

THE IMMUNE FEATURES OF NEUTROPHIL AND NEUTROPHIL IMMUNE DEVIATION INDUCED BY HUMAN SARS-COV-2 INFECTIONS

Abstract

Current published scientific works on human neutrophils have shown them to be heterogeneous in their immune functions. Activated neutrophils perform both up regulation and down regulation processes on other immune cells finalized by either tissue protection or tissue damage in virus human diseases. The objectives of the present opinion were to; I - make a show case analysis for the immune activities of neutrophil compartment both in children and adults with covid-19 illness and ii - to deduce the possible existence of immune deviation mechanisms in this disease. The absolute numbers of circulating neutrophils in childhood and adulthood patients with sars-cov-2 infection were correlated with the infection severity. It was apparent that there was inhibition of T cell mediated immune responses with an elevated sars-cov-2 anti-spike antibodies in continuum with activated immature neutrophil phenotypes that might be induced by the virus antigens and can either preserve or damage the affected tissue. The antibody may directly inhibit T cells or activate neutrophil phenotype to inhibit T cell responses. The features of this immune deviation are ; i – Conditional , ii – reversible, iii – associated with active functional state , iv – the virus antigen induced chemokine that orchestrate neutrophil and induce hyper-cytokemia ,v – acquisition of molecular surface markers variation and appearance of inhibitory markers , vi – the neutrophil/lymphocyte ration dis-proportionated , vii – activation consequences leaves tissue pathology at most ,and viii – The inflammatory circuit stages may serve drug target identification and development. Thus

the opinion suggest that the absolute number of circulating neutrophils is correlated with disease severity and the existence of neutrophil immune deviation in covid-19 human disease .

KEY WORDS: Activated, antibodies ,adult , cell ,child ,deviation ,immature, immune, neutrophil

I - Introduction

The first tempt in understanding Immune deviation[ID] had been; the immune response to different flagellin protein antigens of Salmonella

Adelaide had shown an inverse relationships between delayed type hypersensitivity DTH and antibody response[1].The dictionary definition of immune deviation was the phenomenon of antigen specific exclusion between cell mediated immunity and humoral mediated immunity[2].The TH1 derived cytokines IFN gamma, is the most potent inhibitor of TH2 Immune cells and vice versa IL4,IL10 and to lesser extent TGFB suppress the TH1 cell function [3].In covid-19 severe infection forms the patient have shown immune system deviations, the scientific workers involved in this studies have present three molecular mechanisms and proposed receptor seemed to be out of the norm of outstanding covid-19 workers therein bacterial remanents causing the exaggerated immune reactions in covid-19[4].Ocular immune response to viral invaders can be protective or dangerous depending on the operable immune deviation mechanisms[5].pro-inflammatory cytokine induced immune deviation on immunotherapy of rheumatoid arthritis[6] and Type two immune deviation mechanisms were operable in atopic dermatitis[7].Neutrophil compartment in virus human disease[5] may express either up regulation and/or down regulation processes on the other immune cells which may finalized by tissue preservation or tissue damage. The objective of the present opinion was to make show case analysis for exploring the role of neutrophils in childhood[8] and adulthood[9,10,11,12,13,14] sars-cov-2 infection and to deduce the possible existence of neutrophil immune deviation in covid-19 both in childhood [8] and adulthood[9,10,11,12,13,14].

II - Neutrophil

II-1:Basic Cell And Molecular Biology

Neutrophil leukocytes are being of 12-15 um in diameter in stained preparations as appeared under light microscope. Neutrophil has lobed nuclei with 2-5 lobes .Each lobe separated from the other and connected by nuclear filaments surrounded by chromatin. It appeared that there were neither DNA replication nor DNA repair does happened in these leukocytes with large number of granules in the cytoplasm. These granules are of two basic types azurophilic which contained; myeloperoxidase ,lysosome ,cathapsin acetase G and modified acid enzymes and specific granules that

composed of ;lysosome, collagenase as well as lactoferrin protein-Vitamin B 12. Neutrophil cytoplasm includes ; few polyribosomes in which low level of protein synthesis does happened and little endoplasmic reticulum. Cellular energy secured by glycolytic metabolism of the excessive amount glycogen available in the cytoplasmic continuum during static phase and by hexose monophosphate cycle .Neutrophil cellular skeleton composed of microtubule network part of which extend up to plasma membrane Such network important in phagocytosis and direct cell movement. Parallel to microtubule network there is actin fine filament network connecting in between by actin connecting proteins to the cell periphery. Neutrophil cellular energy derive from the metabolic elements; actin ,myocin ,magnesium and chilfrolin needed for yielding energy eligible for motility, phagocytosis and secretion. The cell surface markers of neutrophil are;CD25 CD11a CD11b CD49b CD32 CD16. They are devoid from FCRR1 and CD23. Neutrophil are the dominant in peripheral blood and the potent professional phagocytes and be the first liner in the local inflammatory foci. They act in combating the invading bacteria and viruses. Neutrophil perform an array of immune-biologic functions like; exocytosis ,extra-cellular killing and respiratory burst. The phagocytic events starts with ,chemo-taxis, opson-ization, ingestion ,killing and digestion. Phagocytosis can be of several forms as; pinocytosis, antibody facilitated, aborted phagocytosis and phagocytic regression[15,16].Bone marrow in healthy subjects differentiate three subsets of neutrophils as; expansive, trafficking and effectors[17].In health ,neutrophil stands as first line cell mediated defense against microbial invasion of human body. They performed an array of immune-physiologic functions such as; phagocytosis ,extracellular trap and reactive oxygen species ROS production .Phagocytic activity and ROS production increased with maturation stage. Mature neutrophil chemokines in circulation orchestrate them to tissue localities and activate their ROS production and L selectin capacities[18,19,20].In disease, however, neutrophil In Disease Excessive neutrophil activation induce synthesis and production of defensin and elastases which in turn lead to increase in vascular permeability[21,22].NET formation[23,24] largely contribute to activation and damage of the endothelial tissues[24] triggering the inflammatory circuits[25].

II – 2 ; Structural Anomalies

In acute covid-19 disease phase and before initiation of therapy ,neutrophil in peripheral blood stained films have showing nucleus and cytoplasmic granules. Three, main forms of structural abnormalities were detected as major hyper-pigmented and minor hypo-pigmented anomalies. First, the hyper-pigmented forms express crowded dark granulations in cytoplasm similar to that of toxic granulation and peripheral light blue agranular areas. The second, minor neutrophil anomalies were showing hypo-pigmented cytoplasm and band form nuclei while the third anomaly was the dys-morphic with total absence of nuclear segmentation consistent with Pseudo-Pelger morphology .An additional form appeared as apoptotic cells with liquid-fied chromatin and granulated deep blue cytoplasm. Immature neutrophil granulocyte appeared as small myelocytes and metamyelocytes were noted in peripheral blood films in early phase cases. These early phase cases were presenting an overall of immaturity ,dys-morphisim and apoptotic degenerative morpho-types. Post-treatment cases have shown shift towards impressive lymphocyte activation. Such anomalies are attributable to hypercytokinemia and hyper –inflammation, therein, initiating disordered granulopoiesis [16].

II-3 : Functional phenotypes

In circulation mature neutrophils in covid-19 patients were showing an array of phenotypes. The homeostatic ,aged and interferon stimulated gene phenotypes .Bone marrow neutrophil phenotypes express as neutrophil committed progenitors, the pro-neu encoded by azurophil granule gene with CD81 CD43 CD15 CD66;the pre-neu is encoded by specific granule gene LTF and express CD11b CD66b CD101CD45d.The immature neutrophil bear surface markers,CD11b CD66b CD101+/- CD10- CD16+/- .Mature neutrophils governed by granule and secretory granules genes with surface markers of CD11b+ CD66b+CD10+ CD16+[18,27,28].

III – Neutrophil In Covid-19

III- 1 : Childhood

Neutrophil from childhood covid-19 cases were analyzed for phenotype and function in comparison to normal subjects. The infected child neutrophil have shown low expression of CD11b CD66b and L selectin with a higher expression of activation markers HLA-DR CD64 and PECAM-1 and inhibitory receptors LAIR-1 and PD-L1. No difference were noted between patient and controls in cytokine production and NETosis. Low expression of adhesion molecules with high expression of inhibitory receptors might prevent tissue infiltration by neutrophil preserving lung function. This is parallels with variable IgG response in patients and baseline titres of controls[8]. IgM and IgG responses in child with covid-19 were high ,but lower than adult covid-19. humoral immune profiles of child similar to that of adult mild covid-19. IgG attenuate neutrophil phagocytosis. IgA is linked to neutrophil activation but to lesser extent than in adult covid-19. RBD specific monocyte enriched lungs .Distinct humoral immune profile to different covid-19 disease forms[10],Table -1.

Table -1 ;Show case study of childhood covid-19.

Feature [8]	[10]
Naïve mature Neutrophil Bone Marrow	Bone Marrow
Activated mature Neutrophil Circulation	Circulation
Immature activated Neutrophil Circulation	Circulation
IgG Functional	Functional
IgA ND	Functional variable concentration
IgA-Neutrophil ND IgG-Neutrophil ND Low expression of inhibitory molecules, preserve lung	Derive active phagocytic function Attenuate neutrophil phagocytosis Preserve lung

III – 2: Adulthood

Neutrophil in adult covid-19 showed; exaggerated oxidative burst, NETosis, and phagocytosis relative to normal control. Besides the elevation of associated cytokines the interleukine IL8 and IL6 and TNF. Increased NETosis correlated with leukocytosis and neutrophilia. Neutrophil and NETs were identified in the airway and alveoli in lung paranchyma in 40% of severe covid-19. Absolute neutrophil count and IL8 increases correlated with disease severity .Increase levels of IL8 alone correlated with death. Thus circulating neutrophil exhibit an active phenotype with enhanced NETosis and oxidative burst[9]Neutrophil express FC receptors for IgG, both IgG and IgA induce different results of neutrophil function IgG derive and may attenuate neutrophil phagocytosis and cytokine secretion. IgA can augment these functions in addition to derive robust degranulation ,cytokine secretion and NETosis.

The study of humoral immune signature in adults with covid-19 have shown higher IgM and IgG isotypes than controls and childhood covid-19. IgM and IgG responses appeared later than in childhood covid. Humoral Immune profiles of mild adult covid similar to that of child covid. IgG attenuate neutrophil phagocytosis .IgA activate neutrophil phagocytosis ,it derives excessive neutrophil activation in severs covid forms which did not appeared in mild and moderate forms. IgA depletion attenuate neutrophil phagocytosis. While ,depletion of both IgG and IgA lead to loss of antibody dependent neutrophil phagocytosis. Distinct humoral immune profiles were noted in accordance with mild ,moderate and severe covid-19 disease[10],Table -2 . The showcase analysis of children-adult covid-19 was depicted in Table -3.

Table-2 :Show case analysis of adult covid-19.

Features	[9]	[10]
Neutrophil phagocytosis	Activated	Activated
Oxidative burst	Active	
NETosis	Activated	Activated
IgG derive of		Active

neutrophil IgG attenuation of neutrophil		Active
IgA activation		Activated
Absolute count of neutrophil and IL8	Correlated with severity	
IL8	Correlated with death	
Leukocytosis	Positive	
Neutrophilia	Positive	

Table 3 :Show case analysis of covid-19 in children and adults[10]

Features	Children	Adult
IgM IgG response	High	Higher
Humoral immune profile	Similar to mild adult response	Expand higher response
Antibody/cell enrichment	RBD specific monocyte	RBD specific neutrophil
IgA-neutrophil activation	Lesser than in adult	Higher than in children
IgA-linked disease severity	Linked to lesser extent than adults	Linked to severely ill patients

IV - Neutrophil Immune Deviation inCovid-19

This opinion tempts to make showcase analysis for covid-19 in childhood[8] and in adulthood[9,10,20,29,30] aiming to deduce if any evidence for the immune deviation of neutrophil in this disease. The disease in both disease groups showed the following immune mechanisms;

I – Conditional Sars-cove-2 antigen induced elevation of neutrophil inhibitory and/or stimulatory cytokines[29]

ii-Appearance of immature neutrophil inhibitory phenotypes[30]

iii-In line with active inflammatory circuit operation[26].

Iv – Inhibition of adaptive T cell response in continuum with enhancement of innate cellular immune responses[29],and elevation of IgG spike specific antibodies[8,10]

v - Rise up of neutrophil/lymphocyte ration[29,30].

vi - Follow a specific kinetic process[20]

vii – Finalized by either tissue pathology, tissue damage or tissue protection in a disease stage dependent manner [8,9,30]

viii-Similar to the case of ocular immune deviation during infection state in having immune protective or immune-pathogenic consequences[5,8,] .

The abovementioned immune mechanisms operable in covid -19 disease both in childhood and adulthood suggest the inclusion of neutrophil compartment activity in this disease in an immune deviation state in which the adaptive cellular immune responses might be suppressed by either the virus specific antibody inhibitory effect on T lymphocyte or by the inhibitory effect of the immature suppressive neutrophil phenotype on T cell after its activation by the virus specific antibody responses with either tissue pathology or tissue protection[1-7].Viewing these mechanism suggested behind neutrophil immune deviation. The features of this immune deviation can be drawn from these deduced and suggested mechanisms are as in the followings; I –Conditional , ii – reversible, iii – associated with active functional state and high IgG antibody responses , iv –Virus antigen induced chemokine orchestrated neutrophils and cytokine activated , v – Co-insides with molecular surface markers change and appearance of inhibitory surface markers , vi – immature neutrophil implicated in higher neutrophil/lymphocyte ration , vii – Activation consequences at most leaves tissue pathology and viii – Inflammatory circuit stages may serve as targets identification and development of immune-therapeutics.

V - Conclusions

The absolute numbers of circulating neutrophils both in human childhood and adulthood patients were correlated with severity of sars-cov-2 infection. Neutrophil compartment suggested to be a representative of

suppression of adaptive cellular immune responses by an innate cellular immune responses and elevation of anit-spike specific antibodies. In which both an immature active neutrophil and elevated IgG responses implicated to be suppressive to T cell responses in covid -19 disease with either affected tissue preservation or affected tissue damage.

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