ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 12 | December - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ORIGIN OF SARS COV-2 AN ALGAE VIRUS WITH ATYPICAL VIRULENCE

Microbiology

Dr Gaurav Kulshrestha MD Pediatrics, FAGE, Department Of Pediatrics, Military Hospital Binnaguri, West Bengal, India.

ABSTRACT

The present pandemic COVID-19 an ongoing havoc with no robust strategy till now despite of aggressive researches and treatment protocols. This could be attributed to poorly understood etiopathogenesis, non-specific hypotheses and theories. Many of the studies have already described its hybrid nature with other Coronaviruses. The SARS-CoV-2, SARS-CoV, and MERS-CoV have similarities in their phenotypic and genomic structure which can influence their pathogenesis. Here we discuss that SARS-COV-2 is an chimeric algae virus (combination of SARS-CoV and microalgae) could be accidentally evolved and leaked in laboratory while making forthcoming vaccines. The *Chlamydomonas reinhardtii* is a unique unicellular microalgae (common ancestor of plants and animals) with extraordinary genetic potential to become a chimera and can manifest in difficult conditions to serve as a platform to produce various recombinant proteins, vaccines, hormones and biofuels. The recent studies also shows the existence of Algae virus and its pathogenicity towards humans. The atypical clinical features like silent hypoxia, loss of taste and smell, vasculotropic and multiorgan inflammatory effect could be easily decoded by this chimera. One should also explore possibility of new genetically modified microalgae based recombinant oral and nasal vaccines.

KEYWORDS

COVID 19, *C.reinhardtii*, SOUL Protein , Chimeric Virus , O- Linked Glycans, SERPINS .

INTRODUCTION

Coronaviruses (CoVs) which are involved in multiple respiratory and gastrointestinal illnesses since early 21st century in humans and vertebrates are members of the subfamily Coronavirinae, family Coronaviridae and the order Nidovirales [1]. The recent 2019 havoc caused by the novel coronavirus SARS-CoV-2 resulting in an unusual clinical manifestations with viral pneumonia and other atypical complications has taken the world by a storm. Based on its genomic configuration and phylogenetic relationships, the SARS-CoV-2 belongs to genera Betacoronavirus. This has almost similar sequences as that of severe acute respiratory syndrome-related coronaviruses (SARS-CoV) and both the viruses use Angiotensin Converting Enzyme 2 (ACE2) as the entry receptor [2].

Chlamydomonas reinhardtii is a unicellular flagellate, eukaryotic green alga also known as last eukatyotic common ancestor (LECA) or plant like animal due to its unique properties. It is commonly found in the soil and can either assimilate CO_2 through photosynthesis or use certain organic compounds such as acetate as carbon sources. It can grow in heterotrophic and fermentative environment. Active research areas involving *C. reinhardtii* include photosynthesis, flagellar proteins, gene regulation and more recently as an excellent platform for recombinant therapeutic proteins synthesis of biological importance like HMBG1, VEGF, vaccines, hormones and biofuels. The genome of *C. reinhardtii* also includes a complex set of non-coding RNA genes, including endogenous microRNAs. Recent discoveries in the field of molecular genetics like indexed insertional mutants, most important targeted gene editing by CRISPR (Clusters of regularly interspaced short palindromic repeats), Optogenetics, Channelrhodopsins chimera, IFT (intraflagellar transport) of granule like particles, kairomones induced chemosensory property have made this organism a perfect role model. It can dispersed to extended distances by wind and survive air transportation [3,4]. Humans and Chlamydomonas shares 706 protein families (806 and 774 proteins, respectively) [5].

Chimeric Virus

The Virus and microalgae interactions exist in the nature. The discovery of Algae virus in throat of human population in John Hopkins center is an example of the same [6]. The Algevir also acts as an expression system for microalgae based on viral vectors [7]. As per our hypothesis, this COVID-19 virus possibly evolved accidentally during passing through the different cell lines of *C. reinhardtii* over the time and leaked while manufacturing future vaccines in laboratory. It seems to be a combination of SARS-CoV and genome of microalgae chloroplast ie *C. reindhardtii*. So it has retained the old properties of SARS-CoV like features with add on features of transgenic microalgae which is responsible for its unpredictable behavior now.

Across the world basic ongoing ' gain of function' researches involving passage of bat SARS-CoV-like coronaviruses in cell culture and/or animal models in biosafety level laboratories has been noticed and there are documented instances of laboratory leaks too [8,9]. The presence of polybasic furin cleavage at spike binding protein also strengthen the possibility of accidental evolution in laboratory due to presence of similar manipulations in Pf25 and CTB domain in nanoparticle vaccine [10], H9 avian influenza reasserting with polybasic cleavage site and H5 avian influenza virus with haemagglutinin cleavage site a highly pathogenic phenotype in chicken which were not present in other beta lineage bat or in pangolins CoVs till now [11,12].

Why *C.reinhardtii* chloroplast genome in SARS-CoV-2 Chimera?

Genetically, SARS-CoV-2 was less similar to SARS-CoV (about 79%) and MERS-CoV (about 50%) but more closer to RaTG13 bat coronavirus (96%). To date a virus identical to SARS-CoV-2 has not been identified in bats or any other nonhuman species, making its origin unclear [13]. Apart from having SARS-CoV genomic features this chimera also exhibits certain exclusive properties of microalgae C. reinhardtii like the presence of 5'UTR and 3'UTR for binding of viral and cellular proteins [14], 12 Radial Spoke Proteins, molecular chaperones also show lot of similarity structurally and functionally. The Radial spoke proteins are 3-D structure 'T' shaped glycoproteins with thin stalk and bulbous head appear to have homologs in other organisms, including Homo sapiens, indicating that not only the structure, but also many of the proteins of it have been preserved throughout evolution. Many of the newly identified proteins in the spoke stalk are predicted to contain domains associated with signal transduction, including Ca2+-, AKAP- and nucleotide-binding domains. This suggests that the spoke stalk is both a scaffold for signaling molecules and itself a transducer of signals [15]. Moreover, in addition to the recently described HSP40/HSP70 family member, a second spoke stalk protein is predicted to be a molecular Chaperone [16,17]. The presence of Sporangin type of serine protease, papain type cysteine protease, Arginine methyltransferase in *C. reinhardtii* further added to its uniqueness [18].

The presence of high catalytic efficiency which was recently proven for 3C like main protease (M^{pro}) in SARS-CoV-2 might be due to presence of above serine and papain type cysteine proteases too in the eye spot of C. reinhardtii [19]. The above mentioned characteristics could be a possibility of high infectivity rate in humans.

High ACE 2 Receptor affinity

SARS-CoV-2 binds to ACE2 receptors which are found on the epithelia of lungs, small intestine, oral and nasal mucosa, nasopharynx by its Spike and allows virions to enter and infect cells. In order to complete entry into the cell, the spike protein has to be primed by a protease called TMPRSS2, similar to SARS-CoV [20]. The high affinity of SARS-CoV-2 towards ACE2 receptors as compared to previous SARS viruses could be due to chimeric structure binding

protein Spike protein (SP) and Receptor binding domain (RBD). C. reinhardtii chloroplast also expressed three antihypertensive peptides (AHP) as a fusion protein [21]. A hypertensive oral vaccine has been developed by ACE2 binding receptors using above microalgae as a part of immunotherapy is an exclusive example for the same [22]. Recent researches which was published in Marine drugs journal in 2018 also established the role of transgenic *C. reinhardtii* as an effective bio-manufacturing platform for production and secretion of human tissue kallikrein which has significant biological implications [23]. The new study which has been conducted by a team recently at Oak Ridge National Laboratory in Tennessee (ORNL) supercomputer theory a RAS mediating ' Bradykinin storm' also explains the same [24] .

Exclusive RNA processing enzymes in C. reinhardtii

Besides RNA dependent RNA polymerase, RNA helicase, and protease activities, which are shared by all RNA viruses, the coronavirus replicase was recently predicted to employ a variety of RNA processing enzymes that are not or extremely rare found in other RNA viruses and include putative sequence-specific endoribonuclease, 30-to-50 exoribonuclease, ADP ribose 10-phosphatase, 20-O-ribose methyltransferase and in a subset of group 2 coronaviruses, cyclic phosphodiesterase activities [25]. These enzymes which are absent in other RNA viruses of coronaviridae have already expressed in *C. reinhardtii* and studied in many in-vitro molecular studies [26].

Inimitable Eyespot Proteome

C. reinhardtii chloroplast eye spot proteome contains an exclusive Haem binding protein (HBPs)/SOUL protein. The first member of this group of HBPs was found to be specifically located in the retina and pineal gland in chicken. Because Descartes considered the pineal gland as the soul, hence named by Zylka and Reppert accordingly [27]. This SOUL protein has two terminals N-terminal and C-terminal. The N-terminal domain could function as a sorting motif for eyespot localization and might be cleaved after sorting, resulting in a smaller protein. The C-terminus has HBP domain, indicating that it may be able to bind heme or its variants. Alignments of amino acid sequences from *C. reinhardtii* SOUL3 and homologous proteins from mammals (mouse and human) shows the highest similarity at this end [28].

The eyespot proteome and phosphoproteome which contains various protein Kinases and phosphatases (Serine/Threonine PPs) which indicates that light signal cascades may involve regulation by reversible protein phosphorylation [29]. The importance of diverse post translational protein modifications ie, methylation beside reversible phosphorylation in the regulation of protein stability, localization, activity and protein-protein interactions in cell organelles are also evident in recent years [30]. The methylation of 23 eyespot proteins have been discovered at Lysine and/or Arginine residues which increases their basicity and hydrophobicity without altering their charge as this important reaction at lysine residue is a key binding strategy of SARS-CoV-2 RBP with ACE2 receptor [31,32].

Presence of O-linked Glycans

A highly conserved and linear hydroxyproline-bound O-glycans were isolated from outer cell wall glycoproteins of the unicellular green alga *C. reinhardtii* [33]. It could create mucin like domain that possible act as an epitopes shield or sugar coated shields in viruses which helps in immunoevasion [34].

Analogous Polybasic Furin cleavage sites

The other prototypical neuropeptide precursors and essential peptide processing enzymes subtilisin-like prohormone convertases and carboxypeptidase B-like (CPB) enzymes were also identified in C. reinhardtii soluble mating secretome. After screening 771 soluble proteins in C. reinhardtii secretome which predicted to contain an N-terminal signal peptide were found to have subtilisin-like prohormone convertase (PC) cleavage sites ((R/K)Xn(R/K)↓, where n = 0 or 2, and ↓ identifies the cleavage site) in 756 proteins and furin-like cleavage sites (RX(K/R)R) in 224 proteins out of which 33 could yield amidated peptides which further strengthen our hypothesis of chimeric virus [35]. The already established role of *C.reinhardtii* in Channelrhodopsins(ChRs) chimera in various human genetic studies [5] and the uniquely evolved molecular mimicry to human epithelial sodium channels alpha subunit ENaC- α by SARS CoV-2 which was absent in previous coronaviruses at S1/S2 furin polybasic cleavage site and recently published further added to the same [36].

Exaggerated Vasculotropic effect

The exaggerated vasculotropic effect likely due to its chimeric nature. Firstly the role of this transgenic microalgae as a manufacturing hub for recombinant bioactive proteins like VEGF, HMBG1 and Fibronectin has been already proved in last two decades. Secondly the presence of various protein kinase cascades in proteome of *C. reinhardtii* likely to be responsible for the same as the role of c AMP-dependent protein kinase A (PKA), VEGF 165 regulates angiogenesis during embryogenesis also supports the same which was studied and published recently [37].

Confirms "Selection through passage" in cell lines

This chimera's imitating features with SARS-CoV-2 like polybasic furin cleavage, O-linked glycans, high population density of reservoir *C.reinhardtii* (plant like animal) host, ACE2 enhancing genes similar to human ortholog, various unique RNA enzymes not only strongly support this hypothesis but also answers the queries of origin of virus in laboratory ie "Selection through passage" in *C. reinhardtii* cell lines and transfer from zoonotic reservoir to humans followed by human to human transmission [38].

Shocking Atypical manifestations

Most of the cases remain asymptomatic or mildly symptomatic only few developed moderate to severe illness. Apart from typical SARS-CoV like features ie-; fever, chills, malaise, bodyache, dry cough, bilateral pneumonia, hepatic, renal, cardiac and deranged coagulation profile [39] the various others atypical presentations which have been frequently popped out and shocked scientists and research lobbies globally [40]. These could be best explained by this chimera. (See table 1).

Advance Immunoevasion techniques and misfiring of our immune cells

There are many acquired factors which helps this smart novel virus to fool our advance immune system. As per this hypothesis spike protein and receptor binding region have already primed with various protiens , sugars and several cell signaling domains due to its chimeric nature with *C.reinhardtii* . The presence of human like analogus ion channels proteins, presence of sulfated polysaccharides in microalgaes, C type lectin domains, Scavenger receptor cysteine-rich domains(SRCR), Flageller associated protein (FAP277) also known as Protease inhibitory protein and Mitogen activated protein kinases (MAPKs) which were studied for various other therapeutic purposes in last few decades and play key roles in the innate immunity system of mammals [56, 57]. SARS-CoV-2 enters not only binding through ACE2 of Nose , lungs and GI tract but also requires cellular heparin sulfate receptors for opening up as mentioned by Thomas Mandel Clausen, et al. in bioRxiv Preprint 2020 Jul 14 (doi: 10.1101/2020.07.14.201616) . Most probabhly initially Toll like-receptor 2 (TLR 2), TLR 5 , Myeloid C-type lectin receptors (CLRs) expressed by macrophages / dendritic cells would get activated by ' Alternate activation pathway ' rather than 'classical pathway' of macrophage activation which leads to decrease interleukin 12(IL 12) , interferon gamma and short-lived upregulation of IL 4,IL 13 and IL 10 response due to which chimera would get easy intracellular acess as a parasite after fooling the innate immunity through immunomodulation and molecular mimicry [58] . After getting inside the cell ssRNA activates intracellular TLR 3, TLR7 , TLR8 and upregulate Th1 cells mediated adaptive immunity response. The Th1 response would take over heavily to remove out from the cell by increasing pro inflammatory cytokines ie- type 1(antiviral), type 3 (anti fungal) initially and type 2(anti helminthes) and chemokines responses later on . This mismatch, confused or maladaptive immune response would become more severe in immunosuppressed or immunocompromised individuals due to other co morbid factors like old age, Obesity, Diabetes and uncontrolled hypertension. In view of above due to high viral load and dysregulatory cytokines storm finally leads to a self destructive tissue or multiorgan damage [59] .

The mystery behind pangolins a toothless , insectivorous mammals of order Pholidota tolerate SARS-CoV-2 virus well despite of presence of ACE2 receptors could be due to deficiency of TLR5 , a receptor for recognization of flagellin protein abuduntly present in human respiratory tract, G I tract , liver,dorsal root ganglion of nervous

system, epithelial cells , immune cells such as monocytes , immature Dendritic cells and the other one Interferon –epsilon an antiviral cytokine of epithelia which was expressed exclusively in female genital tract epithelia.Both in mice and humans its expression was hormonally regulated and already studied in last few decades [60,61] .The later one also helps females to develop immunoregulation against chlamydia, Herpes simplex and other STDs [62] .This could be the reason why Female population better tolerate SARS-CoV-2 and decrease severity of COVID-19 in them .

As per our hypothesis population who have been frequently exposed to different flagellate microorganisms or flagellin protein in the past would definitely show a immune tolerance with controlled response to SARS-CoV-2 virus and decreased number of severe cases or mortality and good recovery response. This is the reason why countries like South East asian and african countries which have poor sanitary conditions and frequent exposure to salmonella typhi, paratyphi, giardia, cholera , nematodes are showing better recovery rate as compare to western countries [63].

SERPIN like effect of FAP277 of *C.reinhardtii*

The most important innate immunity evasion effect is due to presence of Flagellar associated protein (FAP277 gene) also known as protease inhibitor –like protein in *C.reinhardtii* which interferes in classical complement pathway activation which is most advanced defensive system in jawed vertebrates especially mammals .The classical complement arm of complement activation pathways plays a very significant role not only in mounting initial pro inflammatory response against organisms with lectin pathway and Alternate pathway arm but also essential for regulation of complement activation system or inhibitory response which prevents from self tissue destruction . The Serum protease inhibitors (SERPINS) plays a very important role in defense or attack against invading viruses or bacteria [64,65] . The above group of enzymes helps in protection from various Proteases which are required to break down defective or obsolete molecules or proteins inside the cell also known as a "workhorses" of human body and this enzyme family is evolutionary conserved . After entering as a SERPIN it disable the effect of Classical complement pathway and fools the host immunity system to a certain extent . The SERPINS are superantigens and are vulnerable for frequent mutations which leads to various disease states like blood clotting disordrers , lung diseases (emphysema), dementia and liver cirrhosis . Not all SERPINS are protease inhibitors some non inhibitory SERPINS perform diverse functions too[66].

As per this hypothesis when host develops antibodies against this SERPIN proteins of chimeric virus during later part of the illness it causes destruction of other protease inhibitors mimics enzymes like C1 inhibitor (C1INH), alpha 1 antichymotrypsin (ACT) , antitrypsin (AAT), angiotensinogen (ANGT), antithrombin(ANT) , corticosteroid binding globulin (CBG) , Neuroserpin(NEUS) , Maspin , Monocyte or Neutrophil elastase inhibitor(MNEI) or other fluid phase regulators and trigger uncontrolled or poorly controlled alternate pathway or lectin pathway mediated anaphylatoxin reaction(C3a and C5a mediated) inside the host which mimics like acquired autoantibodies to complement components / autoimmune like disease / SLE like syndrome / vasculitis/ Age related macular degeneration or hereditary angioedema .The children have more potential to develop antibodies against this virus protein which manifests as Multisystem inflammatory syndrome (MISC) or Pediatric inflammatory syndrome (PIMS) or antibodies dependent enhancement (ADE) associated with COVID 19. Those have inherited deficiency of C1q , C1r, C1s , C4A orC4b are more susceptible for severe inflammatory anaphylatoxin response and mortality . The C3 and C5 defeciency or anti C3 or C5 therapy would definitely going to shows good response and positive outcome in severe COVID- 19. When we see the race specific distribution pattern of Complement C4 gene copy – number variations in human population monomodular short (mono-SorS) haplotypes with a single shortC4B gene and C4A deficiency is relatively common in White and Black population but almost absent in Asians . The most prevalent haplotype of Whites and Asian Indians is bimodular long-long(LL) and bimodular long-short (LS) in blacks and East Asians [67,68].

Possible Synthesis of Non glycosylated or deglycosylated glycoproteins

After hijacking the cell with high viral load and post translation modification it could produce non glycosylated or deglycosylated non structural proteins(NSPs) due to chimeric nature of this RNA virus being a part of *C.reinhardtii* chloroplast soul protein [69]. Due to production of non glycosylated or deglycosylated non structural proteins the Fc receptor mediating activity become highly compromised but no effect on antigen binding site of antibody molecule after antigen antibody reaction . In view of above the effector mechanisms mediated through the Fc receptors type(FcgRI,FcgRII,FcgRIII) and the C1q component of complement are severly bargained or functionally deficient and produce a acquired C1q deficiency like state , cause ablation of classical pathway and uncontrolled activation of alternate or lectin pathway which further deteriorated by other pro inflammatory states like Obesity,Diabetes mellitus , old age ,Hypertension , Coagulation disorderes, Stress etc . For effective memory B cells / plasma cells or Tcell production the above Fc mediated effector mechanism is very much essential . In severe COVID 19 patients despite of marathon treatment to high viral load there is a decrease clearance from the body and poor antibody or T cell response. The above mechanism also mimics like action of human antiCD20 non glycosylated IgG antibody eg; Rituximab , immunotoxins [70,71] .

By effecting Serotonin / Melatonin synthesis pathway

Melatonin a hormone which synthesized nocturnally in the pineal gland and regulates various physiological functions including sleep induction, circadian rhythm , oxidative stress and immune response . It levels remain under strong genetic influence and have heterogeneous expression among individuals .The presence of enzymes aralkylamine N-acetyl transferase and acetyl serotonin O methyl transferase in *C.reinhardtii* that catalyze one or more key reactions in serotonin / melatonin pathways also added to the above hypothesis[72,73] . The various symptoms of COVID19 like brain fogging, memory loss , learning , sleep problems, depression , Appetite , mood disorders and watery diarrhea due to increase gut motility could be due to monoamine neurotransmitter serotonin CNS and peripheral actions . The recently established role of Selective serotonine reuptake inhibitors (SSRIs) and Melatonin as a repurposing drugs as an immunomodulator in inhibiting SARS CoV-2 virus also strengthen the same[74].

Why Seaweeds outperform Remdesivir ?

Recently while performing in -vitro studies it has been observed that antiviral effects of long chain sulfated polysaccharides like heparins, fucoidans (RPI-27 and RPI-28) extracted from edible seaweeds are higher compare to Drug Remdesivir. They all have been acted as an effective decoy molecules[75] .

Possible future effective Vaccines

In this difficult situation of pandemic and economic crisis the possible operative strategies for COVID-19 vaccines development are limited. The live attenuated oral/nasal spray or recombinant subunit vaccines by using genetically modified *C.reinhardtii* as a biocapsule or nanoparticles would remain very effective if above hypothesis stands correct after research . The antigen which should be used in such vaccines are protein or a portion of the spike protein domain(flagellar associated antigen) and receptor binding domain [76]. In last few months the role of BCG, MMR and OPV like live attenuated vaccines have shown some nonspecific T cell mediated immune response which could be due to phylogenetic relationship of this last eukaryotic common ancestor (LECA) [77,78]. Replacing the wild strain of SARS-CoV-2 with vaccine virus to create a herd immunity could be a robust strategy [79]. The use of Anti proteome/SOUL3/Heam binding protein antibodies could be therapeutically useful. One should also consider air decontamination through *C. reinhardtii* based nanoparticles fogging for mass coverage of containment zones which was effectively executed for air pollution control measures in some countries. The recently developed recombinant vaccines using Cationic pullulan nanogel as a safe and effective nasal vaccine delivery system for respiratory infectious virus should be our future vision [80].

CONCLUSION

Finally to conclude SARS-CoV-2 is a chimeric virus most likely evolved in laboratory over the years accidentally due to passing through the C. reinhardtii cell lines while making new generation vaccines, drugs and therapeutic proteins. The above hypothesis strongly appears to be correct which have excellent research implications in future to explore about SARS-CoV-2 origin and vaccines production. Incorporation of microalgae genes and proteins has conferred this novel Coronavirus-2019 with unique and hitherto

unexplained features that cause atypical clinical presentations of this viral infection. The future vaccine development should also be targeted towards its chimeric spike protein (C. reinhardtii SOUL protein & FAP277 antigen) to prevent its further spread and infectivity in humans. Development of immune tolerance to such viruses not only by balancing our immune system but also require stable gut ecosystem too . A healthy ecofriendly and ego free life style should be our key future strategy.

Financial support and sponsorship: Nil

Table 1-Atypical Manifestation of COVID-19

S No	Atypical Manifestation of COVID-19	GENE/ PROTEIN / Molecules Responsible	PATHOGENESIS	Remarks	Reference
1	Silent Hypoxia or Happy hypoxia without shortness of breath or loss of consciousness	SOUL3 protein Haem binding protein	A heme binding protein present at the edge of the eye spot of Chlamydomonas which allows the cell to phototax. Due to this heme binding protein, iron gets displaced and porphyrin and virus particles get fused. Another possibility can be structural changes and defect in heme, rendering it incapable of carrying oxygen.	This mimics similar to the etiopathogenesis of Acute porphyria like illness which leads to excessive release of porphyrin into the circulation and deposits in various organs can manifest as various dermatological, neuro visceral symptoms . Feeling of excessive tiredness, severe headache and complete exhaustion could be manifestation of histotoxic hypoxia created by this chimeric virus.	[41]
2	Severe cytokine storm or Multisystem inflammatory syndrome or Kawasaki like disease in children or strokes in old .	Increase synthesis of Biological proteins like Kallikrein VEGF 165 FIBRONECTIN HMBG-1	C. reinhardtii serves as an unique platform to produce these bioactive molecules which leads to exaggerated amounts of extracellular HMGB1 causes release of proinflammatory cytokines including TNF, IL-1, and IL-6 . Antibodies dependent enhancement(ADE) against regulatory proteins of complement cascade (SERPINs)cause dysregulatory anaphylatoxins C3a and C5a production	HMGB1 has a strong bipolar charge and is prone to complex-bind other pro inflammatory molecules and their activities amplified in synergistic manner which leads to increased synthesis of above biological molecules in the critically ill patients leads, micoclots/ microplaques formation over already damaged vessels .	[23] ,[42], [43] ,[44]
3	Anosmia or Hyposmia with or without Dysgeusia in patent Nasal Airway	Early (0-3 days) Virus particle activates antigen presenting cells in nasal mucosa induce local immunity	Possibly activation of ACE2 receptors mediating increase kallikrein , VEGF synthesis leads to vasculo-lymphatic inflammation, Oro-nasal lymphoid hyperplasia	This process takes place through supportive sustenicular and other supporting cells not through olfactory nerve and produce oedema associated with subjective ratings of perceived averseness .	[45] , [46]
		Late onset (after 07 days)	Due to neurotrophic chimeric induced neurodegenerative damages in olfactory ciliated neuroepithelium, hypothalamus, amygdala or limbic system .	The role of C. reinhardtii as a source of BDNF, NGF and NTF3 in Optogenetics for various human disorders like stroke, epilepsy, hereditary retinopathies etc are also strengthen the same.	[47], [48]
4	Selective Lymphopenia	Cyclophilin / immunophilin like / Gene CYN71	Reason for selective lymphopenia despite of ongoing T-cell mediated cytotoxic phagocytosis can be explained by release of Peptidyl–prolylcis–trans isomerasecyclophilin type/ immunophilin like molecules from intracellular chimera C. reinhardtii	It mimics as a low dose CyclosporinA an natural immunosuppressant[50].	[49] ,[50],
5	Survives in extreme of cold and heat environment and Secondary waves of pandemic , high spread among population as a biogenic particle . Early dynamics of the outbreak in Wuhan, China, basic reproductive number (R0) of 2.2–2.7	Zygospores like particles	After sexual replication in uncongenial environment it become thick walled highly resistant form known as zygospores	Due to thick walled stage they survive in extreme conditions. In favourable conditions they go for germination and released four vegetative haplotypes mt+ and mt- also known as single nucleotide polymorphisms (SNPs)	[51], [52], [53], [54], [55]

Conflict of Interest: No conflict of interest by author

REFERENCES

1. Chen Y, et al. Emerging Coronaviruses: Genome structure, replication and pathogenesis. J Med Virol 2020;92(4):418-423.
2. Lu R, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395(10224):565-574.
3. Sasso S, Stibor H, Mittag M, Grossman AR. From molecular manipulation of domesticated Chlamydomonas reinhardtii to survival in nature. Elife 2018;7:e39233.
4. Rasala BA, et al. Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of Chlamydomonas reinhardtii. Plant Biotechnol J 2010;8: 1-15.
5. Merchant SS, et al. The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science 2007;5848:245-250.
6. Yolken RH, et al. Chlorovirus ATCV-1 is part of the human oropharyngeal virome and is associated with changes in cognitive functions in humans and mice. Proc Natl Acad Sci 2014;111(45):16106-16111.
7. Bañuelos-Hernández B, et al. Algevir: An Expression System for Microalgae Based on Viral Vectors. Front Microbiol 2017;8:1100.
8. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH,et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. Epub 2013 Oct 30. PMID: 24172901; PMCID: PMC5389864.
9. Lim PL, et al. Laboratory-Acquired Severe Acute Respiratory Syndrome. N Engl J Med

2004;350:1740–1745.
10. Gregory AE, et al. Vaccine delivery using nanoparticles. Front Cell Infect Microbiol 2013;3:13.
11. Gohrbandt S, et al. H9 avian influenza reassortant with engineered polybasic cleavage site displays a highly pathogenic phenotype in chicken. Virol 2011;92(8):1843-1853.
12. Tam JS. Influenza A (H5N1) in Hong Kong: an overview. Vaccine 2002;20Suppl 2:S77-81.
13. Lu R, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395(10224):565-574.
14. Anthonisen IL, Salvador ML, Klein U. Specific sequence elements in the 5¢ untranslated regions of rbcL and atpB genemRNAs stabilize transcripts in the chloroplast of Chlamydomonas reinhardtii. RNA 2001;7:1024–1033.
15. Yang P, Yang C, Sale WS. Flagellar radial spoke protein 2 is a calmodulin binding protein required for motility in Chlamydomonas reinhardtii. Eukaryot. Cell 2004;3:72-81.
16. Yang C, Compton MM, Yang P. Dimeric novel HSP40 is incorporated into the radial spoke complex during the assembly process in flagella. Mol Biol Cell 2005;16:637-648.
17. Nordhues A, et al; New Insights Into the Roles of Molecular Chaperones in Chlamydomonas and Volvox. Int Rev Cell Mol Biol 2010;285:75-113.
18. Takeaki Kubo, Shinsuke Kaida, Jun Abe, Tatsuaki Saito, Hideya Fukuzawa, Yoshihiro Matsuda. The Chlamydomonas Hatching Enzyme, Sporangin, is Expressed in Specific Phases of the Cell Cycle and is Localized to the Flagella of Daughter Cells Within the Sporangial Cell Wall .Plant and Cell Physiology, Volume 50, Issue 3, March 2009, Pages 572–583. https://doi.org/10.1093/pcp/pcp016 k.
19. Jin, Z, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 2020;582:289–293.
20. Hoffmann M, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271-280.
21. Ochoa-Méndez CE, et al. Bioactivity of an antihypertensive peptide expressed in Chlamydomonas reinhardtii. J Biotechnol 2016;240:76-84.
22. Soria-Guerra RE, et al. Expression of an HBcAg-based antigen carrying angiotensin II in Chlamydomonas reinhardtii as a candidate hypertension vaccine. Plant Cell Tiss Organ Cult 2014;116:133–139.
23. Chen, J.; Wu, J.; Wu, Q.; Hu, Z. Biosynthesis and Secretion of Human Tissue Kallikrein in Transgenic Chlamydomonas reinhardtii. Mar. Drugs 2018; 16, 493. https://doi.org/10.3390/md16120493
24. Michael R Garvin, Christiane Alvarez, J Izaak Miller, Erica T Prates et .A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. eLife 2020;9:e59177 DOI: 10.7554/eLife.59177
25. Ziebuhr J. (2005) The Coronavirus Replicase. In: Enjuanes L. (eds) Coronavirus Replication and Reverse Genetics. Current Topics in Microbiology and Immunology, vol 287. Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-26765-4_3.
26. Zimmer SL, Fei Z, Stern DB. Genome-Based Analysis of Chlamydomonas reinhardtii Exoribonucleases and Poly(A) Polymerases Predicts Unexpected Organellar and Exosomal Features. Genetics 2008;179:125-36.
27. Zylka MJ, Reppert SM. Discovery of a putative heme-binding protein family (SOUL/HBP) by two-tissue suppression subtractive hybridization and database searches. Brain Res Mol Brain Res 1999;74(1-2):175-181.
28. Schulze T, et al. The heme-binding protein SOUL3 of Chlamydomonas reinhardtii influences size and position of the eyespot. Mol Plant 2013;6(3):931-944.
29. Wagner V, et al. The phosphoproteome of a Chlamydomonas reinhardtii eyespot fraction includes key proteins of the light signaling pathway. Plant Physiol 2008;146:772–788.
30. Lehtimäki N, Koskela MM, Mulo, P. Posttranslational Modifications of Chloroplast Proteins: An Emerging Field. Plant Physiol 2015;168(3):768-775.
31. Rice JC, Allis CD. Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr Opin Cell Biol 2001;13(3):263-273.
32. Lan J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020;581(7807):215-220.
33. Bollig K, et al. Structural analysis of linear hydroxyproline-bound O-glycans of Chlamydomonas reinhardtii-conservation of the inner core in Chlamydomonas and land plants. Carbohydr Res 2007;342(17):2557-2566.
34. Bagdonaite I, Wandall HH. Global aspects of viral glycosylation. Glycobiology 2018;28:443–467.
35. Dahms SO, Hardes K, Steinmetzer T, Than ME. X-Ray structures of the proprotein convertase furin bound with substrate analogue inhibitors reveal substrate specificity determinants beyond the s4 pocket. Biochemistry 2018;57:925–934.
36. Anand P, Puranik A, Aravamudan M, Venkatakrishnan AJ, Soundararajan V. SARS-CoV-2 strategically mimics proteolytic activation of human ENaC. Elife. 2020;9:e58603. Published 2020 May 26. doi:10.7554/eLife.58603
37. Nedvetsky PI, et al. cAMP-dependent protein kinase A (PKA) regulates angiogenesis by modulating tip cell behavior in a Notch-independent manner. Development 2016;143(19): 3582-3590.
38. Andersen KG, et al. The proximal origin of SARS-CoV-2. Nat Med 2020;26(4):450-452.
39. Chen N, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507-513.
40. Abobaker A, Raba AA, Alzwi A. Extrapulmonary and atypical clinical presentations of COVID-19. J Med Virol. 2020 Jun 10:10.1002/jmv.26157. doi: 10.1002/jmv.26157. Epub ahead of print. PMID: 32520401; PMCID: PMC7300507..
41. Dombeck TA, Satonik RC. The porphyrias. Emerg Med Clin North Am 2005;23(3): 885-899.
42. Soy, M., Keser, G., Atagündüz, P. et al. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol 39, 2085–2094 (2020). https://doi.org/10.1007/s10067-020-05190-5.
43. Andersson U, et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med 2000;192:565–70.
44. Andersson U, Yang H, Harris H. High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells. Semin Immunol 2018;38:40–48.
45. Lee Y, et al. Prevalence and Duration of Acute Loss of Smell or Taste in COVID-19 Patients. J Korean Med Sci 2020;35(18):e174.
46. Patel RM, Pinto JM. Olfaction: anatomy, physiology, and disease. Clin Anat. 2014;27:54-60.
47. Li Y, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol 2020;92(6):552-555.
48. Pendharkar AV, et al. Optogenetic modulation in stroke recovery. Neurosurg Focus 2016;40(5):E6.
49. Liu Y, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci 2020;63(3):364-374.
50. Vallon O. Chlamydomonas Immunophilins and Parvulins: Survey and Critical Assessment of Gene Models. Eukaryot Cell. 2005;4(2):230-41.
51. Renee N. Salas, James M. Shultz and Caren G. Solomon . The Climate Crisis and Covid-19 — A Major Threat to the Pandemic Response. N Engl J Med 2020; 383:e70 .DOI: 10.1056/NEJMp202201
52. Tang X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev 2020;7(6):1012-1023.
53. Goodenough U, Lin H, Lee J-H. Sex determination in Chlamydomonas. Semin Cell Dev Biol 2007;18(3):350-61.
54. Sharma NK, Rai AK, Singh S, Brown RM. Airborne algae: their present status and relevance. J Phycol 2007;43:615–627.
55. Li Q, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020;382:1199–1207.
56. Choudhary, S., Save, S.N. & Vavilala, S.L. Unravelling the inhibitory activity of Chlamydomonas reinhardtii sulfated polysaccharides against α-Synuclein fibrillation. Sci Rep 8, 5692 (2018). https://doi.org/10.1038/s41598-018-24079-7
57. Wheeler GL, Miranda-Saavedra D, Barton GJ. Genome analysis of the unicellular green alga Chlamydomonas reinhardtii Indicates an ancient evolutionary origin for key pattern recognition and cell-signaling protein families. Genetics. 2008;179(1):193-197. doi:10.1534/genetics.107.085936
58. Geijtenbeek, T., Gringhuis, S. Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 9, 465–479 (2009). https://doi.org/10.1038/nri2569
59. Lucas, C., Wong, P., Klein, J. et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 584, 463–469 (2020). https://doi.org/10.1038/s41586-020-2588-y
60. Sharma V, Walther F, Hecker N, Stuckas H, Hiller M. Convergent losses of TLR5 suggest altered extracellular flagellin detection in four mammalian lineages. Mol Biol Evol. (2020) msaa058. doi: 10.1093/molbev/msaa058
61. Choo SW, Rayko M, Tan TK, Hari R, Komissarov A, Wee WY, et al. Pangolin genomes and the evolution of mammalian scales and immunity. Genome Res. (2016) 26:1312–22. doi: 10.1101/gr.203521.115
62. Fung KY, Mangan NE, Cumming H, Horvat JC, Mayall JR, Stifter SA, et al. Interferon-ε protects the female reproductive tract from viral and bacterial infection. Science. (2013) 339:1088–92. doi: 10.1126/science.1233321
63. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-141. doi:10.1016/j.cell.2014.03.011
64. Roberts TH, Hejgaard J, Saunders NF, Cavicchioli R, Curmi PM. Serpins in unicellular Eukarya, Archaea, and Bacteria: sequence analysis and evolution. J Mol Evol. 2004 Oct;59(4):437-47. doi: 10.1007/s00239-004-2635-6. PMID: 15638455.
65. Ahn, Nielson, Hiller, et al. The Sole serpin of chlamydomonas reinhardtii : expression, properties of the recombinant protein, and phenotypes of RNAi knockdown transformants. 2006. Available at: http://hdl.handle.net/1959.14/83518.
66. Lucas A, Yaron J. R., Zhang L, Ambadapadi S. Overview of serpins and their roles in biological systems methods Mol Biol. (2018) 1826:1–7. doi: 10.1007/978-1-4939-8645-3_1
67. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, Atkinson JP and Yu CY (2016) Early Components of the Complement Classical Activation Pathway in Human Systemic Autoimmune Diseases. Front. Immunol. 7:36. doi: 10.3389/fimmu.2016.00036
68. Dunkelberger, J., Song, WC. Complement and its role in innate and adaptive immune responses. Cell Res 20, 34–50 (2010). https://doi.org/10.1038/cr.2009.139
69. Beth A. Rasala & Stephen P. Mayfield (2011) The microalga Chlamydomonas reinhardtii as a platform for the production of human protein therapeutics, Bioengineered Bugs, 2:1, 50-54, DOI: 10.4161/bbug.2.1.13423
70. Arnold J. et al., 2007. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21-50
71. Gurjar SA, Derrick JP, Dearman RJ, Thorpe R, Hufton S, Kimber I, Wadhwa M. Surrogate CD16-expressing effector cell lines for determining the bioactivity of therapeutic monoclonal antibodies. J Pharm Biomed Anal. 2017 Sep 5;143:188-198. doi: 10.1016/j.jpba.2017.06.004. Epub 2017 Jun 9. PMID: 28605680.
72. Zhang, Yujing & Gao, Wenying & Lv, Yawei & Bai, Qingqing & Wang, Yingjuan. (2019). Enhanced melatonin production via aralkylamine N-acetyltransferase overexpression enhances NaCl resistance in transgenic Chlamydomonas reinhardtii (Volvocales, Chlorophyceae). Phycologia. 58. 1-9. 10.1080/00318884.2018.1529971.
73. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. Prog Brain Res. 2010;181:127-51. doi: 10.1016/S0079-6123(08)81008-4. PMID: 20478436.
74. Hamed MGM, Hagag RS. The possible immunoregulatory and anti-inflammatory effects of selective serotonin reuptake inhibitors in coronavirus disease patients. Med Hypotheses. 2020;144:110140. doi:10.1016/j.mehy.2020.110140
75. Kwon, P.S., Oh, H., Kwon, SJ. et al. Sulfated polysaccharides effectively inhibit SARS-CoV-2 in vitro. Cell Discov 6, 50 (2020). https://doi.org/10.1038/s41421-020-00192-8
76. Specht EA, Mayfield SP. Algae-based oral recombinant vaccines. Front Microbiol. 2014;5:60. Published 2014 Feb 17. doi:10.3389/fmicb.2014.00060.
77. Franklin, Robin & Young, Adam & Neumann, Bjoern & Fernandez, Rocio & Joannides, Alexis & Reyahi, Amir & Modis, Yorgo. (2020). Homologous protein domains in SARS-CoV-2 and measles, mumps and rubella viruses: preliminary evidence that MMR vaccine might provide protection against COVID-19. 10.1101/2020.04.10.20053207.
78. Chumakov K, et al. Can existing live vaccines prevent COVID-19? Science 2020;368 (6496):1187-1188.
79. Fine PE. Herd immunity: history, theory, practice. Epidemiol Rev. 1993;15(2):265-302. doi: 10.1093/oxfordjournals.epirev.a036121. PMID: 8174658.
80. Nakahashi-Ouchida R, Yuki Y, Kiyono H. Cationic pullulan nanogel as a safe and effective nasal vaccine delivery system for respiratory infectious diseases. Hum Vaccin Immunother 2018;14(9):2189-2193.