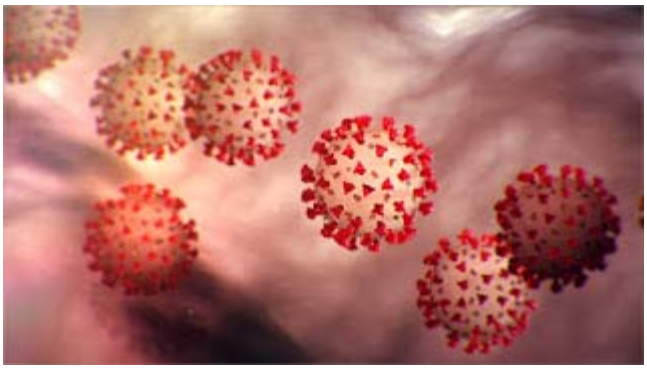

A PRIMER ON COVID-19

ABSTRACT

This is a brief precis regarding the COVID-19 pandemic. The objective is to provide the basis to understand what goes into a model for the progression and spread of the pandemic and why certain measure may be effective.

Terrence McGarty

TGL.173, The Telmarc Group, March 22, 2020

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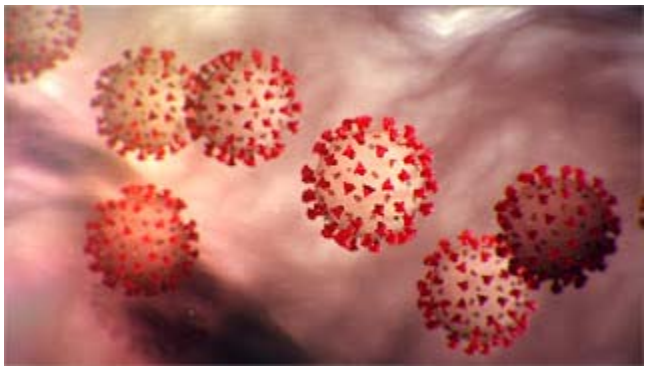
Contents

1 INTRODUCTION

Viruses have been with humans from the beginning. Viruses plague both animals and plants, they even get into most other microorganisms. They are small and we have been able to deal with them only slowly. AIDS is a viral driven disease as are many of the other childhood disorders. Rabies is an animal borne viral infection which is vectored mostly by bats in the US. COVID-19 is the basis pandemic. The initial vector of this pandemic is unknown as of the time of this writing.

Why prepare a document such as this? Simply because it is essential to assemble source based facts as best known as of this writing in an organized manner so as to holistically understand the nature of the pandemic. One suspects it will be studied for several generation to come as to how it was handled and the human interactions related thereto. Moreover, the intent is to depict a simplistic but reasonable model for the spread of the virus and to do so in such a manner that one can grasp the underlying elements. The problem is that most of the current political players and almost all of the Press are grossly ignorant of what they are and thus actions taken must be seriously questioned. The bottom line unfortunately is that without a therapeutic and without an immunization the only way to tamper the pandemic is isolation. Even more unfortunate is that it can start again with a newly infected case.

First, one must have an understanding of the various viruses floating about in our daily lives. In the past century humanity has had a multiplicity of interactions with viruses. The 1918 influenza virus was clearly a significant one. The HIV virus was another. In both cases the treatments were lacking and the very nature of the is ease unknown. The Wuhan virus appears to be from a well-known family but it seems to have some interesting and more pathogenic mechanisms in it which allow its spread. We shall examine these in detail later.



Infection by a virus like coronavirus is often through the nasal passages or the eye. At time it may be inhaled via the mouth or transferred via food.

However, if one looks at the masked Chinese one sees glove-less hands and cell phones. The cell phone is the petri dish for corona. The device is swung about, exposed to everything, holds virions, transfers them to hands and from there to eyes. The mask at best prevents expelling outwards. The eyes are unprotected but they are great sites for internal infection.

When in a potentially infectious site, I would focus first on eyes, then hands, then nose, then mouth, then whatever else can be covered. But when looking at pictures from China the eyes and hands are all exposed and most are carrying cell phones. They become walking transmitters of the virus.

As Zhu et al noted mid-January, 2020, in NEJM:

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China. A previously unknown betacoronavirus was discovered through the use of unbiased sequencing in samples from patients with pneumonia. Human airway epithelial cells were used to isolate a novel coronavirus, named 2019-nCoV, which formed a clade within the subgenus sarbecovirus, Orthocoronavirinae subfamily. Different from both MERS-CoV and SARS-CoV, 2019-nCoV is the seventh member of the family of coronaviruses that infect humans. Enhanced surveillance and further investigation are ongoing.

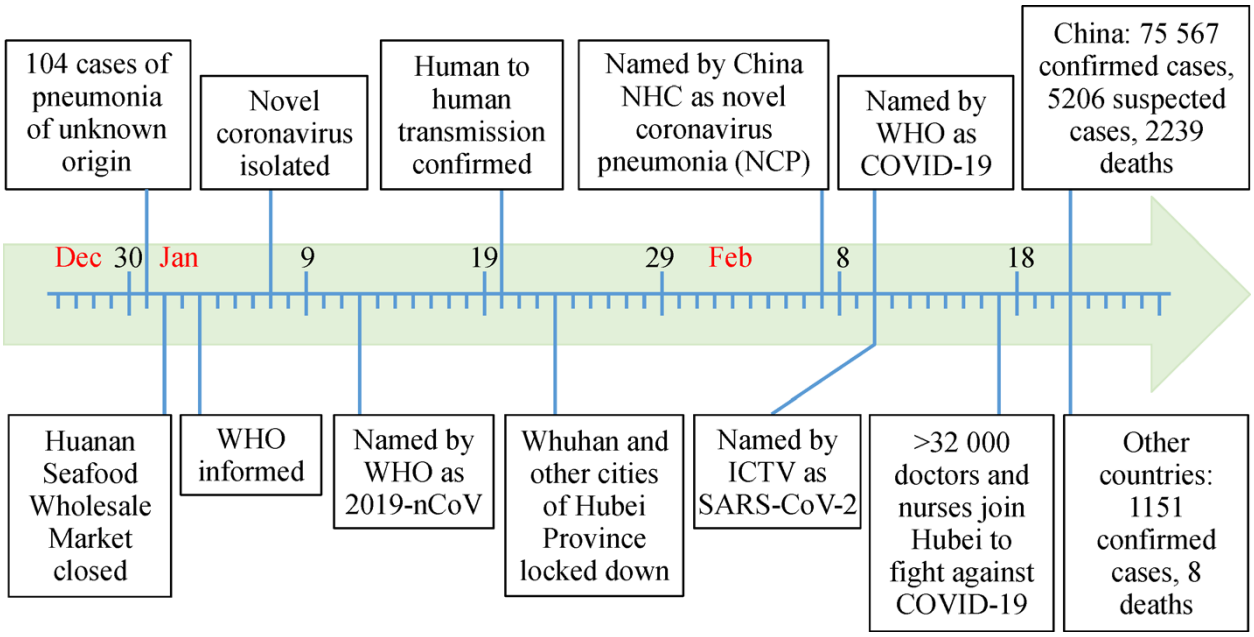
On January 20, 2020, a week before the Chinese announcement, a German group reported in NEJM (see Rothe et al):

The novel coronavirus (2019-nCoV) from Wuhan is currently causing concern in the medical community as the virus is spreading around the world.1 Since its identification in late December 2019, the number of cases from China that have been imported into other countries is on the rise, and the epidemiologic picture is changing on a daily basis. We are reporting a case of 2019-nCoV infection acquired outside of Asia in which transmission appears to have occurred during the incubation period in the index patient

On January 29, 2020, Li et al in NEJM stated:

On the basis of this information, there is evidence that human-to-human transmission has occurred among close contacts since the middle of December 2019. Considerable efforts to reduce transmission will be required to control outbreaks if similar dynamics apply elsewhere. ***Measures to prevent or reduce transmission should be implemented in populations at risk***

From Zhou et al the alleged timeline is:



Thus, the warning for the impending pandemic was clear easily by mid to late January. As we shall note, the details of the putative pandemic were well known, the Chinese had seen it for well more than a month, and outside of China many were left to construct the details for themselves. Our intent is to try to assemble a reasonable first order picture as to what this portends. We will leave it to others to assess the lack of criticality evidenced on the part of the US CDC and its affiliates.

2 VIRAL BASICS

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.

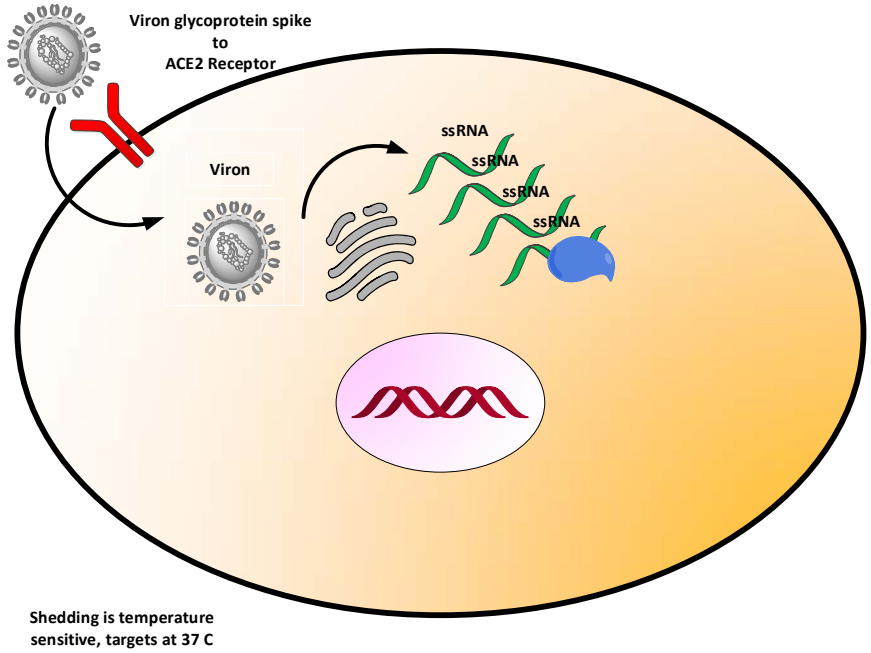
2.2 INFECTION

Infection with the Corona virus is through nasal passages of the nasopharynx. It results from aerosol particles from an infected person or through contact with surfaces infected by similar aerosols.

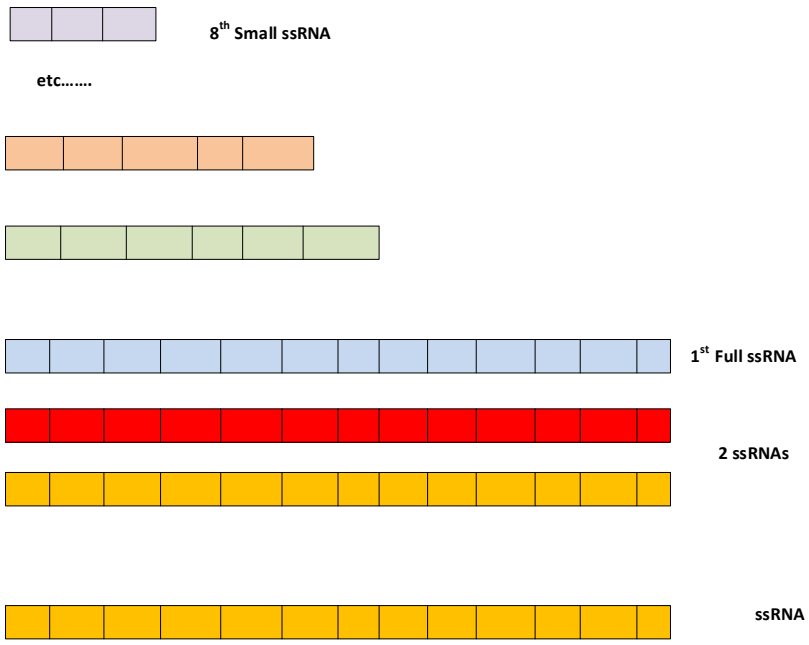
2.3 CORONA SPECIFICS

Let us now examine the specifics of corona. 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.

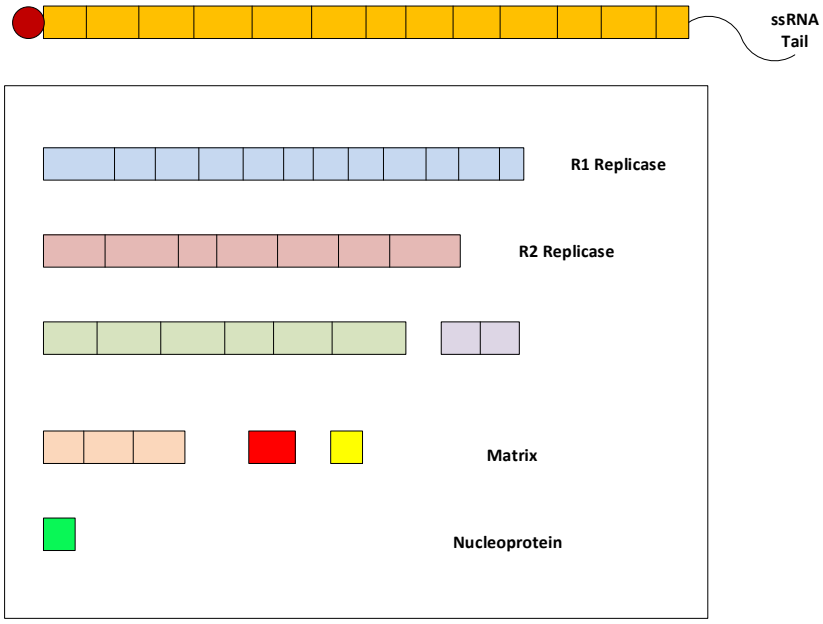
The figure below summarizes this virus. The RNA is about 30,000 base pairs and when translated can produce eight operable regions for the generation of proteins or RNA replication.



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.



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 coro-naviruses; 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 pro-cessed 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).

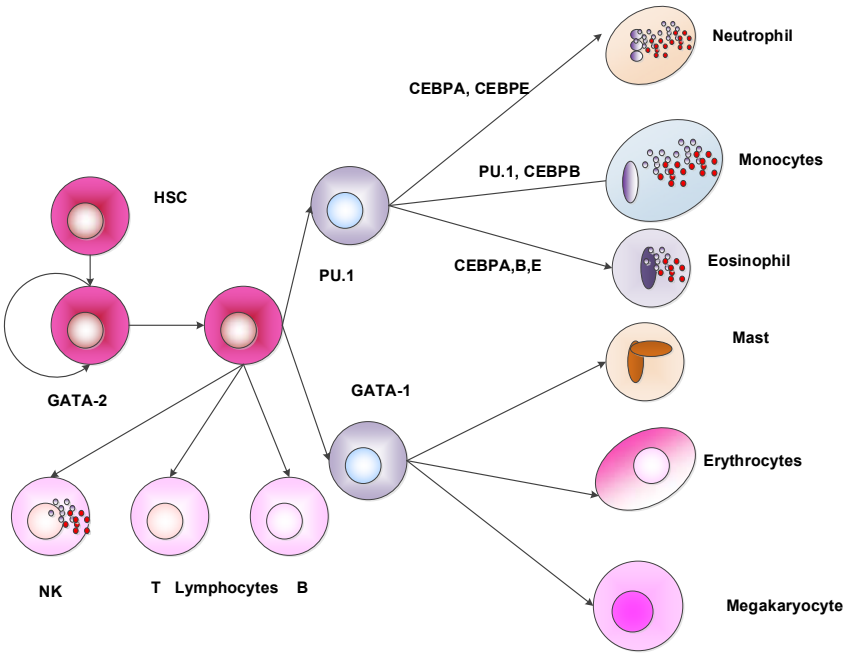
There newly formed RNP interacts with the M protein from the ER, and M interacts with the S and other proteins to form the infectious virus which buds into the Golgi, thereby acquiring a lipid envelope. Envelope proteins are glycosylated in the Golgi. Virions are released by fusion of smooth-walled virion-containing 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 coronavi- ruses and between human viruses. Recombination can allow cor onaviruses to rapidly evolve and adapt to new ecological niches.

3 IMMUNOLOGICAL BASICS

We now provide a brief overview of the immune system. The purpose is to delineate the two stages of immune response; innate and adaptive.

3.1 PRINCIPAL CELLS

First, we present the principal cells of bone origin in the human. They are depicted below, all originating from the hematopoietic stem cell in the bone[1].



There are cells not included above such as the Natural Killer cells and the Dendritic[2] cells which we will also focus on[3].

[1] Mast cell Major effector cell of immediate hypersensitivity (allergic) reactions. Mast cells are derived from the marrow, reside in most tissues adjacent to blood vessels, express a high-affinity Fc receptor for IgE, and contain numerous mediator-filled granules. Antigen-induced cross-linking of IgE bound to the mast cell Fc receptors causes release of their granule contents as well as new synthesis and secretion of other mediators, leading to an immediate hypersensitivity reaction.

[2] Dendritic cells (DCs) Bone marrow–derived cells found in epithelial and lymphoid tissues that are morphologically characterized by thin, membranous projections. Many subsets of DCs exist with diverse functions. Activated (mature) DCs function as APCs for naive T lymphocytes and are important for initiation of adaptive immune responses to protein antigen. Immature (resting) DCs are important for induction of tolerance to self antigens.

[3] Monocyte Type of bone marrow–derived circulating blood cell that is the precursor of tissue macrophages. Monocytes are actively recruited into inflammatory sites, where they differentiate into macrophages.

3.2 INFECTION

Infection are of various types and the immune system attacks the invaders in various manners. We summarize some of these below. First for organisms and the attack by the innate immune system we have:

Organisms	Representative.	Phagocytosis.	Neutrophils	Complement[4]	NK Cells[5].
Viruses (intracellular. cytoplasmic)	Influenza virus				
	Mumps virus				
	Morbillivirus (measles, rubeola)				
	Rhinovirus				
Bacteria (intracellular)	*Listeria monocytogenes*				
	Legionella spp.				
	Mycobacteria				
	Rickettsia				
Bacteria (extracellular)	*Staphylococcus* spp.				
	Streptococcus spp.				
	Neisseria spp.				
	Salmonella typhi				
Protozoa (intracellular)	*Plasmodium malariae*				
	L. donovani				
Protozoa (extracellular)	*Entamoeba histolytica*				
	Giardia lamblie				
Fungi (extracellular)	*Candida* spp.				
	Histoplasma				
	Cryptococcus				

Note how the innate system responds to viruses. It is a combination of phagocytosis and the attack by the Natural Killer cells. This is the first wave of defense. In a sense it is a brutal attack on the invaded cells. As we shall note, the cells invaded have internal endosomes with surface markers call toll like receptors. The ssRNA activates them and this starts the complement system and the production of various responses including cytokines. Thus with a rhinovirus the nose

[4] Complement System of serum and cell surface proteins that interact with one another and with other molecules of the immune system to generate important effectors of innate and adaptive immune responses. The classical, alternative, and lectin pathways of the complement system are activated by antigen-antibody complexes, microbial surfaces, and plasma lectins binding to microbes, respectively, and consist of a cascade of proteolytic enzymes that generate inflammatory mediators and opsonins. All three pathways lead to the formation of a common terminal cell lytic complex that is inserted in cell membranes.

[5] Natural killer (NK) cells Subset of bone marrow–derived lymphocytes, distinct from B or T cells, which function in innate immune responses to kill microbe-infected cells by direct lytic mechanisms and by secreting IFN-γ. NK cells do not express clonally distributed antigen receptors like Ig receptors or TCRs, and their activation is regulated by a combination of cell surface stimulatory and inhibitory receptors, the latter recognizing self MHC molecules.

starts to run and the throat gets sore. With COVID-19 this attack is slower and occurs in the lung tissue which we shall show later.

The adaptive system interacts as shown below. Again we will focus on the viral responses effected principally by the antibodies, Ab, and the cytotoxic T cell, CTL[6].

	gM, IgG, IgA						
Organisms	**Complement Activation**	**Opsonization**	**ADCC**	**Neutralizing Antibody**	**IgE**	**CTL**	**DTH**
Viruses							
Bacteria intracellular							
Bacteria extracellular							
Protozoa intracellular							
Protozoa extracellular							
Fungi							
Flatworms							
Roundworms							

The key observation from above is that the adaptive system will respond by generating antibodies, Ab, which will persist and target the infected cells. Then the cytotoxic T lymphocytes will recognize the targeted cells and destroy them and the remaining virus. It is important to note that the adaptive system takes time. If, as in the common cold, the innate system kills off the cells before the Ab can be generated, then there will be no Ab and no immunity the next time a person is exposed.

It will also be critical to understand the extreme specificity of the Abs. If the virus mutates, as they often do, the immunity provided by the generated Abs will no longer be effective.

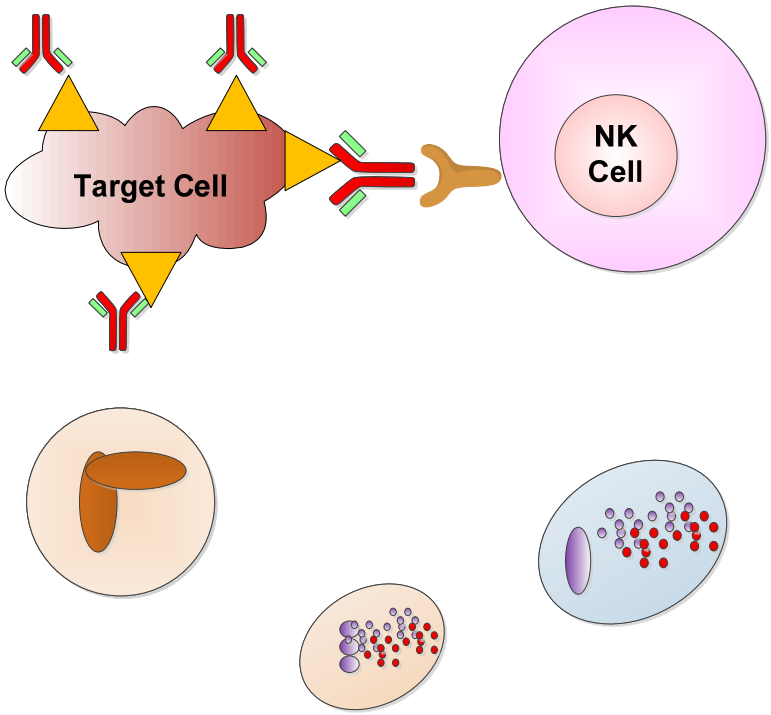
3.3 Innate Immune Response

The innate immune response is the initial response. The two principal types of reactions of the innate immune system are inflammation and antiviral defense. Inflammation consists of the accumulation and activation of leukocytes and plasma proteins at sites of infection or tissue injury. These cells and proteins act together to kill mainly extracellular microbes and to eliminate damaged tissues. Innate immune defense against intracellular viruses is mediated mainly by natural killer (NK) cells, which kill virus-infected cells, and by cytokines called type I interferons, which block viral replication within host cells.

Cells are eliminated via the interaction of phagocytes as well as the Complement system, part of the innate immune system. As we have noted earlier the NK cells can use the Abs as an indicator

[6] Cytotoxic (or cytolytic) T lymphocyte (CTL) Type of T lymphocyte whose major effector function is to recognize and kill host cells infected with viruses or other intracellular microbes. CTLs usually express CD8 and recognize microbial peptides displayed by class I MHC molecules. CTL killing of infected cells involves the release of cytoplasmic granules whose contents include enzymes that initiate apoptosis of the infected cell and proteins that facilitate entry of these enzymes into the target cells.

of targeting. We show this below along with some of the other phagocytes such as macrophages and neutrophils.



The Complement System is what attacks the Target Cell when it is covered with Abs. As Merle et al note:

Complement is a central part of the innate immunity that serves as a first line of defense against foreign and altered host cells. The complement system is composed of plasma proteins produced mainly by the liver or membrane proteins expressed on cell surface. Complement operates in plasma, in tissues, or within cells. Complement proteins collaborate as a cascade to opsonize pathogens and induce a series of inflammatory responses helping immune cells to fight infection and maintain homeostasis.

The complement system can be initiated depending on the context by three distinct pathways – classical (CP), lectin (LP), and alternative (AP), each leading to a common terminal pathway. In a healthy individual, the AP is permanently active at low levels to survey for presence of pathogens.

Healthy host cells are protected against complement attack and are resistant to persistent low-grade activation. The three pathways are activated on the surface of apoptotic cells, which are constantly generated within the body during normal cellular homeostasis This complement activation is tightly regulated to eliminate dying cells without further activation of other innate or adaptive immune components.

Complement is only fully activated in cases of pathogen infection. During an infection, complement leads to inflammation, opsonization, phagocytosis, and destruction of the pathogen

and ultimately results in activation of the adaptive immune response. Both inefficient and over stimulation of complement can be detrimental for the host and are associated with increased susceptibility to infections or non-infectious diseases, including autoimmunity, chronic inflammation, thrombotic microangiopathy, graft rejection, and cancer.

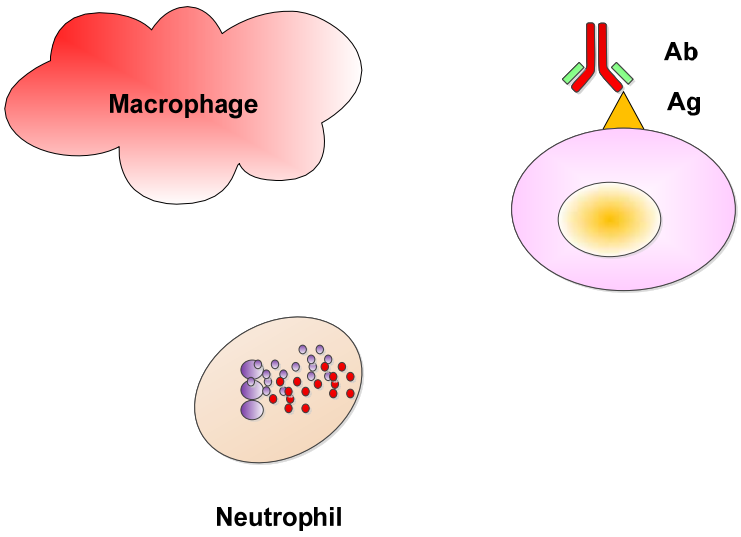
The antibody-dependent cell-mediated cytotoxicity can be described as follows. The “tagging" of an invasive organism can attract phagocytic cells and other cytolytic cells. FcRs on NK cells (FcyRIII) and eosinophils (FcyRI, FcbRI, and FcotRI) are IgG-, IgE-, and IgA-specific. The bound cells may be bacteria, protozoa, or even some parasitic worms. As with phagocytic cells, these receptors allow the cytolytic cells to bind invasive organisms “tagged" with IgG, IgE, or IgA antibodies, but rather than engulfment, they use cytolytic mechanisms to kill the “tagged" organisms. This process is termed ***antibody-dependent cell-mediated cytotoxicity*** (ADCC). The cytolytic mechanisms used by NK cells and eosinophils in ADCC are similar to some of those used by cytotoxic T cells to kill the intruder.

The Complement activation can proceed as follows. The classical pathway of complement is activated by conformational changes that occur in the Fc portion of antibodies upon epitope binding. Antibodies (usually of the IgM and IgG isotypes) facilitate the sequential binding of the C1, C4, C2, and C3 components of the complement system. Like the alternative and mannan-binding lectin pathways, completion of the classical complement pathway results in the production of C3b, a “sticky"

As noted by Merle et al (II):

The main role of complement in pathogen elimination is indirect, namely, the deposition of complement fragments on the surface of pathogen targets, so-called opsonization that allows their recognition, ingestion, and destruction by phagocytic cells, neutrophils, monocytes, and macrophages. Both IgG antibodies and C3 fragments are the classical opsonins. But complement opsonization, resulting from the direct activation of the AP on pathogens surface allows their elimination by phagocytes before the mounting of a response and the appearance of antibodies.

We demonstrate some of these effects below.



Thus, the process is somewhat simple:

1. Target cells produce an antigen

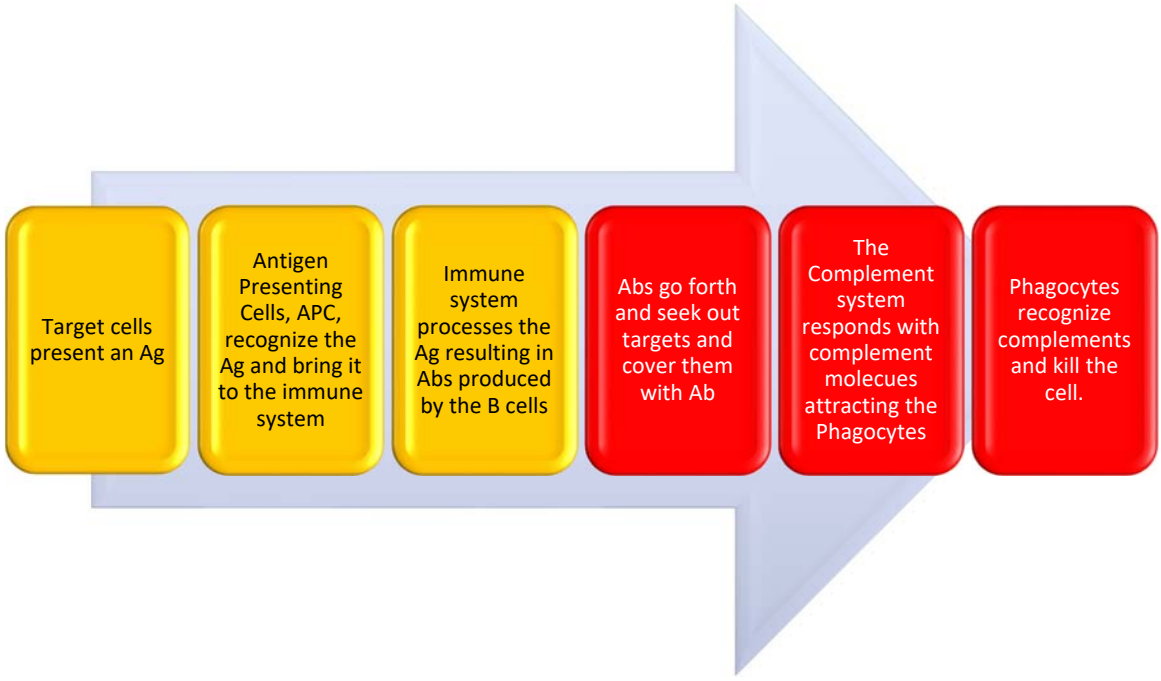
2. Antigen presenting cells see the Ag and carry it to the adaptive system.

3. B cells are activated by the antigen and they produce Abs targeted to the Ag

4. The Abs go out and cover the target cells

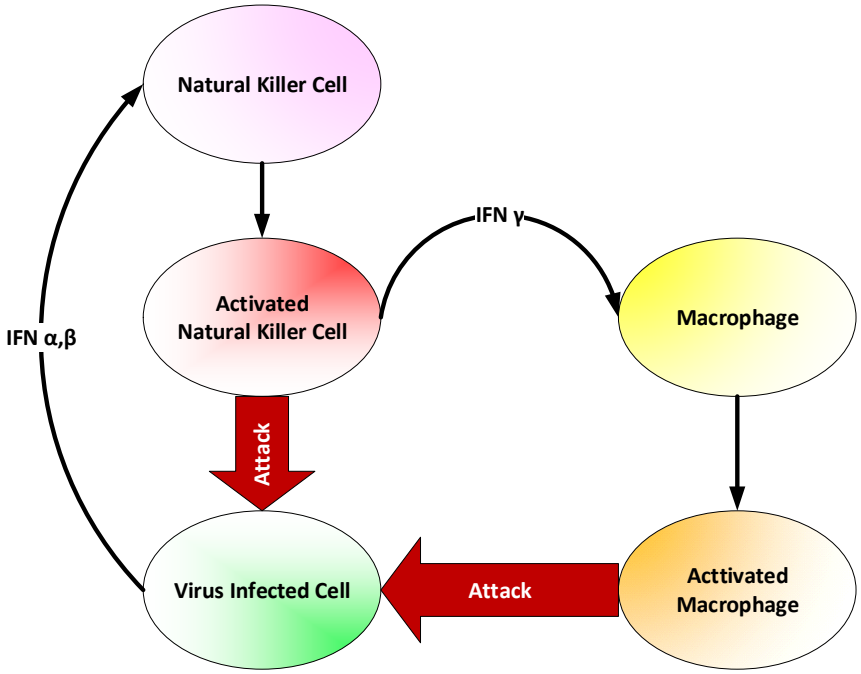
5. The Abs attract the Complement system proteins which cover the target as well

6. The phagocytes are brought out to kill off the complement targeted cells.



Thus, we see this as an orchestrated process between the elements of the immune system all playing parts in seeking out and destroying invaders. Protection of "self" is a key part of this rather aggressive process and that we leave to the well-established literature.

A simplified summary of the innate system is depicted below.

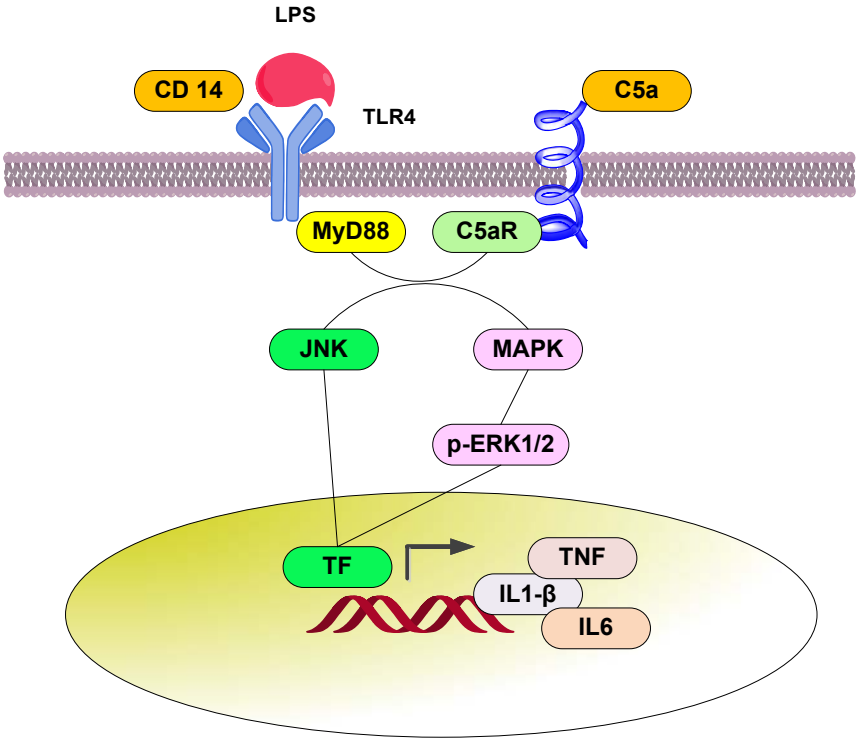


3.4 The Toll Like Receptor Line

We discussed the Toll Like receptors earlier but they also play a role in Ab action and it is worth a brief discussion. Among the most important transcription factors activated by TLR signals are nuclear factor κB (NF-κB), which promotes expression of various cytokines and endothelial adhesion molecules, and interferon regulatory factors (IRFs), which stimulate production of the antiviral cytokines, type I interferons. As Merle et al discuss when examine the Complement system they state:

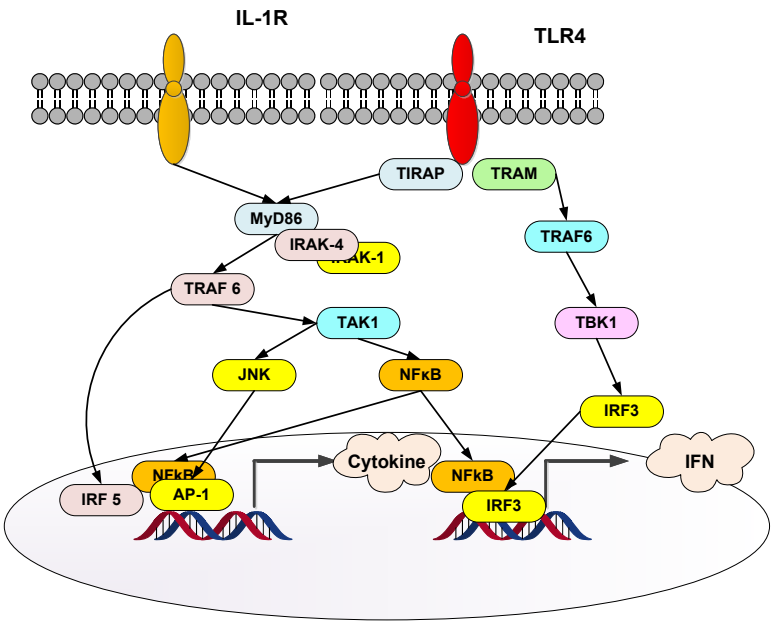
C3a and C5a are able to induce potent inflammatory pathways via their receptors C3aR and C5aR. The implication of intermediates such as NF-kB, MAPK, and c-Jun N-terminal kinase (JNK) in their transduction pathways suggests a potential crosstalk with other pathways, such as those of TLRs. Indeed, complement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



As Lund et al have noted:

Viral infection of mammalian host results in the activation of innate immune responses. Toll-like receptors (TLRs) have been shown to mediate the recognition of many types of pathogens, including viruses. The genomes of viruses possess unique characteristics that are not found in mammalian genomes, such as high CpG content and double-stranded RNA. These genomic nucleic acids serve as molecular signatures associated with viral infections. Here we show that TLR7 recognizes the single-stranded RNA viruses, vesicular stomatitis virus and influenza virus.

The recognition of these viruses by plasmacytoid dendritic cells and B cells through TLR7 results in their activation of costimulatory molecules and production of cytokines. Moreover, this recognition required intact endocytic pathways. Mice deficient in either the TLR7 or the TLR adaptor protein MyD88 demonstrated reduced responses to in vivo infection with vesicular stomatitis virus. These results demonstrate microbial ligand recognition by TLR7 and provide insights into the pathways used by the innate immune cells in the recognition of viral pathogens.

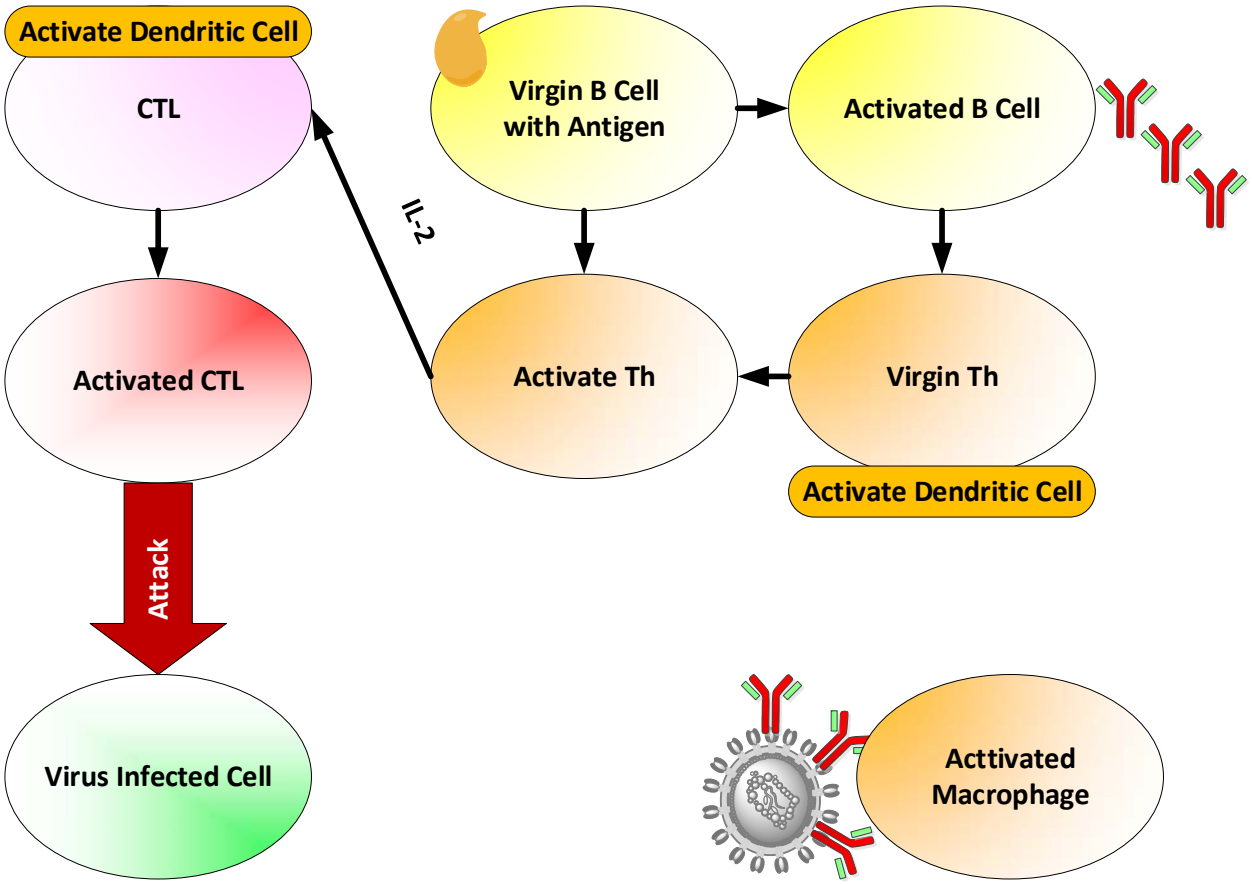
3.5 Adaptive Immune Response

The adaptive immune response if the delayed response. It function by means of antibodies, Ab, proteins produced based upon a unique recognition of the invader ant it function by means of attacking the invading cell before it can do harm.

It typically takes several days to generate the Ab and even more to have a large enough supply to suppress the invasion. Immunization is a relatively harmless mechanism which evokes the development of the Ab for a specific pathogen. The good news is that this system works well, the

bad news is that it takes time to work and is very specific. Thus while the system is working in a human in response to a pathogen the human may continue to spread the pathogen to others, knowingly or unknowingly.

The following Figure is a simplified description of the adaptive system.



Note the following:

1. In order for it to start it must get initiated by antigens

2. The antigens are elements left over from the innate system destruction, namely the viral particles.

3.Then the B cells can be activated to produce antibodies.

4. The Ab then can attack free virus elements by coating them and having them destroyed by activated macrophages.

5. The activated B cells then activate T helper cells which in turn activate Cytotoxic T cells, CTL, which attack the infected cells.

6. The Ab remain in the system after the viral elements have been resolved so that the next attack by a virus will be immediately recognized and neutralized before the innate kicks in.

This is a highly simplified description but it lays out the key factors of temporal effects.

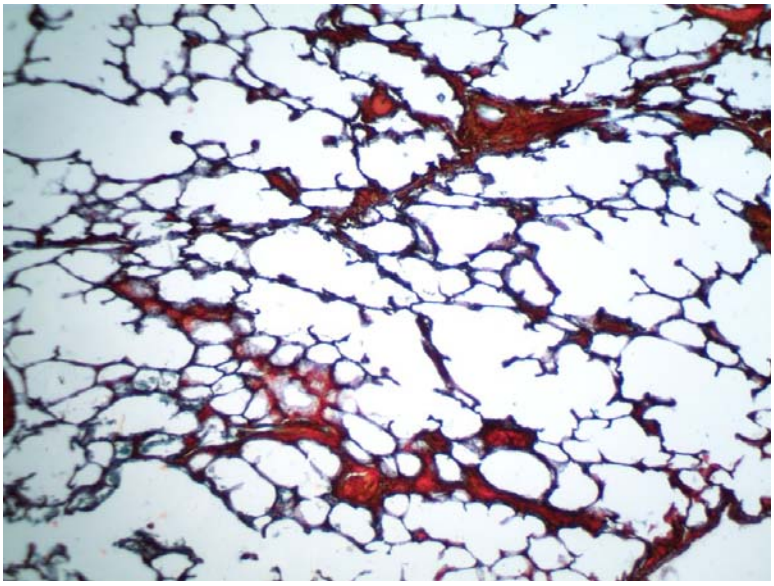
4 COVID-19 BASICS

COVID-19 is a corona virus sourced from Wuhan, China. The actual transfer vector is unknown. It has been hypothesized as a bat or some other mammal but it could equally have been a result of studies in the Chinese Government Virus Studies center in that city. As we shall note, the Center has been actively modifying these class of viruses as evidenced by publications emanating therefrom. At this point, lacking an open environment for information gathering, all one can do is speculate. In this section we examine at a high level what is known regarding this virus.

4.1 LUNG PATHOLOGY

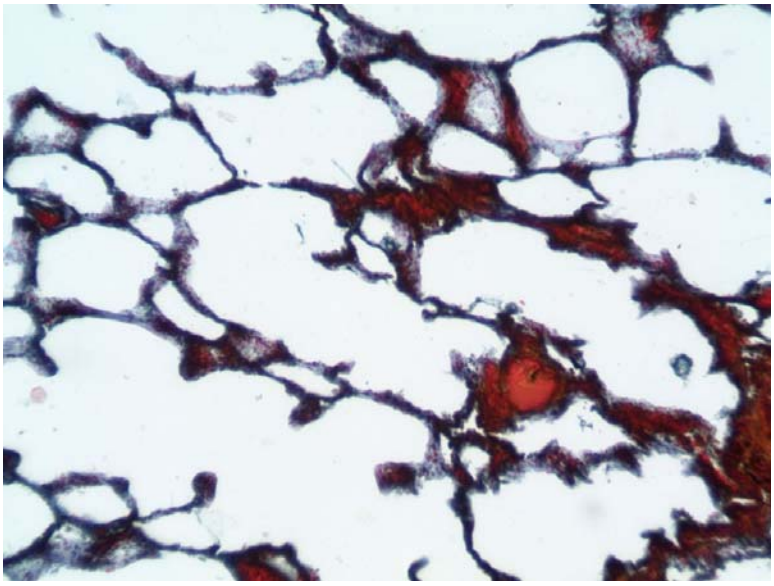
We begin with a brief discussion of the lung and its histology in a normal and inflamed state. This is useful since it clearly indicates what the virus does, namely reduce lung surface area for CO2 elimination and O2 intake. The lung is predominantly an organ filled with air and infiltrated with veins and arteries. It transfers CO2 outward and collects O2 inwards. A normal operating lung has massive amounts of transfer area for this process to occur.

First below is a normal lung tissue at low magnification[7]. There are almost all open spaces. This provides the maximum amount of transfer area for the loss of CO2 and gain of O2.

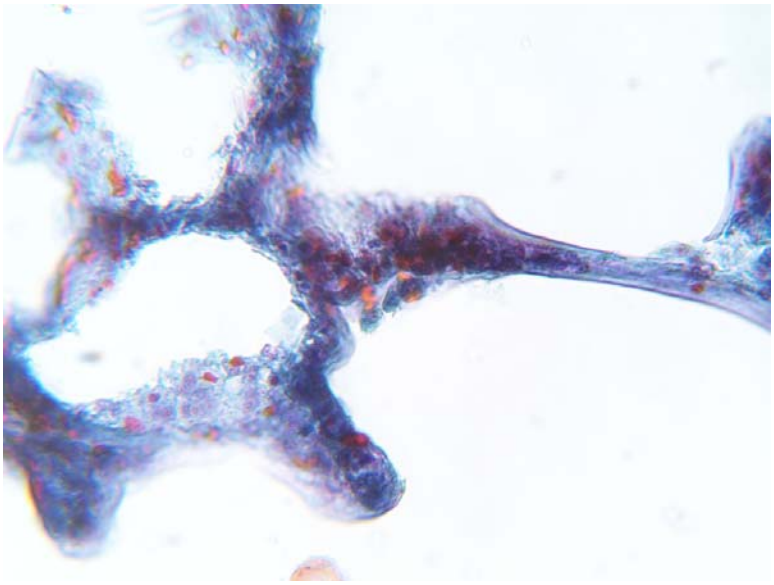


Upon higher magnification we obtain:

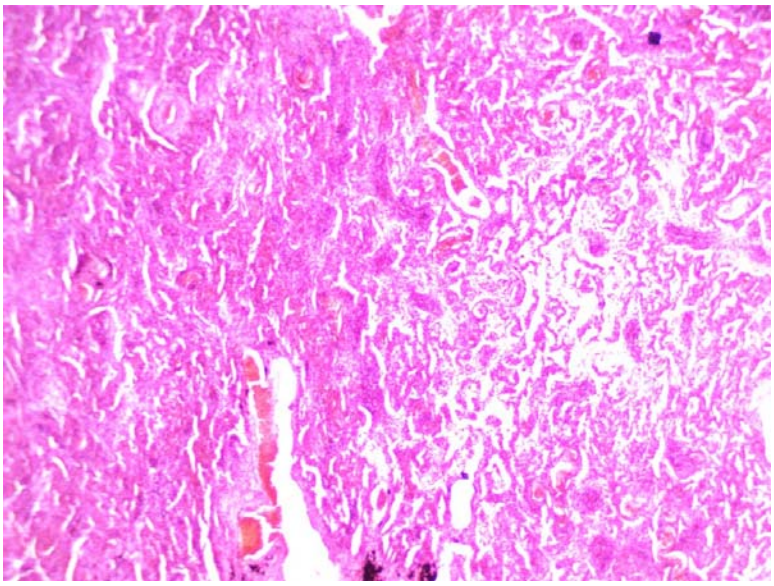
[7] These slides are from the author's collection based upon anonymized patient records.



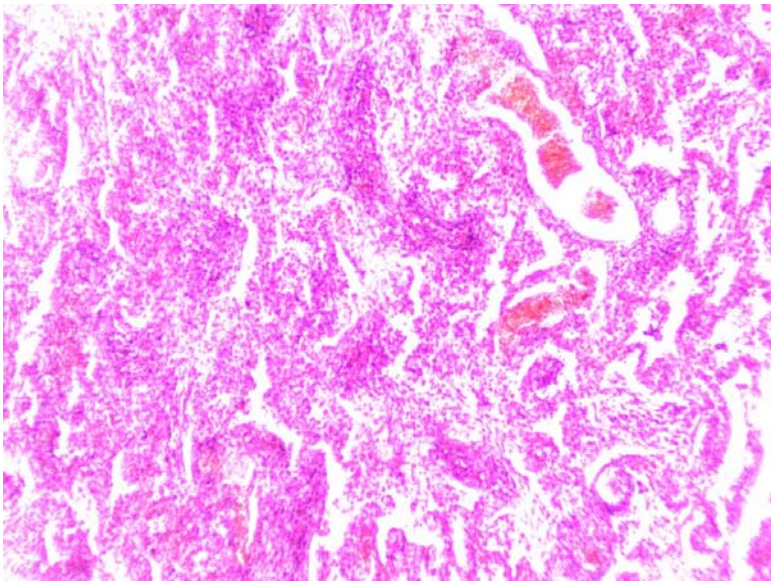
Finally, with the highest magnification we can obtain:



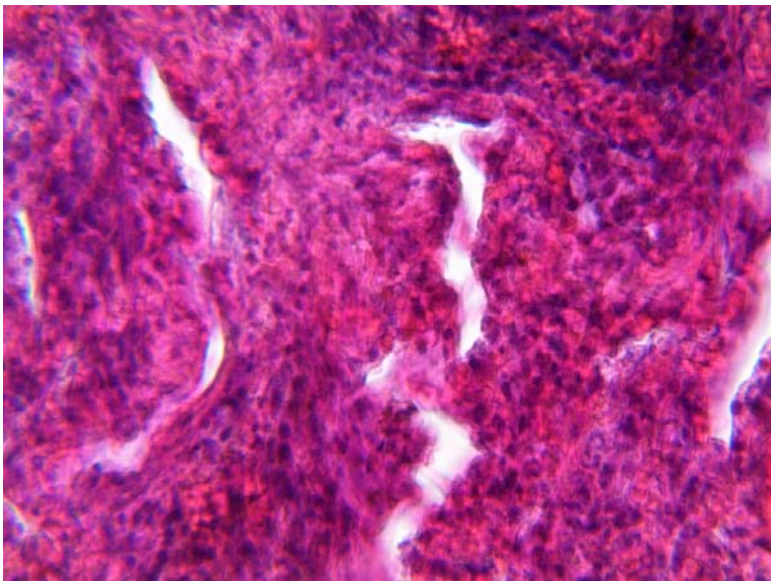
The above clearly shows a clear and health alveoli. That is what we would expect in a health lung. Now consider an inflamed lung with influenza as below:



Note the massive loss of open alveoli. The surface area has almost been lost with the inclusion of inflamed cells. Closer examination is shown below.

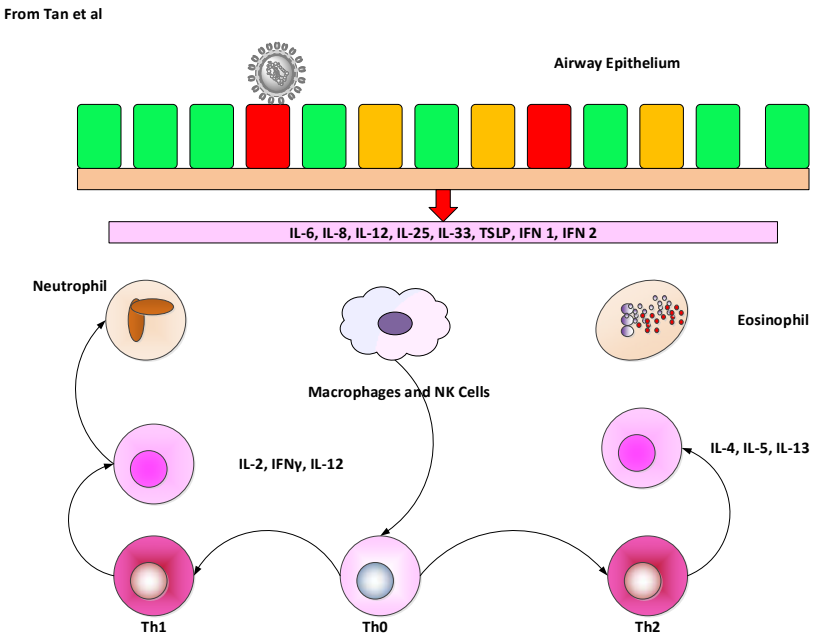


Finally at highest magnification of the inflamed lung we obtain below:



This as seen above is what we must avoid in the viral infections we discuss herein.

The mechanism for this can be seen in Tan et al:



where they note:

Current understanding of viral induced exacerbation of chronic airway inflammatory diseases. Upon virus infection in the airway, antiviral state will be activated to clear the invading pathogen from the airway. Immune response and injury factors released from the infected epithelium normally would induce a rapid type 1 immunity that facilitates viral clearance. However, in the inflamed airway, the cytokines and chemokines released instead augmented the inflammation present in the chronically inflamed airway, strengthening the neutrophilic

infiltration in COPD airway, and eosinophilic infiltration in the asthmatic airway. The effect is also further compounded by the participation of Th1 and ILC1 cells in the COPD airway; and Th2 and ILC2 cells in the asthmatic airway

They then continue:

*On the other end of the spectrum, viruses that induce strong type 1 inflammation and cell death such as IFV and certain CoV (**including the recently emerged COVID-19 virus**), may not cause prolonged inflammation due to strong induction of antiviral clearance. These infections, however, cause massive damage and cell death to the epithelial barrier, so much so that areas of the epithelium may be completely absent post infection. Factors such as RANTES and CXCL10, which recruit immune cells to induce apoptosis, are strongly induced from IFV infected epithelium.*

Additionally, necroptotic factors such as RIP3 further compounds the cell deaths in IFV infected epithelium. The massive cell death induced may result in worsening of the acute exacerbation due to the release of their cellular content into the airway, further evoking an inflammatory response in the airway. Moreover, the destruction of the epithelial barrier may cause further contact with other pathogens and allergens in the airway which may then prolong exacerbations or results in new exacerbations.

Epithelial destruction may also promote further epithelial remodeling during its regeneration as viral infection induces the expression of remodeling genes such as MMPs and growth factors. Infections that cause massive destruction of the epithelium, such as IFV, usually result in severe acute exacerbations with non-classical symptoms of chronic airway inflammatory diseases. Fortunately, annual vaccines are available to prevent IFV infections; and it is recommended that patients with chronic airway inflammatory disease receive their annual influenza vaccination as the best means to prevent severe IFV induced exacerbation.

As Wu et al noted:

As reported by Huang et al,3 patients with COVID-19 present primarily with fever, myalgia or fatigue, and dry cough. Although most patients are thought to have a favorable prognosis, older patients and those with chronic underlying conditions may have worse outcomes. Patients with severe illness may develop dyspnea and hypoxemia within 1 week after onset of the disease, which may quickly progress to acute respiratory distress syndrome (ARDS) or end-organ failure.4 Certain epidemiological features and clinical characteristics of COVID-19 have been previously reported.

However, these studies were based on relatively small sample sizes, and risk factors leading to poor clinical outcomes have not been well delineated. In this study, we report the clinical characteristics and factors associated with developing ARDS after hospital admission and progression from ARDS to death in patients with COVID-19 pneumonia from a single hospital in Wuhan, China …

In this cohort study, we reported the clinical characteristics and risk factors associated with clinical outcomes in patients with COVID-19 pneumonia who developed ARDS after admission, as well as those who progressed from ARDS to death. Patients who received methylprednisolone treatment were much more likely to develop ARDS likely owing to confounding by indication; specifically, sicker patients were more likely to be given methylprednisolone. However, administration of methylprednisolone appeared to reduce the risk of death in patients with ARDS.

These findings suggest that for patients with COVID-19 pneumonia, methylprednisolone treatment may be beneficial for those who have developed ARDS on disease progression. However, these results should be interpreted with caution owing to potential bias and residual confounding in this observational study with a small sample size. Doubleblinded randomized clinical trials should be conducted to validate these results.

Guan et al had depicted the course of the disease in February in NEJM as follows:

During the initial phase of the Covid-19 outbreak, the diagnosis of the disease was complicated by the diversity in symptoms and imaging findings and in the severity of disease at the time of presentation.

Fever was identified in 43.8% of the patients on presentation but developed in 88.7% after hospitalization. Severe illness occurred in 15.7% of the patients after admission to a hospital. No radiologic abnormalities were noted on initial presentation in 2.9% of the patients with severe disease and in 17.9% of those with nonsevere disease.

Despite the number of deaths associated with Covid-19, ***SARS-CoV-2 appears to have a lower case fatality rate than either SARS-CoV or Middle East respiratory syndrome–related coronavirus (MERS-CoV)****. Compromised respiratory status on admission (the primary driver of disease severity) was associated with worse outcomes.*

Approximately 2% of the patients had a history of direct contact with wildlife, whereas more than three quarters were either residents of Wuhan, had visited the city, or had contact with city residents. These findings echo the latest reports, including the outbreak of a family cluster,4 transmission from an asymptomatic patient,6 and the three-phase outbreak patterns.8 Our study cannot preclude the presence of patients who have been termed "super-spreaders."

Conventional routes of transmission of SARSCoV, MERS-CoV, and highly pathogenic influenza consist of respiratory droplets and direct contact, mechanisms that probably occur with SARS-CoV-2 as well. Because SARS-CoV-2 can be detected in the gastrointestinal tract, saliva, and urine, these routes of potential transmission need to be investigated21 (Tables S1 and S2). The term Covid-19 has been applied to patients who have laboratory-confirmed symptomatic cases without apparent radiologic manifestations.

A better understanding of the spectrum of the disease is needed, since in 8.9% of the patients, SARS-CoV-2 infection was detected before the development of viral pneumonia or viral

pneumonia did not develop. In concert with recent studies,1,8,12 we found that the clinical characteristics of Covid-19 mimic those of SARS-CoV.

Fever and cough were the dominant symptoms and gastrointestinal symptoms were uncommon, which suggests a difference in viral tropism as compared with SARS-CoV, MERS-CoV, and seasonal influenza.

***The absence of fever in Covid-19 is more frequent than in SARS-CoV (1%) and MERS-CoV infection (2%)**, **so afebrile patients may be missed if the surveillance case definition focuses on fever detection**. Lymphocytopenia was common and, in some cases, severe, a finding that was consistent with the results of two recent reports.*

We found a lower case fatality rate (1.4%) than the rate that was recently reportedly, probably because of the difference in sample sizes and case inclusion criteria. Our findings were more similar to the national official statistics, which showed a rate of death of 3.2% among 51,857 cases of Covid-19 as of February 16, 2020.

Since patients who were mildly ill and who did not seek medical attention were not included in our study, the case fatality rate in a real-world scenario might be even lower. Early isolation, early diagnosis, and early management might have collectively contributed to the reduction in mortality in Guangdong.

4.2 Prior Corona Viruses

As Wang et al have noted:

Six coronaviruses are known to infect humans and cause respiratory disease, including human coronavirus (HCoV) 229E, OC43, severe acute respiratory syndrome CoV (SARS-CoV), NL63, HKU1 and Middle East respiratory syndrome CoV (MERS-CoV).

SARS-CoV and MERS-CoV are highly pathogenic coronaviruses that caused severe and fatal respiratory infections in humans. The SARSCoV pandemic infected over 8000 people worldwide. As of 9 September 2019, 2458 MERS cases with 848 deaths (34.5% mortality) were reported to World Health Organization (WHO). HCoV-229E, OC43, NL63 and HKU1 are endemic in humans and mainly cause mild respiratory infections worldwide [8,9]. HCoV-NL63 has been prevalent worldwide for many years. The majority of HCoV-NL63 infections in human are mild, although occasionally NL63 causes pneumonia or central nervous system diseases in susceptible individuals including young children, elderly and immunosuppressed patients. HCoVNL63 primarily infects upper respiratory tract and most of HCoV-NL63 infections are acquired during childhood. Neutralizing activity directed against HCoV-NL63 is common in sera from adults and rarely in infant's serum.

During 2009 and 2016, HCoVNL63 accounted for about 0.5% (60/11399) of all acute respiratory tract infections in hospitalized pediatric patients in Guangzhou, China, most of these cases associated with HCoV-NL63 were considered to be evidence of endemic infection and no outbreaks were reported. Here, we identified a cluster of 23 hospitalized pediatric patients with

severe lower respiratory tract infection caused by two subgenotypes (C3 and B) of HCoVNL63, and half of the patients were caused by a new subgenotype C3 which was first reported here. A unique mutation (I507 L) in receptor-binding domain (RBD) was detected in the new subgenotype of HCoV-NL63 associated with increased viral entry into host cells indicating that HCoV-NL63 was undergoing continuous mutation which potentially could enhance HCoV-NL63 virulence and promote transmission.

This study showed that HCoV-NL63 had the potential to cause epidemics in humans and it may be a more important human pathogen than is commonly believed. Efforts should be paid to monitor genetic changes in HCoV-NL63 genome and also its pathogenicity and prevalence in the human population

4.3 A Wuhan Virus Construct

In July 2016 the Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China published a paper discussing the work they did in modifying a SARS like corona virus. The paper appears on Semantic Scholar.

The authors state:

Bats harbor severe acute respiratory syndrome (SARS)-like coronaviruses (SL-CoVs) from which the causative agent of the 2002-2003 SARS pandemic is thought to have originated. However, despite the fact that a large number of genetically diverse SL-CoV sequences have been detected in bats, only two strains (named WIV1 and WIV16) have been successfully cultured in vitro. These two strains differ from SARS-CoV only in containing an extra open reading frame (ORF) (named ORFX), between ORF6 and ORF7, which has no homology to any known protein sequences.

In this study, ***we constructed a full-length cDNA clone of SL-CoV WIV1 (rWIV1), an ORFX deletion mutant (rWIV1-X),*** *and a green fluorescent protein (GFP)-expressing mutant (rWIV1-GFP-X).*

Northern blotting and fluorescence microscopy indicate that ORFX was expressed during WIV1 infection. A virus infection assay showed that rWIV1-X replicated as efficiently as rWIV1 in Vero E6, Calu-3, and HeLa-hACE2 cells.

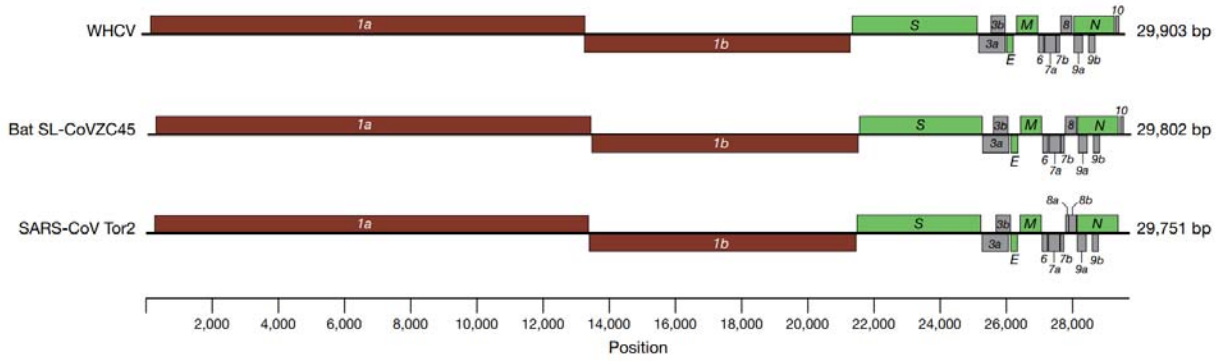
Further study showed that ORFX could inhibit interferon production and activate NF- B. Our results demonstrate for the first time that the unique ORFX in the WIV1 strain is a functional gene involving modulation of the host immune response but is not essential for in vitro viral replication.

One should read through this carefully and cautiously. The mapping of the bases of this virus map well onto what is currently spreading worldwide. There is no claim other than that of coincidence. The paper is worth the read. I want to thank colleagues in California for the reference.

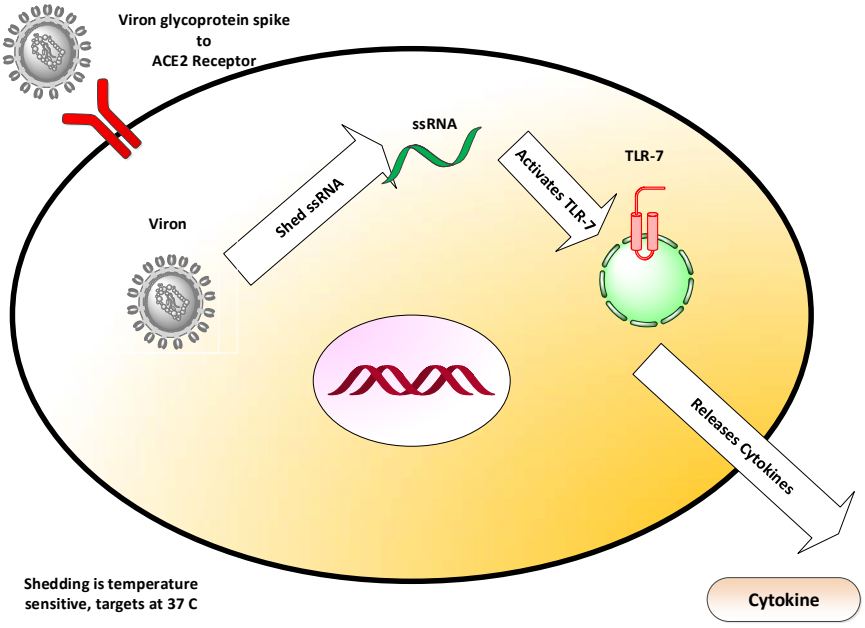
4.4 COVID-19 SPECIFICS

Now we can consider some COVID-19 specifics. The Figure below shows the process of viral infection on a cell in the lung. The cell has a receptor, ACE2, which facilitates the binding of the virion. Then it enters the cell shedding its coat, setting loose the RNA in the virion. This RNA then gets processed and replicated, then rebound in a shell and sent outwards. At the same time the TLR-7 in an endosome releases a mass of cytokines which in a sense do much of the damage we have shown previously.

The RNA is 29,903 nucleotides (see Wu et al). The specifics of this is from Wu et al as shown below:



In the above Wu et al refer to COVID-19 as WHCV.



We can now discuss further details of this process. It is important to note the importance of ACE2. 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 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).

This may be a putative target for a therapeutic.

4.5 Entry and Activation

The entry of the COVID virus has been demonstrated by Sungnak et al as follows:

The SARS-CoV-2 coronavirus, the etiologic agent responsible for COVID-19 coronavirus disease, is a global threat. To better understand viral tropism, we assessed the RNA expression of the coronavirus receptor, ACE2, as well as the viral S protein primingprotease TMPRSS2 thought to govern viral entry in single-cell RNA-sequencing (scRNAseq) datasets from healthy individuals generated by the Human Cell Atlas consortium. We found that ACE2, as well as the protease TMPRSS2, are differentially expressed in respiratory and gut epithelial cells. In-depth analysis of epithelial cells in the respiratory tree reveals that nasal epithelial cells, specifically goblet/secretory cells and ciliated cells, display the highest ACE2 expression of all the epithelial cells analyzed. The skewed expression of viral receptors/entry-associated proteins towards the upper airway may be correlated with enhanced transmissivity.

Finally, we showed that many of the top genes associated with ACE2 airway epithelial expression are innate immune-associated, antiviral genes, highly enriched in the nasal epithelial cells. This association with immune pathways might have clinical implications for the course of infection and viral pathology, and highlights the specific significance of nasal epithelia in viral infection. Our findings underscore the importance of the availability of the Human Cell Atlas as a reference dataset. In this instance, analysis of the compendium of data points to a particularly relevant role for nasal goblet and ciliated cells as early viral targets and potential reservoirs of SARS-CoV-2 infection. This, in turn, serves as a biological framework for dissecting viral transmission and developing clinical strategies for prevention and therapy

Now Holbrook et al have noted the temporal viability of the virus as follows:

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

We evaluated the stability of SARS-CoV-2 and SARS-CoV-1 in aerosols and on various surfaces and estimated their decay rates using a Bayesian regression model. SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) and SARS-CoV-1 Tor2 (AY274119.3) were the strains used. Aerosols (<5 μm) containing SARS-CoV-2 (105.25 50% tissue-culture infectious dose [TCID 50] per milliliter) or SARS-CoV-1 (106.75-7.00 TCID 50 per milliliter) were generated with the use of a three-jet Collison nebulizer and fed into a Goldberg drum to create an aerosolized environment.

The inoculum resulted in cycle-threshold values between 20 and 22, similar to those observed in samples obtained from the upper and lower respiratory tract in humans. Our data consisted of 10 experimental conditions involving two viruses (SARS-CoV-2 and SARS-CoV-1) in five environmental conditions (aerosols, plastic, stainless steel, copper, and cardboard). All experimental measurements are reported as means across three replicates. SARS-CoV-2 remained viable in aerosols throughout the duration of our experiment (3 hours), with a reduction in infectious titer from 103.5 to 102.7 TCID 50 per liter of air.

This reduction was similar to that observed with SARS-CoV-1, from 104.3 to 103.5 TCID 50 per milliliter.

SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard, and viable virus was detected up to 72 hours after application to these surfaces, although the virus titer was greatly reduced … The stability kinetics of SARS-CoV-1 were similar.

On copper, no viable SARS-CoV-2 was measured after 4 hours and no viable SARS-CoV-1 was measured after 8 hours.

On cardboard, no viable SARS-CoV-2 was measured after 24 hours and no viable SARSCoV-1 was measured after 8 hours (Fig. 1A). Both viruses had an exponential decay in virus titer across all experimental conditions, as indicated by a linear decrease in the log10TCID50 per liter of air or milliliter of medium over time.

The half-lives of SARS-CoV-2 and SARS-CoV-1 were similar in aerosols, with median estimates of approximately 1.1 to 1.2 hours and 95% credible intervals of 0.64 to 2.64 for SARS-CoV-2 and 0.78 to 2.43 for SARS-CoV-1.

The half-lives of the two viruses were also similar on copper. On cardboard, the halflife of SARS-CoV-2 was longer than that of SARSCoV-1.

***The longest viability of both viruses was on stainless steel and plastic; the estimated median half-life of SARS-CoV-2 was approximately 5.6 hours on stainless steel and 6.8 hours on plastic**.*

Estimated differences in the halflives of the two viruses were small except for those on cardboard. Individual replicate data were noticeably "noisier" (i.e., there was more variation in the experiment, resulting in a larger standard error) for cardboard than for other surfaces (Fig. S1 through S5), so we advise caution in interpreting this result.

These factors are essential if one is to establish a transmission potential.

5 TESTING FOR VIRUS

One of the greatest misunderstandings in the 2020 Wuhan Plague is the alleged kit for testing. As we shall explain the testing can and should be done extensively and in a before and after manner. Before to see if the virus is present and after to test for antibodies. However the testing is a process not some kit. At most the kit, if we call it that, is a swab in a bottle. The virus kit is really a misnomer and one of the means to tell someone who knows something from someone who does not. Here is the CDC protocol.

It is a cookbook for obtaining the sample and processing the sample. Many Labs can do this, thousands actually. So why the delay. Well we have moved from Federal roadblocks to State roadblocks. So called State Labs are turf protecting.

So let us not kid ourselves, Government is the problem, not the solution. And the kit issue was CDCs way of controlling but no people who have no clue are fixated on kits. It is process and not kits. A process which can be repeated all over the country if States would step aside.

For years I had to look at business which were services or products. Selling a battery is a product business, being a plumber is a service business. I learned that ages ago. The difference is simple. I can shop for batteries from different vendors at different prices. I get this small cylindrical thing which I insert if some electronic device. On the other hand if my sink leaks I get a plumber, the plumber crawls under my sink, twists and turns a bunch of stuff, makes some strange noises, goes out to the truck, comes back with nothing, back under the sink, and then I get the bill. Kind of like a psychiatrist. But the sink now does not leak, can's say as much for a psychiatrist.

Now a process is not a product. The process of testing for the Wuhan Virus is a process. You cannot go out and buy a Wuhan Virus Test, you have to go somewhere, get sampled, it goes to a lab and hopefully comes back negative. Kind of like a plumber.

Now the point. Read this from the Harvard Gazette:

Massachusetts may ultimately need 1.4 million tests for COVID-19 and have to conduct tens of thousands a day, Harvard infectious disease experts said Friday, adding their voices to a nationwide chorus calling to increase dramatically the pace of testing across the country.

It kind of reads as if the test is some product. Like rolls of toilet tissue. It is a process provided as a service. Pick one of the thousands of labs in and around Boston and you will find hundreds which can easily do this test qua process. Throughput is an issue, expendables perhaps, people power, yes, but it is a process. No test kits, now packaging, no shelves to stock.

I just heard some Congressperson state that the VA had on 5 tests. This is why we have a problem; they all seem clueless. If you can do one test you can scale up to whatever, assuming you have some competent people around. Big assumption I know for DC, Albany, Trenton etc.

5.1 TEST FOR WHAT

To test, the first question is to test for what and then to test what? Generally we would test for shedded RNA. Typically one would test the blood. However, in this case testing of nasal secretions is necessary.

As Wolfel et al have noted:

Coronavirus disease 2019 (COVID-19) is an acute respiratory tract infection that emerged in late 20191,2. Initial outbreaks in China involved 13.8% cases with severe-, and 6.1% with critical courses3. This severe presentation corresponds to the usage of a virus receptor that is expressed predominantly in the lung. By causing an early onset of severe symptoms, this same receptor tropism is thought to have determined pathogenicity but also aided the control of severe acute respiratory syndrome (SARS) in 20035.

However, there are reports of COVID-19 cases with mild upper respiratory tract symptoms, suggesting a potential for pre- or oligosymptomatic transmission6-8. There is an urgent need for information on body site - specific virus replication, immunity, and infectivity. Here we provide a detailed virologic analysis of nine cases, providing proof of active virus replication in upper respiratory tract tissues.

Pharyngeal virus shedding was very high during the first week of symptoms (peak at 7.11 X 108 RNA copies per throat swab, day 4). Infectious virus was readily isolated from throat- and lung-derived samples, but not from stool samples in spite of high virus RNA concentration. Blood and urine never yielded virus. Active replication in the throat was confirmed by viral replicative RNA intermediates in throat samples. Sequence-distinct virus populations were consistently detected in throat- and lung samples of one same patient. Shedding of viral RNA from sputum outlasted the end of symptoms. Seroconversion occurred after 6-12 days, but was not followed by a rapid decline of viral loads. COVID-19 can present as a mild upper respiratory tract illness. Active virus replication in the upper respiratory tract puts prospects of COVID-19 containment in perspective

5.2 RNA TEST PROCEDURES

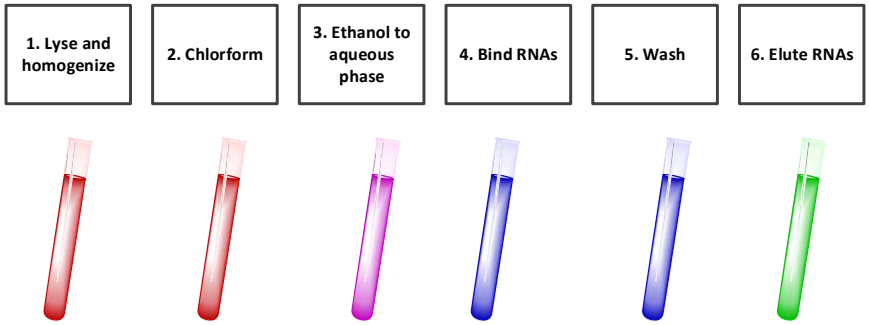
From Korpelainen et al we have:

RNA-seq describes a collection of experimental and computational methods to determine the identity and abundance of RNA sequences in bio-logical samples. Thus, the order of each adenosine, cytosine, guanine, and uracil ribonucleic acid residue present in a single-stranded RNA molecule is identified. The experimental methods involve isolation of RNA from cell, tissue, or whole-animal samples, preparation of libraries that represent RNA species in the samples, actual chemical sequencing of the library, and subsequent bioinformatic data analysis. A critical distinction of RNA-seq from earlier methods, such as microarrays, is the incredibly high throughput of current RNA-seq platforms, the sensitivity afforded by newer technologies, and the ability to discover novel transcripts, gene models, and small noncoding RNA species.

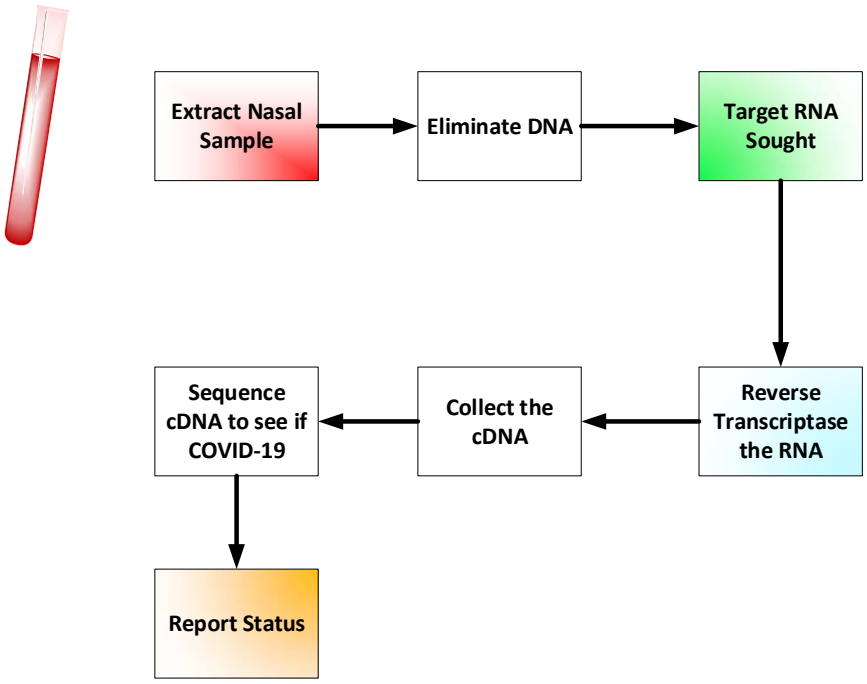
They also note:

RNAs are typically isolated from freshly dissected or frozen cells or tissue samples using commercially available kits such as RNAEasy (Qiagen, Hilden, Germany), TRIZOL (Life Technologies, Carlsbad, CA), or RiboPure (Ambion, Austin, TX), among many others. These kits have the advantage of being easy to use and yielding large amounts of total RNA when used properly. High-throughput RNA isolation systems also exist that relies mainly on RNA attached to magnetic particles which facilitate their washing and isolation.

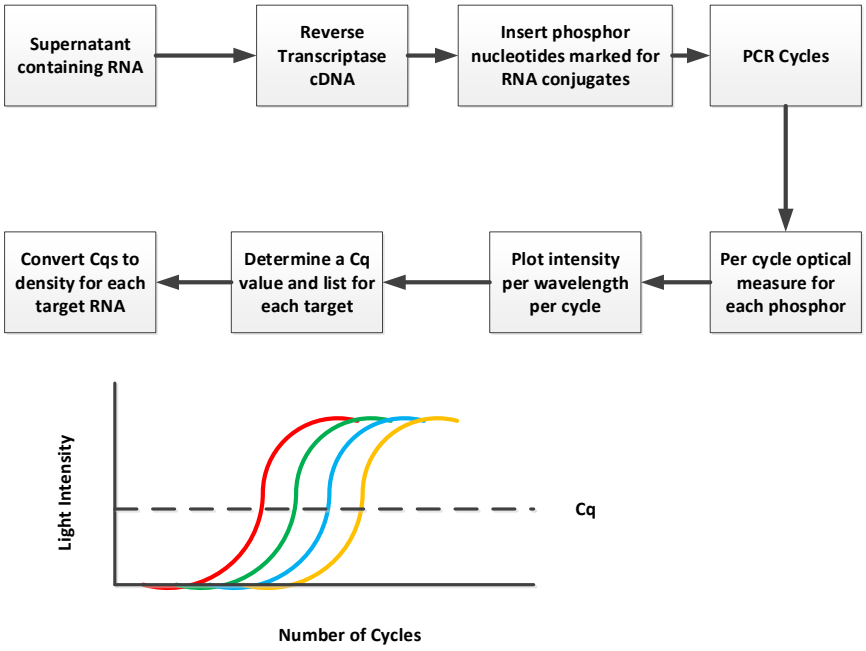
It is also possible, although not ideal, to isolate RNA from formalin-fixed, paraffin-embedded tissues. To prevent degradation of RNA, samples can be immersed in RNA storage reagents such as RNAlater (Ambion), or processed partially and stored as a phenoic emulsion (Trizol). At this stage, RNA samples can also be enriched for size-specific classes such as small RNAs using column systems (miRVana; Ambion). Alternatively, samples can be isolated initially as total RNA and then size selected by polyacrylamide gel electrophoresis.



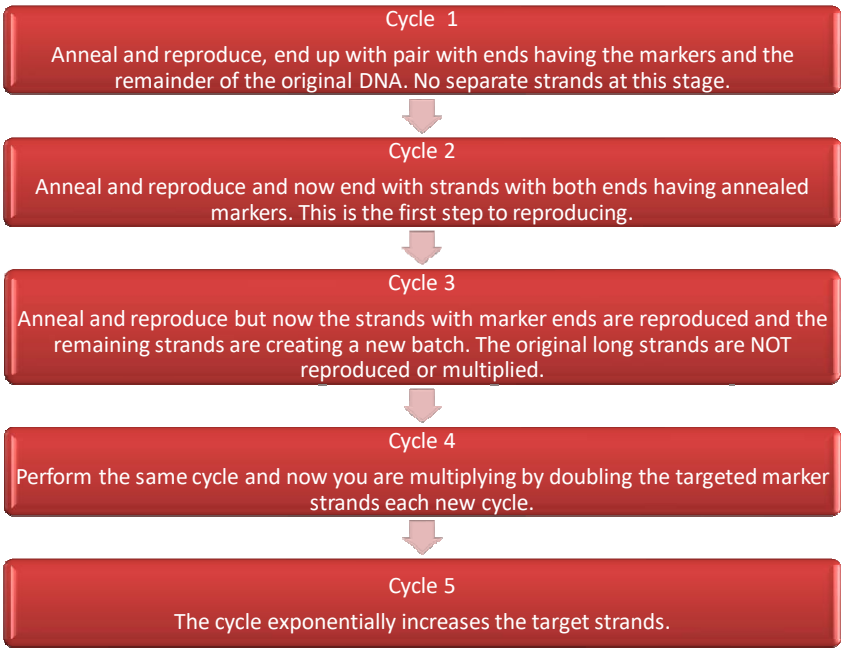
We demonstrate below the process as applied to COVID-19. Note the ssRNA from the virion will be extracted from a nasal sample and not blood or cellular.



Now more detailed flow can be shown below. Note here that this method can be used to measure concentrations of the ssRNA as well. This is not typically used but it is a more advanced process to determine full viral load.



Key to the above is the RT-PCR, real time Polymerase Chain Reaction which we outline in the Figure below. RT-PCR can produce a significant amount of cDNA to allow for an adequate measurement of ssRNA from the Corona virus.



Wu et al have described their original test protocol as follows (abbreviated):

1. *Total RNA was extracted from the BALF sample using the RNeasy Plus Universal Mini kit (Qiagen) following the manufacturer's instructions.*

2. *The quantity and quality of the RNA solution was assessed using a Qbit machine and an Agilent 2100 Bioanalyzer (Agilent Technologies) before library construction and sequencing.*

3. *An RNA library was then constructed using the SMARTer Stranded Total RNA-Seq kit v.2 (TaKaRa).*

4. *Ribosomal RNA depletion was performed during library construction following the manufacturer's instructions.*

5. *Paired-end (150-bp reads) sequencing of the RNA library was performed on the MiniSeq platform (Illumina).*

6. *All of these assembled contigs were compared (using BLASTn and Diamond BLASTx) against the entire non-redundant (nr) nucleotide and protein databases, …*

7. *Non-human reads (23,712,657 reads), generated by filtering host reads using the human genome (human release 32, GRCh38.p13, downloaded from Gencode) by Bowtie235, were used for the RSEM abundance assessment.*

8. *As the longest contigs generated by Megahit (30,474 nt) and Trinity (11,760 nt) both showed high similarity to the bat SARS-like coronavirus isolate bat SL-CoVZC45 and were found at a high abundance, the longer sequence (30,474 nt)…*

9. *The viral loads of WHCV in BALF were determined by quantitative real-time RT–PCR using the Takara One Step PrimeScript RT–PCR kit (Takara RR064A)…*

10. *The PCR product covering the Taqman primers and probe region was cloned into pLB vector …*

11. *The viral genes were aligned … using the nucleotide sequences of various CoV gene datasets: (1) whole genome, (2) ORF1a, (3) ORF1b, (4) nsp5 (3CLpro), (5) RdRp (nsp12), (6) nsp13 (Hel), (7) nsp14 (ExoN), (8) nsp15 (NendoU), (9) nsp16 (O-MT), (10) spike (S) and (11) nucleocapsid (N).*

12. *Phylogenetic trees were inferred using the maximum likelihood method implemented …*

13. *The best-fitting model of nucleotide substitution was determined using MEGA (v.5)39. Amino acid identities among sequences were calculated using the MegAlign program implemented in the Lasergene software package (v.7.1, DNAstar). Genome recombination analysis Potential recombination events in the history of the sarbecoviruses were assessed using both the RDP419 and Simplot (v.3.5.1)40.*

14. *The RDP4 analysis was conducted based on the complete genome (nucleotide) sequence, using RDP, GENECONV, BootScan, maximum chi square, Chimera, SISCAN and 3SEQ methods. Putative recombination events were identified with a Bonferroni corrected P-value cut-off of 0.01. Similarity plots were inferred using Simplot to further characterize potential recombination events, including the location of possible breakpoints. Analysis of the RBD domain of the spike protein of WHCV An amino acid sequence alignment of RBD sequences from WHCV, SARS-CoVs and bat SARS-like CoVs was performed using MUSCLE41.*

15. *The predicted protein structures of the RBD of the spike protein were estimated based on target–template alignment using ProMod3 on SWISS-MODEL server*

5.3 ANTIBODY TESTING

After an infected person is cured they should have antibodies, Ab, in their system. It will be essential to measure these Ab as a matter of course. It give one an ex post facto measure to compare to the assumed infection rate. Frankly if an adequate sample of this is not done we may find ourselves back again in a short period of time. The following Figure depicts a typical Ab detection system, Simply:

1. We line a container with a protein or antigen to bind to the COVID Ab.

2. We then rinse the container to wash out unbound Ab. In the container with the Ab they stay and in the container with no Ab they wash clean.

3. We then add another protein market to the solution which binds to the Ab.

4. We wash and fill with a reactant solution which turns color based on the last added protein.

5. Measuring color and density we can detect Abs and their density.

See: Clark and Pazdernik, p198

1. Add Ab from patients to jar with protein for binding to the target Ab.

2. Rinse jars, Abs stay fixed if bound to target protein

3. Add additional protein marker

4. Added proteins turns the solution a color and can measure titer of Ab

5.4 OTHER SCREENING

As Lethko et al have noted:

Over the past 20 years, several coronaviruses have crossed the species barrier into humans, causing outbreaks of severe, and often fatal, respiratory illness. Since SARS-CoV was first identified in animal markets, global viromics projects have discovered thousands of coronavirus sequences in diverse animals and geographic regions. Unfortunately, there are few tools available to functionally test these viruses for their ability to infect humans, which has severely hampered efforts to predict the next zoonotic viral outbreak.

Here, we developed an approach to rapidly screen lineage B betacoronaviruses, such as SARS-CoV and the recent SARS-CoV-2, for receptor usage and their ability to infect cell types from different species. We show that host protease processing during viral entry is a significant barrier for several lineage B viruses and that bypassing this barrier allows several lineage B viruses to enter human cells through an unknown receptor. We also demonstrate how different lineage B viruses can recombine to gain entry into human cells, and confirm that human ACE2 is the receptor for the recently emerging SARS-CoV-2.

6 PANDEMIC DYNAMICS

The dynamics of a pandemic have been studied extensively over the past century. An excellent description is given in Murray. We consider a simpler model which just highlights some key variables. The basis of the variables relies upon some recent analysis on the corona virus outbreak. The analysis here is descriptive and not dispositive.

6.1 BASICS

To build a simple model we can consider the following.

1. The virus spreads by contact. Contact means that an infected individual deposits the virions on a surface, most likely, or aerosolizes them, often rarely.

2. Another individual come along and touches the surface where the virus has been deposited. We assume the virus deposited has a lifetime of n hours. Studies referenced here indicate that this may be up to 5 hours.

3. The individual on contact transfers the virus to their hand. Hands inhibit internal transfer but the virus can now survive longer.

4. The individual then touches their face. Transfer occurs upon touching eyes, nose or mouth.

5. The virus will not proliferate at the temperature of the initial new infected. Namely, the virus need 37 C to multiple but at 35 C it can survive and move.

6. The new infected person remains asymptomatic for up to ten days. The first couple of days the virus is migrating and when it reaches the epithelial tissues of the lung at 37C it enters and begins massive proliferation.

7. At this point the new patient is infected and begins sloughing off virions. Namely they become a carrier and are contagious. This may last several days until symptoms are presented. This is the most serious period since this is when the virus is transferred to others.

8. At day ten the patient now has symptoms and get removed from the pool of carriers and is quarantined.

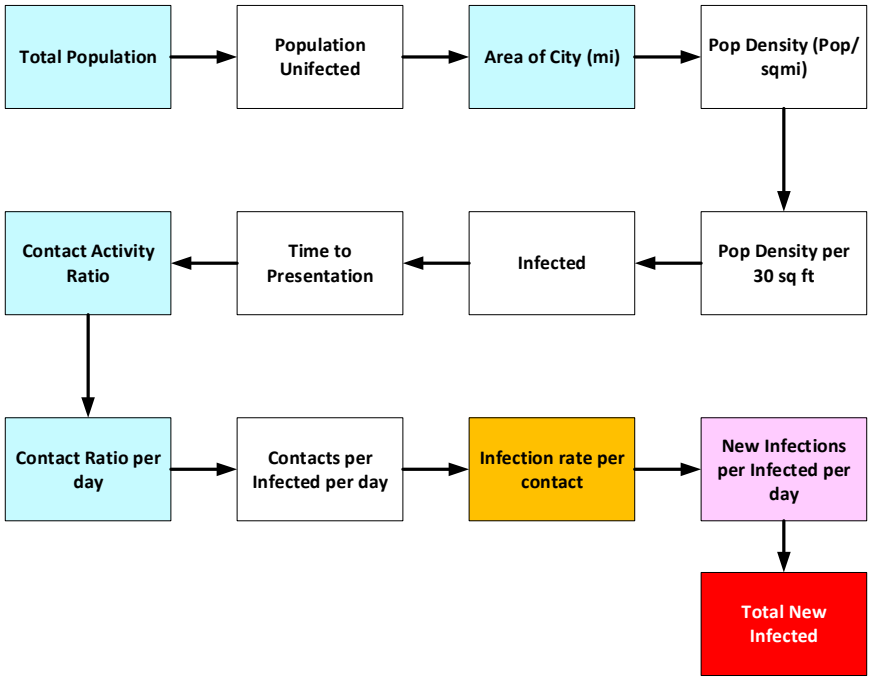
9. The patient if they survive then recovers in 8-14 days and re-enters but now immune.

The issue is; how do we depict these dynamics? We discuss this in the next section.

6.2 A SIMPLE MODEL

This pandemic has many interesting features which have been seen before. It is worthwhile to provide a descriptive model which is not dispositive. The intent is to try to delineate the key variables and how perhaps mitigation would work.

The following is a simple model:



It goes as follows:

1. Total Population is the total pop in some defined area. We start with a large metro area.

2. Population infected is the number of people infected day N. This becomes the factor which spreads the virus about.

3. Area is the sq. miles in the city. Some are dense and some are sparse

4. We then get the Pops per sq. mile

5. The important number is the prolixity density. We assume that is Pops/30 sq. ft. No great reason other than proximity but it does drive the infection rate.

6. Number Infected. We always start with a Patient Zero

7. Time to Presentation. Here is the worst part about this virus as I understand it. The common cold, Rhinovirus, presents in 24-48 hours. Nose runs, throat sore, etc. This character enters most likely through the oral pharynx and then multiplies there but does not activate the immune system with the emission of cytokines. It works its way to the lungs where it is there it gets activated with the immune system. Interesting! Almost a sleuth type virus, wonder who

engineered this one. The time to presentation may be 10 days thus allowing an infected person to spread.

8. The Contact Activity Ratio is the amount of time an infected person gets to infectively interact with others.

9. Contact Ratio per day is the per person interaction resulting from the above

10. Using the above and the pops we get the Contacts per infected person per day

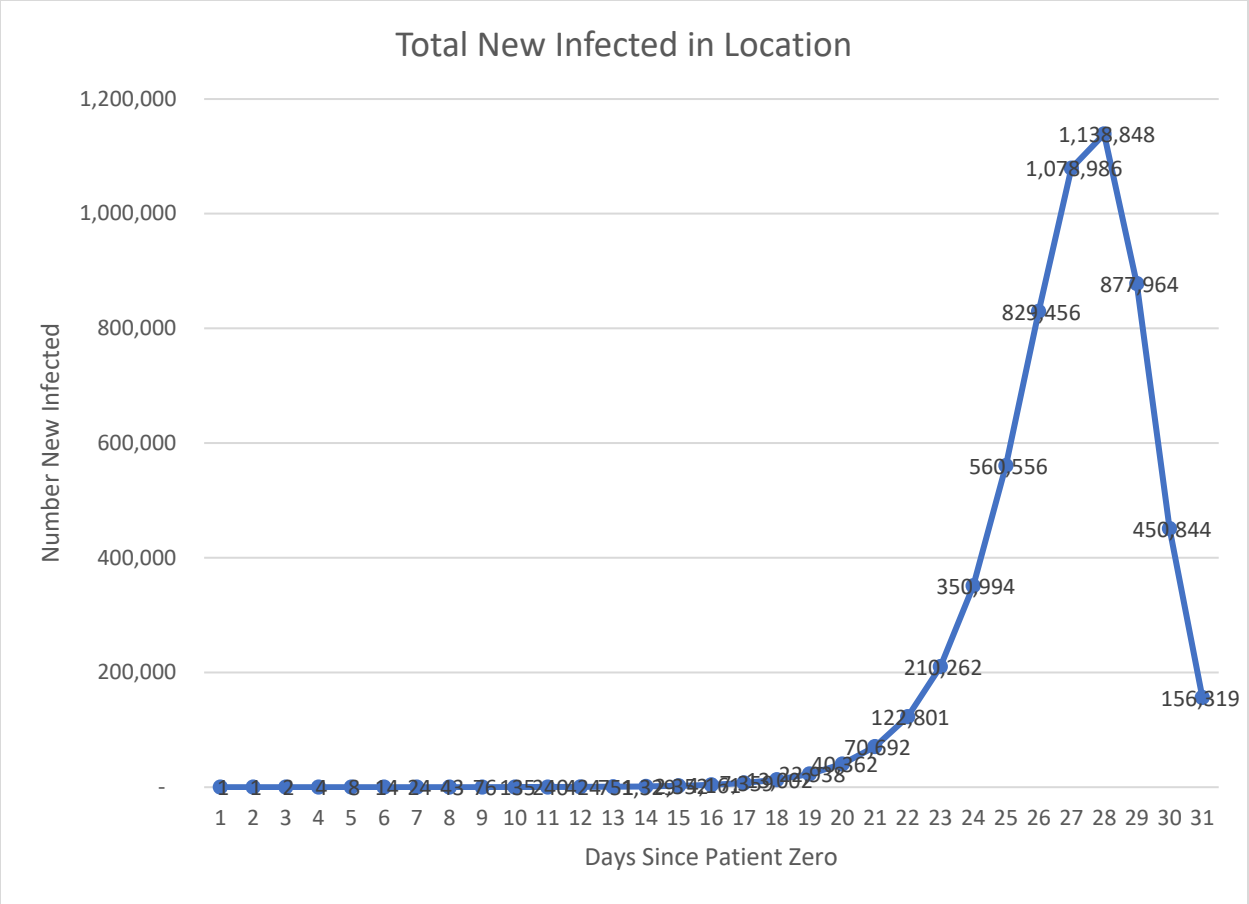
11. Infection Rate. This is key, it is the rate at which an infected person infects another uninfected person. This is a handshake, then a face contact just at the right time and then off we go! We can drive this down by lower person to person contact and personal sanitation.

12. New Infections results from before.

The following are some person observations guesses (see Li et al for data).

Population	6,000,000
Population Uninfected	6,000,000
Number Cities	1
Total Sub Pop	6,000,000
Total Unifected Pop	6,000,000
Area	100
Density (Pop per Sq mi)	60,000
Density (pop per 30 sq ft)	1.94
Infected per city	1
Time to Presentation	10
Contact Activity Ratio	0.08
Contact Ratio/Day	0.000003
Contatcts per person per Day	15
Infection Rate	0.05
New Infections per day per infected	0.77
Total new Infected per city	1
Total New Infected	1

Finally we can display the results from above set of numbers and the simple model we developed in the following figure. Here we assume that it starts with just one infected person. Here it takes more than 20 days for any appreciable incidence. Then it explodes over a short period as the entire population is infected. In this example in about a two week period the entire population is infected.



We now can ask:
what can be done to slow this process or actually even stop it? We consider some simple examples as follows.

Kind of what we have been seeing. Now we can then ask: how do we stop this? From this simple model many factors emerge:

1. Test: This means testing as many as possible as soon as possible. The ten day gestation and shedding time is the dominant factor. It keeps the virus in the community.

2. Reduce the Density by staying in some localized areas.

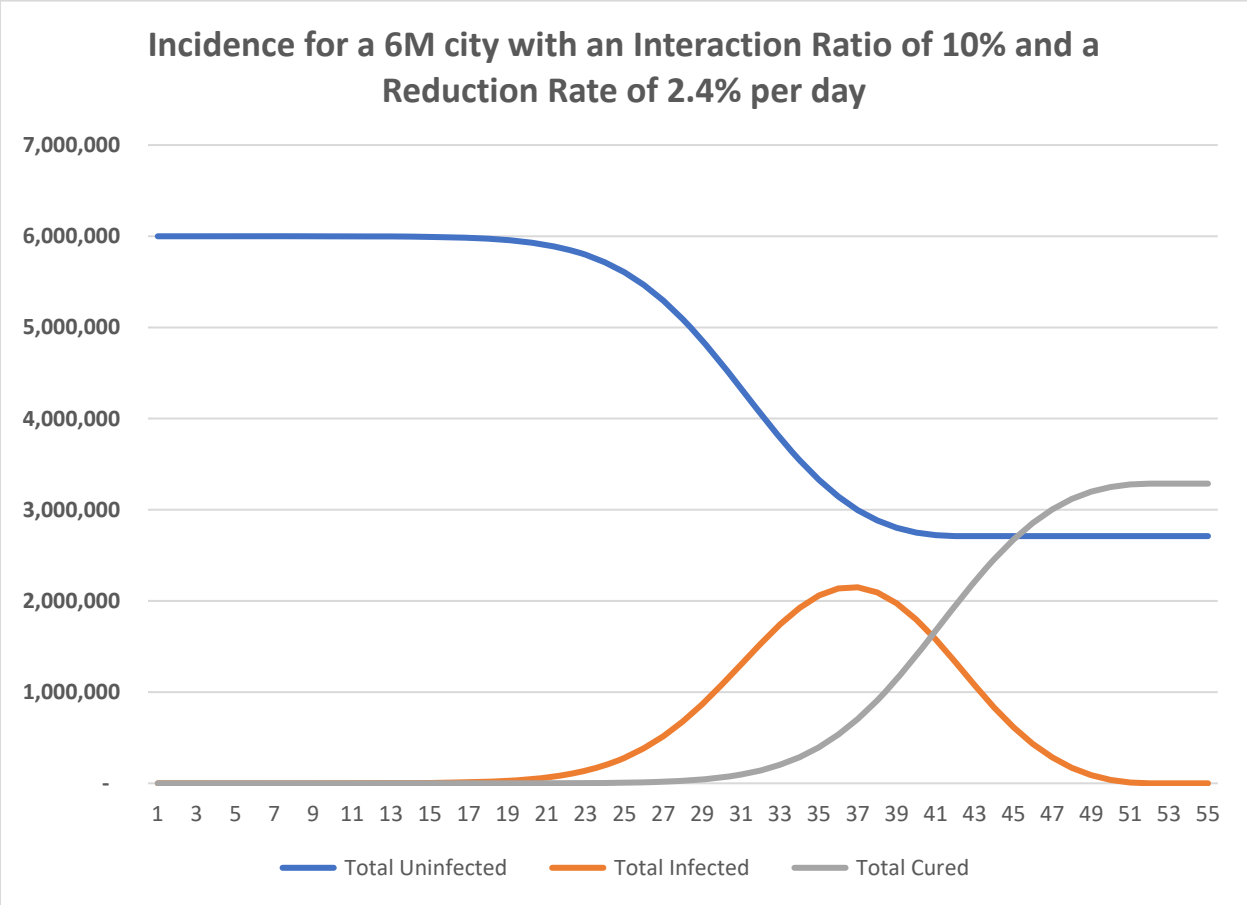
3. Avoid personal contact to get that Contact Ratio down.

4. Do not make personal contacts. No hand shaking and wash hands. The entry point is usually oral or through the eyes. Wear gloves! I saw a woman in Italy on television with a mask, bare hands and then stuffing some food inside the mask. Really!

5. Measure, measure, measure.

Hopefully this makes some sense. Each element in the pandemic model is controllable. Yet once Patient Zero gets started they are all too often careless, clueless and they go on. Also we must be aware of the Typhoid Mary syndrome, namely a viral carrier.

This is a personal opinion and observation. Its intent is to elucidate some key variables and actions.



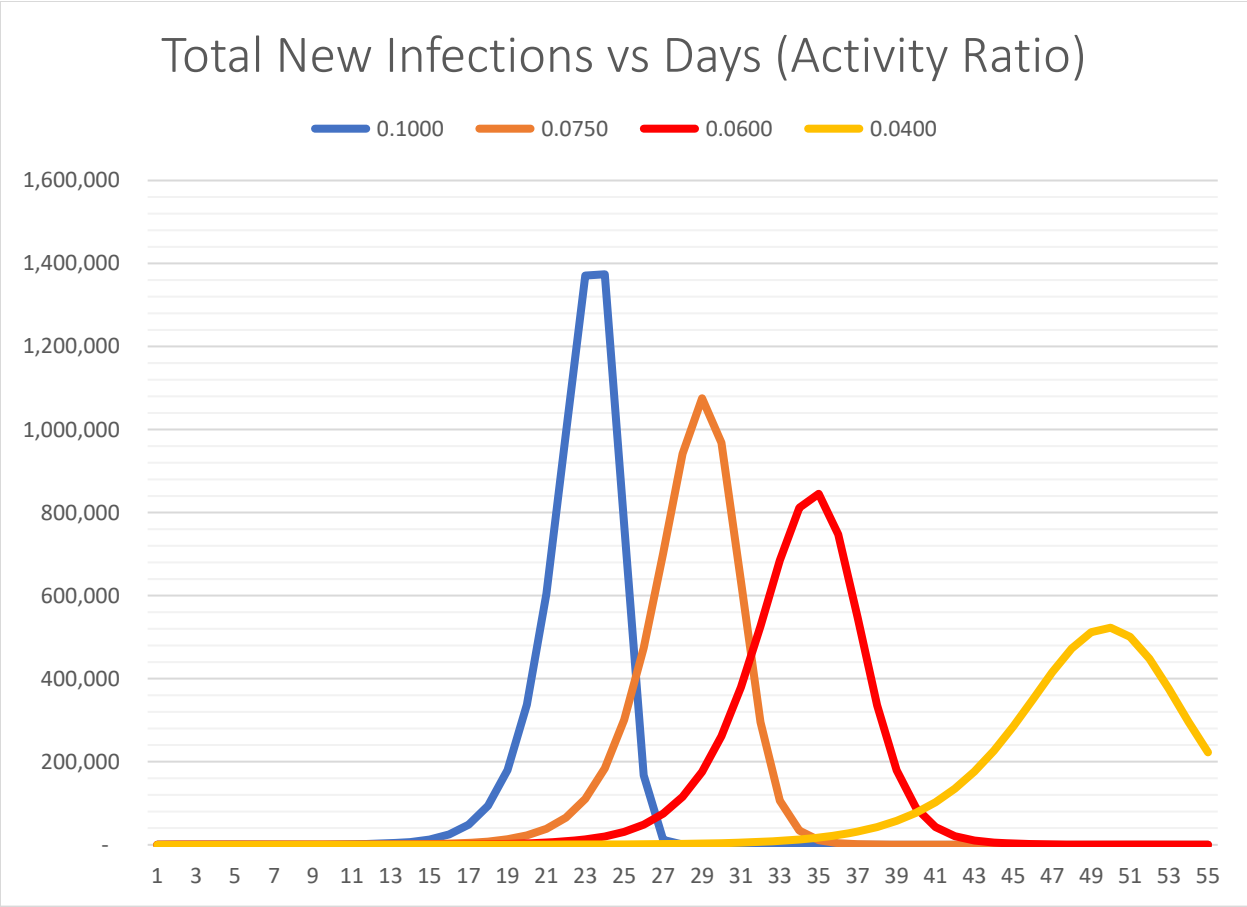
The above is an analysis of the infection of the virus in a city of 6 million and 100 sq. mi. It assumes an interaction rate of 10% as we had defined before. It then assumes that distancing and interaction is reduce by 2.4% each day, namely people are not assembling in groups. The result is burnout of the infection.

It would be nice if somehow the politicians were a bit more attuned to the system. Instead of throwing stones in glass houses. Burnout can be achieved but only by reducing interaction rates. Also it can happen quickly if people follow the guidelines and panic does not ensue.

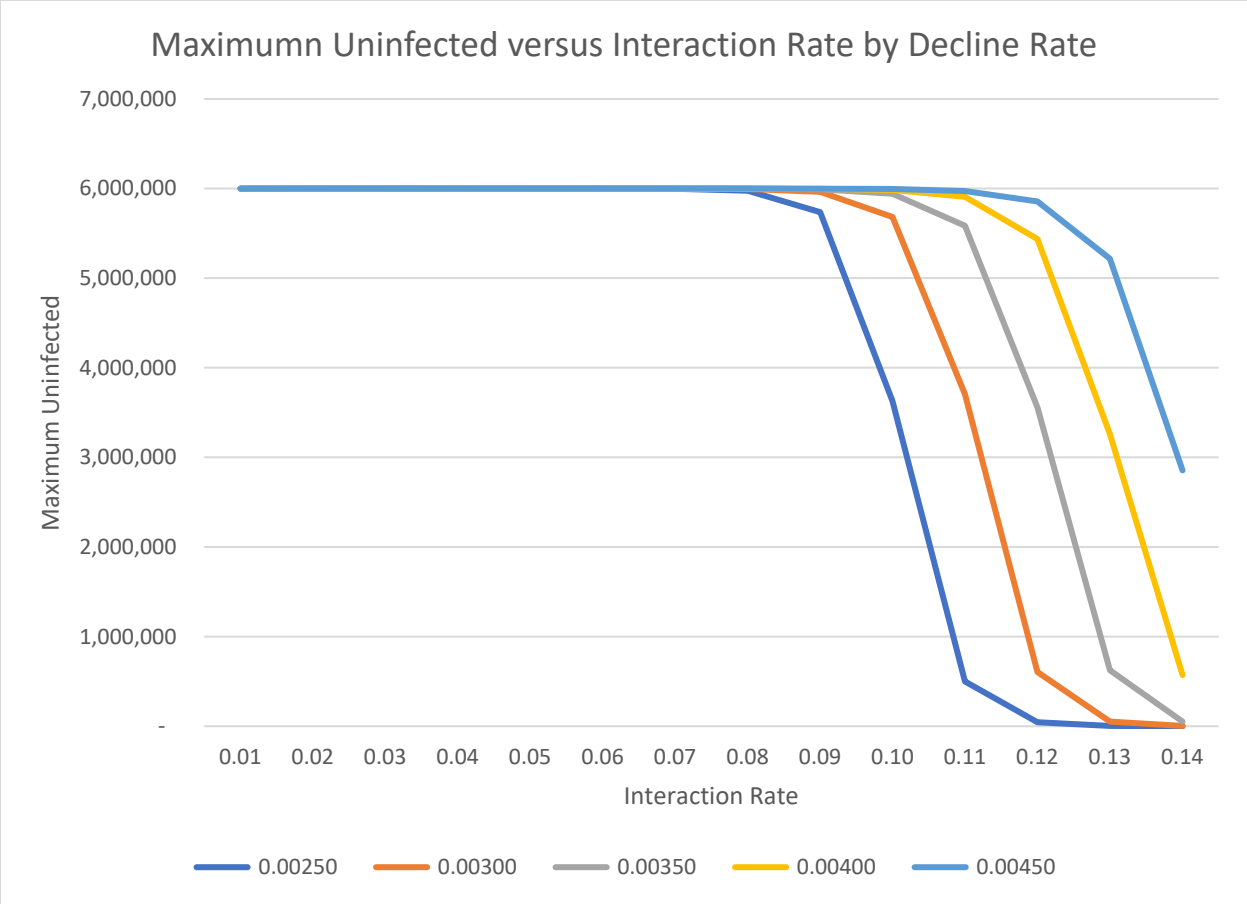
I also assume, and this is critical, that once infected and over the infection one is no longer an active agent. This could be a big assumption.

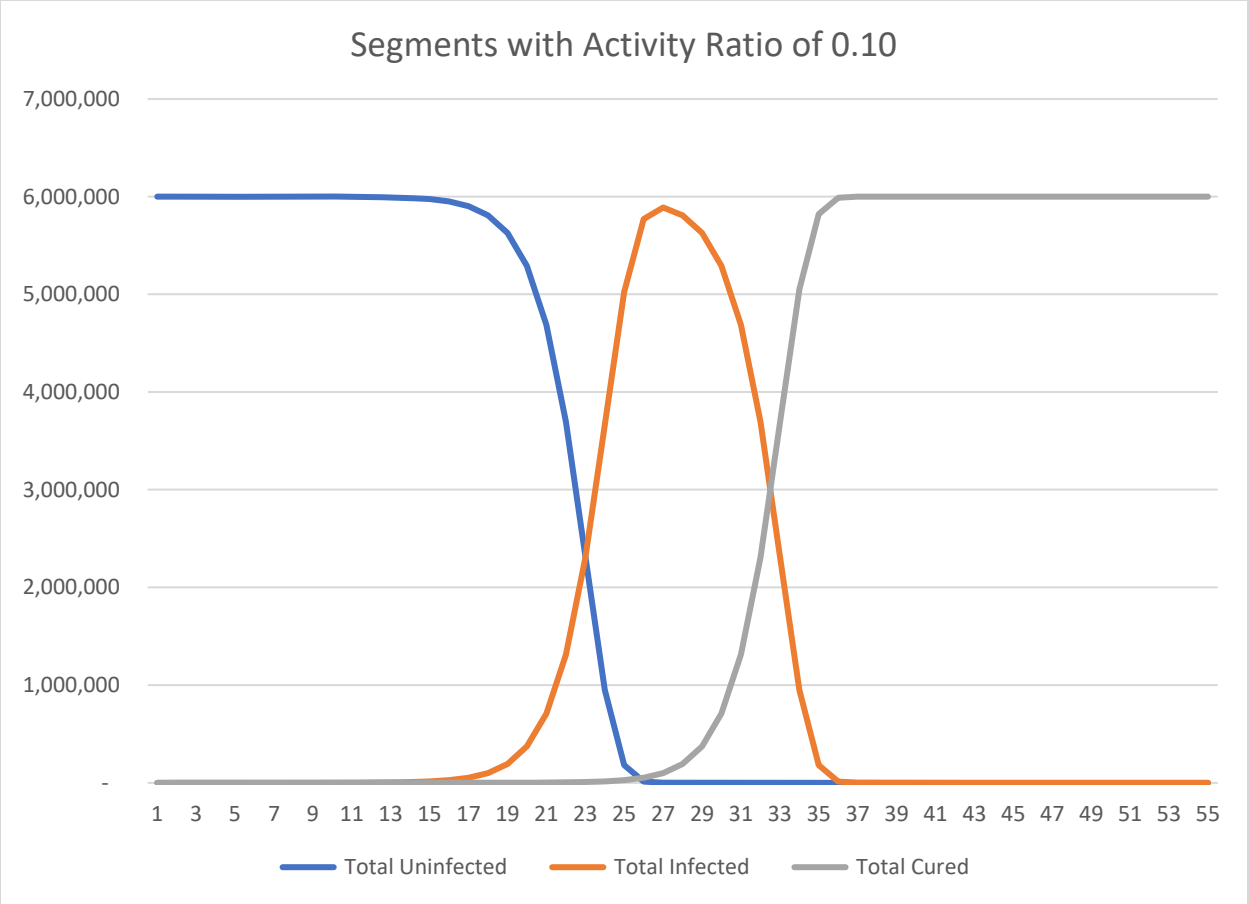
6.3 Implications and Sensitivities

We now examine several of the key sensitivities. The following Figure depicts the new infections as a function of the sensitivity ratio.



Note that as we lower the activity ratio or contacts we see the peak drop and the curve spread. The total new infected can also be reduced. The following Figure depicts the maximum uninfected versus the interactions parameterized on activity ratio. Not that is you keep people far enough apart and washing hands you can control the pandemic.


Maximumn Uninfected versus Interaction Rate by Decline Rate
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
-
Maximum Uninfected
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
0.12
0.13
0.14
Interaction Rate
0.00250
0.00300
0.00350
0.00400
0.00450



6.4 IMMUNIZATION

In a recent Nature article, the authors note[9]:

Do people develop immunity?

Vaccines help a person to generate an immune response against an infection without first being exposed to the pathogen. Studies of other coronaviruses, such as the four that cause some common colds, lead most researchers to assume that people who have recovered from SARS-CoV-2 infection will be protected from reinfection for a period of time. But that assumption needs to be backed by evidence, says Michael Diamond, a viral immunologist at Washington University in St. Louis, Missouri. "We don't know that much about immunity to this virus."

A preprint1 posted online on 14 March by a team based in China looked at two rhesus macaques (Macaca mulatta) that had recovered from SARS-CoV-2 infection, which caused them only mild illness. The monkeys did not seem to become re-infected when researchers exposed them to the virus for a second time four weeks after their initial exposure. Researchers will be looking for

[9] https://www.nature.com/articles/d41586-020-00798-8

evidence that humans react in the same way, for instance by studying people potentially exposed multiple times, Diamond says.
If humans do develop immunity, how long does it last?

That's another big unknown. Immunity is short-lived for the coronaviruses that cause common colds; even people who have high levels of antibodies against these viruses can still become infected, says Stanley Perlman, a coronavirologist at the University of Iowa in Iowa City.

The evidence is more equivocal for the two other coronaviruses that have triggered epidemics: those that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Perlman says his team has found that after people recover from MERS, their antibodies against the virus drop precipitously. He also says that his team has gathered data — not yet published — showing that SARS antibodies are still present in the body 15 years after infection. But it's not clear whether this immune response is enough to prevent reinfection. "We don't have good evidence of long-lasting immunity, but we also don't have really good data from both SARS and MERS," Perlman adds.

What kind of immune response should vaccine developers look for?

The phase 1 trial that began this week focuses on the safety of a vaccine developed by Moderna, a company based in Cambridge, Massachusetts. But researchers will also look closely at the nature of the immune response the vaccine summons.

The Moderna vaccine consists of an RNA molecule. Like many of the other SARS-CoV-2 vaccines in development, it is designed to train the immune system to make antibodies that recognize and block the spike protein that the virus uses to enter human cells.

"I think it's reasonable as a first pass, but we will learn that, perhaps, antibody responses to the spike exclusively may not be the whole story," says Diamond. A successful SARS-CoV-2 vaccine might need to prompt the body to generate antibodies that block other viral proteins, for instance, or make T cells that can recognize and kill infected cells.
How do we know if a vaccine is likely to work?

Normally, vaccines go into human trials after tests for safety and effectiveness in animals. But the Moderna vaccine and another being developed by Inovio Pharmaceuticals in Plymouth Meeting, Pennsylvania, are being tested in animals at the same time as human phase 1 trials are happening. Inovio plans to begin its first human trial in April.

"In a non-emergency situation you might do this in a more serial way, but in this case a lot of things are being done in parallel," says Barney Graham, deputy-director of the US National Institutes of Health (NIH) Vaccine Research Center in Bethesda, Maryland, which is sponsoring the Moderna vaccine trial.

In a 2 March preprint2, researchers reported injecting Inovio's vaccine — a DNA molecule carrying instructions to make the spike protein — into mice and guinea pigs. They found that the animals produced both antibodies and T cells against the virus. Study leader Kate Broderick,

Inovio's senior vice-president for preclinical research and development, says that her team has now given the vaccine to monkeys and is soon to start studies in which vaccinated animals are infected with the virus to see whether they are protected. Such 'challenge' studies are also in the works for the Moderna vaccine, says Graham.

He adds that large, costly trials of whether a vaccine can prevent infections in people won't proceed without such data from animals. Diamond expects that as researchers learn more about the infection from both human and animal studies, they will get a better sense of which vaccines are likely to work best. "It may not be the most efficient way to do it. But it may be the most expedient way to generate a vaccine," says Diamond.
Will it be safe?

Because they are given to large numbers of healthy people, vaccines usually have a higher bar for safety than do drugs administered to people who are already ill. With SARS-CoV-2 vaccines, researchers' main safety concern is to avoid a phenomenon called disease enhancement, in which vaccinated people who do get infected develop a more severe form of the disease than people who have never been vaccinated. In studies of an experimental SARS vaccine reported3 in 2004, vaccinated ferrets developed damaging inflammation in their livers after being infected with the virus.

7 OBSERVATIONS

We now make several observations regarding the state of this virus and its implications. Furthermore, what results from this effort is a clear marker for what must be done going forward. As I have noted previously, the world does not need a new app, it needs a better understanding of the biological world we inhabit. We could have approached this pandemic as was done in 1348 and yersinia pestis, just watch the bodies build up and pray one could escape. That should be unthinkable this time. However the approach is somewhat akin to it. We have no way to deal with it and the genie had gotten out of the bottle despite the clear evidence of its approach.

Clear biological threats can be more devastating that even nuclear. The panic and terror builds with everybody dragged. It slowly and painfully changes people lives and it lay bare the incompetence of many whose responsibility it is to have seen and mitigated this.

7.1 RESPONSES

From NEJM:

Given what historians have learned about past epidemics, it's hard not to be jaded now. This particular coronavirus may be new, but we have seen it all before. A novel pathogen emerged in China? That's no surprise: China has given rise to many past pandemics. People were slow to recognize the threat? That dynamic is what Camus described so well. Officials tried to suppress early warnings? Of course. Governments have reacted with authoritarian interventions? They often do — though the scale of China's interventions may be unprecedented. A quarantine fails to contain the pathogen? That has happened more often than not, especially with pathogens like influenza virus and SARS-CoV-2 that render people contagious before they're symptomatic. This does not mean that interventions are futile.

When influenza struck the United States in 1918, different cities responded in different ways. Some were able to learn from the mistakes of those that had been hit first. Cities that implemented stringent controls, including school closures, bans on public gathering, and other forms of isolation or quarantine, slowed the course of the epidemic and reduced total mortality. ***China's aggressive response may have delayed the global spread of the current outbreak.***

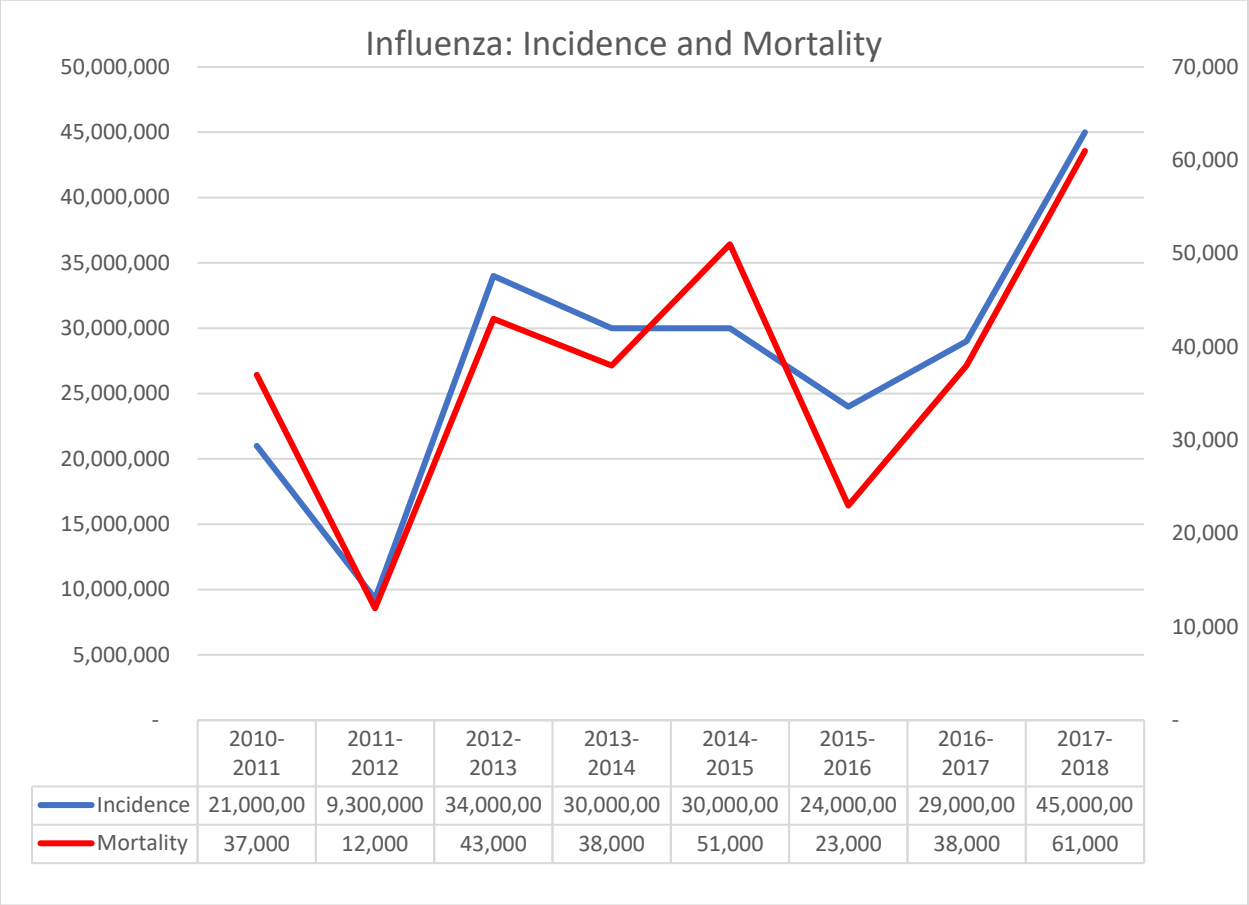
History has a way of repeating. Yet one must remember:

"Delay is the deadliest form of denial" Ken Curtin.

7.2 MORTALITY RATES

From the South China Post we have the above mortality rates as current. It is critical to use non US News since often is of a dramatically more reliable quality. Not with the first death the US rate is 1.6% and China is 3.6%. Iran is 7.5%. The Diamond Princess, being a Petri dish experiment on the part of Japan to see how bad it could be was only 0.86%.

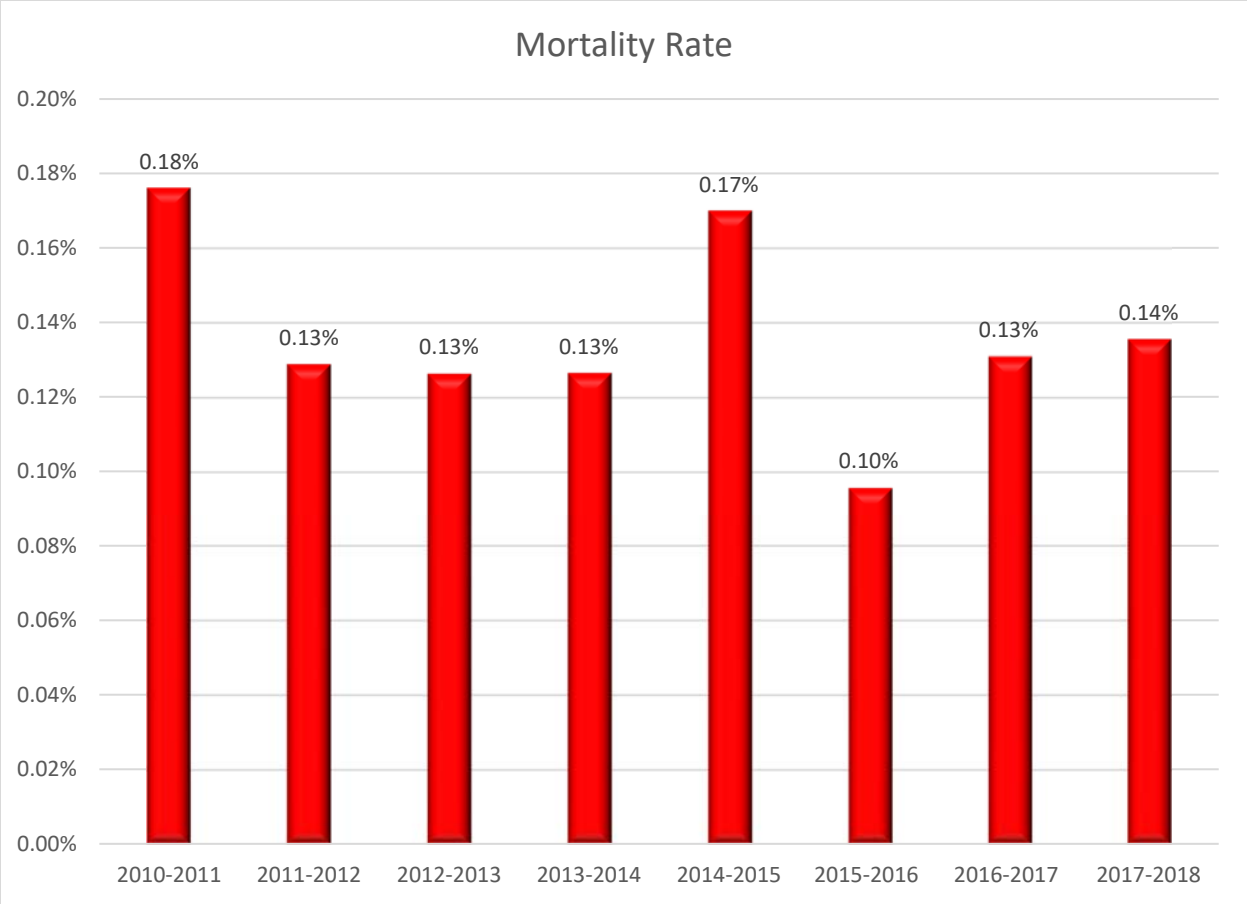
Just an update on the flu stats from the CDC:



	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018
Incidence	21,000,00	9,300,000	34,000,00	30,000,00	30,000,00	24,000,00	29,000,00	45,000,00
Mortality	37,000	12,000	43,000	38,000	51,000	23,000	38,000	61,000

The above are the incidence and mortality rates. Roughly incidence, 2017-2018, is about 12% and that is with flu shots. The incidence without flu shots is difficult to assess give the data. The CDC says there were about 160 million shots of vaccine. Thus given a population of about 330 million, there are 170 million without and thus the infection rate is about 35%.

The mortality rate is:



Thus a 35% infection rate without vaccine is about 100 million, and a mortality rate say ten times influenza, which seems a stretch, means 1 million dead. Just as a side note, the NCI notes: *In 2018, an estimated 1,735,350 new cases of cancer will be diagnosed in the United States and 609,640 people will die from the disease.*

That is about 17 times the flu and twice that of all cancer deaths. That is if it is transmitted as efficiently as the flu and it has as high a mortality rate as has been projected thus far.

It will be interesting to see what the cable folks brew up.

Mortality rates for such things as the current virus issue are theoretically simple if one has the data. Namely the mortality rate is the number of deaths among those infected divided by the number of those infected.

We appear to know the numerator. For those who have a BA degree or who are in the media, that is the top number in the ratio, the fraction, oh whatever.

Then we need to know the denominator. That again is the bottom number. Now recall that the number is the total number infected. Well how do we get that? Simple, kind of. One must sample a large random population, say thousands of people, to get a reasonable number. And one must do that daily for some period knowing the temporal dynamics of the mortality and morbidity.

Are we doing that? No way. In fact the system established prevents that very critical set of information.

Now recall we mentioned six weeks ago when we started commenting on this that RNA identification is trivial. However the Federal regulations prohibited third year Biology students from doing this even though it was part of their weekly labs. The CDC Government employees said only then could decide. Think Medicare for All!

Now it seems that we finally can do what we should be doing. BUT! Why not random testing of say a thousand or so per day? Public Health centers a century ago did this. Yes we closed them all down, but why not open them up again? For example, some sixty years or so ago one need a TB test as part of employment. I recall needing one to get my working papers as a Lifeguard in the 1950s. What happened? HIPPA and a multiplicity of Government regulations as well as a complete demolition of Public Health systems. New York at one time had a good one, if not the best, in the world. With all the immigrants the possibility of the introduction of new diseases was significant. However as things evolved it was easy to cut costs on Public Health, push it off to the hospital ERs. Below is my NYC Public Health card. It really worked then.

To understand the spread one must have data. We do not have data. When we see 3% mortality we really mean 3% die who have a severe recognizable and isolateable infection. That folks is not the mortality rate, it is another rate, mortality amongst those infected. We still have no clue on the denominator. Yet it is so easy to do. Just do it! Bu alas it is an election year and we all seem to find the advantage of collapsing the economy based upon zero data!

One should read the NY Times piece on Patient Zero in New York. This all seems to have happened a whole month after the official announcement of the virus as a putative pandemic, and a month after we had marked it as such.

7.3 Undetected Pools

Nature has an interesting discussion on the undetected pools[10]. Namely people with little or no symptoms and carrying the virus. They note:

Many scientists have suspected that there is an undetected pool of covert cases showing limited to no symptoms, because an increasing number of infected people cannot be linked to known COVID-19 cases or travel to epidemic hotspots. Most people with mild infections would not be ill enough to seek medical help, and would probably slip past screening methods such as temperature checks, so the extent of the phenomenon and its role in virus transmission has remained elusive….In a preprint posted online on 6 March, the group suggests1 that by 18 February, there were 37,400 people with the virus in Wuhan whom authorities didn't know about. Most of those unreported cases were in people who had mild or no symptoms but could still be contagious, according to the authors. "By our most conservative estimate, at least 59% of the infected individuals were out and about, without being tested and potentially infecting

[10] https://www.nature.com/articles/d41586-020-00822-x

others," says Wu Tangchun, a public-health expert at Huazhong University of Science and Technology in Wuhan, who led the study. "This may explain why the virus spread so quickly in Hubei and is now circulating around the world."

The team's results are within the range of the estimates of several other studies based on much smaller data sets, says Adam Kucharski, a disease modeller at the London School of Hygiene and Tropical Medicine. "It's the most recent analysis of the best data set we have," he says, and the methodology is sound. But the model assumes that everyone in the community has the same opportunity to be in contact with anyone else. In reality, "you have more chances of interacting with a small fraction of people: your family, your friends and your colleagues", says Gerardo Chowell, a mathematical epidemiologist at Georgia State University in Atlanta. By assuming there is homogeneous mixing, he says, the model probably overestimates the transmission rate and exaggerates the number of infections with mild or no symptoms.

But the result is in the right ballpark, he says. But probably the best-documented evidence for asymptomatic cases has come from the Diamond Princess cruise ship, which had a COVID-19 outbreak in early February while in Japanese waters, says Chowell. The ship was quarantined and the 3,711 passengers and crew members were repeatedly tested and closely monitored. Chowell's modelling study3, published on 12 March in Eurosurveillance, shows that about 18% of some 700 infected individuals on Diamond Princess never showed symptoms. "You have to keep in mind that this was a special population" with lots of elderly people, says Chowell.

Older people tend to fare badly when infected with the new coronavirus, so he suspects the rate of asymptomatic infections in a general population might be closer to the 31% that the Japanese team reported. Taking the results from several studies into account, Chowell thinks that asymptomatic or mild cases combined represent about 40–50% of all infections. Another team, in China, detected high viral loads in 17 people with COVID-19 soon after they became ill. Moreover, another infected individual never developed symptoms but shed a similar amount of virus to those who did, the researchers report5 yesterday in The New England Journal of Medicine. These are the first detailed analyses of the extent of viral shedding at different stages of the disease, says Osterholm.

The data confirm what many scientists have suspected: that some infected people "can be highly contagious when they have mild or no symptoms", he says. But he stresses that the scale of the problem is still unclear. Many scientists fear that this might also have led to an underestimate of kids' susceptibility to the virus. A study of more than 700 infected children in China found that 56% had mild or no symptoms. If the findings hold water, urgent measures are needed to curb mild and asymptomatic cases that are fuelling the pandemic, researchers say. They call for closing schools, cancelling public gatherings and generally keeping people at home and out of public spaces.

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