B cells over-activation by viral proteins <70 kDa causes Th2 immune suppression in COVID-19 sepsis

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Abstract:

COVID-19 sepsis immune response remains unclear. Here we propose a new perspective in host response against pathogenic proteins that may lead to a vaccine design by polymerization of antigens of <70 kDa.

In COVID-19, initial Th1 response kills infected cells releasing viral proteins.

SARS-CoV-2 viral structural proteins are Spike (140 kDa), Nucleocapsid (50 kDa), Membrane (25 kDa) and Envelope (10 kDa).

B cell receptor cannot capture antigens >70 kDa.

The Spike protein (140 kDa) cannot be captured by B cells and triggers inflammatory Th1 response via the macrophages.

Only proteins with a size <70 kDa can activate B cell receptor and trigger Th2 adaptative humoral response.

Moreover, M-25 kDa and E-12 kDa glycoproteins can activate IgM-BCR like oligovalent or monovalent antigens.

The sustained infected cells lysis overfeeds high levels of viral proteins <70 kDa, increases B cells activation and, in the shift from Th1 to Th2 immune response, triggers the cytokine storm.

The continuous BCR activation increases IL-10 releasing and may lead to immune paralysis.

Keywords:

COVID-19, SARS-CoV-2, antigen, monovalent, oligovalent, protein, kilodalton (kDa), Th1 response, Th2 response, B cell activation, B cell receptor (BCR), macrophage, dendritic cell, apoptosis, subcapsular sinus, immunoglobulin, interleukin, cytokine, Cytokine Storm Syndrome (CSS), allergen, immune paralysis, vaccine, polymer.

Introduction:

COVID-19 is a disease caused by novel Severe Acute Respiratory Syndrome Coronavirus-CoV-2 (SARS-CoV-2) which pathogenesis still remains under investigation.

C. Huang et al. (The Lancet, January 2020), has reported that patients infected with SARS-CoV-2 had high levels of inflammatory cytokines, probably because of activated T-helper-1 (Th1) cells response. Those patients that required ICU (Intensive Care Unit) admission had higher concentrations of inflammatory cytokines than those not requiring ICU admission, suggesting that cytokines storm was associated with disease severity. (1)

However, COVID-19 infection also initiated increased secretion of T-helper-2 (Th2) cytokines, interleukin 4 (IL-4) and interleukin 10 (IL-10), that suppress inflammation. The significant progressive elevation of T helper 2 (Th2) cytokines was correlated with the immune suppression and fatal infection in patients. (1)

The pro-inflammatory phase Th1 and the following dysregulated Th2 immune response was characterized by reduction of the peripheral lymphocyte counts that was associated with a high risk of developing secondary bacterial infection. (1, 2)

C. Huang et al. consider that it is needed to elucidate the responses shifts Th1 to Th2 and cytokine storm associated with COVID-19 infection severity.

Regarding shift Th1 to Th2 cytokines and immune suppression in COVID-19:

In sepsis, the increase of Th2 anti-inflammatory cytokines, such as IL-10 and Transforming Growth Factor beta (TGF β) could accelerate apoptosis. (3, 4)

The Th2 immune response polarization is promoted by human dendritic cells (DCs) upon interaction with activated B cells. (6)

The binding of specific antigens to B cells triggers activation of its receptors (BCR) and IL-10 releasing, playing a critical role in the immune system balance by negatively regulating inflammatory response increasing Th2 immune response. (7)

Roozendaal R et al. (Immunity, 2009) has reported that antigen recognition by circulating naïve B cells occurs in follicles present in peripheral lymphoid organs (lymph nodes and spleen), which receive lymphatic fluid originating in peripheral tissues and delivered to lymph nodes through the afferent vessels or via blood through the trabecular artery. Antigens smaller <70 kDa quickly reach follicular B cells through conduits that penetrate the follicle. Larger antigens >70 kDa are retained within the subcapsular sinus by macrophage and follicular dendritic cells (FDCs) that capture and present antigen to B cells by tethering complement or Fc γ receptor. The follicular conduits provide an efficient and rapid mechanism for delivery small antigens <70 kDa to B cells. (8, 9)

It is obvious that antigens size is critical in the increase of IL-10 release and Th2 immune response, that is modulated by B cells activation restricted to the capture of <70 kDa antigens. (10)

SARS-CoV-2 viral structural proteins are: Spike protein (140 kDa), Nucleocapsid protein (50 kDa), Membrane glycoprotein (25 kDa) and Envelope glycoprotein (10 kDa). (11, 12)

Moreover, Membrane glycoprotein (25 kDa) and Envelope glycoprotein (10 kDa), which are similar or minor in size to immunoglobulins fragment Fab arm, can make a engage, oligovalent or monovalent, with the immunoglobulin M of B cells receptor (IgM-BCR) but not with IgD-BCR. (13, 14)

Monovalent membrane-bound of the antigen is efficient at triggering BCR signals, and this antigen-B cell interaction increases Th2 type response, but does not promote immune memory. (13, 14, 15)

Small antigens, which acquire enhanced crosslinking capacity by aggregation in the IgM-BCR and the expression of repeated epitopes, may gain antigenicity. The isotype-specific compartmentalization of the B-cell plasma membrane may be the reason for the different signalling in the IgM-BCR and IgD-BCR when exposed to monovalent antigens.

Therefore, the shift Th1/Th2 immune response in COVID-19, over the time, could be estimated in the relation between the number and size (>70 kDa/<70 kDa) of the viral protein burden:

$$\text{Balance } \frac{\text{Th1}}{\text{Th2}} = f\frac{n\,(\text{S 150 kDa})}{1} + g\frac{1}{n(\text{N 50 kDa})} + h\frac{1}{n\,(\text{M 25 kDa})} + i\frac{1}{n\,(\text{E 10 kDa})}$$

f, g, h i: function avidity, affinity and host genetic, n: proteins amount

The balance between viral proteins >70 kDa and viral proteins <70 kDa defines the type of predominant Th1 or Th2 immune response.

Regarding cytokines storm and immune paralysis in COVID-19:

The Cytokine Storm Syndrome (CSS) is a robust form of Systemic Inflammatory Response Syndrome (SIRS) triggered by a variety of factors such as antigens, superantigens, allergens and, sometimes, adverse effects of therapies. The Cytokine Storm, in the critical pathologic of these processes, have a similar cytokines network: Tumor Necrosis Factor alpha (TNFα), IL-6 and IL-1 β, among others. (5, 16)

In general, initial pro-inflammatory Th1 immune response is thought to be responsible for collateral tissue damage and severe sepsis, whereas anti-inflammatory Th2 immune response, that limits tissue injury, is implicated in the enhanced susceptibility to secondary infections.

The systemic production of IL-10 following the onset of a cytokine storm is a marker of an anti-inflammatory response termed "immune paralysis". (5)

In the COVID-19 sepsis, the increase of Th2 anti-inflammatory cytokines, such as IL-10 and Transforming Growth Factor beta (TGF β), could accelerate apoptosis. (3, 4)

The shift from Th1 to Th2 cytokine production, during apoptosis, inhibits pro-inflammatory cytokines and activates anti-inflammatory factors. (3)

The cytokine storm is a pathway triggered by a strong Th1 pro-inflammatory innate immune response that shifts to a strong and sustained Th2 anti-inflammatory response.

The virulence of the infection causes cell lysis, and the Th1 immune activity kills infected cells, releasing strong amounts of disassembled viral proteins which number, size and immunogenicity are critical in the immune response type shift.

Spike protein (140 kDa), that is the only structural protein >70 kDa in the SARS-CoV-2, promotes inflammatory Th1 immune response via the macrophage in the subcapsular sinus and via the phagocytosis of the dendritic cells.

Since its first exposure, the size of the other proteins <70 kDa, that are majority, can only activate B cell receptor and trigger Th2 adaptive humoral response, that expand specific B and T cell clones.

The sustained overfeed of viral particles, released by viral lysing of the cells that are also killed by Th1 pro-inflammatory response, increases the levels of proteins <70 kDa that activate a great amount of B cells.

Along time, the repeated immune stimulation of B cells may lead to an Activation-Induced Cell Death (AICD), accelerating apoptosis and lymphopenia by the release of anti-inflammatory cytokines, such as interleukin IL-10, increasing Th2 response shift, and leading to immune suppression, sepsis and death.

The response magnitude of the Cytokine Storm Syndrome depends on the viral protein burden and the host genetic characteristics plus its coexisting illnesses.

In COVID-19, the cytokines network caused by the over stimulation of the immune response during the shift from Th1 to Th2 is what triggers the cytokines storm.

Conclusions:

B cell receptor cannot capture antigens >70 kDa.

The binding of antigens <70 kDa to B cells triggers the activation of its receptors.

Activated B cells can induce Th2 polarization and immune suppression.

Cytokine storm is caused by the over stimulated cytokines network during the shift from Th1 to Th2 immune response.

It can be deduced that, the balance between antigenic proteins >70 kDa/<70 kDa in a pathological process defines the type of predominant Th1 or Th2 host immune response.

The perspective here described may be important for polymer-based vaccines design.

Proteins N (50kDa) + M (25 kDa) + E (10 kDa) →   Polymer N-M-E (85 kDa)

Experimental works:

The perspective here presented is based on the works of Amit AG et al. (Science 1986) regarding crystallographic analysis on the lysozyme antigen interaction with the Fab arm of the receptor B. (19)

Also, on statistical research of this author about the similar sizes (around 25 kDa) of the allergens and antigens. Later, these data were correlated with the clinical signs, interleukins and immunoglobulins levels that manifest in viral infections, like flu and others, and allergic syndromes. (20, 21)

Experimental works regarding this paper were conducted in 1996 by the Epidemiology Service and Immunology Laboratory of the Hospital Universitario Infanta Cristina de Badajoz (Spanish National Health Service). (21)

These experimental results were described by this author on the patent *Polymerized vaccines* (International free patent number: PCT/ES1996/000145), and referred in the book *Vacunas de Nueva Generación*, page 107. (21, 22)

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