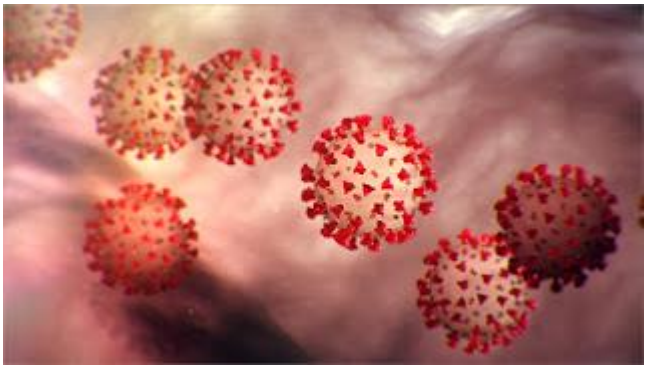

IL-6, COVID-19, Cytokine Storms and Galen

ABSTRACT

We examine the co-morbidities from COVID-19 and develop a logical but purely speculative therapeutic approach using a classic "cocktail" model targeting a multiplicity of the co-morbidities. This document is purely speculative and is not intended to be dispositive of any specific therapeutic approach.

Terrence McGarty

TGL 175, May 2020

Notice

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Contents

1 INTRODUCTION

There has been a recent flurry of reports that the COVID-19 response is consistent in many ways with what is seen in the cytokine storms of the type observed in CAR-T cell therapy. Specifically the release of massive amounts of IL-6 and the resultant attack on the local epithelium of the lung. Massive release of IL-6 can not only cause damage to the epithelial cells but can also have secondary severe co-morbidities in cardiac tissue and other parts of the body as well. IL-6 is a powerful cytokine and as such any extensive over expression must be dealt with first in treating many of these infections.

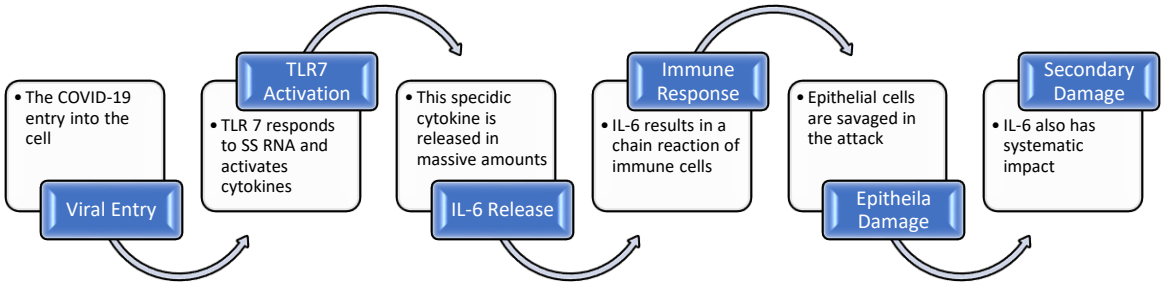
This note is an attempt at Galenic speculation with Roger Bacon attempts to utilize a modicum of scientific factual observations. The complete story of COVID-19 will be a long time in the telling, and at best we can speculate based on observations and known facts regarding the elements therein. However I believe it is worth the effort while one has the time to try to place some perspective on the table for discussion.

Unlike prior Notes which comment on some recent topic highlighted in research, this Note is an attempt to make an argument for an approach that follows a path in immunological understanding and not just a well-trod viral path.

My Galen/Bacon remark is based upon a previous examination of the development of medicine in the 14th century[1]. Namely following the mandate of Galen to observe logic while recognizing the Baconist new world view of phenomenon and facts as sounding boards for avoiding logically consistent but erroneous paths. I will avoid any debate regarding the use of existing therapeutics and their efficacy and attempt to establish a set of arguments hopefully both logically and factually consistent. In a Popperian sense they should have the capacity of being proven wrong not just alleged to be correct.

The logic we follow is as follows:

[1] https://www.researchgate.net/publication/322937399_A_Franciscan's_Life_1295-1385_DRAFT



Our approach is as follows:

1. Understand the basics of the virus. In this case it is a single stranded RNA positive virus, the corona virus.

2. Examine the immune system interactions resulting from this virus. Namely we know that cells have toll like receptors inside in endosomes that can be activated by viral RNA when it is un wrapped. The TLRs then excite a set of immune paths setting off a cascade of events. One of those events is the release of a strong cytokine, IL-6 and the other is an activation of NF-kB.

3. The entry of the virion is assisted by a receptor on the cells surface. Understanding this receptor allows for a grasp of another putative control point.

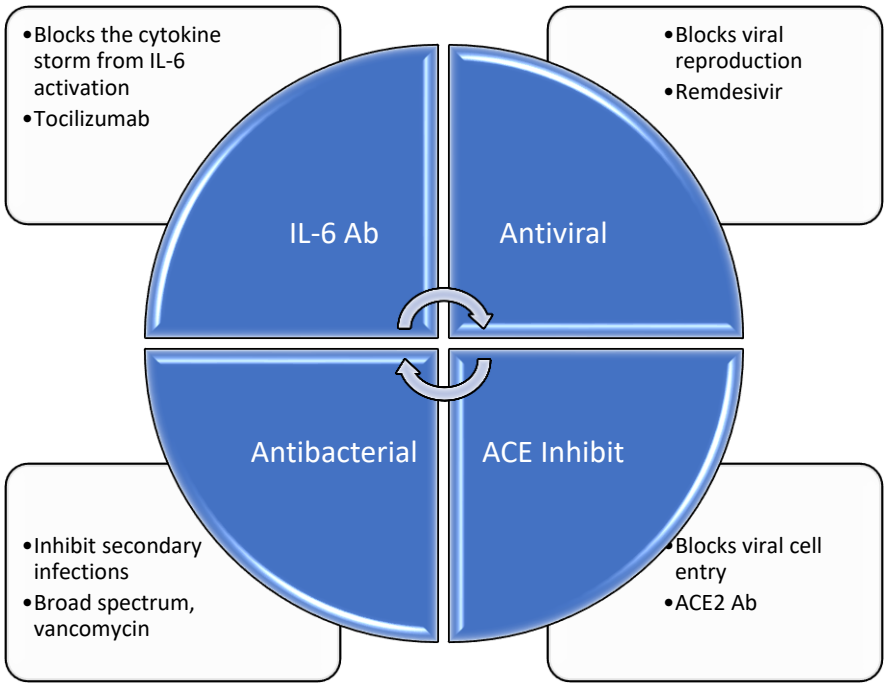
4. Apparently cytokine storms can be quite severe and are often fatal. Our understanding of these events has improved especially as we better understand immune responses.

5. There are a multiplicity of co-morbidities related to the release of IL-6. These co-morbidities are common but in a COVID-19 infection that may oftentimes be an added burden and result in increased mortality.

6. Therapeutic targets may be understood as a means to control the virus as well as mitigate against the cytokine release and alleviate the problems of comorbidities.

7. Finally, we propose a putative "cocktail" approach to therapeutics based upon this set of observations using in most cases existing therapeutic agents.

The proposed approach is shown below and we shall develop the logic to justify it in what follows:



One of the key questions we keep asking is; how does COVID-19 kill a patient. A Nature article notes[2]:

Some of the earliest analyses of coronavirus patients in China suggested that it might not be only the virus that ravages the lungs and kills; rather, an overactive immune response might also make people severely ill or cause death. Some people who were critically ill with COVID-19 had high blood levels of proteins called cytokines, some of which can ramp up immune responses. These include a small but potent signalling protein called interleukin-6 (IL-6).

IL-6 is a call-to-arms for some components of the immune system, including cells called macrophages. Macrophages fuel inflammation and can damage normal lung cells as well. The release of those cytokines, known as a cytokine storm, can also occur with other viruses, such as HIV. The ideal counter, then, would be a drug that blocks IL-6 activity and reduces the flow of macrophages into the lungs. Such drugs, known as IL-6 inhibitors, already exist for the treatment of rheumatoid arthritis and other disorders. One called Actemra (tocilizumab), made by the Swiss pharmaceutical firm Roche, has been approved in China to treat coronavirus patients, and researchers around the world are working furiously to test it and other drugs of this type.

The author continues:

[2] https://www.nature.com/articles/d41586-020-01056-7

A combination of damage from both a virus and the immune response to it is not uncommon, says Rafi Ahmed, a viral immunologist at Emory University in Atlanta, Georgia. The effects of 'hit-and-run' viruses such as norovirus, which make people sick almost immediately after infection, are more probably due to the virus itself, he says. By contrast, people infected with viruses such as coronavirus do not show symptoms until several days after infection. By then, collateral damage from the immune response often contributes to the illness.

Indeed this is what we are considering but in an even broader context[3].

[3] https://www.forbes.com/sites/nathanvardi/2020/04/08/handicapping-the-most-promising-of-267-potential-coronavirus-cures/#44f8273d7f23 This is an example of the massive numbers of purported "cures" seen in the popular press.

2 VIRAL INFECTIONS

Let us begin with a summary presentation of viruses. The intent is to emphasize the critical factors in the pandemic of 2020.

2.1 FAMILIES AND GENUS

There is a multiplicity of viruses in nature and a large group impact humans. The classification is in families and genus. The basic classification is between DNA and RNA viruses. There are approximately 6 major DNA virus families and 15 RNA families. The Corona virus is in the RNA family. Namely the virus is an RNA strand.

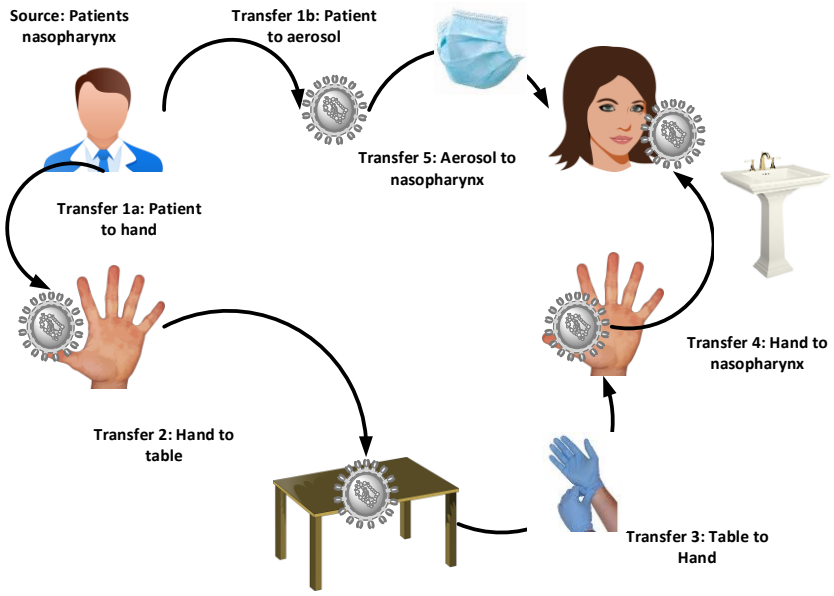
They can be broken down into single and double strands. Thus, Corona is a single strand RNA virus. Moreover, the strands can be positive or negative. Positive strands have tails on the 3' ends and have a small virus protein on the 5' end. Negative strands are the opposite. Corona virus are thus single stranded positive RNA viruses.

The virus is also enveloped. The envelope contains three major proteins. They are: (i) a transmembrane glycoprotein, (ii) a surface peplomer which neutralizes antibodies, does receptor binding, membrane fusion, and other activities, (iii) a haemagglutinin and esterase activity unit. The genome size is quite large, about 30,000 bases.

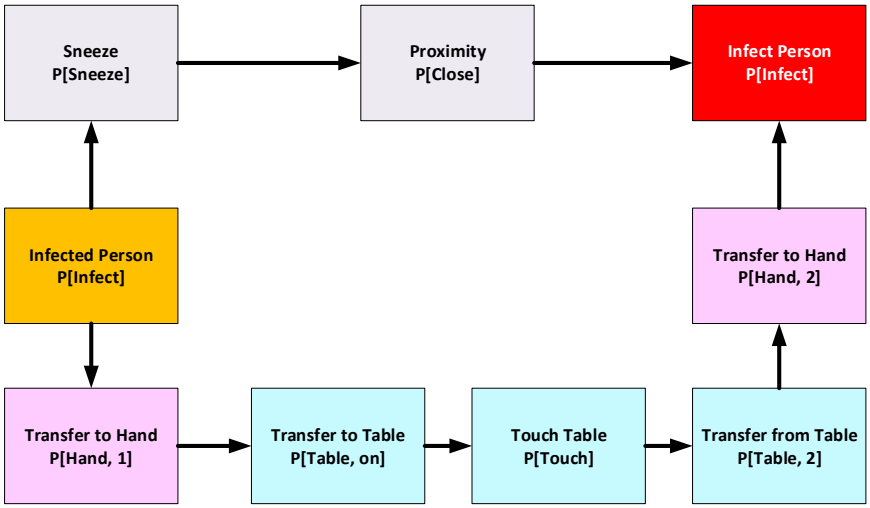
2.2 INFECTION

Infection with the Corona virus is considered to be generally through nasal passages of the nasopharynx. It results mostly from aerosol particles from an infected person or through contact with surfaces infected by similar aerosols. However, the exact infection process is somewhat still poorly understood.

Let's give a simple yet complex example. Namely how does one model the transfer of the virus. Consider the example below:



Here we have an example of person to person infection. The infected individual can infect an uninfected by two means. First by aerosolizing the virus and transferring by air directly. That could be stopped by a mask. However, proximity and time continuity is essential for this path to function. Second, by secondary touching. Namely the virus transferred say by hand to a surface, then the second person must touch the surface at the right spot in the right time period and transfer to their hand and then to their face to start the process. Gloves and hand washing stops that transfer.



We now above show a model for this process. Namely each step has a probability and each step has a mitigation element. We can then estimate the probability of infection from one person to another at a specific time period in a specific table surface at a specific temperature. Lots of assumptions.

Then if we want a model we have to assume that there exists an ensemble of people al functioning in some statistically similar manner. I think one starts to get the point. Assume, assume, assume etc

The issues continue since how this happens in a NYC subway is not how this happens in Scranton, and less so say in Bangor. But, and this is critical, this simple example is at the very heart of the model.

Problems like this were considered a century ago when examining statistical thermodynamics. It became an element of Einstein's Nobel Prize winning paper on Brownian Motion. Unfortunately the application to pandemics is grossly wanting.

As the NY Times article states[4]:

The models used by the White House team are standard epidemiological tools but are not precise, as the results can vary widely depending on how closely people follow the guidelines. In other words, the assumptions built into the models can shape the results.

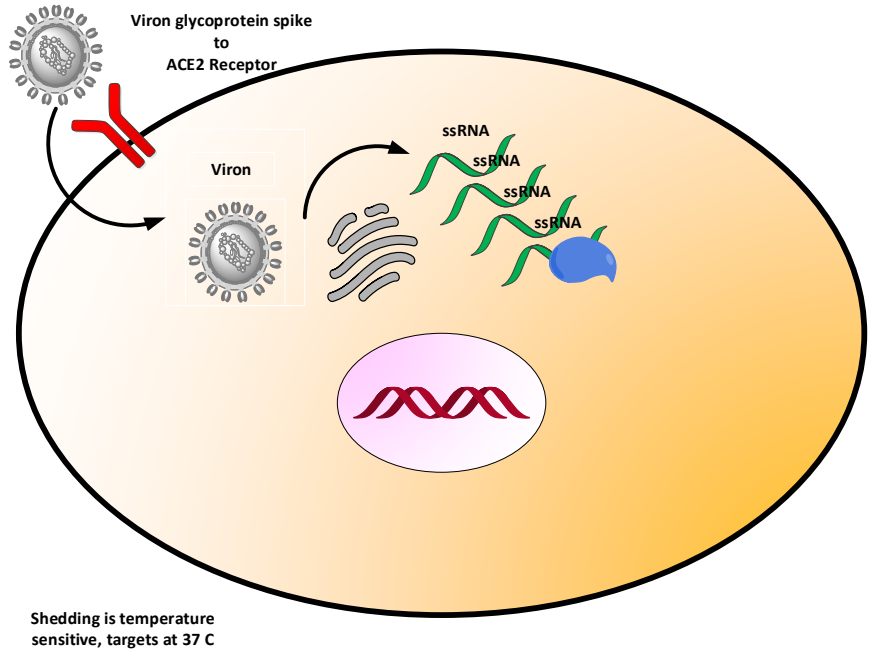
This is an understatement. Having designed and tested such models and examined hundreds of others, and also understanding some of the dynamics of this virus, the simple answer is that no one has a real clue. Yet the two steps shown above of distance and hand protection are the best we can come up with now.

2.3 CORONA SPECIFICS

Let us now examine the specifics of the corona virus and in turn the specifics of COVID-19. Corona are large positive single strand RNA viruses with surface ligands that bind to ACE2 receptors on epithelial cells and then progress to multiply internally at a temperature of 37 C. Unlike rhinoviruses which are epithelial but multiply at 35 C, the nasal passageways, the corona needs to move to the lungs and the higher temperature to fully expand.

The figure below summarizes this virus. The RNA is about 30,000 nucleotides in length, about 120 nm in diameter and when translated can produce eight operable regions for the generation of proteins or RNA replication. We show below the simplified version.

[4] https://www.nytimes.com/2020/03/31/world/coronavirus-news.html?action=click&module=Spotlight&pgtype=Homepage#link-a737c70



The corona virus envelope protein has been described by Schoeman and Fielding. They note:

The CoV E protein is a short, integral membrane protein of 76–109 amino acids, ranging from 8.4 to 12 kDa in size. The primary and secondary structure reveals that E has a short, hydrophilic amino terminus consisting of 7–12 amino acids, followed by a large hydrophobic transmembrane domain (TMD) of 25 amino acids, and ends with a long, hydrophilic carboxyl terminus, which comprises the majority of the protein.

The hydrophobic region of the TMD contains at least one predicted amphipathic α- helix that oligomerizes to form an ion-conductive pore in membranes. Comparative and phylogenetic analysis of SARS-CoV E revealed that a substantial portion of the TMD consists of the two nonpolar, neutral amino acids, valine and leucine, lending a strong hydrophobicity to the E protein . The peptide exhibits an overall net charge of zero, the middle region being uncharged and flanked on one side by the negatively charged amino (N)-terminus, and, on the other side, the carboxy (C)-terminus of variable charge.

The C-terminus also exhibits some hydrophobicity but less than the TMD due to the presence of a cluster of basic, positively charged amino acids. Computational predictions regarding the secondary structure of E suggest that the C terminus of β- and γ-CoVs also contains a conserved proline residue centred in a β-coil-β motif. This motif likely functions as a Golgi-complex targeting signal as mutation of this conserved proline was sufficient to disrupt the localization of a mutant chimeric protein to the Golgi complex and instead localized the protein to the plasma membrane.

The spikes on the protein coat, which are critical to attachments to cells, have been described by Li as follows

The coronavirus spike protein is a multifunctional molecular machine that mediates coronavirus entry into host cells. It first binds to a receptor on the host cell surface through its S1 subunit and then fuses viral and host membranes through its S2 subunit. Two domains in S1 from different coronaviruses recognize a variety of host receptors, leading to viral attachment. The spike protein exists in two structurally distinct conformations, prefusion and postfusion.

The transition from prefusion to postfusion conformation of the spike protein must be triggered, leading to membrane fusion. This article reviews current knowledge about the structures and functions of coronavirus spike proteins, illustrating how the two S1 domains recognize different receptors and how the spike proteins are regulated to undergo conformational transitions. I further discuss the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion, in the context of the corresponding functions from other viruses and host cells.

The RNA is composed of eight regions. These eight regions of the RNA of the Corona virus are depicted below. First the ssRNA is combines with it complement creating a double strand and within that double strand we have sub units which will give rise to the protein elements necessary for its replication. Key to that will be a polymerase allowing for the production of the elements. As Schoeman and Fielding have noted regarding these structural elements:

The coronaviral genome encodes four major structural proteins:

(i) the spike (S) protein,

(ii) nucleocapsid (N) protein,

(iii) membrane (M) protein, and the

(iv) envelope (E) protein,

all of which are required to produce a structurally complete viral particle.

These four segments take from the virus RNA what is necessary to construct a new virion structure. The viral RNA is then replicated in toto and a single strand enters the new virion. The authors continue:

More recently, however, it has become clear that some CoVs do not require the full ensemble of structural proteins to form a complete, infectious virion, suggesting that some structural proteins might be dispensable or that these CoVs might encode additional proteins with overlapping compensatory functions. Individually, each protein primarily plays a role in the structure of the virus particle, but they are also involved in other aspects of the replication cycle.

The S protein mediates attachment of the virus to the host cell surface receptors and subsequent fusion between the viral and host cell membranes to facilitate viral entry into the host cell. In some CoVs, the expression of S at the cell membrane can also mediate cell-cell fusion between infected and adjacent, uninfected cells. This formation of giant, multinucleated cells, or syncytia, has been proposed as a strategy to allow direct spreading of the virus between cells, subverting virus-neutralising antibodies.

Unlike the other major structural proteins, N is the only protein that functions primarily to bind to the CoV RNA genome, making up the nucleocapsid. Although N is largely involved in processes relating to the viral genome, it is also involved in other aspects of the CoV replication cycle and the host cellular response to viral infection. Interestingly, localisation of N to the endoplasmic reticulum (ER)-Golgi region has proposed a function for it in assembly and budding. However, transient expression of N was shown to substantially increase the production of virus-like particles (VLPs) in some CoVs, suggesting that it might not be required for envelope formation, but for complete virion formation instead.

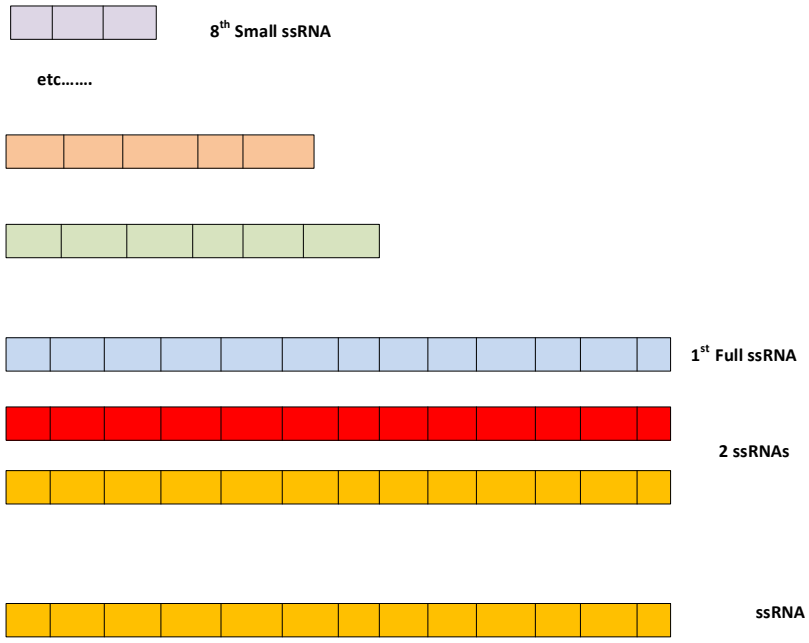
The M protein is the most abundant structural protein and defines the shape of the viral envelope. It is also regarded as the central organiser of CoV assembly, interacting with all other major coronaviral structural proteins. Homotypic interactions between the M proteins are the major driving force behind virion envelope formation but, alone, is not sufficient for virion formation [54–56].

Interaction of S with M is necessary for retention of S in the ER-Golgi intermediate compartment (ERGIC)/Golgi complex and its incorporation into new virions, but dispensable for the assembly process. Binding of M to N stabilises the nucleocapsid (N protein-RNA complex), as well as the internal core of virions, and, ultimately, promotes completion of viral assembly.

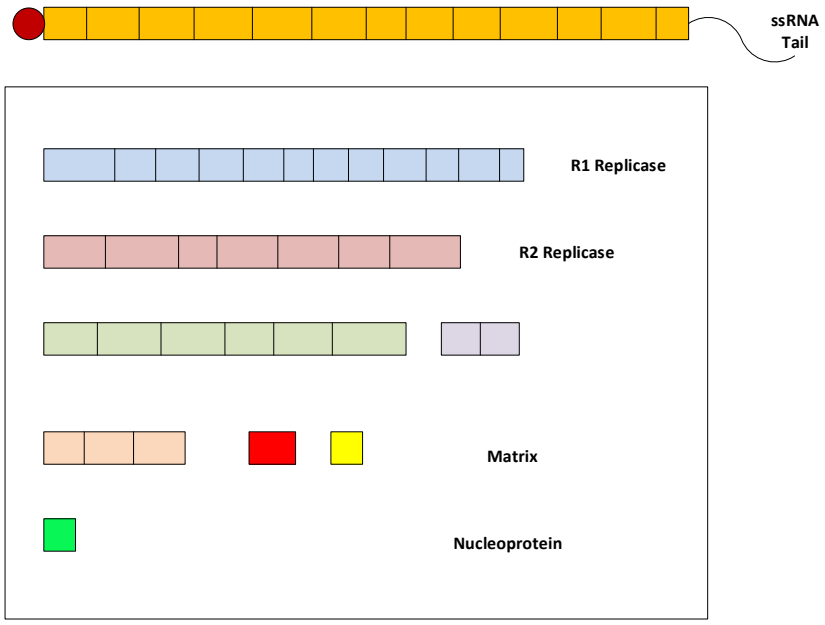
Together, M and E make up the viral envelope and their interaction is sufficient for the production and release of VLPs.

The E protein is the smallest of the major structural proteins, but also the most enigmatic. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is incorporated into the virion envelope. The majority of the protein is localised at the site of intracellular trafficking, viz. the ER, Golgi, and ERGIC, where it participates in CoV assembly and budding. Recombinant CoVs have lacking E exhibit significantly reduced viral titres, crippled viral maturation, or yield propagation incompetent progeny, demonstrating the importance of E in virus production and maturation.

Now the total RNA in the virus can be separated into multiple parts. The above structural parts have been discussed. In addition the RNA can have the necessary elements for self-replication withing a host cell. We demonstrate some of these parts below, where we have structure segments as noted above and polymerase type sections to reproduce itself in terms of the RNA. Thus the viral RNA has the ability to reproduce not only the RNA but the structure which the RNA is transported in. It does so at the expense of the host. The example below is interpreted in that sense.



The details of these eight elements are shown as below. The RNA has a protein on one end and a tail at the other end. The eight active sections are depicted including the two replicase regions essential for reproducing the ssRNA and other smaller segments involved in the process.



There is a reasonable understanding as to the virologic processes associated with the COVID virion.

The replication of the virus is described by Oxford et al (5th Ed):

Virions initially attach to the cell plasma membrane through specific receptors. These have been identified for several corona viruses; for example, human coronavirus uses the membrane-bound metalloproteinase, aminopeptidase N (APN), whereas OC43 simply binds to sialic acid groups on cell-surface proteins. SARS CoV uses the host-cell receptor angiotensin-converting enzyme 2 (ACE2) to gain entry into cells whereas MERS CoV uses the host receptor dipeptidyl peptidase 4 (DPP4). Uptake into cells is rapid and temperature-dependent, involving fusion with the plasma membrane or via endocytosis followed by a spike-mediated fusion in the endosome. Large multinucleated giant cells, syncytia, can be formed both in the laboratory and in an infected host.

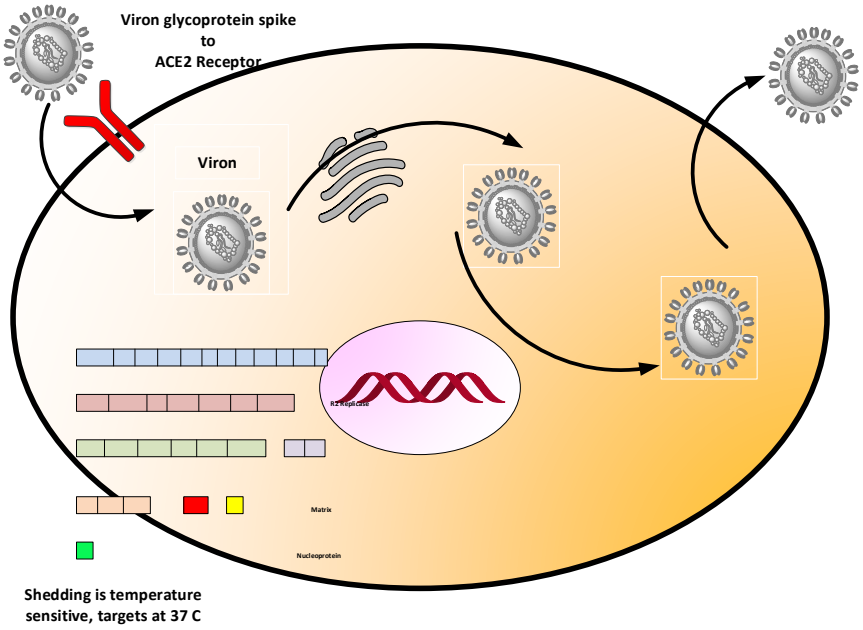
Once released into the cytoplasm the virus positive-strand RNA is translated directly into two polypeptides: ORFla and ORFlb at the 5' end of the genome. These are processed to form a replicase-transcriptase complex that possesses RNA polymerase activity. The RNA polymerase transcribes a full-length negative RNA strand, which acts as the template for transcription of multiple subgenomic virus mRNAs. Coronavirus mRNAs are unusual in that they all terminate at the common 3' end of the genome, but start at various places from the 5' end to produce a nested set of 3' co-terminal transcripts.

Each of the eight mRNAs, except for the smallest, therefore encode for multiple proteins, with the longest one being, in effect, full-length coronavirus genome RNA and the others in descending order of size being S, E, M, and N. Generally, each subgenomic virus mRNA is the template for translation into one protein. There are 16 non-structural proteins (l-16nsp), some of which have proteinase functions or are polymerases, including RNA-dependent RNA polymerase (nspl2) and endoribonuclease (nspl5).

Virus proteins that constitute the virus particle, namely N, M, and S, are produced in the infected cell and new virion assembly occurs initially in the cytoplasm on smooth-walled vesicles located between the ER and the Golgi known as ERGIC (endoplasmic reticulum Golgi intermediate compartment).

There newly formed RNP interacts with the M protein from the ER, and M interacts with the S and other proteins to form the infectious virus which buds into the Golgi, thereby acquiring a lipid envelope. Envelope proteins are glycosylated in the Golgi. Virions are released by fusion of smooth-walled virion-containing vesicles with the plasma membrane. As with other RNA viruses, the lack of proofreading functions in the virus RNA polymerase leads to a high rate of mutation in the new virus genomes. The very long genomes, together with the discontinuous RNA replication, can favour recombination leading to new genotypes with varying pathogenicity. There remains also the possibility of recombination between zoonotic coronaviruses and between human viruses. Recombination can allow coronaviruses to rapidly evolve and adapt to new ecological niches.

We demonstrate the above in the figure below:



Shedding is temperature sensitive, targets at 37 C

3 IL-6

We examine the impact of IL-6 as a prime driver of the cytokine storm. We first review the key elements of the immune system, then consider IL-6 specifically and finally consider the impact on NF-kB, a significant control element.

3.1 IMMUNE SYSTEM ISSUES

As Abbas et al note regarding Toll Like Receptors, TLRs, and their control capabilities in the immune system. TLRs are found on the cell surface and on intracellular membranes and are thus able to recognize microbes in different cellular locations. The authors then note:

TLRs 1, 2, 4, 5, and 6 are expressed on the plasma membrane, where they recognize various PAMPs in the extracellular environment. Some of the most potent microbial stimuli for innate immune responses bind to these plasma membrane TLRs, such as bacterial LPS and lipoteichoic acid, which are recognized by TLRs 4 and 2, respectively.

In contrast, TLRs 3, 7, 8, and 9 are mainly expressed inside cells on endoplasmic reticulum and endosomal membranes, where they detect several different microbial nucleic acids. These include double-stranded RNA, which binds to TLR3; single-stranded RNA, which binds to TLR7 and TLR8; and unmethylated CpG motifs in DNA, which bind to TLR9. Single- and double-stranded RNA are not unique to microbes, but their location in endosomes likely reflects origin from microbes. This is because host cell RNA is not normally present in endosomes, but microbial RNA may end up in endosomes of neutrophils, macrophages, or DCs when the microbes are phagocytosed by these cells.

TLR7 seems to be the primary TLR for this virus. We shall assume that but it does not seem to make a material difference. No literature seems to exist on this fact as of this time.

Enzymatic digestion of the microbes within endosomes will release their nucleic acids so these are able to bind TLRs in the endosomal membrane. Thus, the endosomal TLRs may distinguish nucleic acids of normal cells from microbial nucleic acids on the basis of the cellular location of these molecules. A protein in the endoplasmic reticulum called UNC-93B is required for the endosomal localization and proper function of TLRs 3, 7, 8, and 9.

Genetic deficiency in UNC-93B leads to susceptibility to certain viral infections, especially herpes simplex virus encephalitis, demonstrating the importance of the endosomal location of TLRs for innate defense against viruses.

TLR recognition of microbial ligands results in the activation of several signaling pathways and ultimately transcription factors, which induce the expression of genes whose products are important for inflammatory and antiviral responses (Fig. 4.3). The signaling pathways are initiated by ligand binding to the TLR at the cell surface or in the endoplasmic reticulum or endosomes, leading to dimerization of the TLR proteins. Ligand-induced TLR dimerization is predicted to bring the TIR domains of the cytoplasmic tails of each protein close to one another.

This is followed by recruitment of TIR domain–containing adaptor proteins, which facilitate the recruitment and activation of various protein kinases, leading to the activation of different transcription factors.

***The major transcription factors that are activated by TLR signaling pathways are nuclear factor κB (NF-κB)**, activation protein 1 (AP-1), interferon response factor 3 (IRF3), and IRF7. NF-κB and AP-1 stimulate the expression of genes encoding many of the molecules required for inflammatory responses, including inflammatory cytokines (e.g. tumor necrosis factor [TNF] and IL-1), chemokines (e.g., CCL2 and CXCL8), and endothelial adhesion molecules (e.g., E-selectin) (discussed later). IRF3 and IRF7 promote production of type I interferons (IFN-α and IFN-β), which are important for antiviral innate immune responses.*

NF-kB is a major player in activating a variety of genes both for viral as well as malignant paths.

The endosomal TLRs 7 and 9, which are most highly expressed in plasmacytoid Dendritic Cells, signal through a MyD88-dependent, TRIF-independent pathway that activates both NF-κB and IRFs. Therefore, TLR7 and TLR9, like TLR4, induce both inflammatory and antiviral responses.

1. *The innate immune system uses cell-associated pattern recognition receptors, present on plasma and endosomal membranes and in the cytosol, to recognize structures called PAMPs, which are shared by microbes, are not present on mammalian cells, and are often essential for survival of the microbes, thus limiting the capacity of microbes to evade detection by mutating or losing expression of these molecules. In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called DAMPs.*

2. *TLRs, present on the cell surface and in endosomes, are the most important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids. Cytosolic pattern recognition receptors exist that recognize microbial molecules. These receptors include the RLRs, which recognize viral RNA, CDSs which recognize microbial DNA, and NLRs, which recognize bacterial cell wall constituents and also serve as recognition components of many inflammasomes.*

3. *Pattern recognition receptors, including TLRs, NLRs, and RLRs, signal to activate the transcription factors NF-κB and AP-1, which stimulate expression of cytokines, costimulators, and other molecules involved in inflammation, and the IRF transcription factors, which stimulate expression of the antiviral type I interferon genes.*

4. *The inflammasome, a specialized caspase-1 containing enzyme complex that forms in response to a wide variety of PAMPs and DAMPs, includes recognition structures, which are often NLR family proteins, an adaptor, and the enzyme caspase-1, the main function of which is to produce active forms of the inflammatory cytokines IL-1 and IL-18.*

We believe that the immune system is a major player in this disease. To date most of the effort seems to be focused by infectious disease experts such as those at NIH. However immunological understanding we believe is critical.

3.2 IL-6 DETAILS

IL-6 is a powerful cytokine. It has been linked closely to cytokine storms and is often activated via viral infections. As NCBI notes[5]:

This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. The functioning of this gene is implicated in a wide variety of inflammation-associated disease states, including suspectibility to diabetes mellitus and systemic juvenile rheumatoid arthritis. Alternative splicing results in multiple transcript variants.

As Puel and Casanova have recently observed:

The time has come for IL-6 to begin to reveal its true nature. IL-6, first identified as B cell stimulatory factor 2, or BSF2, was cloned in 1986 and has been one of the most intensively studied cytokines ever since.

Its pleiotropy is legendary. It is produced by and acts on many cell types by binding to a receptor composed of the transmembrane IL-6R (or its soluble form, sIL-6R) and the transmembrane GP130 protein. The result is a tremendous diversity of effects, in cis and trans, on the development and function of many leukocyte subsets and various other cell types.

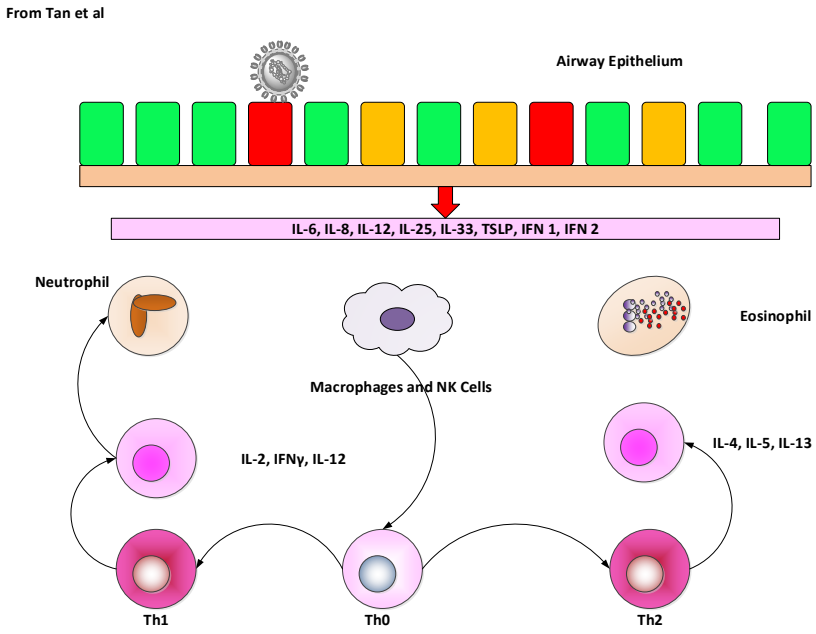
IL-6–producing cells include bone marrow stromal cells, T cells, macrophages, dendritic cells, fibroblasts, synovial cells, endothelial cells, glia cells, and keratinocytes, whereas IL- 6–responsive cells include B cells, T cells, hepatocytes, monocytes, vascular endothelial cells, and synoviocytes.

Studies of mouse IL-6 at the whole-organism level have revealed multiple roles, the most frequently studied of which relates to inflammation, host defense, metabolism, bone homeostasis, and tissue regeneration. Mice with knockouts of IL-6 or IL-6R display a wide range of immunological and nonimmunological phenotypes, including impaired inflammatory responses to localized tissue damage, impaired induction of acute-phase proteins, and impaired responses to or enhanced susceptibility to various microbes (e.g., vesicular stomatitis virus, vaccinia virus, and Listeria monocytogenes).

Following these studies, therapeutic agents blocking IL-6 activity have been introduced into clinical practice for various inflammatory conditions. These studies raised questions about the

[5] https://www.ncbi.nlm.nih.gov/gene/3569

essential immunological functions of human IL-6 and the most likely clinical phenotype of humans with genetic defects resulting in a lack of IL-6 immunity



From Garbers et al we have a discussion of the interaction between IL-6 and the TLR. Specifically they note:

IL-6 is a four-helical cytokine of 184 amino acids that can be secreted by many cell types upon appropriate stimulation during infection, inflammation or cancer. IL-6 is secreted by monocytes and macrophages after engagement of Toll-like receptors (TLRs) by, for example, lipopolysaccharides (LPS); by fibroblasts, keratinocytes, astrocytes and endothelial cells after IL-1 stimulation; and by subsets of activated B cells and T cells and by microglial cells after viral infection. IL-6 is important for regulating B cell and T cell responses and for coordinating the activity of the innate and the adaptive immune systems. Moreover, IL-6 is needed for regeneration of the liver.

Although under normal conditions, the IL-6 concentration in the circulation is around 1–5 pg per ml, IL-6 concentrations in the serum can easily increase into the nanograms per millilitre range in pathological states16. IL-6 is strongly induced during most, if not all, inflammatory processes, infection and cancer. In sepsis, IL-6 levels of several microgram per millilitre have been reported. In the brain, high IL-6 levels lead to astrocytosis and neurodegeneration.

IL-6 binds to IL-6R — an 80 kDa receptor devoid of signalling capacity13 — and the complex of IL-6 and IL-6R binds to a second membrane protein, glycoprotein 130 (gp130; also known as IL-6R subunit-β), which dimerizes and initiates intracellular signalling20. Although gp130 is expressed on all cells, IL-6R is found only on a few cells, such as hepatocytes, some leukocytes and epithelial cells17. IL-6 exhibits measurable affinity only for IL-6R but not for gp130;

consequently, cells expressing gp130 but not IL-6R are unresponsive to IL-6 per se. The gp130 protein has been shown to act as a signalling receptor for additional cytokines, including IL-11, oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin 1 (CT1), leukaemia inhibitory factor (LIF) and the cardiotrophin-like cytokine factor 1 (CLCF1), which, together with IL-6, form the IL-6 family of cytokines.

In addition, gp130 is one component of the heterodimeric receptor complexes for some heterodimeric IL-12 family members, including IL-27 (which is composed of subunits p28 (IL-27p28) and IL-27 subunit-β (EBI3)) and IL-35 (composed of IL-12p35 and EBI3).

In a similar manner Su et al note:

Interleukin-6 (IL-6) is a pleiotropic cytokine that not only regulates the immune and inflammatory response but also affects hematopoiesis, metabolism, and organ development. IL-6 can simultaneously elicit distinct or even contradictory physiopathological processes, which is likely discriminated by the cascades of signaling pathway, termed classic and trans-signaling. Besides playing several important physiological roles, dysregulated IL-6 has been demonstrated to underlie a number of autoimmune and inflammatory diseases, metabolic abnormalities, and malignancies.

As Tanaka and Kishimoto note:

Interleukin-6 (IL-6), initially designated as a B cell differentiation factor, is a representative cytokine featuring redundancy and pleiotropic activity. In the early phase of infectious inflammation, IL-6 is produced by monocytes and macrophages immediately after the stimulation of Toll-like receptors (TLRs) with distinct pathogen-associated molecular patterns (PAMPs). In noninfectious inflammations, such as burn or traumatic injury, damage-associated molecular patterns (DAMPs) from damaged or dying cells stimulate TLRs to produce IL-6.

As Velazquez-Salinas et al note

IL-6 is a pleotropic cytokine produced in response to tissue damage and infections. Multiple cell types including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T and B cells are associated with the production of this cytokine.

After targeting its specific receptor, IL-6 starts a cascade of signaling events mainly associated with the JAK/STAT3 activation pathway promoting the transcription of multiple downstream genes associated with cellular signaling processes, including cytokines, receptors, adaptor proteins, and protein kinases. …It also controls the production of proteins implicated in regulation of gene expression …. The number of genes regulated by IL-6 activity may explain the pleotropic nature of this interleukin.

Accordingly, the biological consequences of IL-6 production have been associated with both pro- and anti-inflammatory effects, highlighting IL-6's pivotal role in the activation and regulation of the immune response. Biological activities affected by production of IL-6 include:

control of the differentiation of monocytes into macrophages by regulating the expression of macrophage colony-stimulating factor, increasing B-cell IgG production by regulating the expression of IL-21, negative regulation of dendritic cell maturation by activation of the STAT3 signaling pathway, as well as the promotion of the Th2 response by inhibiting Th1 polarization.

Two different mechanisms have been described to promote the inhibition of Th1 polarization by IL-6:

(1) IL-6 stimulates CD4 T cells to secrete IL-4 and direct the response to Th2, and

(2) IL-6 affects the secretion of IFNγ by CD4 T cells, an essential interferon to promote Th1 polarization.

Thus IL-6 is truly a powerful initiator and supporter of a variety if immune elements. They continue:

As a warning signal during viral infections, different immune cellular pathogen recognition receptors, including toll-like receptors ***(TLR:2, 3, 4, 7, 8, and 9)****, nucleotide-binding oligomerization domain-like receptors, DNA receptors, and retinoic acid-inducible gene-1-like receptors, are able to sense a variety of pathogen-associated molecular patterns displayed by viruses (envelope glycoproteins, single and double-stranded RNA, and unmethylated CpG DNA), which stimulate transcription of IL-6 among other proinflammatory cytokines.*

In this context, it has been shown that specific amino acid substitutions in a TLR-like structure in the NS4B protein of a highly virulent classical swine fever virus (CSFV) strain resulted in a completely attenuated phenotype in pigs. Infection of pigs with this mutant CSFV was characterized by the sustained accumulation of IL-6 in tonsils.

Further in vitro experiments using exogenous IL-6 confirmed the ability of this cytokine to repress the replication of CSFV in swine peripheral blood mononuclear cells, the natural target cell during CSFV infection in pigs.

Similarly, evidence of the antiviral effect of IL-6 was described during in vitro studies conducted with hepatitis B virus (HBV) where the direct ability of exogenous IL-6 to suppress the replication of this virus was described. Disruption of HBV replication was characterized by a marked decrease in the number of viral genome-containing nucleocapsids, an effect mediated in an interferon-independent manner.

The above observation of the anti-viral properties of IL-6 is one of those "on the one hand, on the other hand" observations. Namely it responds well to virus intrusion but it may respond too well. They continue:

Furthermore, IL-6 was able to block HBV infection in hepatocytes by inhibiting expression of HBV receptor in the human liver, i.e., the bile acid transporter Na (+)/taurocholate co-transporting polypeptide, and effectively disrupted epigenetic control of the nuclear cccDNA mini-chromosome, inhibiting HBV transcription and the expression of hepatocyte nuclear

transcription factors 1 and 4 alpha. However, experimental scientific evidence also suggests potential negative consequences that increased levels of IL-6 might have on the cellular immune response against viruses. In this context different potential mechanisms involving this cytokine might affect viral clearance, ultimately favoring the establishment of a viral persistent state in infected hosts.

As von Essen et al have noted for Multiple Sclerosis:

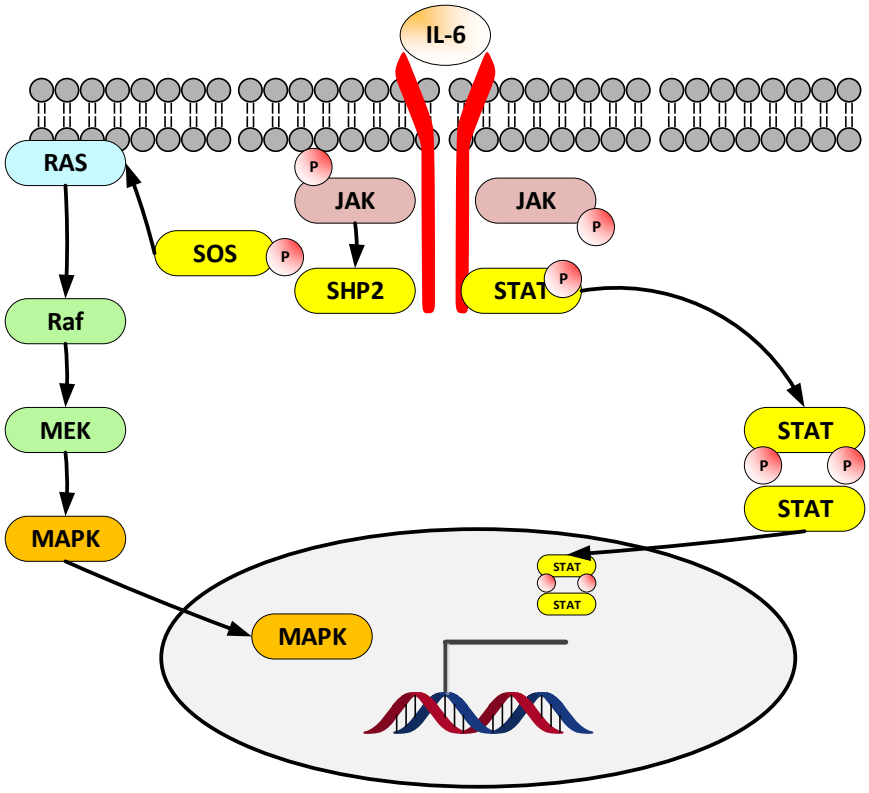
In addition to IL-23, IL-6 induces activation of STAT3. IL-6 signals through JAK1/JAK2/TYK2/STAT3 to induce expression of the IL-23 receptor and stabilizes the expression of RORγt, favoring Th17 differentiation. Although both IL-6 and IL-23 employ STAT3 as their principal signaling moiety, and induce a Th17 phenotype of T cells, their downstream cellular effects are not identical. In addition to the MS-risk alleles shared with the IL-23/STAT3-pathway, the IL-6/STAT3-pathway includes MS-risk alleles near genes encoding the IL-6 receptor subunit IL6ST (gp130) and signaling molecule JAK1

In a similar manner Heinrich et al noted:

Dysregulation of IL-6-type cytokine signalling contributes to the onset and maintenance of several diseases, such as rheumatoid arthritis, inflammatory bowel disease, osteoporosis, multiple sclerosis and various types of cancer (e.g. multiple myeloma and prostate cancer). IL-6-type cytokines exert their action via the signal transducers gp (glycoprotein) 130, LIF receptor and OSM receptor leading to the activation of the JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogenactivated protein kinase) cascades.

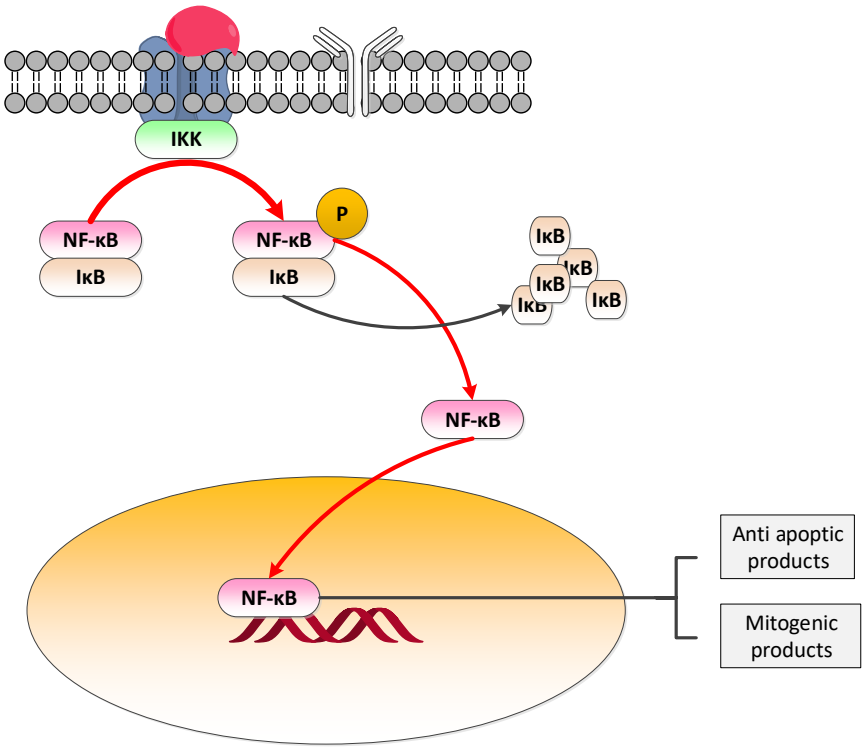
This review focuses on recent progress in the understanding of the molecular mechanisms of IL-6-type cytokine signal transduction. Emphasis is put on the termination and modulation of the JAK/STAT signalling pathway mediated by tyrosine phosphatases, the SOCS (suppressor of cytokine signalling) feedback inhibitors and PIAS (protein inhibitor of activated STAT) proteins. Also the cross-talk between the JAK/STAT pathway with other signalling cascades is discussed

We demonstrate several of these above pathways below.



3.3 NF-KB AND ITS IMPLICATIONS

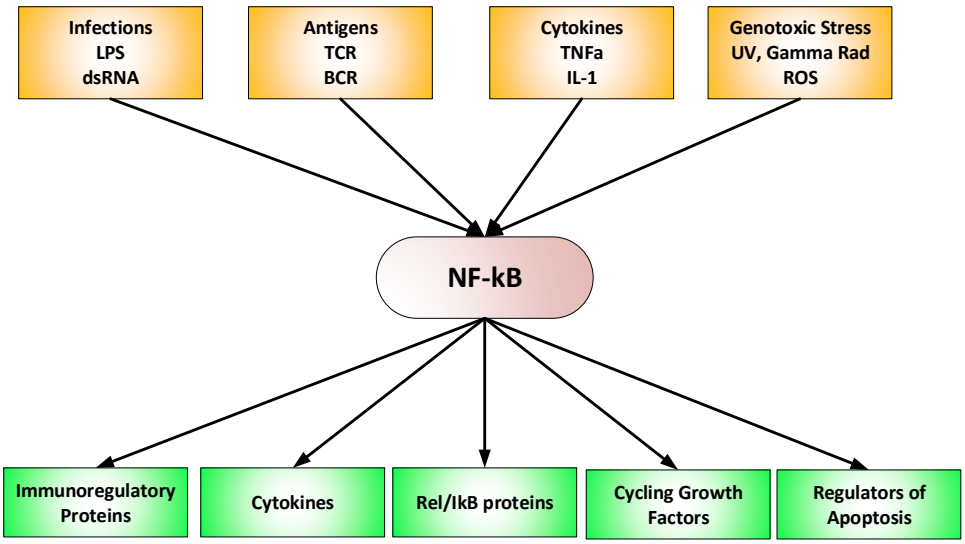
The NF-kB dimer is a powerful transcription factor that plays a role in the function of the immune system and in dealing with inflammation. It also has a significant role in cancer development. We briefly summarize this significant factor and highlight the key elements that relate to the conjunction between inflammation and cancer.



From Gorlach et al we have:

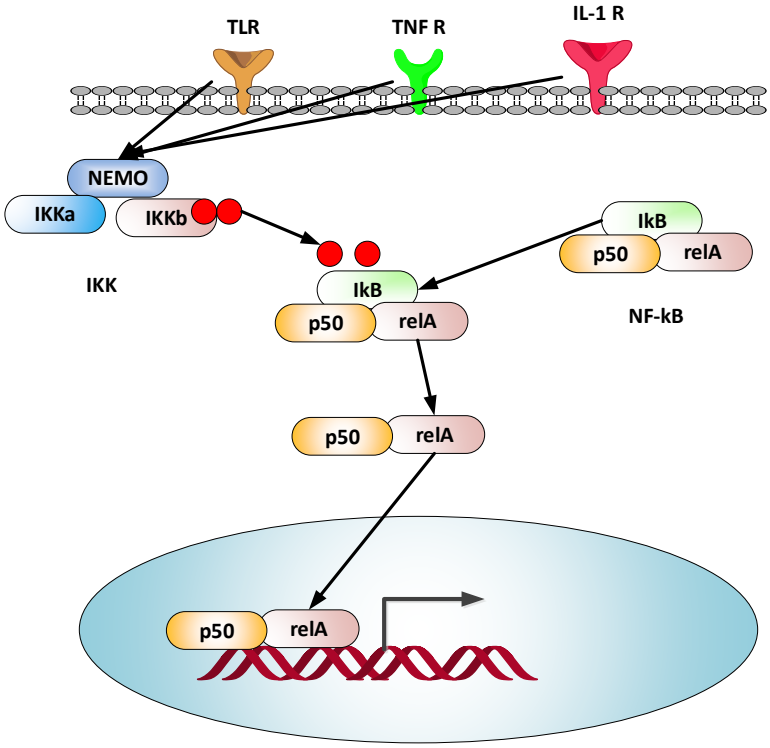
The activation of NF-κB is closely linked with ROS generation during inflammation and obesity. ROS were found to mediate inhibitor of NF-κBα (IκBα) kinase (IKKα and IKKβ) phosphorylation and release of free NF-κB dimers. Tumor necrosis factor α (TNFα), a bona fide NF-κB activator, was shown to mediate a redox- dependent activation of protein kinase A which subsequently phosphorylated Ser276 on RelA (v-rel avian reticuloendotheliosis viral oncogene homolog A). By contrast, the NF-κB member p50 was found to have reduced DNA binding activity when oxidized at Cys62.

We demonstrate the impact of NF-kB below. There are a multiplicity of drivers as well as impacts on cells.

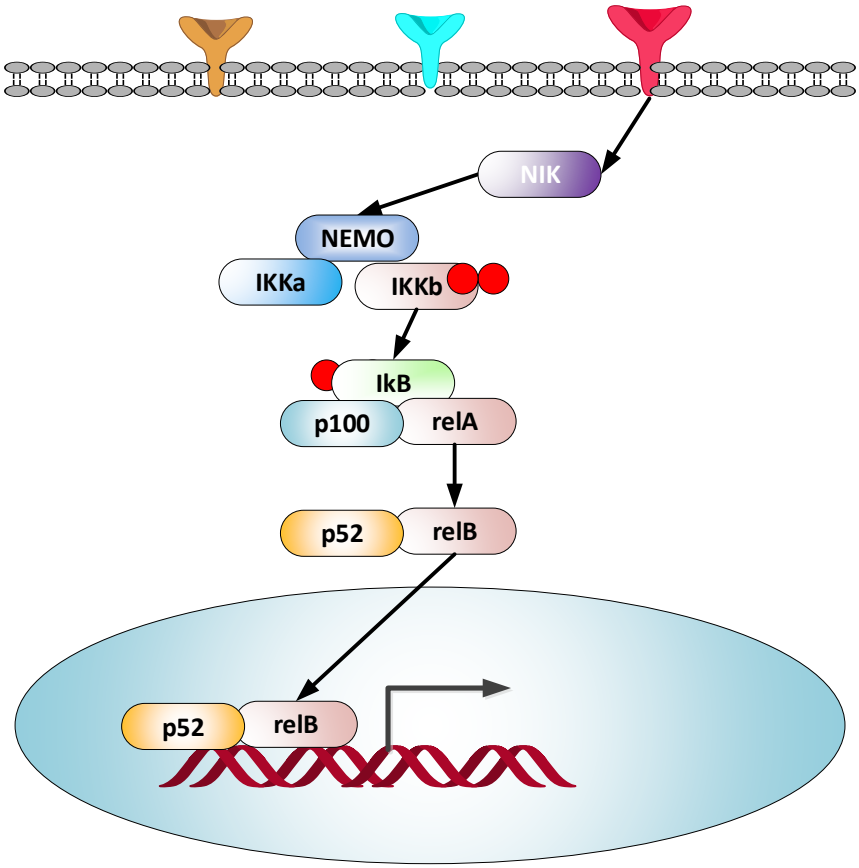


Note that NF-kB can be activated by the very things that are part of the inflammatory response. In turn, NF-kB as a promoter can then release more of this drivers, increase growth factor expression, and stop normal apoptosis. NF-kB is one of the most significant intracellular drivers that connects inflammation, the immune system, and unregulated growth.

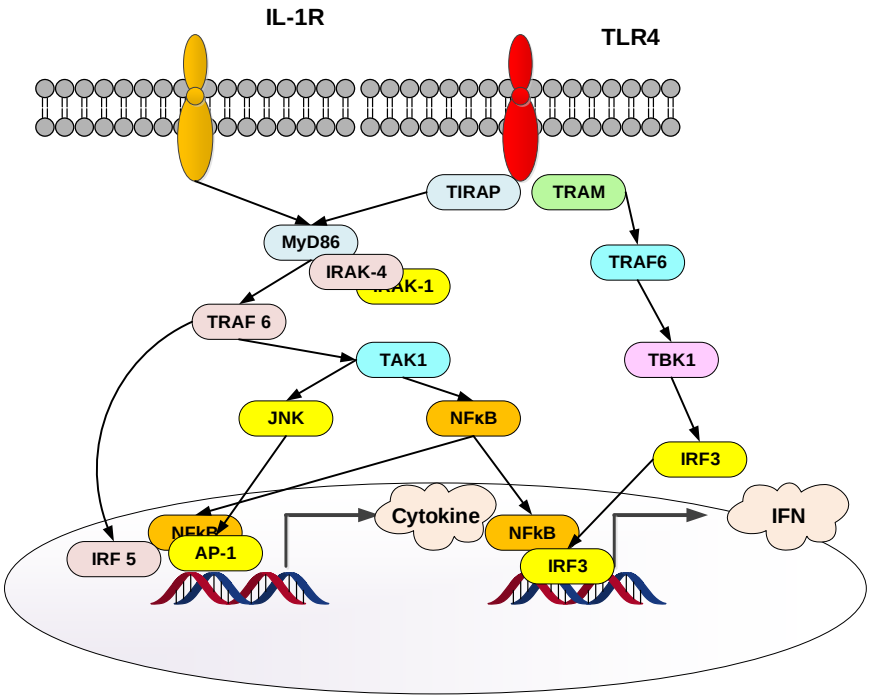
Now as we noted NF-kB is a dimer, namely a combination of two proteins which in turn, when activated, result in a molecule which in a very effective promoter. We show this below with a set of examples:



Here in the above is one of the dimer expressed, namely a relA along with a p50. Below we show the relB and the p52 expression.



The integrated combination is shown below.



To understand NF-kB we need to see how it functions as a powerful promoter. From NCBI we have the following description[6]:

NF-kappa-B is a ubiquitous transcription factor involved in several biological processes. It is held in the cytoplasm in an inactive state by specific inhibitors. Upon degradation of the inhibitor, NF-kappa-B moves to the nucleus and activates transcription of specific genes. NF-kappa-B is composed of NFKB1 or NFKB2 bound to either REL, RELA, or RELB. The most abundant form of NF-kappa-B is NFKB1 complexed with the product of this gene, RELA. Four transcript variants encoding different isoforms have been found for this gene.

From Nature we have the following description[7]:

The canonical pathway is induced by tumour necrosis factor-alpha (TNFalpha), interleukin-1 (IL-1) and many other stimuli, and is dependent on activation of IKKbeta. This activation results in the phosphorylation (P) of IkappaBalpha at Ser32 and Ser36, leading to its ubiquitylation (Ub) and subsequent degradation by the 26S proteasome. Release of the NF-kappaB complex allows it to relocate to the nucleus. Under some circumstances, the NF-kappaB–IkappaBalpha complex shuttles between the cytoplasm and the nucleus (not shown).

IKK-dependent activation of NF-kappaB can occur following genotoxic stress. Here, NF-kappaB essential modifier (NEMO) localizes to the nucleus, where it is sumoylated and then ubiquitylated, in a process that is dependent on the ataxia telangiectasia mutated (ATM)

[6] https://www.ncbi.nlm.nih.gov/gene/5970

[7] https://www.nature.com/nrm/journal/v8/n1/box/nrm2083_BX1.html

checkpoint kinase. NEMO relocates back to the cytoplasm together with ATM, where activation of IKK-beta occurs. IKK-independent atypical pathways of NF-kappaB activation have also been described, which include casein kinase-II (CK2) and tyrosine-kinase-dependent pathways.

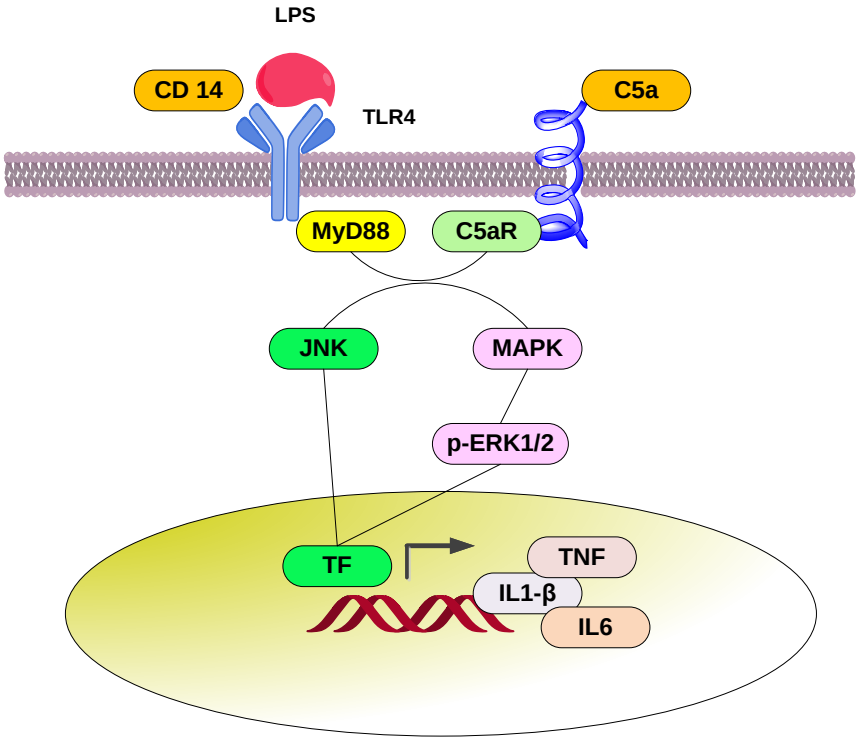
The non-canonical pathway results in the activation of IKK alpha by the NF-kappa B-inducing kinase (NIK), followed by phosphorylation of the p100 NF-kappa B subunit by IKK alpha. This results in proteasome-dependent processing of p100 to p52, which can lead to the activation of p52–Rel B heterodimers that target distinct kappa B elements. Phosphorylation of NF-kappa B subunits by nuclear kinases, and modification of these subunits by acetylases and phosphatases, can result in transcriptional activation and repression as well as promoter-specific effects.

Moreover, cooperative interactions with heterologous transcription factors can target NF-kappa B complexes to specific promoters, resulting in the selective activation of gene expression following cellular exposure to distinct stimuli.

As Merle et al discuss when examine the Complement system they state:

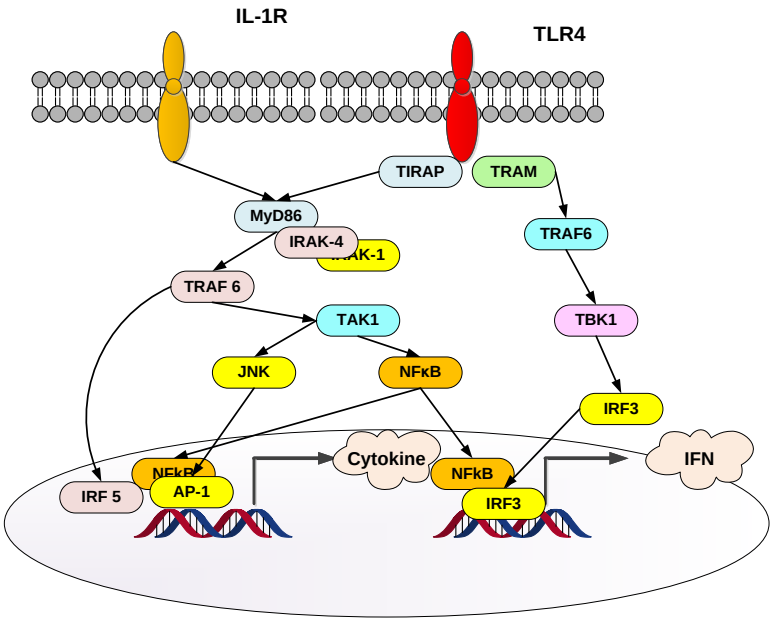
C3a and C5a are able to induce potent inflammatory pathways via their receptors C3aR and C5aR. The implication of intermediates such as NF-kB, MAPK, and c-Jun N-terminal kinase (JNK) in their transduction pathways suggests a potential crosstalk with other pathways, such as those of TLRs. Indeed, complement is involved in TLR-induced inflammation.

They show in the following Figure how this does function:



C5a/C5aR signalingpathwaycancooperatewithTLR-4activationby LPS on macrophages. Intermediate signaling pathways JNK and MAPK are activated and thus lead to proinflammatory effect by TNF-α, IL6, and IL1-β synthesis. On dendritic cells (DCs),TLR-4 and C5aR cooperate in different manner between mice and human. In vivo experiments have demonstrated an implication in Th1cells expansion, whereas in human, an anti-inflammatory role of TLR-4/C5aR collaboration has been described by an antagonized effect on IL-12 and IL-23 synthesis by DC.

Thus, when examining the effects of the complement proteins one must also examine the interactions with other receptors. Further details on this interaction are shown below. Here we show the Toll like receptors, TLR as initiations. These are powerful initiators in the innate response.



As Amiri and Richmond state:

Nuclear Factor-kappa B (NF-κB) is an inducible transcription factor that regulates the expression of many genes involved in the immune response. Recently, NF-κB activity has been shown to be upregulated in many cancers, including melanoma. Data indicate that the enhanced activation of NF- κB may be due to deregulations in upstream signaling pathways such as Ras/Raf, PI3K/Akt, and NIK. Multiple studies have shown that NF-κB is involved in the regulation of apoptosis, angiogenesis, and tumor cell invasion, all of which indicate the important role of NF-κB in tumorigenesis. Thus, understanding the molecular mechanism of melanoma progression will aid in designing new therapeutic approaches for melanoma.

They continue:

Constitutive activation of NF-κB is an emerging hallmark of various types of tumors including breast, colon, pancreatic, ovarian, and melanoma. In the healthy human, NF-κB regulates the

expression of genes involved in normal immunologic reactions (e.g. generation of immunoregulatory molecules such as antibody light chains) in response to proinflammatory cytokines and by-products of microbial and viral infections. NF-κB also modulates the expression of factors responsible for growth as well as apoptosis. However, increased activation of NF-κB results in enhanced expression of proinflammatory mediators, leading to acute inflammatory injury to lungs and other organs, and development of multiple organ dysfunctions as well as cancer.

They then summarize NF-kB's role as:

3.1. Apoptosis resistance and cell proliferation*: In processes such as tumor initiation and promotion where prolonged survival of cells is a crucial event, NF-κB plays an important role as a mediator of inhibition of apoptosis. In melanoma, NF-κB has been shown to activate expression of anti-apoptotic proteins such as tumor necrosis factor receptor-associated factor 1 (TRAF1), TRAF2, and the inhibitor-of apoptosis (IAP) proteins c-IAP1, c-IAP2, and melanoma inhibitor of apoptosis (ML-IAP), survivin as well as Bcl-2 like proteins…*

3.2. Invasion and metastasis*: In invasion and metastasis of melanoma, NF-κB may regulate the production of prostaglandins via cyclooxygenase-2 (COX-2), which has been shown to be overexpressed in melanoma [44,45]. It was shown that COX-2 is expressed in the majority of primary malignant melanoma, as well as in five human malignant melanoma cell lines….*

However, as Liu et al (2006) state:

Malignant melanoma is the most lethal skin cancer, whose ability to rapidly metastasize often prevents surgical cure.

Furthermore, the systemic treatment of melanoma is largely ineffective due to the intrinsic resistance of melanoma cells to numerous anticancer agents. Increased survival of melanoma cells is primarily attributed to the constitutive activation of the transcription factor nuclear factor kB (NF-kB), which regulates the expression of many anti-apoptotic, pro-proliferative and pro-metastatic genes.

Canonical activation of the NF-kB pathway occurs when NF-kB switches its localization from the cytoplasm, where it is maintained inactive by assembly with the inhibitor IkB protein, to the nucleus, where NF-kB regulates gene expression. NF-kB activation relies upon the phosphorylation dependent ubiquitination and degradation of IkB mediated by the IkB kinase (IKK) complex and b-Trcp E3 ubiquitin ligases.

Consequently, both IKK activity and the levels of b-Trcp regulate the extent of IkB degradation and hence NF-kB activation. The genetic basis that underlies the elevated NF-kB activity in malignant melanoma largely remains elusive.

Constitutively active IKK has been demonstrated to sustain NF-kB activation in human melanoma cells, resulting in induction of the chemokine CXCL1. CXCL1, in turn, is capable of activating IKK and NF-kB and promoting cell survival and tumorigenesis …. However, the

original genetic alterations that initiate this feed-forward mechanism in melanoma remain unclear.

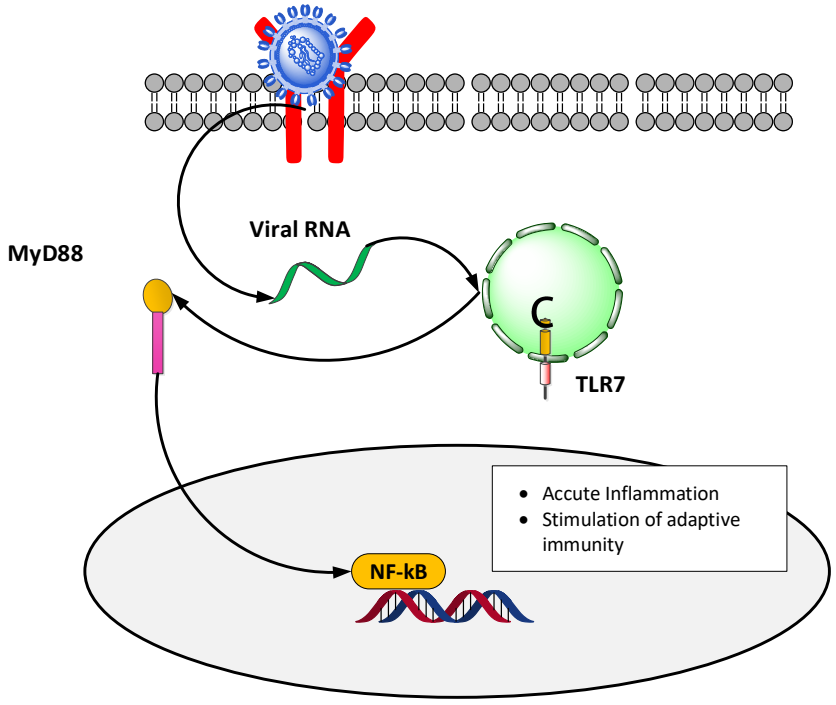
One of the major oncogenic events described in the genesis of malignant melanoma is constitutive activation of the Ras-regulated RAF-MEK-ERK mitogen-activated protein kinase (MAPK) pathway. This is achieved most frequently by activating mutations in either BRAF (e.g. V600E substitution) or, less frequently, in N-RAS … Recent evidence indicates that oncogenic BRAF activity is essential for human melanoma cell growth and survival …

However, despite prior reports that RAF can activate NF-kB …, the mechanism(s) by which $BRAF_{V600E}$ ($BRAF_{VE}$) may elicit NF-kB signaling in melanoma cells have not yet been elucidated. Activation of the canonical NF-kB pathway depends on both IKK activity, which has been shown to be elevated in human melanomas….

Liu et al conclusion is speculative but telling:

Taken together, these data support a model in which mutational activation of BRAF in human melanomas contributes to constitutive induction of NF-κB activity and to increased survival of melanoma cells.

Again we have the issue of speculation as to where and why the mutations occur. Here they speculate about the BRAF mutation resulting in the antiapoptotic control with NF-κB.



4 CYTOKINE STORMS

COVID-19 is a viral infection. As we have noted previously, the virus starts off an immune response by binding with TLR7, a toll like receptor in the invaded cell. It then sets off a massive set of cytokine releases. As we have also noted when the immune system is triggered there can be massive cytokine storms. Namely the release of massive amounts of immune related molecules which can run havoc in the body.

As we get to better understand COVID-19 and its dynamics we apparently are seeing is more as a massive immune reaction and less as a classic viral disease. Let us briefly examine this observation.

As Vaninov had recently noted:

Not all patients with COVID-19 develop the same symptoms, but the immunological determinants of a poor prognosis are unknown. In this preprint article, Yang, Y et al. followed a cohort of 53 clinically moderate and severe patients; they conducted a multiplex screen for 48 cytokines and correlated these results with lab tests, clinical characteristics and viral loads. They found a marked increase of 14 cytokines in patients with COVID-19 compared with healthy controls. Continuously high levels of three of these cytokines (CXCL10, CCL7 and IL-1 receptor antagonist) were associated with increased viral load, loss of lung function, lung injury and a fatal outcome. These observations offer key insights into the immunopathology of COVID-19 and provide new avenues for prognosis and therapy.

The implication seems to be to find therapeutics which may be counter-intuitive, namely immune repressors. Thus the classic virologist may have to be replaced by the immunologist, those dealing with immunotherapy. For example in the use of CAR-T cells, we can create these cytokine storms. Not always, but often enough to have some concerns.

As Tisoncik et al note:

Inflammation associated with a cytokine storm begins at a local site and spreads throughout the body via the systemic circulation. Rubor (redness), tumor (swelling or edema), calor (heat), dolor (pain), and "functio laesa" (loss of function) are the hallmarks of acute inflammation. When localized in skin or other tissue, these responses increase blood flow, enable vascular leukocytes and plasma proteins to reach extravascular sites of injury, increase local temperatures (which is advantageous for host defense against bacterial infections), and generate pain, thereby warning the host of the local responses.

These responses often occur at the expense of local organ function, particularly when tissue edema causes a rise in extravascular pressures and a reduction in tissue perfusion. Compensatory repair processes are initiated soon after inflammation begins, and in many cases the repair process completely restores tissue and organ function. When severe inflammation or the primary etiological agent triggering inflammation damages local tissue structures, healing occurs with fibrosis, which can result in persistent organ dysfunction.

The multi-organ attack seems to be now a part of COVID-19 and results in significant co-morbidities.

As Tisoncik et al note:

Cytokines are a diverse group of small proteins that are secreted by cells for the purpose of intercellular signaling and communication. Specific cytokines have autocrine, paracrine, and/or endocrine activity and, through receptor binding, can elicit a variety of responses, depending upon the cytokine and the target cell. Among the many functions of cytokines are the control of cell proliferation and differentiation and the regulation of angiogenesis and immune and inflammatory responses

The following are common in a cytokine storm:

Interferons: The interferons (IFNs) are a family of cytokines that play a central role in innate immunity to viruses and other microbial pathogens (45, 75). They are classified into three major types (types I, II, and III) on the basis of their receptor specificity.

Interleukins: IL-1α and IL-1β are proinflammatory cytokines that mediate the host response to infection through both direct and indirect mechanisms. Among their biological functions, these cytokines increase acute-phase signaling, trafficking of immune cells to the site of primary infection, epithelial cell activation, and secondary cytokine production.

CSFs: Colony-stimulating factors (CSFs), such as granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte colony stimulating factor (G-CSF), stimulate hematopoietic progenitor cell proliferation and differentiation. Colony-stimulating factors are also associated with inflammation, and there is evidence that these factors may be part of a mutually dependent proinflammatory cytokine network that includes IL-1 and tumor necrosis factor (TNF)

TNF: Tumor necrosis factor (TNF) is perhaps the best known and most intensely studied of the proinflammatory cytokines, and it plays a prominent role in the cytokine storm literature. The name “tumor necrosis factor” was first used in 1975 for a cytotoxic serum factor capable of inducing tumor regression in mice (23), which soon thereafter was reported to play a role in the pathogenesis of malaria and sepsis (14, 30, 31). TNF is now considered a central cytokine in acute viral diseases, including those caused by influenza virus, dengue virus, and Ebola virus.

As Mehta et al note regarding this syndrome in previous viral attacks:

As during previous pandemics (severe acute respiratory syndrome and Middle East respiratory syndrome), corticosteroids are not routinely recommended and might exacerbate COVID-19-associated lung injury. However, in hyperinflammation, immunosuppression is likely to be beneficial. Re-analysis of data from a phase 3 randomised controlled trial of IL-1 blockade (anakinra) in sepsis, showed significant survival benefit in patients with hyperinflammation, without increased adverse events A multicentre, randomised controlled trial of tocilizumab (IL-6

receptor blockade, licensed for cytokine release syndrome), has been approved in patients with COVID-19 pneumonia and elevated IL-6 in China (ChiCTR2000029765). Janus kinase (JAK) inhibition could affect both inflammation and cellular viral entry in COVID-19. All patients with severe COVID-19 should be screened for hyperinflammation using laboratory trends (eg, increasing ferritin, decreasing platelet counts, or erythrocyte sedimentation rate) and the HScore11 to identify the subgroup of patients for whom immunosuppression could improve mortality. Therapeutic options include steroids, intravenous immunoglobulin, selective cytokine blockade (eg, anakinra or tocilizumab) and JAK inhibition.

We believe that a rethinking of the COVID-19 treatments in this context can have a significant advantage. As regards to IL-6 signalling Flynn et al have noted specifically:

Signaling of the pleiotropic cytokine Interleukin-6 (IL-6) via its soluble IL-6R (sIL-6R) has been termed trans-signaling and is thought to be responsible for the pro-inflammatory properties of IL-6. The sIL-6R can be generated by alternative mRNA splicing or proteolytic cleavage of the membrane-bound IL-6R.

However, which stimuli induces IL-6R release and which endogenous signaling pathways are required for this process is poorly understood.

Here, we show that activation of Toll-like receptor 2 (TLR2) on primary human peripheral blood mononuclear cells (PBMCs) and on the monocytic cell line THP-1 induces expression and secretion of IL-6 and the generation of sIL-6R. We show by flow cytometry that monocytes are a PBMC subset that expresses TLR2 in conjunction with the IL-6R and are the major cellular source for both IL-6 and sIL-6R. Mechanistically, we find that the metalloproteases ADAM10 and ADAM17 are responsible for cleavage of the IL-6R and therefore sIL-6R generation. Finally, we identify the Extracellular-signal Regulated Kinase (ERK) cascade as a critical pathway that differentially regulates both IL-6 and sIL-6R generation in monocytes.

5 THERAPEUTICS

One approach to a therapeutic for the target we have discussed herein is an antibody to block IL-6 and its actions.

5.1 IL-6 ANTIBODIES

The most logical approach to mitigating IL-6 is the application of an antibody, Ab, to block activation. As Le et al note:

Tocilizumab is a recombinant humanized monoclonal antibody directed against the interleukin-6 receptor (IL-6R). It binds both soluble and membrane-bound IL-6R and inhibits IL-6- mediated signaling through these receptors. Herein, we summarize key review findings that supported the approval of tocilizumab for treatment of severe or life-threatening CAR T cell induced cytokine release syndrome

From Garbers et al we have the following Table detailing several available such therapeutics:

Name	*Target*	*Company*	*Disease (clinical phase)*
Sirukumab	IL-6	Centocor (Janssen)	• Rheumatoid arthritis * Depression « Lupus nephritis (phase II)
Olokizumab	IL-6	UCB	Rheumatoid arthritis and Crohn's disease (phase II)
Ciazakizumab	IL 6	Bristol Myers Squib and Alder	Organ transplant rejection (phase II)
Siltuximab	IL-6	Janssen	Castleman disease (approved) and multiple myeloma
EBI-031	IL 6	Eleven Biotherapeutics and Roche	Diabetic macular oedema and uveitis (preclinical)
Tocilizumab (Intravenous and subcutaneous)	IL-6R	Chugai, Roche and Genentech	Rheumatoid arthritis, juvenile idiopathic arthritis, Castleman disease, giant cell arteritis and cytokine release syndrome (approved) • NMO (phase II) • Systemic lupus erythematosus (phase 1)
Saritumab	IL-6R	Sanofi and Regeneron	Rheumatoid arthritis (approved)
NI-1201	IL-6R	Novimmuneend liziana	Not specified (preclinical)
Vobarilizumab	IL 6R	Ablynx	Rheumatoid arthritis and systemic lupus erythematosus (phase II)
Olamkicept	IL-6 and sIL-GR	Conaris Research Institute. Feiring Pharmaceuticals and l-Mflb Biopharma	Ulcerative colitis and IBD (phase II)
Tofacilinib	JAK3 > JAKl »JAK2	National Institutes of Health and Pfizer	Rheumatoid arthritis and Crohn's disease (approved) • Psoriasis and ulcerative colitis (phase III)
Ruxolitinrb	JAKland JAK2	Novartis and Incyle	Myelofibrosis and polycythaemia vera (approved)
Filgotinib	JAKl	Galapagos and Gilead	Rheumatoid arthritis, Crohn’s disease and ulcerative colitis (phase III)
Baricitinib	JAK1and JAK2	Eli I illy and Incyle	Rheumatoid arthritis (phase III) Psoriasis and systemic lupus erythematosus (phase II)
Upadacitinib	JAKl	AbbVie	Ankylosing spondylitis, atopic dermatitis, juvenile rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, rheumatoid arthritis and Crohn s disease (phase 111)
PF 04965842	JAKl	Pfizer	Atopic dermatitis (phase III)

5.2 ACE INHIBITORS

ACE2 is a key gene product associated with attaching the COVID-19 virus to a cell., As NCBI notes[8]:

The protein encoded by this gene belongs to the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases and has considerable homology to human angiotensin 1 converting enzyme. This secreted protein catalyzes the cleavage of angiotensin I into angiotensin 1-9, and angiotensin II into the vasodilator angiotensin 1-7. The organ- and cell-specific expression of this gene suggests that it may play a role in the regulation of cardiovascular and renal function, as well as fertility. In addition, the encoded protein is a functional receptor for

[8] https://www.ncbi.nlm.nih.gov/gene/59272

the spike glycoprotein of the human coronavirus HCoV-NL63 and the human severe acute respiratory syndrome coronaviruses, SARS-CoV and SARS-CoV-2 (COVID-19 virus).

As Yan et al have noted:

Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for severe acute respiratory syndrome– coronavirus (SARS-CoV) and the new coronavirus (SARS-CoV-2) that is causing the serious coronavirus disease 2019 (COVID-19) epidemic. Here, we present cryo–electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B0AT1 with or without the receptor binding domain (RBD) of the surface spike glycoprotein (S protein) of SARS-CoV-2, both at an overall resolution of 2.9 angstroms, with a local resolution of 3.5 angstroms at the ACE2-RBD interface.

The ACE2-B0AT1 complex is assembled as a dimer of heterodimers, with the collectrin-like domain of ACE2 mediating homodimerization. The RBD is recognized by the extracellular peptidase domain of ACE2 mainly through polar residues. These findings provide important insights into the molecular basis for coronavirus recognition and infection.

As Vaduganathan et al have noted:

SARS-CoV-2 appears not only to gain initial entry through ACE2 but also to subsequently downregulate ACE2 expression such that the enzyme is unable to exert protective effects in organs. It has been postulated but unproven that unabated angiotensin II activity may be in part responsible for organ injury in Covid-19.

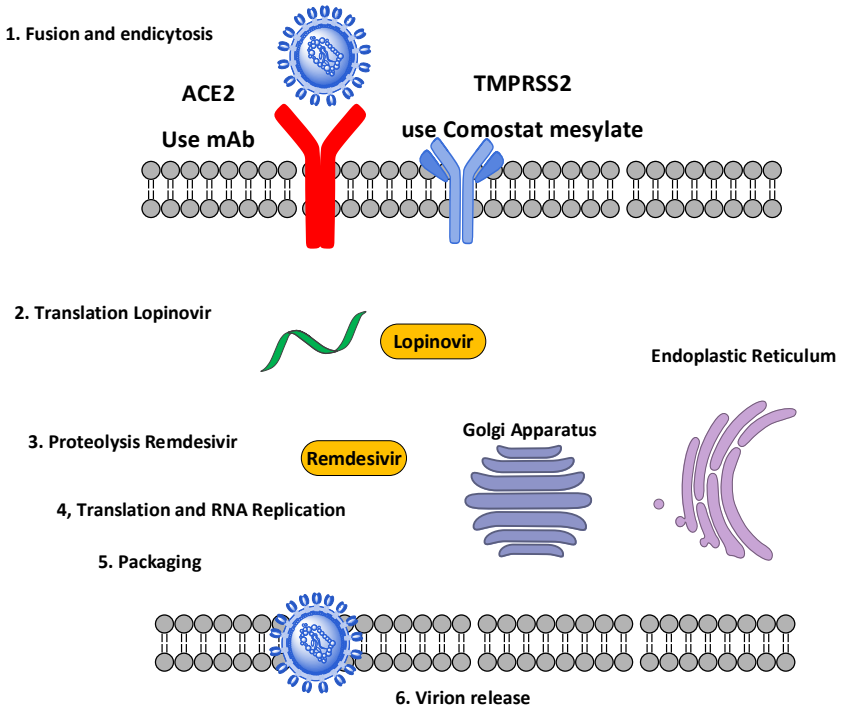
After the initial engagement of SARS-CoV-2 spike protein, there is subsequent down-regulation of ACE2 abundance on cell surfaces. Continued viral infection and replication contribute to reduced membrane ACE2 expression, at least in vitro in cultured cells. Down-regulation of ACE2 activity in the lungs facilitates the initial neutrophil infiltration in response to bacterial endotoxin and may result in unopposed angiotensin II accumulation and local RAAS activation. Indeed, in experimental mouse models, exposure to SARS-CoV-1 spike protein induced acute lung injury, which is limited by RAAS blockade.

Other mouse models have suggested that dysregulation of ACE2 may mediate acute lung injury that is secondary to virulent strains of influenza and respiratory syncytial virus.50 In a small study, patients with Covid-19 appeared to have elevated levels of plasma angiotensin II, which were in turn correlated with total viral load and degree of lung injury.44 Restoration of ACE2 through the administration of recombinant ACE2 appeared to reverse this devastating lung-injury process in preclinical models of other viral infections and safely reduced angiotensin II levels in a phase 2 trial evaluating acute respiratory distress syndrome in humans….

On the basis of the available evidence, we think that, despite the theoretical concerns and uncertainty regarding the effect of RAAS inhibitors on ACE2 and the way in which these drugs might affect the propensity for or severity of Covid-19, RAAS inhibitors should be continued in patients in otherwise stable condition who are at risk for, are being evaluated for, or have Covid-19, a position now supported by multiple specialty societies. Although additional data

may further inform the treatment of high-risk patients with Covid-19, clinicians need to be cognizant of the unintended consequences of prematurely discontinuing proven therapies in response to hypothetical concerns that may be based on incomplete experimental evidence.

From Kupferschmidt and Cohen, the authors have graphically outlined several targets including the ACE2 target as below:



As Hoffman et al have recently noted:

The recent emergence of the novel, pathogenic SARS-coronavirus 2 (SARS-CoV-2) in China and its rapid national and international spread pose a global health emergency. Cell entry of coronaviruses depends on binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases. Unravelling which cellular factors are used by SARS-CoV-2 for entry might provide insights into viral transmission and reveal therapeutic targets.

Here, we demonstrate that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. A TMPRSS2 inhibitor approved for clinical use blocked entry and might constitute a treatment option. Finally, we show that the sera from convalescent SARS patients cross-neutralized SARS-2- S-driven entry. Our results reveal important commonalities between SARS-CoV-2 and SARS-CoV infection and identify a potential target for antiviral intervention.

5.3 ANTI VIRALS

Antivirals have been developed extensively over the past decades. Some are quite effective. As Kupferschmidt and Cohen note:

Remdesivir, developed by Gilead Sciences to combat Ebola and related viruses, shuts down viral replication by inhibiting a key viral enzyme, the RNA polymerase. It didn't help patients with Ebola in a test during the 2019 outbreak in the Democratic Republic of the Congo. But in 2017, researchers showed in test tube and animal studies that the drug can inhibit the SARS and MERS viruses. The drug, which is given intravenously, has been used in hundreds of COVID-19 patients in the United States and Europe under what's known as compassionate use, which required Gilead to review patient records; some doctors have reported anecdotal evidence of benefit, but no hard data. Gilead says it is now starting to supply remdesivir under a simpler "expanded use" designation.

As Gilead notes[9]:

Remdesivir is an investigational nucleotide analog with broad-spectrum antiviral activity both in vitro and in vivo in animal models against multiple emerging viral pathogens, including Ebola, Marburg, MERS and SARS. In vitro testing conducted by Gilead has demonstrated that remdesivir is active against the virus that causes COVID-19. The safety and efficacy of remdesivir to treat COVID-19 are being evaluated in multiple ongoing Phase 2 and 3 clinical trials. Initial clinical trial data are expected in mid-April.

As a nucleotide analog it gets inserted in the replication of the RNA but since it is a defective nucleotide it then block the replication. Acyclovir is an early example of such an approach. Ongoing trials with this antiviral are expected to be complete soon.

As Grein et al have noted:

Remdesivir is a prodrug of a nucleotide analogue that is intracellularly metabolized to an analogue of adenosine triphosphate that inhibits viral RNA polymerases. Remdesivir has broad-spectrum activity against members of several virus families, including filoviruses (e.g., Ebola) and coronaviruses (e.g., SARS-CoV and Middle East respiratory syndrome coronavirus [MERS-CoV]) and has shown prophylactic and therapeutic efficacy in nonclinical models of these coronaviruses.

In the trial reported by Grein et al the results indicated:

Over a median follow-up of 18 days (interquartile range, 13 to 23) after receiving the first dose of remdesivir, 36 of 53 patients (68%) showed an improvement in the category of oxygen

[9] https://www.gilead.com/news-and-press/press-room/press-releases/2020/4/data-on-53-patients-treated-with-investigational-antiviral-remdesivir-through-the-compassionate-use-program-published-in-new-england-journal-of-medici

support, whereas 8 of 53 patients (15%) showed worsening (Figure 1). Improvement was observed in all 12 patients who were breathing ambient air or receiving low-flow supplemental oxygen and in 5 of 7 patients (71%) who were receiving noninvasive oxygen support (NIPPV or high-flow supplemental oxygen).

It is notable that 17 of 30 patients (57%) who were receiving invasive mechanical ventilation were extubated, and 3 of 4 patients (75%) receiving ECMO stopped receiving it; all were alive at last follow-up. Individual patients' changes in the category of oxygen support... By the date of the most recent follow-up, 25 of 53 patients (47%) had been discharged (24% receiving invasive ventilation [8 of 34 patients] and 89%....

28 days of follow-up, the cumulative incidence of clinical improvement, as defined by either a decrease of 2 points or more on the six-point ordinal scale or live discharge, was 84% (95% confidence interval [CI], 70 to 99) by Kaplan–Meier analysis.

Clinical improvement was less frequent among patients receiving invasive ventilation than among those receiving noninvasive ventilation (hazard ratio for improvement, 0.33; 95% CI, 0.16 to 0.68) (Figure 3B) and among patients 70 years of age or older (hazard ratio as compared with patients younger than 50 years, 0.29; 95% CI, 0.11 to 0.74) Sex, region of enrollment, coexisting conditions, and duration of symptoms before remdesivir treatment was initiated were not significantly associated with clinical improvement.

Seven of the 53 patients (13%) died after the completion of remdesivir treatment, including 6 of 34 patients (18%) who were receiving invasive ventilation and 1 of 19 (5%) who were receiving noninvasive oxygen support... The median interval between remdesivir initiation and death was 15 days (interquartile range, 9 to 17).

Overall mortality from the date of admission was 0.56 per 100 hospitalization days (95% CI, 0.14 to 0.97) and did not differ substantially among patients receiving invasive ventilation (0.57 per 100 hospitalization days; 95% CI, 0 to 1.2]) as compared with those receiving noninvasive ventilation (0.51 per 100 hospitalization days; 95% CI, 0.07 to 1.1]).

Risk of death was greater among patients who were 70 years of age or older (hazard ratio as compared with patients younger than 70 years, 11.34; 95% CI, 1.36 to 94.17) and among those with higher serum creatinine at baseline (hazard ratio per milligram per deciliter, 1.91; 95% CI, 1.22 to 2.99). The hazard ratio for patients receiving invasive ventilation as compared with those receiving noninvasive oxygen support was 2.78 (95% CI, 0.33 to 23.19)

Although a small study the results are highly promising.

5.4 TMPRSS2 INHIBITORS

TMPRSS2 is a gene which is activated by androgens and which produces transmembrane serine proteases[10]. As noted in NCBI[11]:

This gene encodes a protein that belongs to the serine protease family. The encoded protein contains a type II transmembrane domain, a receptor class A domain, a scavenger receptor cysteine-rich domain and a protease domain. Serine proteases are known to be involved in many physiological and pathological processes. This gene was demonstrated to be up-regulated by androgenic hormones in prostate cancer cells and down-regulated in androgen-independent prostate cancer tissue.

The protease domain of this protein is thought to be cleaved and secreted into cell media after autocleavage. This protein also facilitates entry of viruses into host cells by proteolytically cleaving and activating viral envelope glycoproteins. Viruses found to use this protein for cell entry include Influenza virus and the human coronaviruses HCoV-229E, MERS-CoV, SARS-CoV and SARS-CoV-2 (COVID-19 virus).

Serine proteases are a class of enzymes which cleave proteins. TMPRSS2 is a transmembrane protein, crossing the cell membrane and thus it does have interaction with viral infiltrates as well as many other key functions.

We briefly discuss TMPRSS2 as a gene and gene product as a standalone. Unfused it has regulatory properties but fused it become an aggressive assist in activating the remaining portion of ERG. As Chen et al state:

TMPRSS2 is an androgen responsive gene that encodes a type II transmembrane serine protease (TTSP). The members of the TTSP family share common protein structures including a transmembrane domain at the N terminus, linker regions with a variety of protein–protein interaction domains, and a canonical serine protease domain at the C terminus.

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[10] See https://www.healio.com/endocrinology/reproduction-androgen-disorders/news/online/%7B967d41c4-7696-4364-999f-413dd814fed2%7D/endocrine-related-targets-may-drive-treatments-for-covid-19 which states:

The TMPRSS2 gene, located on human chromosome 21, has several androgen receptor elements, or AREs, located upstream of the transcription start site and the first intron. The gene encodes a protein of 492 amino acids that is highly expressed in both normal prostate epithelial cells and in prostate cancer cells. There is also low-level expression of TMPRSS2 in the lungs, colon, liver, kidneys and pancreas. Notably, in both prostate and lung cancer cells, TMPRSS2 is expressed in an androgen-dependent manner. Androgen-regulated TMPRSS2 in prostate cells plays a role in both normal male reproduction and in prostate cancer progression and metastases. The highly related SARS-CoV1, often called SARS, also utilizes TMPRSS2 for spike protein priming and also exhibits a male predominance in terms of morbidity and mortality. Given the seminal role of TMPRSS2 in SARS-CoV2 viral uptake and priming, and the data indicating that men with the disease exhibit higher case fatality rates, it is plausible that androgen-driven TMPRSS2 expression among men may explain the sex discrepancy in this disease.

[11] https://www.ncbi.nlm.nih.gov/gene/7113

TTSPs have been found to play important roles in the development and homeostasis of mammals, and the aberrant expression of TTSP genes are reported to contribute to the etiology of several human disorders, including cancer.

The importance of TMPRSS2 in vivo remains unclear because homozygous TMPRSS2-null mice are essentially phenotypically normal.

However, TMPRSS2 was reported to regulate epithelial sodium channel (ENaC) activity in vitro, implying a possible role in epithelial sodium homeostasis. TMPRSS2 may play a role in angiogenesis and tubulogenesis in microvesicular endothelial cells, potentially modulating several aspects of prostate tumor biology. In addition to its proteolytic activity, TMPRSS2 may also serve as a cell receptor, conducting external signaling or interacting with the extracellular matrix through its extracellular protein binding domains.

Overexpression of TMPRSS2 has been demonstrated in poorly differentiated prostate cancer with significant increase in the mRNA level.

The discussion above demonstrates that TMPRSS2 has multiple functions in cell regulation. The ultimate fusion of TMPRSS2 with ERG would thus inhibit the function of the gene product by itself. It is not clear that the loss of the functionality is a key factor in developing aggressive PCa. Clearly the androgen activation is a key factor in normal operation and the question is why does the gene lose this functionality?

As Iwata-Yoshikawa et al have noted[12]:

Transmembrane serine protease TMPRSS2 activates the spike protein of highly pathogenic human coronaviruses such as severe acute respiratory syndrome related coronavirus (SARS-CoV) and Middle East respiratory syndrome-related coronavirus (MERS-CoV). In vitro, activation induces virus-cell membrane fusion at the cell surface. However, the roles of TMPRSS2 during coronavirus infection in vivo are unclear.

Here, we used animal models of SARS-CoV and MERS-CoV infection to investigate the role of TMPRSS2. Th1-prone C57BL/6 mice and TMPRSS2-knockout (KO) mice were used for SARS-CoV infection, and transgenic mice expressing the human MERS-CoV receptor DPP4 (hDPP4-Tg mice) and TMPRSS2-KO hDPP4-Tg mice were used for MERS-CoV infection. After experimental infection, TMPRSS2-deficient mouse strains showed reduced body weight loss and viral kinetics in the lungs. Lack of TMPRSS2 affected the primary sites of infection and virus spread within the airway, accompanied by less severe immunopathology.

The above observation is a critical one in terms of our discussion of COVID-19. It facilitates the activation along with ACE2. Thus it may be useful to block this transmembrane protein as well as ACE2. However we must always be cognizant of the unanticipated effects that may result from such a process. They continue:

[12] https://www.rndsystems.com/resources/articles/ace-2-sars-receptor-identified This article is of interest and worth reading.

However, TMPRSS2-KO mice showed weakened inflammatory chemokine and/or cytokine responses to intranasal stimulation with poly(I·C), a Toll-like receptor 3 agonist. In conclusion, TMPRSS2 plays a crucial role in viral spread within the airway of murine models infected by SARS-CoV and MERS-CoV and in the resulting immunopathology. …

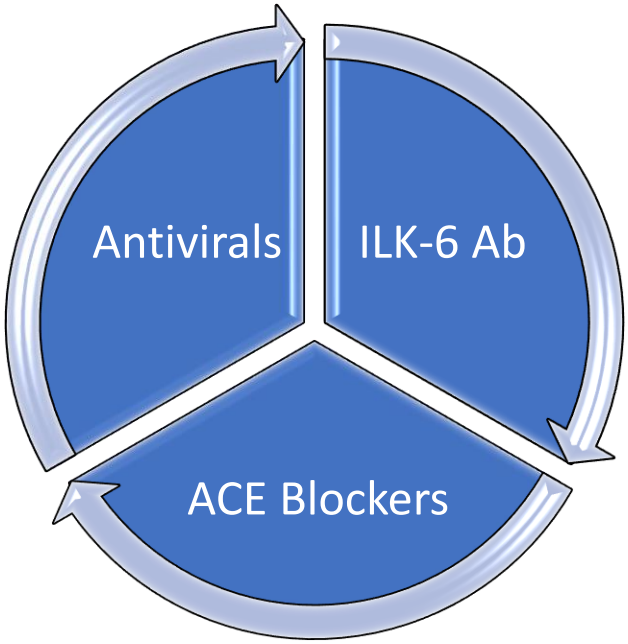
Broad-spectrum antiviral drugs against highly pathogenic coronaviruses and other emerging viruses are desirable to enable a rapid response to pandemic threats. Transmembrane protease serine type 2 (TMPRSS2), a protease belonging to the type II transmembrane serine protease family, cleaves the coronavirus spike protein, making it a potential therapeutic target for coronavirus infections.

Here, we examined the role of TMPRSS2 using animal models of SARSCoV and MERS-CoV infection. The results suggest that lack of TMPRSS2 in the airways reduces the severity of lung pathology after infection by SARS-CoV and MERS-CoV. Taken together, the results will facilitate development of novel targets for coronavirus therapy.

Indeed, this does represent a novel target.

5.5 Multi Theraputic Approach

As we have seen from the early days of chemotherapy and now with immunotherapy, multi therapeutic regimens are often more effective.



The above is a multi-therapeutic approach which we shall discuss later. Not that we try to combat each of the three key elements; cytokine storms, viral replication and viral entry. The

tools available are antibodies for blocking entry, antivirals for mitigating RNA replication and antibodies for inhibiting the IL-6 activated storms. We are NOT saying that this specific approach is dispositive of any accepted methodology. We are arguing for a logical fact based process for therapeutic development.

6 COMORBIDITIES

We now examine several of the comorbidities that may exacerbate COVID-19 presentations.

6.1 IMMUNE COMORBIDITIES

First, there are a multiplicity of co-morbidities related to immune states such as excess IL-6. As Wu et al noted amongst COVID-19 patients, IL-6 was overexpressed in nearly 50%. This group also had high cytokine morbidities as well. The profile of the patients relative to IL-6 are:

Test	***Reference Range***	***No Patients***	***Values***	***No Pt with Deviation and %***
IL-6, pg/L	0-7	123	6.98 (5.46-9.02)	60 (48.8%)

The comorbidities are of interest for two reasons. First, they present the predisposing conditions that we all too often seem to see in the presentation of COVID-19 patients. Second, they also present existing morbidities where we must treat them as a immunological process as well as a fundamental disease state. Now from Garbers et al we list some of the disease states and the targets for mitigating these states along with currently accepted therapeutics. These may be arguable approaches for COVID-19 morbidity treatments.

Disease	**Targets**	**Drugs approved**
Rheumatoid arthritis	IL-6 and IL-6R	Tocilizumab and sarilumab
Castleman disease	IL-6 and IL-6R	Tocilizumab and siltuximab
Erdheim-Chester disease (non-Langerhans cell histiocytosis)	IL-6R	NA
Neuromyelitis optica	IL-6R	NA
Cytokine release syndrome	IL-6R	Tocilizumab
Multiple myeloma	IL-6R	NA
Giant cell arteritis	IL-6R	Tocilizumab

6.2 AGE AND STRESS FACTORS

There are other factors besides disease that place a stress on the COVID-19 patient. Namely we have age and other stress related conditions. As Kiecolt-Glaser et al have noted:

Overproduction of IL-6, a proinflammatory cytokine, is associated with a spectrum of age-related conditions including cardiovascular disease, osteoporosis, arthritis, type 2 diabetes, certain cancers, periodontal disease, frailty, and functional decline. To describe the pattern of change in IL-6 over 6 years among older adults undergoing a chronic stressor, this longitudinal community study assessed the relationship between chronic stress and IL-6 production in 119 men and women who were caregiving for a spouse with dementia and 106 noncaregivers, with a mean age at study entry of 70.58 (SD 8.03) for the full sample.

On entry into this portion of the longitudinal study, 28 of the caregivers' spouses had already died, and an additional 50 of the 119 spouses died during the 6 years of this study. Levels of IL-6 and health behaviors associated with IL-6 were measured across 6 years. Caregivers' average rate of increase in IL-6 was about four times as large as that of noncaregivers. Moreover, the mean annual changes in IL-6 among former caregivers did not differ from that of current caregivers even several years after the death of the impaired spouse.

There were no systematic group differences in chronic health problems, medications, or health-relevant behaviors that might have accounted for caregivers' steeper IL-6 slope. These data provide evidence of a key mechanism through which chronic stressors may accelerate risk of a host of age-related diseases by prematurely aging the immune response

Production of IL-6 and other proinflammatory cytokines can be directly stimulated by depression and other negative emotions and stressful experiences (16–20). Indeed, both physical and psychological stressors can provoke transient increases in proinflammatory cytokines (21, 22). Additionally, negative emotions contribute to greater risk for infection, prolonged infection, and delayed wound healing (1–5), all processes that can fuel sustained proinflammatory cytokine production.

Thus, stressors can directly affect the cells of the immune system and modulate the secretion of proinflammatory cytokines. Accordingly, we argue that distress-related immune dysregulation may be one central mechanism behind a large and diverse set of health risks associated with caregiving and other chronic stressors.

In this study, we tested the hypothesis that caregivers would show a steeper increase in IL-6 levels over time than noncaregiving controls. Additionally, we assessed the question of whether the cessation of caregiving would have beneficial consequences for IL-6 levels.

6.3 TYPE 2 DIABETES

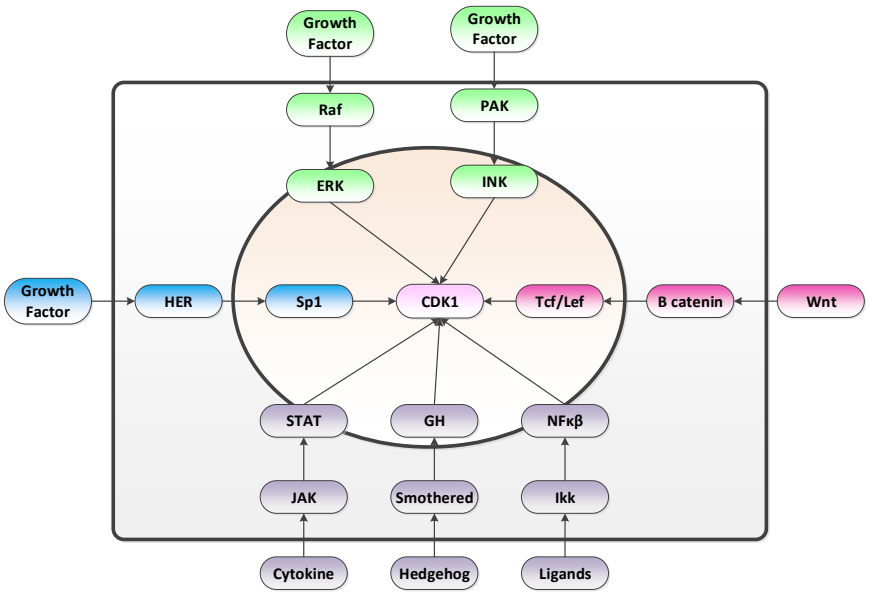
Type 2 diabetes is rampant in the United States and in most developed nations. We have examined this in detail a while back and indicated that its prevalence will not portent well for any stressed health environment[13]. In the case of COVID-19 patients a co-morbidity of Type 2 Diabetes places a significant burden at presentation and in treatment. As Rhodes notes:

A variety of cytokines play a role in the pathogenesis of type 2 diabetes. The discovery that there is local induction of IL-1b production within islets in response to chronic glucose implies that IL-1b plays a role in inducing b-cell apoptosis in type 2 diabetes, as well as in type 1 diabetes. In obesity-linked diabetes, certain adipocyte-derived cytokines are elevated in the circulation, including leptin, tumor necrosis factor a (TNFa), and IL-6. Intriguingly, leptin has recently been shown to modulate IL-1b–induced apoptosis in human b cells. Some of these cytokines can induce b-cell apoptosis through induction of signaling pathways that activate the transcription factor NFkB.

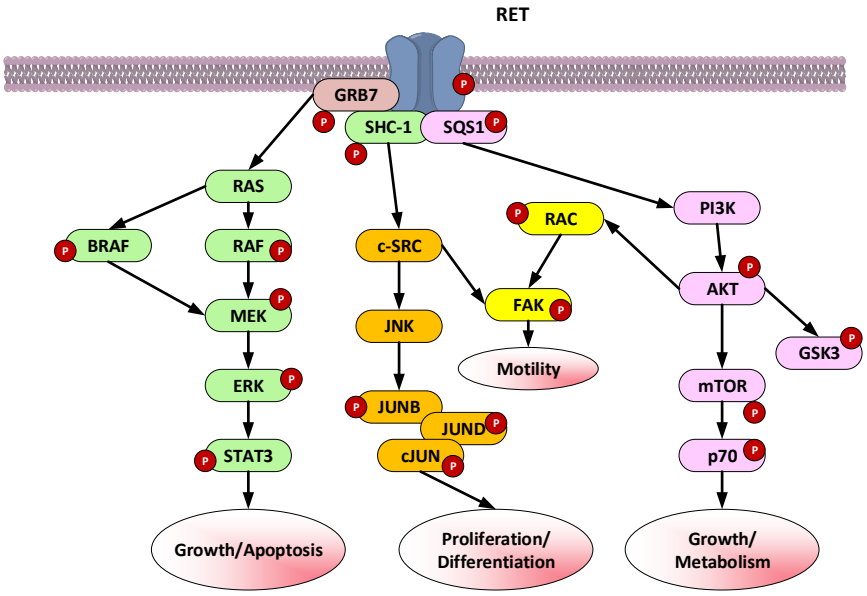
[13] https://www.researchgate.net/publication/265206539_Obesity_and_Type_2_Diabetes_Cause_and_Effect

However, they may also activate signaling pathways that trigger increased degradation of IRS-2. Certain cytokines, such as leptin, IL-6, and IFN-g activate the Janus Kinase- 2/Signal Transducer and Activator of Transcription (JAK/STAT) postreceptor signaling pathway. This leads to increased expression of Suppressor of Cytokine Signaling-1 (SOCS-1) and SOCS-3 proteins, which normally bind to the leptin, IL-6, and IFN-g receptors and inhibit JAK-2/STAT signaling. SOCS-1 and SOCS-3 have also been shown to bind to the C terminus of IRS molecules, leading to their ubiquitination and subsequent degradation. Thus, it is conceivable that leptin and/or IL-6 may cause b-cell apoptosis by decreasing b-cell IRS-2 levels through a similar mechanism.

As noted above the JAK/STAT pathway is important. We demonstrate its effect below on CDK1 activation.



The figure below further depicts the STAT pathway and its impact.



6.4 ARTERITIS

Arteritis is an inflammatory condition of the veins and arteries. It can result in significant morbidity and mortality and requires immediate identification and remediation. It is a fundamental immunological disorder. From Shmerling:

If vascular competency is sufficiently compromised due to intimal hyperplasia or, more rarely, thrombosis, ischemic complications may follow, including vision loss or scalp and tongue necrosis. Meanwhile, cytokines derived from macrophages, including IL-1 and IL-6,18 may contribute to the prominent systemic features of temporal arteritis, such as fever, anorexia and, perhaps, PMR…

It is of concern to mitigate the arteritis effects promptly. This is especially as regards to cerebral deficits resulting in massive and fatal cerebrovascular events.

6.5 KIDNEY

The kidneys are especially sensitive to a variety of immune assaults. As Su et al note:

Interleukin-6 (IL-6) is a pleiotropic cytokine that not only regulates the immune and inflammatory response but also affects hematopoiesis, metabolism, and organ development. IL-6 can simultaneously elicit distinct or even contradictory physiopathological processes, which is likely discriminated by the cascades of signaling pathway, termed classic and trans-signaling. Besides playing several important physiological roles, dysregulated IL-6 has been demonstrated to underlie a number of autoimmune and inflammatory diseases, metabolic abnormalities, and malignancies.

This review provides an overview of basic concept of IL-6 signaling pathway as well as the interplay between IL-6 and renal-resident cells, including podocytes, mesangial cells, endothelial cells, and tubular epithelial cells. Additionally, we summarize the roles of IL-6 in several renal diseases, such as IgA nephropathy, lupus nephritis, diabetic nephropathy, acute kidney injury, and chronic kidney disease.

6.6 BREAST CANCER

Cancers are fundamentally a failure of the immune system. Breast cancer is a typical example. As Studebaker et al have noted:

We found a direct correlation between the ability of breast, lung, and bone fibroblasts to enhance ERA-positive breast cancer cell growth and the level of soluble interleukin-6 (IL-6) produced by each organ-specific fibroblast, and fibroblast-mediated growth enhancement was inhibited by the removal or inhibition of IL-6. Interestingly, mice coinjected with MCF-7 breast tumor cells and senescent skin fibroblasts, which secrete IL-6, developed tumors, whereas mice coinjected with presenescent skin fibroblasts that produce little to no IL-6 failed to form xenograft tumors.

We subsequently determined that IL-6 promoted growth and invasion of breast cancer cells through signal transducer and activator of transcription 3–dependent up-regulation of Notch-3, Jagged-1, and carbonic anhydrase IX. These data suggest that tissue-specific fibroblasts and the factors they produce can promote breast cancer disease progression and may represent attractive targets for development of new therapeutics.

7 OBSERVATIONS

Our objective herein was to logically explores the elements of the COVID-19 viral assault on a patient and then to address the putative therapeutics currently available to mollify the attack. As noted earlier there is no intent to suggest that what is considered herein is the promotion or endorsement of any specific therapeutic. The intent is solely an exploration based upon logical interconnections between facts.

7.1 VIRAL INFECTION MODES

The greatest problem that we seem to face in this pandemic is to understand how the infection occurs. We have stream views ranging from surface contacts to 8 m plumes of virion infected clouds. The latter have been promulgated by academics but lack any significant validation. The surface contacts we have discussed previously but there is a constantly moving set of observations[14].

7.2 IMMUNE SYSTEM RESPONSES

One of the most significant findings is that this virus initiates a cytokine storm response. It is that specific response that we have tried to address focusing on IL-6 as a target cytokine. This target may or may not be correct. Yet it is clear that the immune response often is more deleterious than the viral load itself. Thus correlating viral load and immune response would be of interest.

We have laid out several mechanisms for this cytokine storm response focusing on the literature currently available. No definitive result is posited.

7.3 THERAPEUTIC APPROACH

Based upon our analysis of the virus, the viral attack, and the immune response, we have proposed a straw man therapeutic approach. We do so in the figure below. Specifically we note:

1. Barrier to Virus: ACE2 and TMPRSS2 are surface proteins, receptors, that enable the attachment and entry of the virus into a cell where it can proliferate. Recall that viruses need the mechanisms in the cell to reproduce and they are effectively dormant outside. The dormancy may be a long-term issue if the virus is not attacked by the adaptive immune system. Thus a barrier may actually both inhibit the viral reproduction while also inhibiting the adaptive immune response. The result is the classic "Typhoid Mary" syndrome.

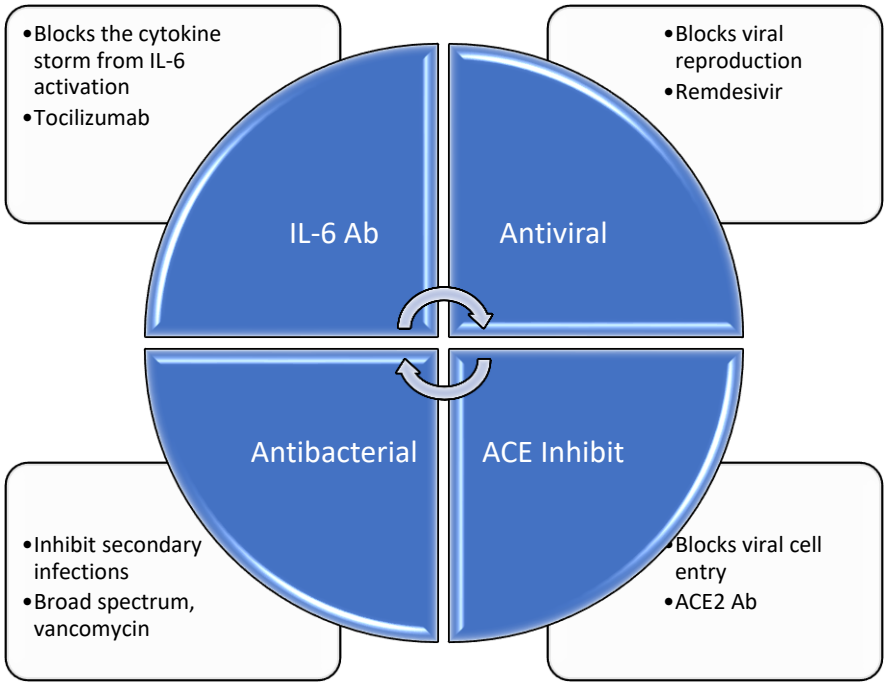
2. Barrier to Reproduction: This is the antiviral strategy. Generally, antivirals attempt to block the replication of the RNA or the structural elements of the virus inside the cell. As with the initial approach this may also slow down the adaptive response.

[14] https://www.telmarc.com/Documents/White%20Papers/173Corona.pdf

3. Inhibition of Cytokine Storm: Here we have selected blocking of IL-6 a reasonable target along with an overactivation of NF-kB. The IL-6 target has been used in the context of CAR-T cell storm behavior and thus appears to be a reasonable target.

4. Antimicrobial: Here we throw in the metaphorical "kitchen sink" to prevent any secondary bacterial infection on top of the damaged tissues and immune suppression.

We demonstrate this "cocktail" therapeutic below along with putative existing therapeutics.



7.4 THERAPY VS IMMUNIZATION

The current problem is as follows: if immunization of a nation is a slow process, can one move forward using therapeutics as infections may occur as a strategy? Consider the simple fact. In New Jersey at just past the peak we have but 0.6% prevalence. Namely 99.4% of the population is purportedly disease free. Must we keep the nation in an economic stand still until we can eliminate the disease? This is an economic tradeoff. Is the cost of treatment less than the cost of a closed economy. Add to that the death rate of 3.75% of those infected and we then must make a reasonable economic study.

Let us assume that immunization is 18 months away. Then one could ask what the cost of therapeutics would be assuming some modicum of viral avoidance. Also we can ask what is that mode of viral avoidance.

We believe that a broad based therapeutic approach should be taken. However, the most important issue is measuring via tests what the actual prevalence is. Secondarily, we must be aware of the "Typhoid Mary" syndrome in both those infected and no signs, and those no longer infected but act as a carrier.

7.5 A Proposal for Going Forward

Given the previous presentation we must ask; now what. Clearly the ultimate end point is herd immunity via some form of vaccine. On the other hand, we have just discussed therapeutic choices which will be necessary notwithstanding. We now make a simple proposal for moving forward and addressing economic realities and the ongoing threat of the pandemic.

One of the questions that must be answered is; what next. We have to make certain assumptions.

1. It will take 12-18 months to get a vaccine and distribute it. Worse if we rely on the Government. Remember the CDC.

2. We cannot just do nothing everywhere for that period. First it would kill the economy and second it makes no sense, Government Doc excluded.

3. We must have the ability to test on a large scale on an ongoing basis.

4. Right now only about 0.6 %% of the people are or have been infected. So why punish the other 99.4 %!

5. Solution, simple lay out a plan to get back to work. Carefully.

Now the solution to the problem is simple. We propose that we do the following:

1. Recognize that there are 3000 counties in the US

2. Recognize that there are 330,000,000 people

3. That means on average there are 110,000 people per county. Yes I know this is just an average so we can adjust accordingly.

4. We then need to do 500 tests per week per county to get a reasonable confidence interval for the tests. This number of tests will yield an assurance that we have some compliance of prevalence.

5. Set the prevalence compliance to say 0.6 % Frankly you can choose whatever you like.

6. If a county is in compliance with a 95% confidence interval then they can stay open until next test.

7. If not then mitigation is needed, namely some form of shut down.

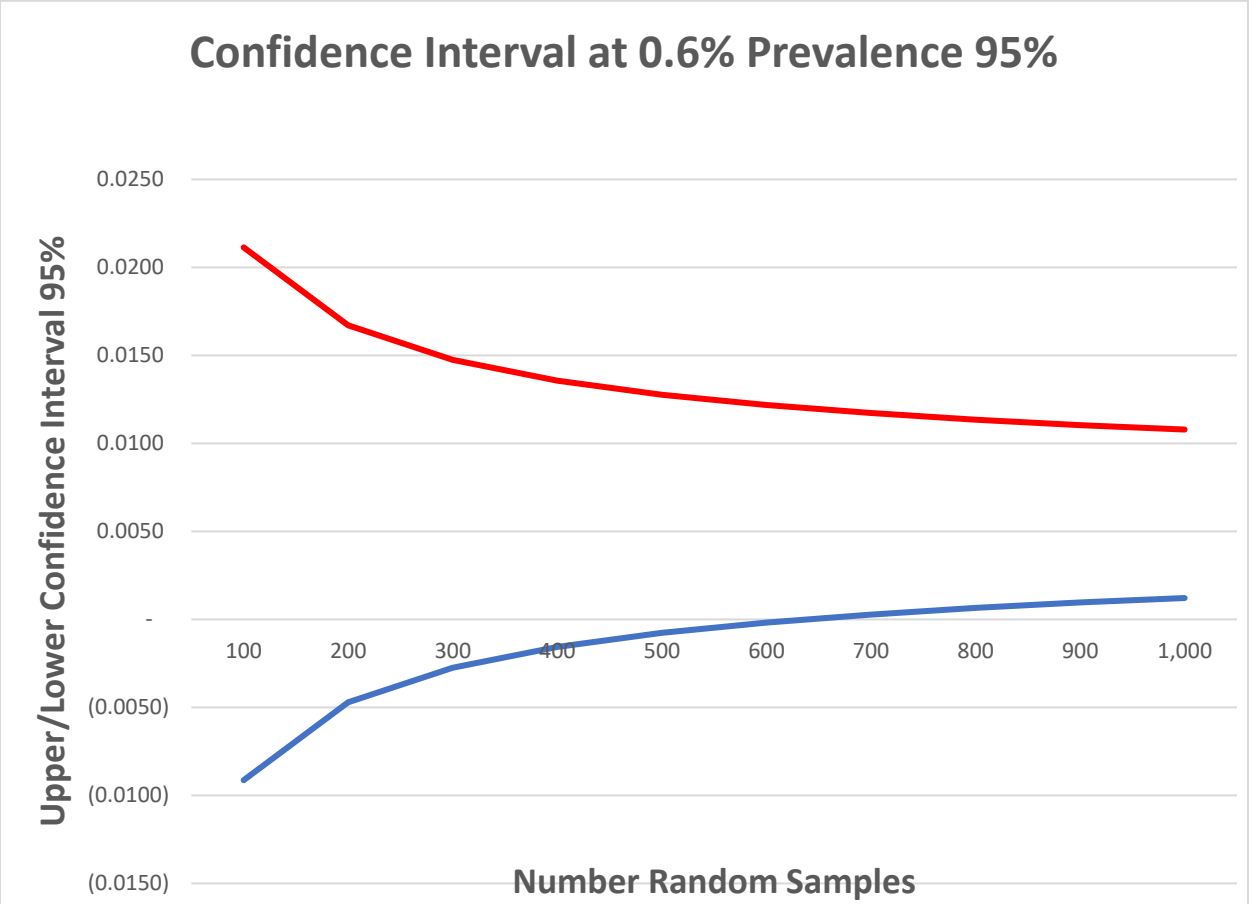
8. This process lasts until we get to 80% immunized when Herd Immunity kicks in.

9. Each community knows their score so no surprises.

Now where did we get this? Simple, from basic statistics we have a confidence interval for a binomial test in large numbers as[15]:

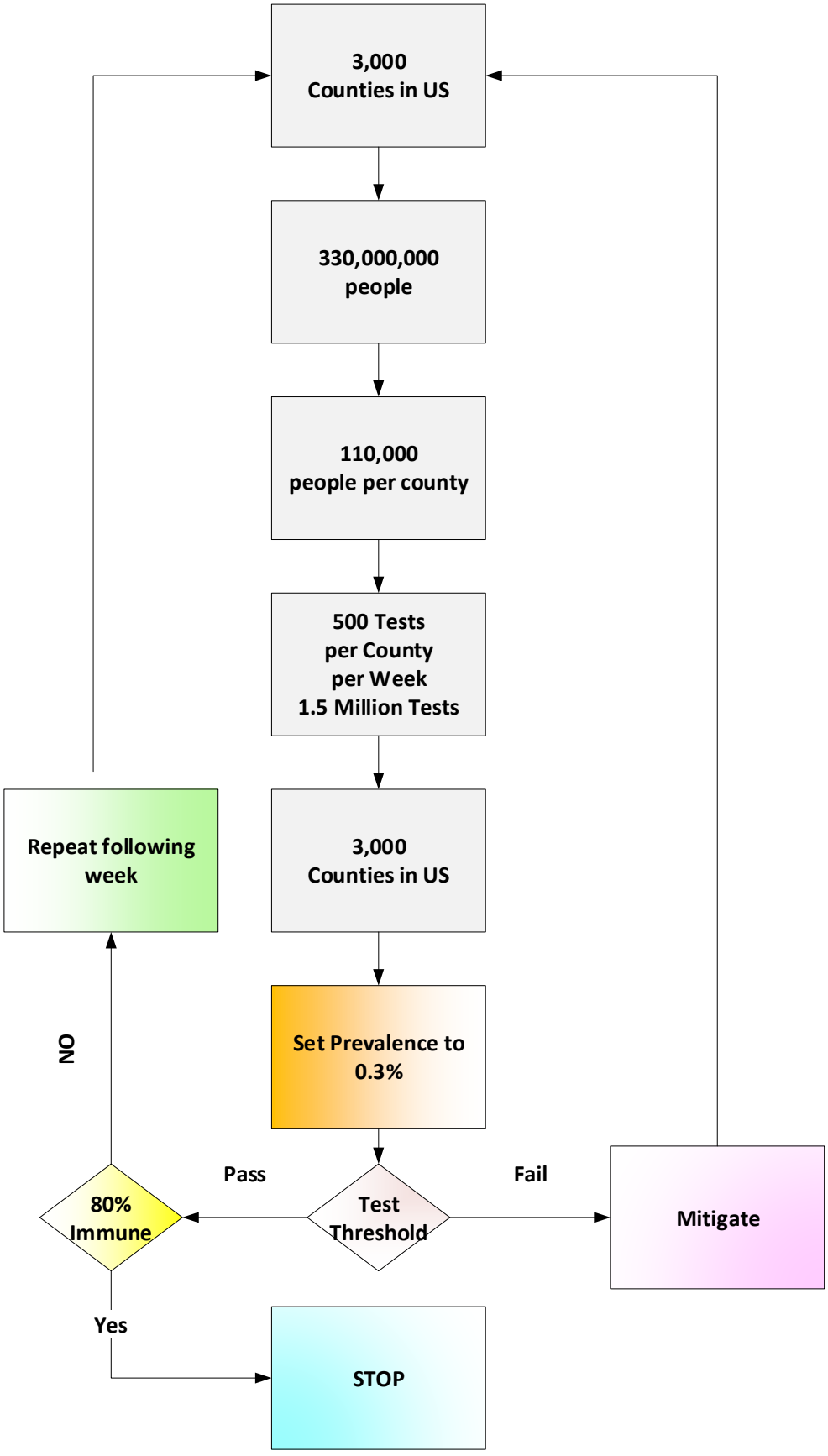
$$P\left[\hat{p}-1.96\sqrt{\frac{\hat{p}(1-\hat{p})}{N}} \le p \le \hat{p}+1.96\sqrt{\frac{\hat{p}(1-\hat{p})}{N}}\right]=0.95$$

Then we would get confidence intervals at 0.6% as follows:



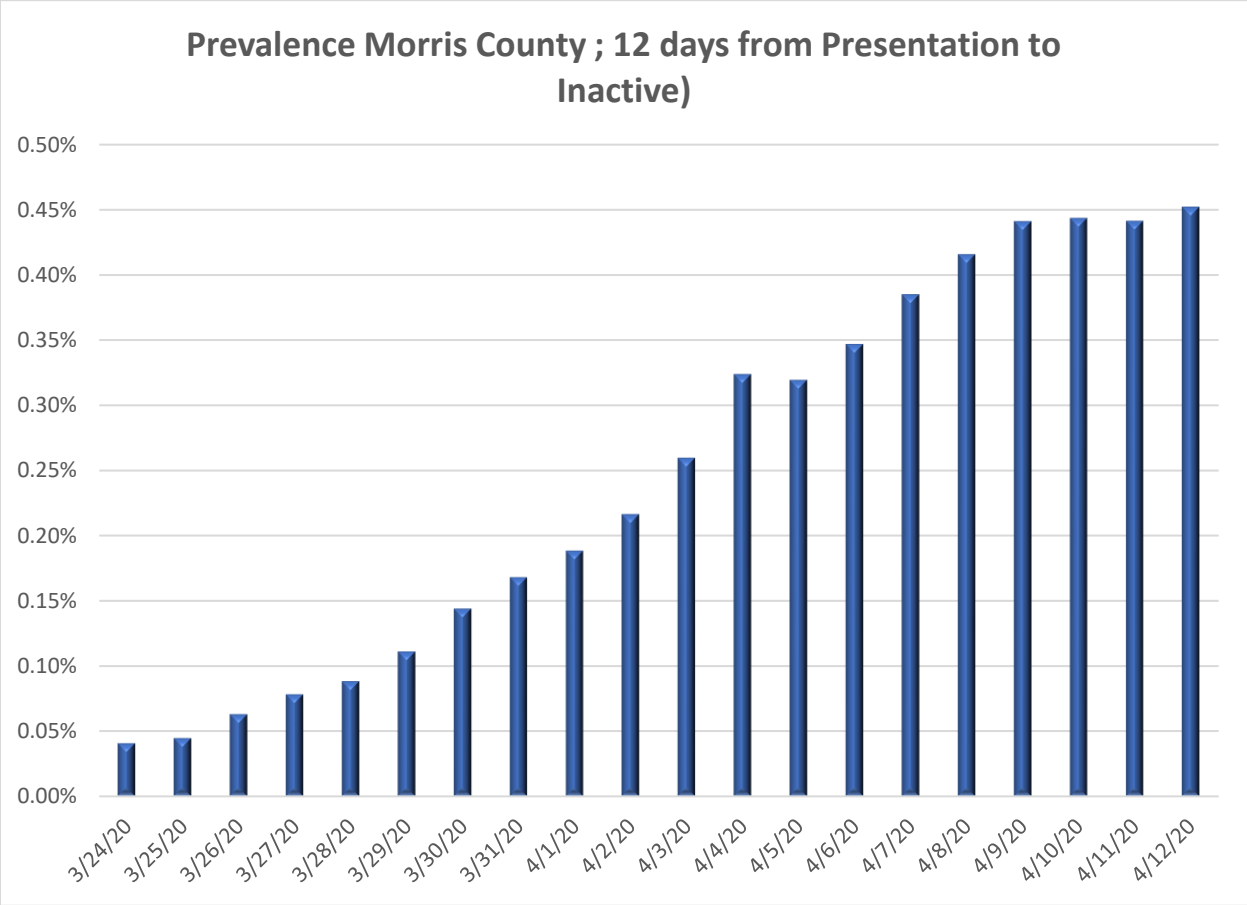
The algorithm then looks as follows:

[15] See Mood and Graybill, Introduction to the Theory of Statistics, 2nd Ed, McGraw Hill, 1963, p. 263


3,000
Counties in US
330,000,000
people
110,000
people per county
500 Tests
per County
per Week
1.5 Million Tests
3,000
Counties in US
Repeat following
week
Set Prevalence to
0.3%
NO
Pass
Test
Threshold
Fail
80%
Immune
Mitigate
Yes
STOP

Namely we have a simple feedback algorithm where each county knows its status as described above. Any county in compliance remains open. Otherwise we enter mitigation, namely stay at home orders as we have been doing. The information must be publicly available and totally transparent. I know it is difficult when the Government is involved but let's try.

Let us take Morris County NJ as an example. Here is the graph of the prevalence. Note that it has leveled off at 0.45%. If this is within the limit then we open the county.



This plan opens up most likely a few thousand counties in a week. If they people wash their hands etc. it can remain open. If not, then they are at home until the number returns to the acceptable limit.

This plan is simple, readily understood like baseball scores, and everyone knows where they are. It also puts the country back to work based upon facts, simple facts. However the plan requires simple and universal and periodic random testing.

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