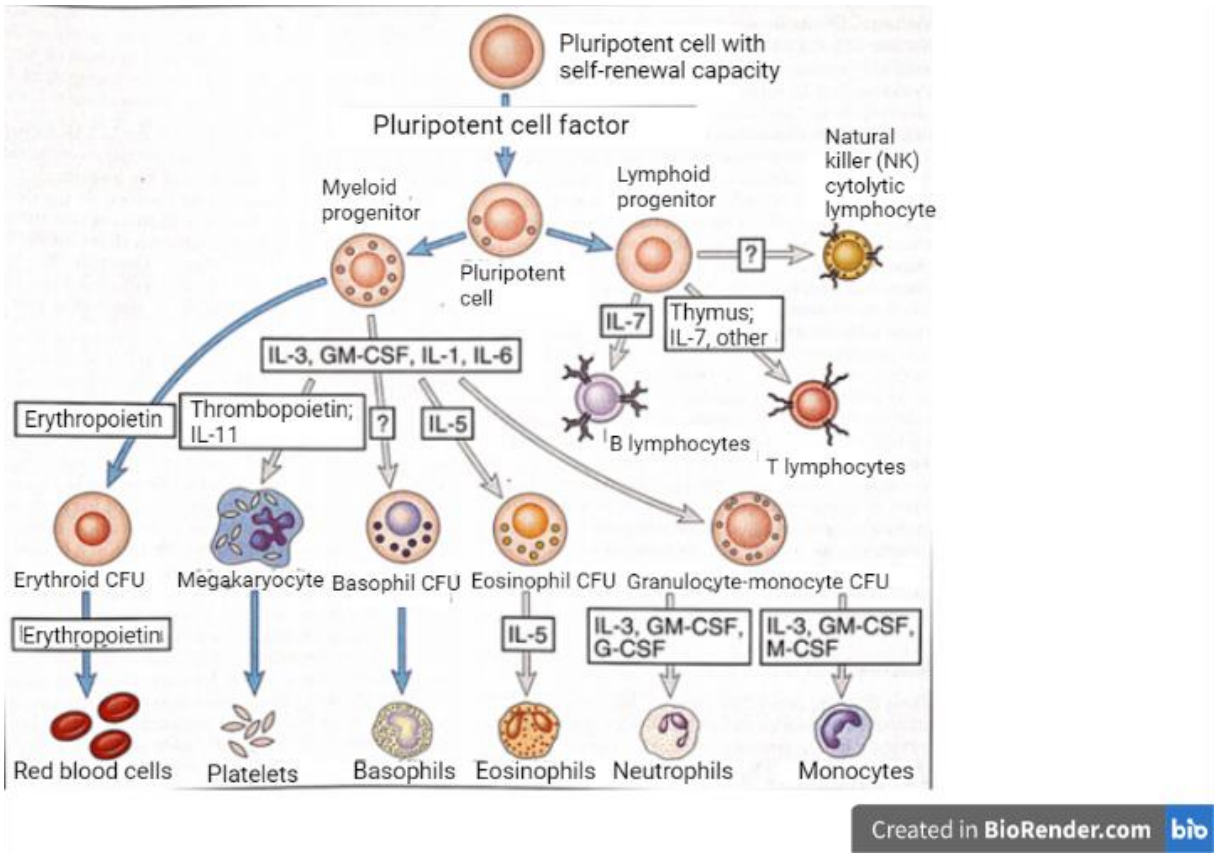

Figure 5.3. Functions of cytokines in haematopoiesis. CFU (colony forming unit); CSF (colony stimulating factors). (Abbas and Lichtman, 2004).

Shortly after the onset of the infectious process, macrophages noticeably increase the production of IL-1, TNF-α and IL-6, in response to their stimulation by PAMP (pathogen-associated molecular pattern), cytokines or complement components.

These three cytokines mediate a set of activities that tend to develop an acute local and systemic inflammatory case, called an acute phase reaction, which attempts to resolve the infectious process by eliminating the causative agent (Fainboim and Geffner, 2005).

Local inflammatory actions mediated by IL-1, TNF-α and IL-6 are exerted on the different cell populations in the immediate environment of the infectious focus.

Systemic inflammatory actions mediated by IL-1, TNF-α and IL-6 are exerted at three different levels:

- Hepatic: induce the production of acute phase proteins.
- Hypothalamic: induce an increase in body temperature.
- In bone marrow and marginal neutrophil pool they induce neutrophilia.

Chemokines produced by activated macrophages, together with those produced by other cell types involved in the antimicrobial inflammatory reaction (endothelial cells, mast cells, neutrophils and dendritic cells), induce the recruitment of leukocytes to the site of injury.

Chemokines produced in response to pathogenic microorganisms include primarily those interacting with CCR1, CCR2, CCR3, CCR5, CXCR2 and CXCR3 receptors (Fainboim and Geffner, 2005).

Each of the above receptors recognises more than one chemokine, so different chemokines may mediate identical biological responses. On the other hand, many may interact with different receptors, mediating different biological responses.

In addition, activated macrophages synthesise other cytokines that promote the production and differentiation of different cell types.

Through the secretion of IL-15, macrophages play an important role in the production of different cell types belonging to innate immunity, such as NK cells, NKT cells and T lymphocytes (Fainboim and Geffner, 2005). On the other hand, through the

production of IL-12 and IL-18 they promote the differentiation of CD4+ T cells towards the TH1 profile.

Dendritic cells, that have captured antigen in peripheral tissues and have sensed the presence of an infectious process by recognising PAMP or inflammatory cytokines produced within the infectious focus, migrate to secondary lymphoid organs. These secondary lymphoid organs present T cell-secreting cytokines, such as IL-12, that induce T cells to differentiate into antigen-specific effector T cells (e.g. Th1, Th2 or Th17) (Gordon et al, 2014).

A critical factor is the presence of IL-12, and to a lesser extent IL-18. In the presence of IL-12, CD4+ T cells tend to differentiate into a Th1 profile (development of inflammatory responses). In its absence, they differentiate into a Th2 profile (development of humoral responses). The latter will depend on whether they detect the presence of IL-4 in their environment.

IL-12 has a second very important role. It is responsible for inducing the production of interferon-γ by NK cells.

In conclusion, the activation of the immune response brings into play a wide variety of humoral and cellular responses with destructive potential. In order to modulate the development of the immune response and avoid harmful effects on the host itself, each of the various effector mechanisms that make up the immune response is controlled by inhibitory mechanisms (Fainboim and Geffner, 2005).

When activated, macrophages produce cytokines capable of inhibiting their inflammatory potential. The most important of these are IL-10 and transforming growth factor beta (TGF-β).

5.3. Signaling pathways.

Downstream signal transduction is mediated by JAKs (Janus kinases) and STAT3 (signal transducer and activator of transcription 3), as well as by Akt–mTOR (mammalian target of rapamycin) and MAPK–ERK (mitogen-activated protein kinase–extracellular signal-regulated kinase) pathways (Fajgenbaum and June, 2020).

NF-κB → The NF-κB transcription factor (nuclear factor kappa B) has the ability to regulate:

- The activation of T and B lymphocytes.
- The secretion of chemokines and proinflammatory cytokines.
- The expression of enzymes and adhesion molecules involved in inflammatory processes.

The NF-κB family consists of five different proteins. The p65 protein is found under basal conditions (resting lymphocytes) in the cytoplasm, associated with inhibitory proteins called IκB (Fainboim and Geffner, 2005). Different inflammatory or activating stimuli result in the activation of IκB kinases, leading to the cleavage of active p65.

In these circumstances, subunit association and translocation to the nucleus occurs, where the assembled transcription factors will exert their biological effects by interacting with their specific sequence, promoting the expression of genes involved in the inflammatory response (Fainboim and Geffner, 2005).

NF-κB inhibitors (sulfasalazine) are being tested as specific immunosuppressants or anti-inflammatories.

MAPK → Mitogen-activated protein kinase (MAPK) containing three sequentially activated protein kinases are key components of a series of vital signal transduction pathways that regulate processes such as cell proliferation, cell differentiation, and cell death in eukaryotes from yeast to humans (K. Morrison, 2012).

The MAP kinases can be grouped into three main families. In mammals, these are ERKs (extracellular-signal-regulated kinases), JNKs (Jun amino-terminal kinases), and p38/SAPKs (stress-activated protein kinases).

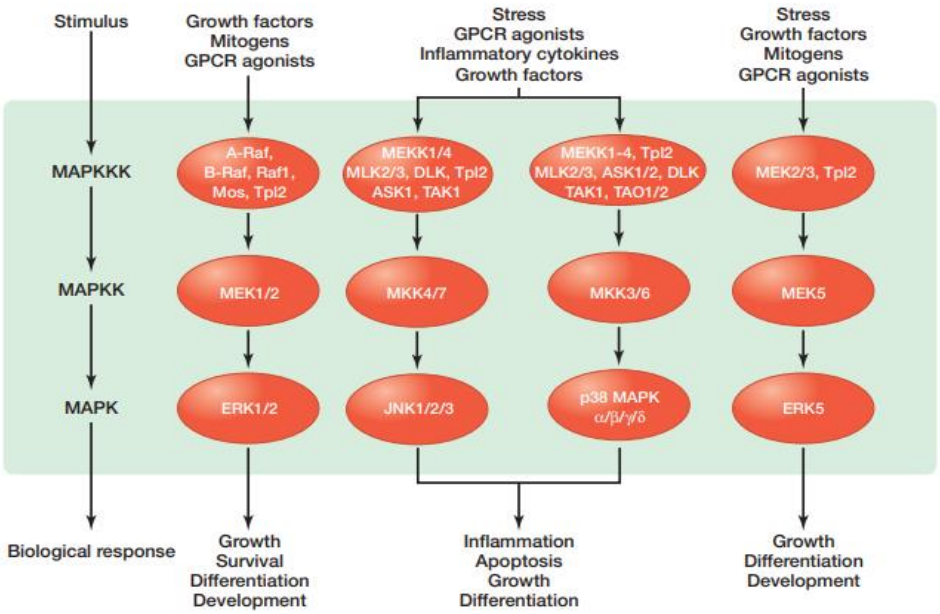

Figure 5.4. MAPK pathways (K. Morrison, 2012).

JNK family members contain a TPY motif in the activation segment and include JNK1, JNK2, and JNK3. The JNK module (Fig. 3) is activated by environmental stresses (ionizing radiation, heat, oxidative stress, and DNA damage) and inflammatory cytokines, as well as growth factors, and signaling to the JNK module often involves the Rho family GTPases Cdc42 and Rac (Johnson and Nakamura, 2007). The JNK module plays an important role in apoptosis, inflammation, cytokine production, and metabolism (Dhanasekaran and Reddy 2008; Huang et al. 2009; Rincon and Davis 2009).

Like JNK modules, p38 modules are strongly activated by environmental stresses and inflammatory cytokines. p38 activation also contributes to inflammation.

JAK/STAT → More than 50 cytokines signal via the JAK/STAT pathway to orchestrate haematopoiesis, induce inflammation and control the immune response (Morris et al, 2018). The JAK/STAT pathway is a signalling cascade through which cytokines trigger specific responses in target cells. This pathway requires the involvement of enzymes called Janus Kinases (JAKs) and transcription factors called Signal Transducers and Activators of Transcription (STATs).

Each cytokine binds to a specific receptor on the surface of its target cell. These receptors contain intracellular domains which are constitutively associated with members of the JAK (Janus Kinase) family of tyrosine kinases (Morris et al, 2018).

Once activated, JAKs phosphorylate the intracellular tails of the receptors on specific tyrosines which in turn act as docking sites for members of the Signal Transducers and Activators of Transcription (STAT) family of transcription factors (Morris et al, 2018). Receptor-localized STATs are then phosphorylated by JAK, which leads to their disassociation from the receptor and translocation to the nucleus, where they drive the expression of cytokine-responsive genes, often leading to proliferation and/or differentiation (Morris et al, 2018).

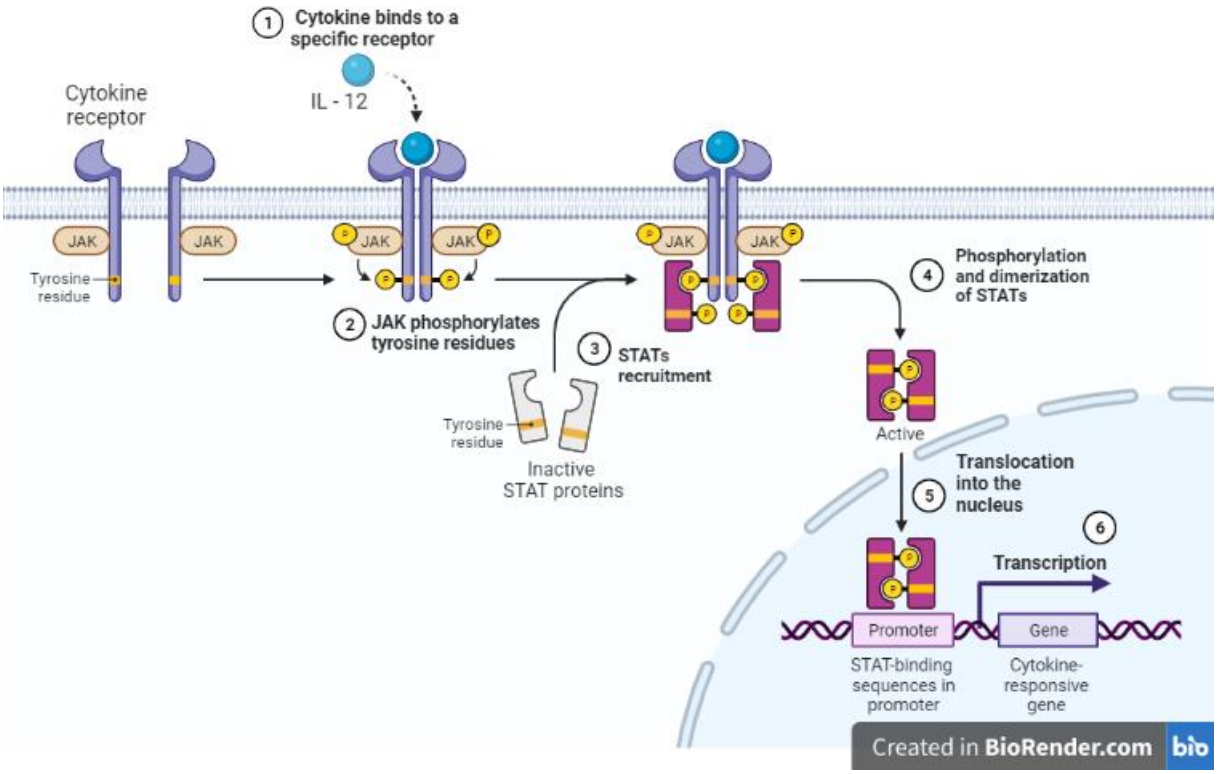

Figure 5.5. The IL-12 receptor signals through a JAK/STAT pathway, in which cytokine binding to the receptor activates receptor-associated tyrosine kinases called Janus kinases (JAKs) and ultimately triggers activation of STATs. Many cytokines use this pathway to induce responses in target cells and different cytokines activate different combinations of JAK and STAT (Abbas and Lichtman, 2004). Created with BioRender.com.

mTOR → The mTOR (mammalian target of rapamycin) pathway has been implicated in immune functions (Zhang et al, 2017). The mammalian target of rapamycin (mTOR) is an evolutionary conserved serine/threonine kinase that is present in two complexes, mTORC1 and mTORC2. mTORC1 is the main energy and nutrient sensor of the cell: it senses the presence of amino acids, glucose, lipids and ATP to allow efficient activation of the network in response to growth factors, Toll-like receptor (TLR) ligands and cytokines (Weichhart et al, 2015).

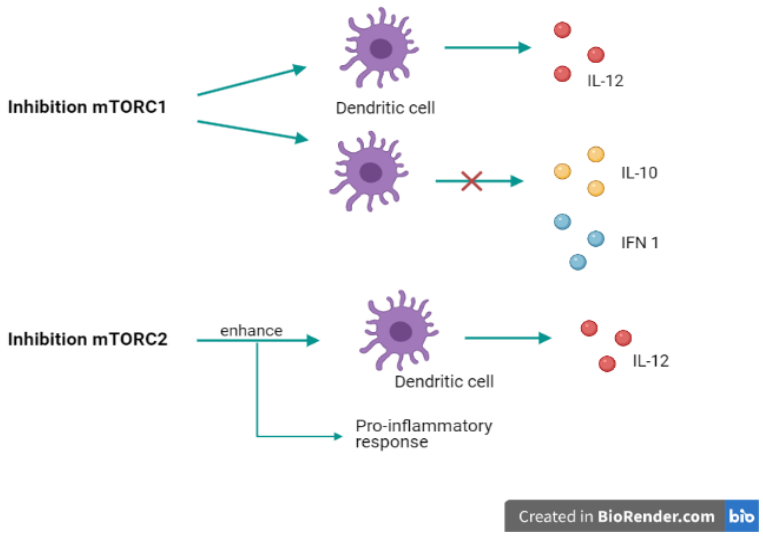

Figure 5.6. Inhibition of mTORC1 during TLR triggering generally promotes IL-12 production and inhibits IL-10 and type I interferon expression by dendritic cells (DCs) and augments their T cell stimulatory capacity. Inhibition of mTORC2 enhances a pro-inflammatory response and IL-12 production in DCs (Weichhart et al, 2015).

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6. CYTOKINE STORM.

The immune system is expected to recognize foreign invaders, respond proportionally to the pathogen burden, and then return to homeostasis. This response requires a balance between sufficient cytokine production to eliminate the pathogen and avoidance of a hyperinflammatory response in which an overabundance of cytokines causes clinically significant collateral damage. Cytokines play a key role in coordinating antimicrobial effector cells and providing regulatory signals that direct, amplify, and resolve the immune response. Cytokines have short half-lives, which normally prevents them from having effects outside lymphoid tissue and sites of inflammation. Although typically considered to be pathologic, sustained production of cytokines that leads to elevated circulating levels may be necessary to appropriately control some disseminated infections. (Fajgenbaum and June, 2020).

Given the lack of an unifying definition for cytokine storm and disagreement about the distinction between cytokine storm and a physiologic inflammatory response, we propose the following three criteria for identifying cytokine storm (Fajgenbaum and June, 2020):

- Elevated circulating cytokine levels.
- Acute systemic inflammatory symptoms.

- Secondary organ dysfunction (often renal, hepatic, or pulmonary) due to inflammation beyond that which could be attributed to a normal response to a pathogen (if a pathogen is present), or any cytokine-driven organ dysfunction (if no pathogen is present) (Fajgenbaum and June, 2020).

Cytokine release syndrome (CRS) or “cytokine storm” is a form of uncontrolled systemic inflammatory reaction that can be triggered by a variety of factors. It starts fairly quickly in cases of severe (mainly respiratory) infections, in other types of massive immune activation such as severe courses of some systemic diseases (RA, SLE), rarely anaphylaxis. It can be frequently mentioned in connection to medical interventions such as transplantation or administration of certain drugs (monoclonal antibodies, adoptive T-cell therapies). Subtle CRS may occur following immunization against rubella, human papillomavirus, or hepatitis B (Lukan, 2020).

Synonymous with cytokine release syndrome (CRS), cytokine storm syndrome (CSS) is a cytokine-mediated systemic inflammatory response induced by a variety of initiating factors, resulting in a clinical presentation of unremitting high fever, lymphadenopathy, hepatosplenomegaly, cytopaenia, hyperferritinaemia and central nervous system (CNS) abnormalities, and, if untreated, progression to multiple organ failure (MOF) is almost inevitable (Gao et al, 2020).

Such broad spectrum of etiological factors leading to clinical and laboratory manifestation of CRS like hypotension, hypoxia, fever, fatigue, myalgias, malaise, nausea, hypoxia, coagulopathy, capillary leakage, tachycardia, tachypnea, hemophagocytic lymphohistiocytosis/macrophage activation syndrome, organ toxicity, pulmonary edema, pneumonitis, and renal insufficiency support the basic scientific postulate: cytokine storm results from excessive pro-inflammatory stimuli, inadequate regulation of inflammation, immune cells or both (Lukan, 2020).

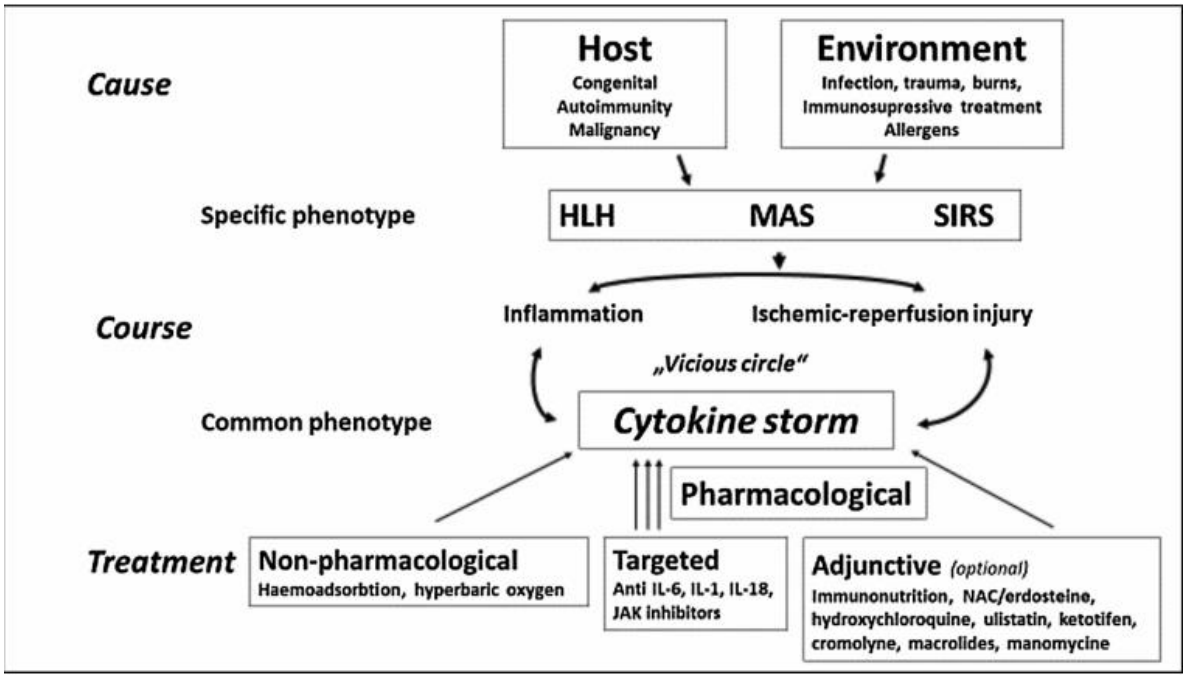

Figure 6.1. Vicious circle of cytokine storm in a view of causes, course and treatment. (HLH-hemophagocytic lymphohistiocytosis, MAS - macrophage activation syndrome, SIRS - systemic inflammatory response syndrome, NAC – N-acetylcysteine) (Lukan, 2020

The hallmark of CSS is an unchecked feedforward activation and amplification of host immune, causing the massive release of a wide range of cytokines (Gao et al, 2020), including the IL-1 family, IL-6, IL-8, IL-10, TNF-α, and interferon (IFN-γ), are involved in the development of a cytokine storm, although the key pathogenic cytokines appear to differ depending on the disease. IFN-γ is a key cytokine in primary HLH. IL-1ß plays a major role in systemic juvenile idiopathic arthritis (SJIA), while in MAS (macrophage activation syndrome) associated with SJIA, IL-18 may be a key cytokine (Seok Kim et al, 2021). Another representative cytokine, IL-6, is a key inflammatory molecule in CRS (cytokine release syndrome) (Seok Kim et al, 2021).

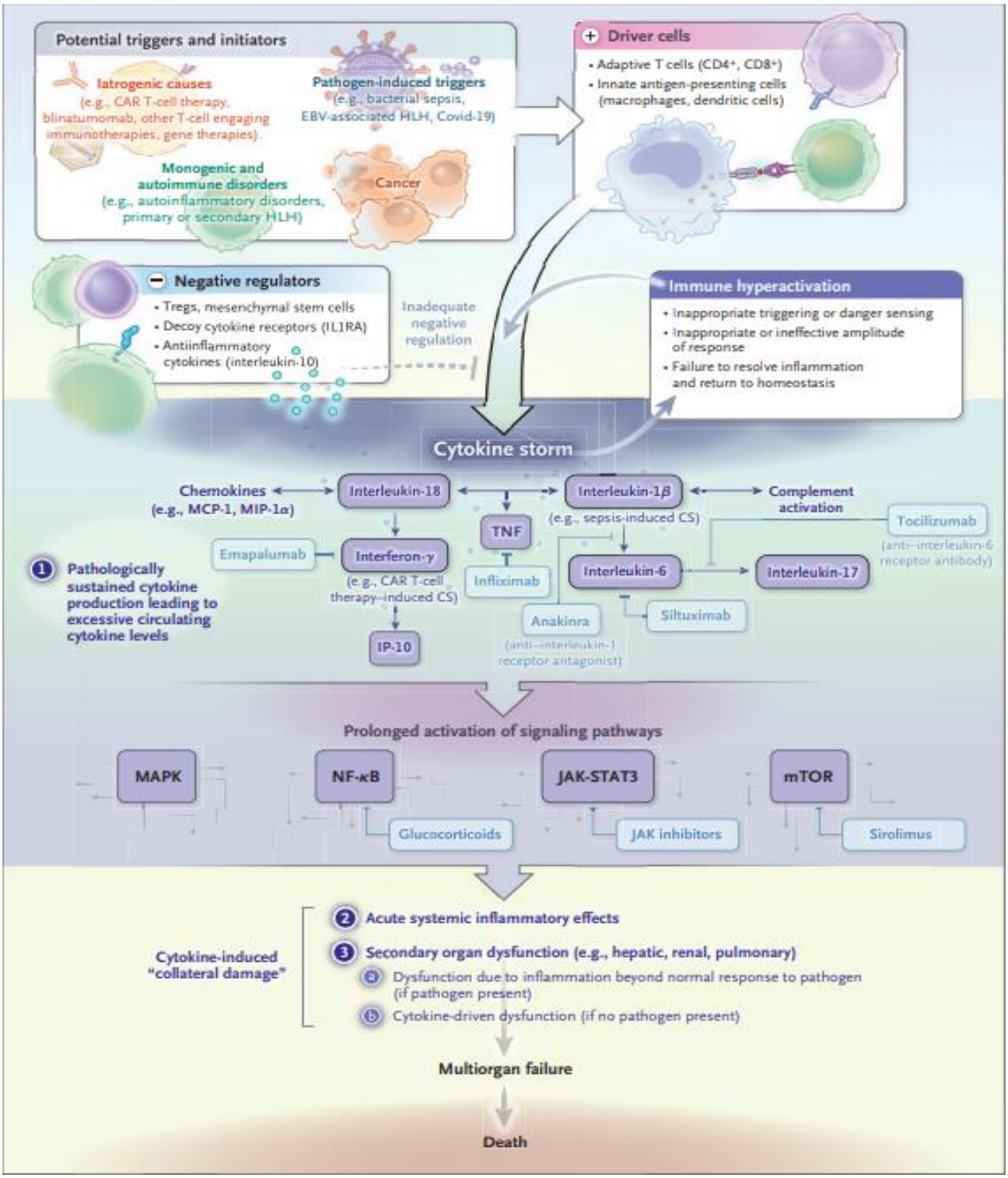

Figure 6.2. Cytokine storm. (Fajgenbaum and June, 2020).

6.1. Types of cytokine storm.

- Iatrogenic Cytokine Storm.

Infusion of CAR T cells engineered to recognize and eliminate CD19+ lymphoma cells can induce cytokine storm, with supraphysiologic levels of interferon-γ and interleukin-6 (Fajgenbaum and June, 2020).

The highly activated CAR T cells are clearly the initiators of the cytokine storm. However, the cytokines mediating the severity of cytokine storm are produce by macrophages instead of by the CAR T cells. This can be reversed by interleukin-1 and interleukin-6 blockade.

Cytokine storm can be observed with other T-cell–engaging immunotherapies as well, such as blinatumomab, a bispecific antibody that binds to CD19+ and CD3+ T cells. Like CAR T cells, activated T cells initiate the cytokine storm, and macrophage activation propagates blinatumomabinduced cytokine storm, which also responds to anti–interleukin-6 antibody therapy (Fajgenbaum and June, 2020).

However, not all patients treated with CAR T cells or blinatunomab develop cytokine storm. This suggests that additional factors play a part in the development of iatrogenic cytokine storm such as disease burden, CAR structure and design, and host genomic background.

- Pathogen-Induced Cytokine Storm.

Infections are most likely the most common trigger of cytokine storm. Although distinguishing between an excessive cytokine production and an appropriate cytokine production for controlling a widespread infection is challenging.

The quick and early prediction of hypercytokinemia through specific biological markers would prevent many deaths (Rowaiye et al, 2021).

The collateral damage caused by the immune response as it attempts to clear the pathogen can be more deadly than the pathogen itself (Fajgenbaum and June, 2020).

Disseminated bacterial infections causing sepsis induce the production of many cytokines that can lead to fever, cell death, coagulopathies, and multiorgan dysfunction. In sepsis-associated cytokine storm, it is unclear which immune cell types and cytokines may be responsible for propagating the pathologic hyperinflammation (Fajgenbaum and June, 2020).

Disseminated viral infections can also induce profound cytokine storm. Patients with hyperinflammatory responses to microbes often have defects in pathogen detection, effector and regulatory mechanisms, or resolution of inflammation. For example, patients lacking functional perforin, which is critical for resolving infections and inflammation, have prolonged CD8+ T-cell production of interferon-γ and TNF, and HLH-associated cytokine storm develops in such patients when they are infected with EBV or cytomegalovirus (Fajgenbaum and June, 2020).

For some viral infections, administering proinflammatory cytokines in the early stages of infection can help to avoid detrimental effects of the immune response

- Monogenic or Autoimmune Cytokine Storm.

In rare cases, a pathogen triggers cytokine storm in patients with monogenic disorders, and in other cases, cytokine storm has autoimmune, neoplastic, or idiopathic causes (Fajgenbaum and June, 2020).

Autoinflammatory diseases are characterized by seemingly unprovoked inflammation and cytokine storm without signs of infection or autoimmunity. Affected patients have germline mutations in genes regulating the innate immune system and activation of the inflammasome (Fajgenbaum and June, 2020).

Monogenic or autoimmune cytokine storm is shown in diseases such as primary and secondary HLH, and idiopathic multicentric Castleman’s disease.

- Covid-19 –Associated Cytokine Storm.

Covid-19, which is caused by SARS-CoV-2, is characterized by heterogeneous symptoms ranging from mild fatigue to life-threatening pneumonia, cytokine storm, and multiorgan failure (Fajgenbaum and June, 2020).

Although SARS-CoV is abortive in macrophages and DCs, the virus induces an increase in levels of pro-inflammatory cytokines and chemokines (Tang et al, 2020): interleukin-6, IP-10. interleukin-1β, interferon-γ, VEGF, TNF, and macrophage inflammatory protein (MIP) 1α and 1β.

Severely ill patients tended to have higher interleukin-6 levels.

Importantly, even if the clinical symptoms caused by a cytokine storm exhibit a common pattern, treatment must be individualized because the degree to which each cytokine contributes to the development of the disease can differ. On the other hand, cytokine storm in sepsis involves multiple factors, and thus, multi-directional suppression of inflammatory reactions is necessary (Seok Kim et al, 2021).

6.2. Pathogenesis of CSS (Cytokine Storm Syndrome).

To date, the pathogenesis of CSS has not been fully elucidated. Previous studies have shown that the development of CSS involves the imbalance of proinflammatory and anti-inflammatory mechanisms and the interaction of a variety of cells and cytokines, resulting in immune regulation disorder, causing a series of clinical manifestation (Gao et al, 2020).

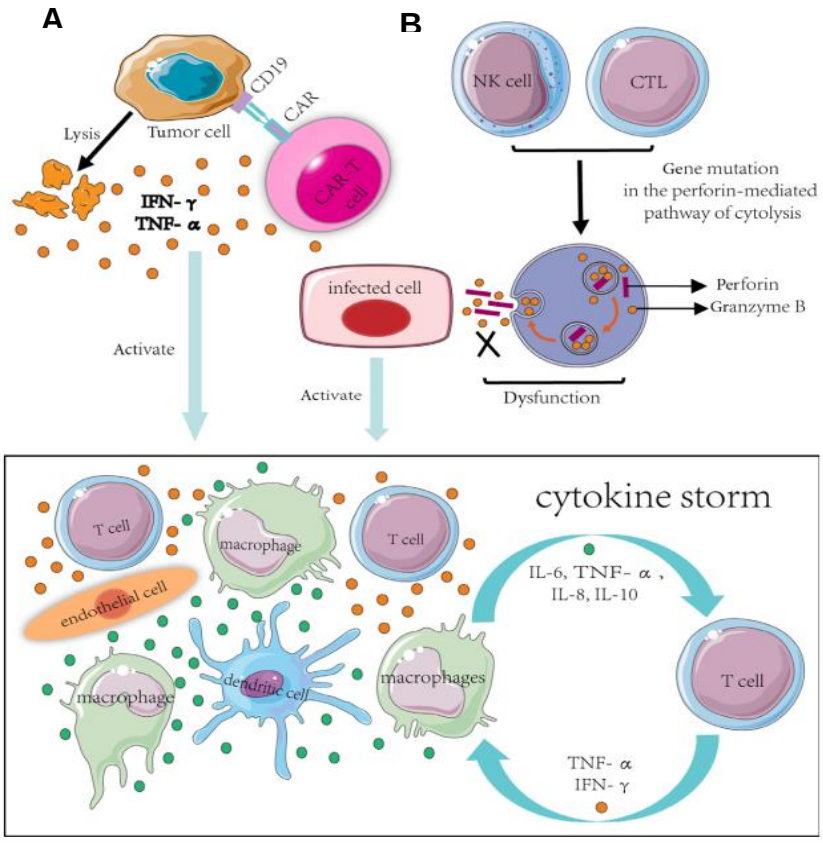

Figure 6.3. Pathogenesis of CSS. **A.** In the setting of CAR T-cell therapy, CAR T cells can recognize target cells (tumour cells) and induce the lysis of target cells, along with the activation of CAR T cells and T cell, causing a consecutive release of cytokines including IFN-γ or TNF-α. These cytokines trigger a cascade reaction by activation of innate immune cells including macrophages, DCs and endothelial cells with further cytokine releasing, which finally leads to a cytokine storm. **B.** In the setting of HLH, mutations in perforin coding gene or genes essential for perforin transport will cause a failure of normal cytolytic function and inability to clear the antigenic stimulus, leading to the persistent activation of macrophages and T cells by the infected cells, accompanied by excessive secretion of proinflammatory cytokines, which finally leads to a cytokine storm. (Gao et al, 2020)

Cytolytic cell dysfunction.

The most progress in the understanding of the mechanism of CSS has been made in FHL (primary haemophagocytic lymphohistiocytosis), which is a result of mutations in genes involved in the perforin-mediated pathway of cytolysis shared by NK cells (innate immunity) and cytotoxic CD8+ T cells (adaptative immunity) (Crayne et al, 2019) (Gao et al, 2020). Normally, perforin in NK cells and cytotoxic $CD8^+$ cells is packaged into cytolitic granules and is trafficked along the actin cytoskeleton to the immunologic synapse between the antigen presenting cell (APC) or target cell (infected or tumour cells) and the cytolytic lymphocyte. The polarized granules then allow release of perforin into the immunologic synapse to form a pore between the lytic cell and the target cell, allowing granzyme B, co-packaged with perforin, to induce the apoptotic cell death of the target cell (Gao et al, 2020).

Therefore, mutations in perforin coding gene or genes essential for perforin transport will cause a failure of normal cytolytic function. The inability to lyse the infected APC results in a prolonged hyperactivation of macrophages and T helper 1 (Th1) cells accompanied by excessive secretion of proinflammatory cytokines, causing a self-amplifying hyperinflammatory state known as the cytokine storm.

Similarly, gene mutations in sHLH (secondary haemophagocytic lymphohistiocytosis) alter cytolytic function in cytotoxic CD8+ T cells and NK cells as well (Gao et al, 2020).

Activation of immune and nonimmune cells.

- Macrophages

The acute phase of MAS (macrophage activation syndrome) is often associated with markedly elevated levels of pro-inflammatory cytokines. This cytokine storm triggers a cascade of inflammatory pathways that, if untreated, leads to tissue damage and death (Crayne et al, 2019).

Known inducers of macrophage activation include toll-like receptor (TLR) ligands and cytokines. The type of TLR stimuli and cytokines in the inflammatory milieu define the genetic programs, either pro- or anti-inflammatory adopted by macrophages in response to inflammatory stimuli (Crayne et al, 2019).

Haemophagocytic macrophages were found to produce the proinflammatory cytokine TNF-α and IL-6 in the liver of MAS patients, suggesting that activated macrophages may participate in the pathogenesis of MAS. As we know, activated macrophages can produce a variety of cytokines, especially TNF-α and a variety of interleukins (IL-6, IL-1 and IL-18), which may trigger the cascade reaction of inflammatory factors, and finally form a cytokine storm. Macrophages also play a crucial role in the CSS induced by chimeric antigen receptor (CAR) T-cell therapy.

Monocyte-derived cells are responsible for IL-6 secretion that results in response to CAR-mediated T-cell activation (Singh et al, 2017) and IL-6 blockade did not affect the therapeutic effect of CAR T cell.

Both monocyte depletion and blocking IL-6 or IL-1 receptor could avoid the occurrence of CSS. All of these findings suggest that monocytes/macrophages play a crucial role in the development of CSS after CAR T-cell therapy (Gao et al, 2020).

Macrophages infected by SARS and MERS-CoV showed delayed but elevated levels of IFN and other proinflammatory cytokines, suggesting macrophages could play an important role in SARS and MERS pathogenesis (Gao et al, 2020).

- Dendritic cells

The role of dendritic cells (DC) in the pathogenesis of CSS is mainly mediated by the ability of these cells to present antigen to T cells. When dendritic cells are pulsed with

antigen and exposed to cognate naïve or effector T cells, they present their processed antigen peptides in the context of MHCII (Gordon et al, 2014).

Patients with pHLH or some patients with MAS have cytolyic cell dysfunction, which means cytotoxic T cells may fail to clear the antigen loaded DCs (Gao et al, 2020).

DC mediate disease pathogenesis in hosts with cytotoxic dysfunction. In cases where there is an infectious trigger, such as a viral infection, cytotoxic CTL fail to clear virus-infected DC. This leads to constant DC activation and antigen-presentation of viral antigens to T cells, which in turn respond by hyperproliferation and overproduction of pro-inflammatory cytokines responsible for multi-organ failure seen in MAS (Crayne et al, 2019).

- Endothelial cells

Endothelial activation can also participate in the pathogenesis of CSS.

On the one hand, the excessive production of proinflammatory cytokines such as IL-6 and IFN-γ can induce endothelial activation in severe CSS, characterized by the release of stored von Willebrand factor and angiopoietin-2. Angiopoietin-2 can further amplify endothelial activation (Gao et al, 2020). Moreover, vascular endothelial cells are also a key source of IL-6 (Obstfeld et al, 2017).

<u>Excessive production of cytokines.</u>

A variety of cytokines can be markedly increased in patients with CSS, which may vary according to the heterogeneity of the disease background (Gao et al, 2020).

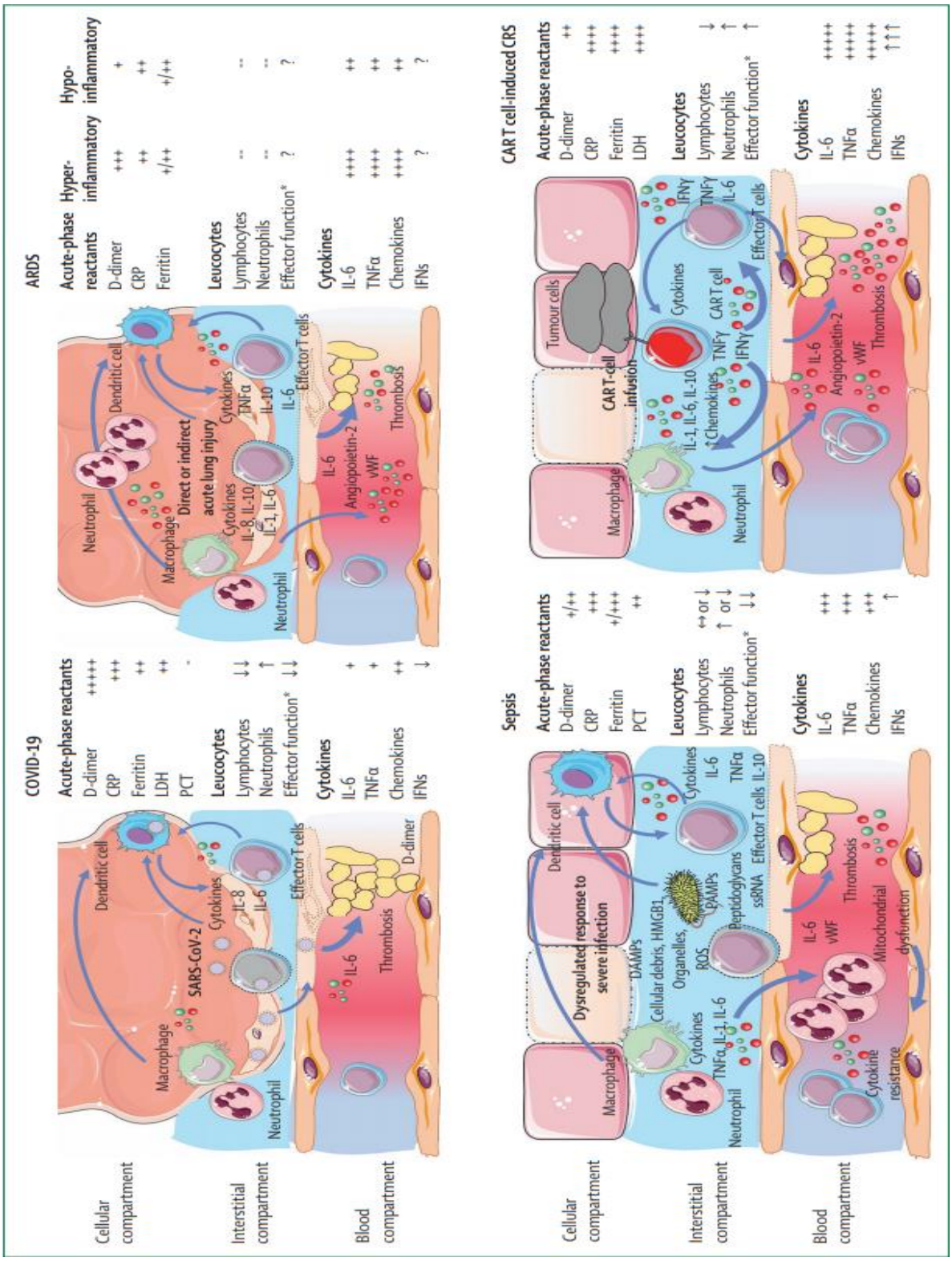

Figure 6.4. **Mechanistic comparison of inflammatory processes in patients with COVID-19 versus ARDS, sepsis, and CAR T cell-induced CRS.** ARDS=acute respiratory distress syndrome. CAR T cell-induced CRS=chimeric antigen receptor T cell-induced cytokine release syndrome. CRP=C-reactive protein. DAMPs=damage-associated molecular patterns. IFN=interferon. IL=interleukin. LDH=lactate dehydrogenase. PAMPs=pathogen-associated molecular patterns. PCT=procalcitonin. ROS=reactive oxygen species. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. ssRNA=single-stranded RNA. vWF=von Willebrand factor. (Leisman et al, 2020).

7. DEVELOPMENT AND CONTROL OF CYTOKINE STORM IN DIFFERENT PATHOLOGIES.

7.1. Haemophagocytic lymphohistiocytosis.

Hemophagocytic lymphohistiocytosis (HLH) is a heterogeneous hyperinflammatory syndrome with different pathways of pathogenesis resulting in similar clinical presentations that includes: fever, hyperferritemia, neurologic dysfunction, cytopenias, hypertriglyceridemia, hemophagocytosis and diminished NK cell activity (Wegehaupt et al, 2020) (Chu et al, 2021). These clinical features of HLH are explained in large part by the cytokine storm that results from defective lymphocyte cytotoxicity (Henderson and Cron, 2020).

It is classically subdivided between primary (familial or lymphoproliferative disorder) and secondary (acquired) HLH (Chu et al, 2021). Its primary form is mostly seen in childhood and is considered a genetic disease of impaired perforin-dependent cytotoxic function (Soy et al, 2020) (Mehta et al, 2020). Secondary HLH can present at any age and is often triggered by viral infection (commonly Epstein-Barr virus), autoimmune or autoinflammatory disorders, haematological malignancy or iatrogenic causes, including haematopoietic stem cell transplantation (Mehta et al, 2020).

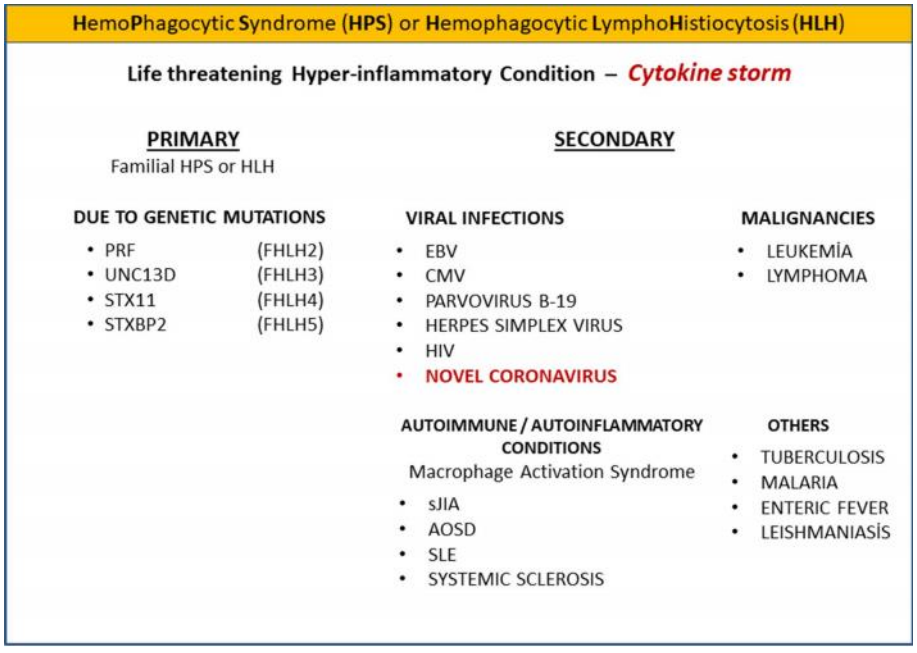

Figure 7.1. Classification of HLH. (Soy et al, 2020).

PATHOGENESIS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS.

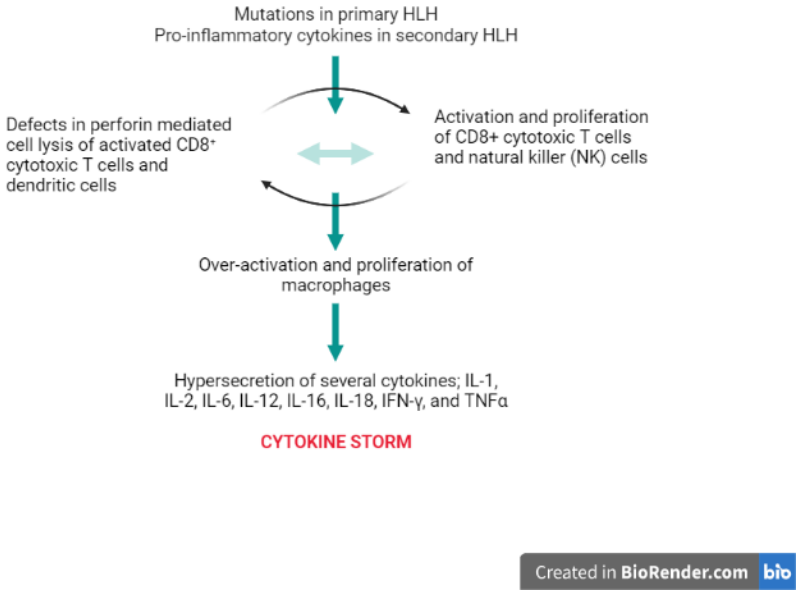

Figure 7.2. Pathogenesis of HLH. Created with BioRender.com.

- Pathogenesis of primary HLH.

Primary HLH (FHL) is caused by bi-allelic mutations, is divided into four subtypes based on the gene affected by mutation: perforin 1 (PRF1), unc-13 homolog D (UNC13D), syntaxin binding protein 2 (STXBP2), and syntaxin11 (STX11), all of which are required for cytotoxic granule release by $CD8^+$ T cells and NK cells (Henderson and Cron, 2020) (Chu et al, 2021).

PRF1 encodes perforin, lower levels of which result in decreased NK cell cytotoxicity and reduced effector cell lysis due to impaired pore formation. The genes UNC13D, STX11 and STXBP2 encode vesicle priming and fusion proteins, the loss of which results in impaired cytotoxic granule exocytosis (Chu et al, 2021).

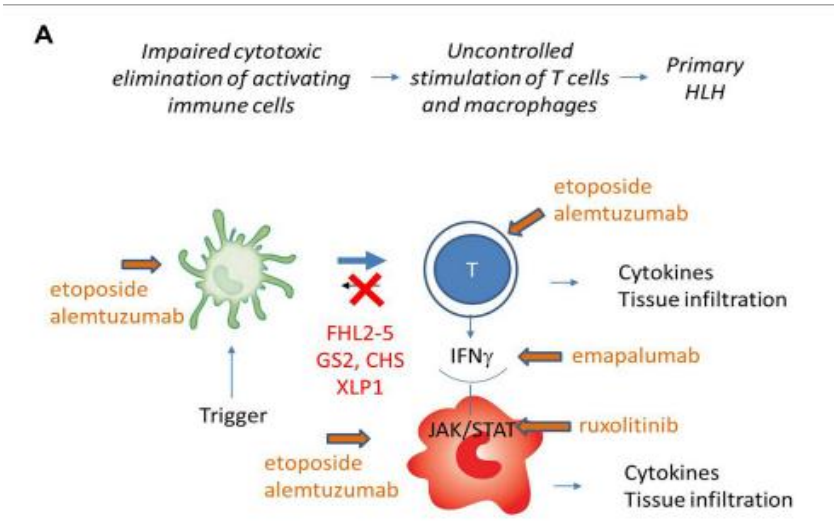

Figure 7.3. Pathogenesis of "primary" HLH (simplified). Impaired cytotoxicity (red) leads to uncontrolled T cell activation by APC. T cell secreted IFN-γ is the key driver of macrophage activation (Wegehaupt et al, 2020).

Under normal physiological conditions, immune stimulation such as a viral infection leads to priming of cytotoxic T-lymphocytes by APC, followed by their activation and proliferation (Wegehaupt et al, 2020). These activated T cells and NK cells can recognize virus-infected target cells and subsequently eliminate these through polarized release of granules that contain perforin and granzyme (protein involved in triggering apoptosis in the target cell). Entry of granzymes into target-cells by membrane pores, established by perforin activity, mediates apoptotic cell death (Wegehaupt et al, 2020).

This cytotoxic activity also act against APCs, providing a negative feedbackloop that limits T cell activation. Disruption of this process with genetic mutations leads to the development of primary HLH. Defective clearance of antigenic stimulus for any reason results in persistence and strengthening of the immune response (Soy et al, 2020).

Immune activation is further driven by the high levels of macrophage activation, with proinfammatory cytokines, released by activated immune cells, including IL-1β, IL-6, IL-8, IL-18, interferon gamma (IFNγ), and tumour necrosis factor (TNF), resulting in clinical sequelae including unremitting fever (the cardinal feature of cytokine storm syndromes) (Mehta et al, 2020).

- Pathogenesis of secondary HLH.

While FHL is rare, acquired or secondary HLH is increasingly recognized in patients with infections and rheumatologic conditions.

Secondary HLH may result from multiple individual triggers (malignant trigger, autoimmune trigger, infectious trigger and an unknown trigger), that breach a threshold level of disease that the immune system is no longer able to counteract (Henderson and Cron, 2020) (Chu et al, 2021).

Hematologic malignancies such as T/NK-cell disorders, acute leukemias, lymphomas, myelodysplastic syndrome, and lymphoproliferative diseases, are the most common triggers of malignancy derived HLH (Chu et al, 2021).

Chemotherapeutic triggers cell lysis and necrosis, resulting in a release of IL-1β, IL-5, IL-6, IL-10, IL-13, IFN-γ and TNF-α, further potentiated by tumour infiltrating

lymphocytes. Chemotherapeutic can also increase subclinical mutation leading to development of HLH (Chu et al, 2021).

Epstein–Barr virus (EBV) infects nearly all people worldwide without serious sequelae. However, it is recognized as the leading cause of infection associated HLH, with other herpes viruses also playing a prominent role (Chu et al, 2021) (Mehta et al, 2020).

Influenza viruses have also been associated with the development of HLH.

Infection-associated HLH have been demonstrated to have elevated IL-6, IL-10, IL-18, s-IL-2r (soluble IL-2) and IFN-γ. The interleukin-2 receptor (IL-2R, CD25) is primarily secreted by activated T-helper lymphocytes, but is also expressed by granulocytes, NK cells and B cells. Therefore, the release of soluble IL-2r (sIL-2r) reflecting the activation of the immune system (Chu et al, 2021).

7.2. Control of cytokine storm in HLH.

The heterogeneity in pathophysiology of primary HLH caused by cytotoxicity defects versus acquired HLH makes it obvious that there is no “one fits all” therapeutic strategy. Treatment must be targeted to the pathophysiology of each HLH case and results from treatment studies obtained in one group of diseases cannot simply be transferred to another (Wegehaupt et al, 2020).

Therapeutic regimens in primary HLH are either directed at the immune cells involved, or at the cytokines secreted by these cells. The goal is to disrupt ongoing immune stimulation and to limit severe hyperinflammation and tissue damage (Wegehaupt et al, 2020).

Prompt diagnosis and timely treatment with the elimination of inciting triggers, and rapid initiation of immunosuppression are essential for effective management and a good prognosis of HLH (Henderson and Cron, 2020).

- Treatment of Triggers.

Both genetic and acquired HLH are often triggered by infections. In patients with EBV-driven HLH, B-cell depletion with rituximab has been shown to reduce viral load,

serum ferritin levels, and to improve clinical parameters of the disease when used in combination with traditional HLH therapies (Henderson and Cron, 2020) (Soy et al, 2020).

Rarely, patients with acquired HLH improve only with eradication of the infection and may not require further immunosuppression.

- Targeting cells.

Etoposide-Based Protocols → The HLH-94 protocol is based on immuno-chemotherapy including dexamethasone, etoposide and CSA to achieve remission of the hyperinflammatory state (Wegehaupt et al, 2020). Subsequently, patients with FHL or recurrent disease proceeded to HSCT (hematopoietic stem cell transplantation). These agents target lymphocytes, macrophages and APCs. Etoposide induces cell death mainly in activated T cells, but also in macrophages and dendritic cells (Wegehaupt et al, 2020). Steroids slow down inflammation by reducing cytokine secretion, but in addition, they have a moderate cytotoxic effect on activated T cells (Wegehaupt et al).

Antithymocyte Globulin (ATG) → Antithymocyte globulin directly targets T cells and other lymphocytes, to a minor extent also monocytes and granulocytes (Wegehaupt et al, 2020).

Alemtuzumab (Anti-CD52) → Humanized monoclonal anti-CD52 antibody alemtuzumab has been used in patients with FLH. It is directed against the CD52 antigen, a surface protein on mature lymphocytes and APCs (Wegehaupt et al, 2020).

- Cytokine-Targeted Therapies.

HLH is associated with excessive production of a large number of cytokines, so block of a single cytokine could not have significant therapeutic effects. However, pivotal studies in mouse models of primary HLH have indicated that some key cytokines, in particular IFN gamma, are drivers of the immune dysregulation and that their neutralization can interrupt the inflammatory circle and restore immune homeostasis (Wegehaupt et al, 2020).

As a consequence, therapeutic approaches targeting IFN-gamma is a possible strategy that is at different stages of evaluation in clinical trials of primary HLH. Preliminary results from a phase III open-label trial of emapalumab (recombinant human monoclonal antibody against interferon gamma) confirm that neutralization of IFN-γ is a promising option (Henderson and Cron, 2020).

An alternate strategy for blocking cytokine effects is to target signaling pathways. Many cytokine receptors signal through JAK/STAT pathways (Henderson and Cron, 2020). Ruxolitinib is a JAK1/2 inhibitor that blocks signaling of type I interferons, IFN-γ, and IL-6, among other cytokines (Wegehaupt et al, 2020).

Others key cytokines to target are: IL-18, IL-1, IL-6 and TNF-α.

Several reports have found elevated free IL-18 concentrations (i.e., IL 18 not bound to its binding protein IL-18BP) in the serum of patients with both HLH. Treatment with IL-18BP can reduce severe organ damage.

IL-1, IL-6, and TNF alpha can be targeted by monoclonal antibodies and other blocking agents (Wegehaupt et al, 2020). The recombinant human IL-1 receptor antagonist, anakinra, blocks the activity of both IL-1α and IL-1β (Henderson and Cron, 2020). Some studies of anakinra, canakinumab, and rilonacept reported that IL-1 blockade alone is not sufcient to prevent MAS in the course of sJIA (Soy et al, 2020).

7.3. Ebola Virus Disease.

Ebola virus disease (EVD) is a severe and frequently lethal disease, with mortality rates up to 90%, caused by Ebola virus (EBOV) (Jacob et al, 2020).

Ebolavirus is part of the Filoviridae family, which consists of three genera: Cuevavirus, Ebolavirus and Marbugvirus (Lalle et al, 2019). Zaire Ebola virus (EBOV) is an enveloped, negative-sense RNA virus that causes an often fatal hemorrhagic disease in humans (Johnson et al, 2014).

In severe and fatal forms, death occurs between 6 and 16 days after symptoms appeared because of shock, seizures, severe metabolic complications, and bleeding (Van der Ven et al, 2015).

Most human cells can become infected, but dendritic cells (DC) and macrophages, both antigen-presenting cells (APCs), are early and sustained targets of EBOV infection. As the primary target cells become infected, they probably facilitate further virus dissemination and migrate to the regional lymph nodes and to the liver and spleen (Jacob et al, 2020).

Ebola Virus Disease (EVD) is commonly associated with multiple organ systems, including the liver, renal organs, and lungs. So far, little is known about the involvement of the respiratory tract and EBOV pathogenesis in the lung (Lalle et al, 2019).

Over the years, there has been an increasing concern regarding the possible involvement of the lung in EBOV infection.

The lung is a vulnerable organ. Airway epithelial cells regulate both innate and adaptive immunity through production of functional molecules and physical interactions with cells of the immune system. Despite these defensive mechanisms, viruses have found ways to evade the immune system, resulting in severe respiratory disease (Lalle et al, 2019).

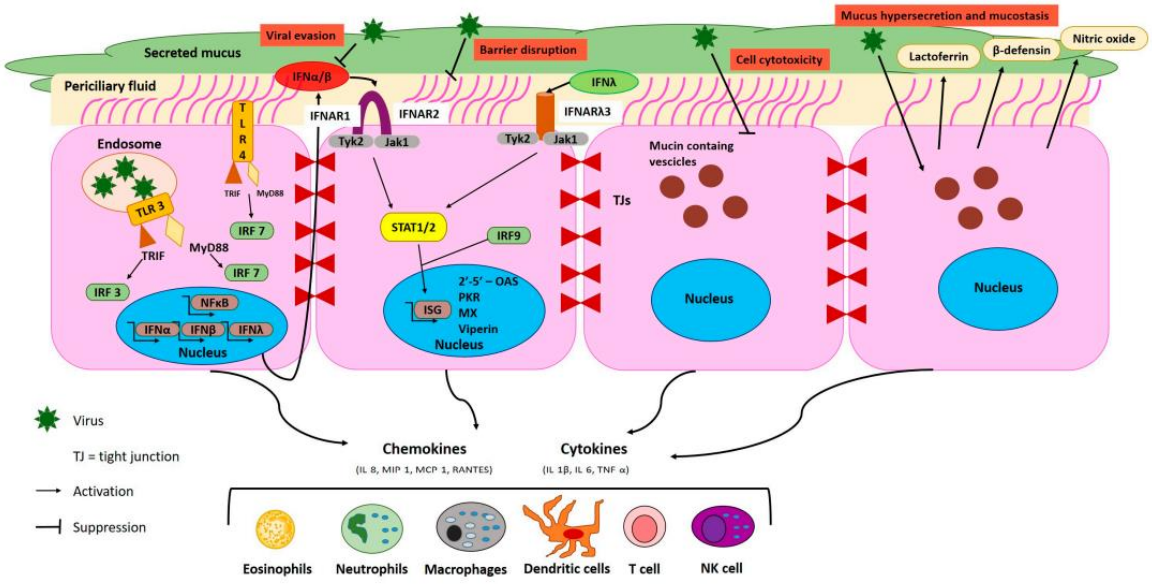

Figure 7.4. Direct and indirect effects of viral infections of the airway epithelium (Lalle et al, 2019).

Infected macrophages are activated to secrete pro-inflammatory cytokines, in particular interleukins IL-1β, IL-6 and IL-8, and tumour necrosis factor (TNF). These secretions probably result in the recruitment of additional EBOV-susceptible

macrophages to the site of infection and, ultimately, the breakdown of endothelial barriers (Jacob et al, 2020) spreading to different organs and causing systemic manifestation of cardiovascular, coagulation, or inflammatory disturbances (Lalle et al, 2019).

The high virulence of EBOV is attributed to the ability to cause endothelial and epithelial inflammation and the hyperactivation of the immune system, through the activation of soluble mediators (cytokines and chemokines).

EBOV infection induces MAPK signaling cascades, and p38 MAPK is thought to play dual roles in DC maturation and induction of inflammatory cytokines (Johnson et al, 2014).

Severe dysregulation of the innate immune response, resulting in a cytokine storm, is associated with severe disease and fatal outcome (Hill-Batorski et al, 2015). The dysregulation of immune mediators in humans has been associated with the secretion of other inflammatory mediators, such as IL-1β, IL-6, IL-8, CXCL1,CXCL10, CXCL11, CXCL12, CCL2, CCL3, CCL13 (Lalle et al, 2019). In addition, severe inflammatory cytokines/chemokines may spill over into the circulation and result in systemic cytokine storms, which are responsible for multi-organ dysfunction and for the impairment of the vascular system and disseminated intravascular coagulation (Lalle et al, 2019).

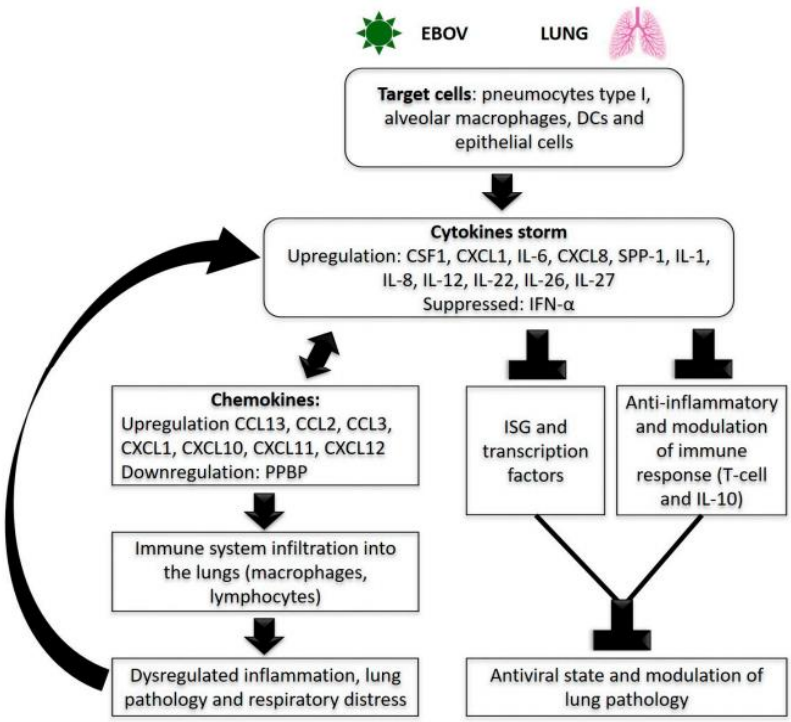

Figure 7.5. EBOV pulmonary disease pathogenesis. Arrows show the proposed sequence of events and inverted Ts show the blocked mechanisms due to the consequences of viral infection (Lalle et al, 2019).

Death was associated with elevated concentrations of cytokines such as IL-1α, IL-1RA, IL-6, MCP-1, MCSF, MIP-1α) (Van der Ven et al, 2015).

IL-1Ra (interleukin 1 receptor antagonist) competes with IL-1 for binding to the IL-1 receptor, blocking IL-1–induced proinflammatory signaling, and may, through this mechanism, affect viral pathogenicity (Hill-Batorski et al, 2015).

7.4. Control of cytokine storm in EVD.

EBOV activates mitogen activated protein kinase (MAPK) signaling upon infection of APCs. (Johnson et al, 2014).

Pyridinyl imidazole p38 MAPK inhibitors exhibit anti-inflammatory properties and may serve as leads for the development of therapeutics to treat EBOV infection (Johnson et al, 2014). The p38 MAPK inhibitors suppressed EBOV-induced cytokine, chemokine and growth factor production.

The identification of HLH as part of severe EVD brings the possibility for a pathophysiologically targeted approach of therapy, such as cytokine directed therapy in the form of recombinant interleukin-1 receptor antagonist (anakinra). Treatment with anakinra has the advantage to interrupt the deleterious IL-1-mediated hyperinflammatory loop in MAS. (Van der Ven, 2015).

Atorvastatin, pravastatin, and rosuvastatin have been reported to alleviate inflammation, reduce C-reactive protein and TNFα levels (Dhama et al, 2018).

8. DEVELOPMENT AND CONTROL OF CYTOKINE STORM IN SARS-COV-2.

The Coronavirus Disease 2019 (COVID-19), first reported in Wuhan, China, has raised acute and grave global concern since December 2019 (Tang et al, 2020).

The most common symptoms of COVID-19 were fever, cough, shortness of breath, fatigue, and myalgia (Tang et al, 2020). Although most patients with COVID-19 had mild and moderate symptoms, severe and critically ill patients progressed rapidly to acute respiratory failure, ARDS, metabolic acidosis, coagulopathy, septic shock, and MOF (multiple organ failure) (Tang et al, 2020).

SARS-CoV-2 is an enveloped, positive sense, single-stranded RNA virus. Its spike (S) proteins facilitate viral entry into their target cells via the interaction with functional cellular receptor identified as angiotensin-converting enzyme 2 (ACE2). Generally, ACE2 was highly expressed in intestinal epithelial cells, alveolar epithelial cells, renal proximal tubular cells, vascular endothelial cells and cardiomyocytes (Gao et al, 2020).

Severe coronavirus disease 2019 (COVID-19) is characterized by systemic hyper-inflammation, acute respiratory distress syndrome, and multiple organ failure (Kim et al, 2021). In serious cases, clinical deterioration is often rapid, and in a large proportion the severe disease course is caused by cytokine storm (Kim et al, 2021).

PATHOPHYSIOLOGY OF CYTOKINE STORM.

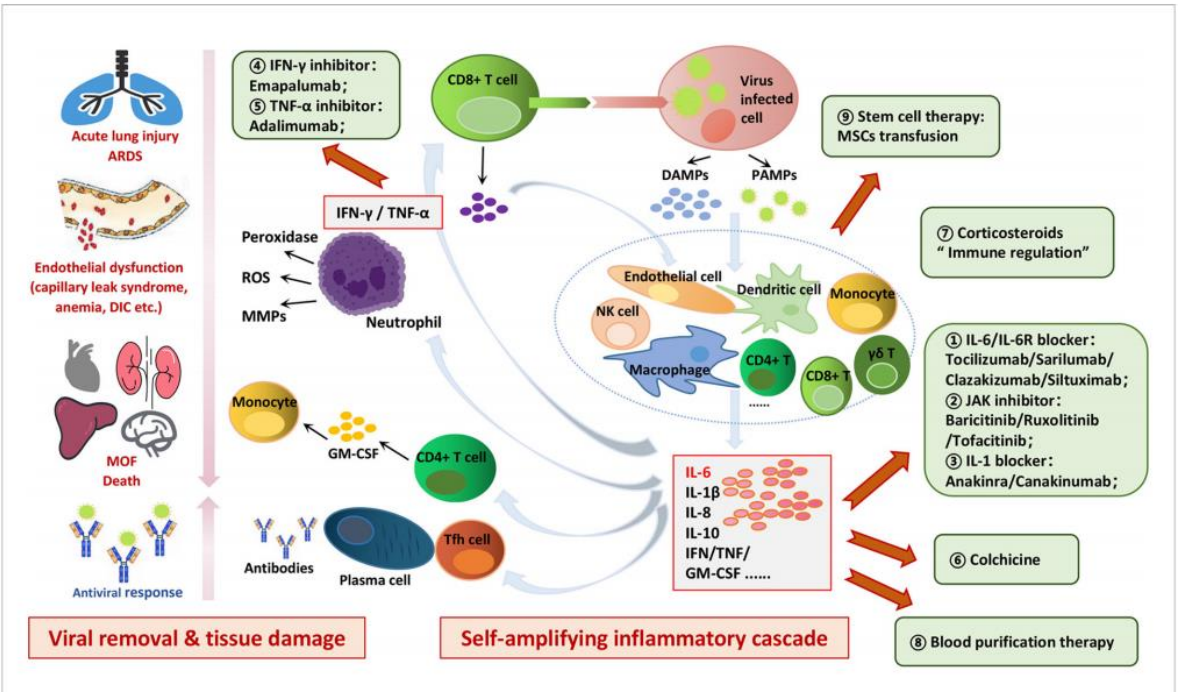

Figure 8.1. Mechanisms and hazards of cytokine storms induced in COVID-19 and potential therapeutic targets (Tang et al, 2020). Downstream production of interferons promotes intracellular antiviral defenses in neighbouring epithelial cells which may limit viral dissemination, while the release of IL-6 and IL-1β. Subsequently, the activation of T cells or lysis of immune cells induces secretion of IFN-γ and TNF-α, leading to the activation of immune cells and endothelial cells with further release of pro-inflammatory cytokines (Tang et al, 2020).

Both damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) are produced upon viral infection, which can induce antiviral responses in neighbouring cells as well as recruit innate and adaptive immune cells (Tang et al), such as NK cells, T cells, macrophages, B cells and

dendritic cells, leading to self-amplifying inflammatory cascade in a positive feedback loop manner.

The major subsets of the T lymphocytes (T cell) (CD3+ CD4+ T cell and CD3+ CD8+ T cells) are reduced in the COVID-19 and are significantly lower in the severe cases (Tang et al, 2020). In this context, it is worth considering that the less pronounced cytokine elevations in COVID-19 could reflect a regulated, or even inadequate, inflammatory response to overwhelming viral infection (Leisman et al, 2020).

Cytokine storm can be divided into two stages:

- The first stage is a temporary immune-deficient condition that is similar to FHL.
- The subsequent secondary stage is an overactive immune state to compensate for the target clearance failure, which appears as a clinical manifestation of a cytokine storm (Kim et al, 2021).

High serum levels of proinflammatory cytokines and chemokines were found in SARS-CoV-2 indicating that exaggerated cytokine and chemokine responses played an important role in the pathogenesis of CoV infection (Gao et al, 2020).

COVID-19 patients display high levels of both inflammatory cytokines and chemokines (IL-1a/β, IP-10, MCP-1), with severe cases showing elevation in MCP-1, MIP-1A, IL-1, IL-6, IL-8, IL-10, IL-18, TNF-α (Chu et al, 2021).

Coagulation is intricately linked with inflammation, in particular elevated levels of the pro-inflammatory cytokine, interleukin 6 (IL-6) and C reactive protein (CRP). The IL-6 plays a crucial role in the pathologic of COVID19, including the chemotaxis of neutrophils and lymphocyte necrosis (Tang et al, 2020).

8.1. Control of cytokine storm in SARS-CoV-2.

Even though a large variety of treatments to mitigate cytokine storm have been introduced, no concrete treatment recommendations have been issued so far for COVID-19 (Kim et al, 2020).

Table 8.1. Therapeutic options for the treatment of cytokine storm in COVID-19 (Kim et al, 2020).

Targeted inhibition	Drugs or Interventions	Previous established or alternative indications
IL-1	Anakinra, Canakinumab	RA, MAS-HLH
IL-6	Tocilizumab, Sarilumab, Siltuximab	RA, SJIA, CRS, Castleman disease
TNF-α	Etanercept	RA, SJIA
IFN-γ	Emapalumab	Primary HLH
JAK	Baricitinib, Ruxolitinib	RA, MF, PV
Non-selective	Glucocorticoid	Various autoimmune diseases and hematologic malignancies
Non-selective	Colchicine	Gout
Non-selective	Mesenchymal stem cell	Not yet documented, Regenerative medicine
Non-selective	Plasma exchange	Various Ab-mediated diseases, Hyperviscosity syndrome
Non-selective	Intravenous immunoglobulin	Various autoimmune and infectious diseases, Ab deficiency disorders
Non-selective	Convalescent plasma	Not yet documented, Rescue therapy in severe infectious diseases
Non-selective	Radiation	Tumor

Ab: antibody; COVID-19: coronavirus disease 2019; CRS: cytokine release syndrome; HLH: hemophagocytic lymphohistiocytosis; IFN: interferon; IL: interleukin; JAK: Janus kinase; MAS: macrophage activation syndrome; MF: myelofibrosis; PV: polycythemia vera; RA: rheumatoid arthritis; SJIA: systemic juvenile idiopathic arthritis; TNF-α: tumor necrosis factor-alpha.

Treatments with specific cytokine inhibitors.

In light of successes in the treatment of various types of cytokine storm, a few cytokine blockades have been explored in the treatment of severe COVID-19 (Kim et al, 2020).

- IL-6 Inhibition

IL-6 is an important pro-inflammatory cytokine that has pleiotropic effects. IL-6 promotes the production of various acute phase proteins in hepatocytes and induces the differentiation of immune cells, such as B and T cells (Kim et al, 2020).

Tocilizumab (TCZ) is an anti-human IL-6 receptor monoclonal antibody, preventing IL-6 binding to its receptor to exert the immunosuppression promoted by IL 6 (Tang et al, 2020). One clinical trial from China (ChiCTR2000029765) reported that tocilizumab made rapid improvements in fever control and respiratory functions in 21 severe patients with COVID-19 (Tang et al, 2020).

Sarilumab is another kind of IL-6R blocker that is being investigated in SARS-CoV-2 infection (Tang et al, 2020).

- IL-1 Inhibition

There are three important cytokines of IL-1 family in cytokine storms: IL-1β, IL-18, and IL-33.

Signaling IL-1 is one of the major pro-inflammatory cytokines. It is comprised of two types of ligands, IL-1α and IL-1β, of which IL-1β plays a major role with systemic effects (Kim et al, 2020).

Anakinra, a modified form of the human IL-1 inhibitor, can be used in the treatment of cytokine storms caused by infection. Canakinumab, a monoclonal antibody selectively targeting IL-1β, is also being investigated in the treatment for COVID-19. (Tang et al, 2020).

- Inhibition of TNF-α

TNF-α is a cytokine primarily produced by activated macrophages in the acute inflammatory phase (Kim et al, 2020). Anti-TNF-α therapy has not been actively attempted in COVID-19.

- Inhibition of IFN-γ

IFN-γ is secreted by several immune cells and plays a role in stimulating the main inflammatory effector cells directly (Kim et al, 2020). Several studies have documented clinical effects for emapalumab (Kim et al, 2020).

- Inhibition of JAK pathway

JAK is an intracellular tyrosine kinase that mediates signals from cytokines, hormones, and growth factors. JAK/STAT signal transduction pathway can block multiple targeted cytokines at the same time if inhibited (Tang et al, 2020). On the other hand, non-selective inhibition of the immune response poses a risk of secondary infection (Kim et al, 2020).

JAK inhibitors, such as tofacitinib, baricitinib, and ruxolitinib (selective inhibitors of JAK1/JAK2) are currently being investigated to determine whether they can be applied to the treatment of COVID-19 (Tang et al, 2020).

Corticosteroids.

Glucocorticoid induces gene transcription and protein synthesis of NF-κB inhibitors and lipocortin-1. Through inhibition of NF-κB signaling, glucocorticoids induce inhibition of synthesis of downstream proteins such as granulocyte-macrophage colony-stimulating factor, IL-6 and IL-1, Glucocorticoids reduce also the proliferation, activation, differentiation, and survival of T cells and macrophages (Tang et al, 2020).

The TH1 and macrophage-based pro-inflammatory cytokines TNF-α , IL-17, IL-2, IL-6 and IL-1 (Tang et al, 2020).

Blood purification therapy.

The application of blood purification technology is helpful to the removal of cytokines and may be beneficial to improve the prognosis of critically ill patients. Commonly used extracorporeal blood purification treatments in CSS include plasma exchange, blood/plasma filtration, adsorption, perfusion and continuous renal replacement therapy (Gao et al, 2020).

9. CONCLUSION.

Accumulating evidence has shown that cytokine storm might be one of the most important and deadly complications in severe patients with HLH, Ebola and Covid-19.

Cytokine storm development in infection diseases remain a leading cause of bad prognosis and mortality.

By elucidating the key role of cytokines in driving the pathophysiology of the disease, entirely new classes of medications have become available. Cytokines can be directly neutralized with biologic agents. Alternately, the action of cytokines can be inhibited by blocking signaling pathways with JAK inhibitors. These cytokine-directed therapies could be the future optional treatment to control inflammation.

Suppression of cytokine release at an early stage of disease as treatment is still controversial, the timing and the doses of the intervention still need to be inspected clearly.

However, knowledge of the pathophysiology of diseases such as HLH and Ebola has led to the development of approaches for the treatment SARS-CoV-2 severe cases.

10. BIBLIOGRAPHY.

Abbas, A. K., & Lichtman, A. H. (2004): Inmunología celular y molecular (5.ª ed.). Elsevier.

Ana Copaescu, A., Smibert, O., Gibson, A., Phillips E. J., & Trubiano, J. A. (2020). The role of IL-6 and other mediators in the cytokine storm associated with SARS-CoV-2 infection. J Allergy Clin Immunol 146, pp. 518-534.

Bastard, P., Rosen, L. B., Zhang, Q., Michailidis, E., Hoffmann, H. H., Zhang, Y. et al. (2020). Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 370, 423.

Channappanavar, R. & Perlman, S. (2017): Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol, 39, pp. 529–539.

Chu, R., van Eeden, C., Suresh, S., Sligl, W. I., Osman, M. & Tervaert, J. W. (2020). Do COVID-19 Infections Result in a Different Form of Secondary Hemophagocytic Lymphohistiocytosis. International Journal of Molecular Sciences, 22, 2967.

Crayne, C. B., Albeituni, S., Nichols, K. E. & Cron, R. Q. (2019). The Immunology of Macrophage Activation Syndrome. Frontiers in Immunology, 10, 119.

Dhama, K., Karthik, K., Khandia, R., Chakraborty, S., Munjal, A., Latheef, S. K. et al. (2018). Advances in Designing and Developing Vaccines, Drugs, and Therapies to Counter Ebola Virus. Frontiers in Immunology, 9(1803).

Dhanasekaran, D. N. & Reddy, E. P. (2008). JNK signaling in apoptosis. Oncogene, 27, pp. 6245 –6251.

Fainboim, L., & Geffner, J. (2005): Introducción a la Inmunología Humana (5ª ed.). Medica Panamericana.

Fajgenbaum, D. C. & June, C. H. (2020). Cytokine Storm. The New England Journal of Medicine, 383, pp. 2255-73.

Filella, X., Molina, R. & Ballesta, AM. (2002): Estructura y función de las citocinas. Med Integral, 39(2), pp. 63-71.

Gao, Y. M., Xu, G., Wang, B & Liu, B. C. (2020). Cytokine storm syndrome in coronavirus disease 2019: A narrative review. Journal of Internal Medicine, 289(2), p. 147-161.

Gordon, J. R., Ma, Y., Churchman, L., Gordon, S. A., & Dawicki, W. (2014). Regulatory Dendritic Cells for Immunotherapy in Immunologic Diseases. Frontiers in Inmunology, 5.

Henderson, L. A. & Cron, R. Q. (2020). Macrophage Activation Syndrome and Secondary Hemophagocytic Lymphohistiocytosis in Childhood Inflammatory Disorders: Diagnosis and Management. Paediatr Drugs, 22(1), pp. 29–44.

Hill-Batorski, L., Halfmann, P., Marzi, A., Lopes, T., Neumann, G., Feldmann, H. & Kawaoka, Y. (2015). Loss of Interleukin 1 Receptor Antagonist Enhances Susceptibility to Ebola Virus Infection. The Journal of Infectious Diseases, 212, pp. 329–35.

Huang, G., Shi, L. Z. & Chi, H. (2009). Regulation of JNK and p38 MAPK in the immune system: Signal integration, propagation and termination. Cytokine, 48, pp. 161 –169.

Jacob, S. T., Crozier, I., Fischer, W. A., Hewlett, A., Kraft, C. S., de La Vega, M. A. et al. (2020). Ebola virus disease. Nature Reviews, 6(13).

Johnson, G. L., & Nakamura, K. (2007). The c-Jun kinase/stress-activated pathway: Regulation, function and role in human disease. Biochim Biophys Acta, 1773, pp. 1341–1348.

Johnsona, J. C., Martinez, O., Honkoa, A. N., Hensleya, L. E., Olingera, G. G. & Baslere, C. F. (2014). Pyridinyl imidazole inhibitors of p38 MAP kinase impair viral entry and reduce cytokine induction by Zaire ebolavirus in human dendritic cells. Antiviral Res, 107, pp. 102–109.

Kim, K. J., Lee, J. Y., Yang, J. W., Lee, K. H., Effenberger, M., Szpirt, W. et al. (2021). Immunopathogenesis and treatment of cytokine storm in COVID-19. Theranostics, 11, pp. 316-329.

Lalle, E., Biava, M., Nicastri, E., Colavita, F., Di Caro, A., Vairo, F. et al. (2019). Pulmonary Involvement during the Ebola Virus Disease. Viruses, 11, 780.

Leisman, D., Ronner, L., Pinotti, R., Taylor, M., Sinha, P., Calfee, C., et al. (2020). Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. Lancet Respir Med, 8, pp. 1233–44.

Lukan, N. (2020): “Cytokine storm”, not only in COVID-19 patients. Mini-review. Immunology Letters, 228, pp. 38-44.

Mehta, P., Cron, R. Q., Hartwell, J., Manson, J. J. & Tattersall, R. S. (2020). Silencing the cytokine storm: the use of intravenous anakinra in haemophagocytic lymphohistiocytosis or macrophage activation syndrome. Lancet Rheumatol, 2, pp. 358–367.

Morris, R., Kershaw, N. J. & Babon, J. J. (2018). The molecular details of cytokine signaling via the JAK/STAT pathway. Protein Science, 27, pp. 1984-2009.

Morrison, D. K. (2012). MAP Kinase Pathways. Cold Spring Harb Perspect Biol, 4.

Obstfeld, A. E., Frey, N. V., Mansfield, K., Lacey, S. F., June, C. H., Porter, D. L. et al. (2017). Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. Blood, 130 (23), pp. 2569-72.

Opoka-Winiarska, V., Grywalska, E. & Roliński, J. (2020). Could hemophagocytic lymphohistiocytosis be the core issue of severe COVID-19 cases? BMC Medicine, 18:214.

Quan, C., Li, C., Ma, H., Li, Y. & Zhang, H. (2021). Immunopathogenesis of Coronavirus-Induced Acute Respiratory Distress Syndrome (ARDS): Potential InfectionAssociated Hemophagocytic Lymphohistiocytosis. Clinical Microbiology Reviews, 34.

Rincon, M. & Davis RJ. (2009). Regulation of the immune response by stress-activated protein kinases. Immunol Rev, 228, pp. 212 –224.

Rowaiye, A. B., Okpalefe, O. A., Adejoke, O. O., Ogidigo, J. O., Oladipo O. H., Chidinma Ogu, A. C. et al. (2021). Attenuating the Effects of Novel COVID-19 (SARS-CoV-2) Infection-Induced Cytokine Storm and the Implications. Journal of Inflammation Research, 14, pp. 1487–1510.

Singh, N., Hofmann, T. J., Gershenson, Z., Levine, B., Grupp, S., Teachey, D. T. et al. (2017). Monocyte lineage-derived IL-6 does not affect chimeric antigen receptor T-cell function. Cytotherapy, 19(7), pp. 867–880.

Sinkala, M., Nkhoma, P., Mulder, N. & Martin, D. P. (2021). Integrated molecular characterisation of the MAPK pathways in human cancers reveals pharmacologically vulnerable mutations and gene dependencies. Communications Biology, 4:9.

Soy, M., Atagündüz, P., Atagündüz, I. & Sucak, G. T. (2020). Hemophagocytic lymphohistiocytosis: a review inspired by the COVID-19 pandemic. Rheumatology International.

Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J. & Wen, C. (2020). Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. Frontiers in Immunology, 11, 1708.

Van der Ven, A. J. A. M., Netea, M. G., van der Meer, J. W., & de Mast, Q. (2015). Ebola virus disease has features of hemophagocytic lymphohistiocytosis syndrome. Frontiers in Medicine, 2(4).

Wang, J., Jiang, M., Xin Chen, X. & Montaner, L. 2020. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. Journal of Leukocyte Biology, pp. 1–25.

Wegehaupt, O., Wustrau, K., Lehmberg, K. & Ehl, S. (2020). Cell Versus Cytokine – Directed Therapies for Hemophagocytic Lymphohistiocytosis (HLH) in Inborn Errors of Immunity. Frontiers in Immunology, 11, 808.

Weichhart, T., Hengstschläger, M. & Linke, M. (2015). Regulation of innate immune cell function by mTOR. Nat Rev Immunol., 15(10), pp. 599–614.

Zhang, Q., Bastard, P., Liu, Z., Le Pen, J., Moncada-Velez, M., Chen, J. et al. (2020). Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 370, 422.

Zhang, Y., Jing, Y., Qiao, J., Luan, B., Wang, X., Wang, L. & Song, Z. (2017). Activation of the mTOR signaling pathway is required for asthma onset. Scientific Reports, 7, 4532.