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Immunology: The science of pandemics, infectious disease and COVID-19

The secret of change is to focus all of your energy, not on fighting the old, but on building the new.

Socrates

1. Introduction

This chapter is being written early in the year 2021. As we are all painfully aware, the world was struck by a virulent and highly contagious form of a coronavirus, referred to as the "novel coronavirus" or SARS-CoV-2 at the end of 2019. During the months that followed, this (Severe Acute Respiratory Syndrome [SARS]-CoV-2) virus spread to create a global pandemic referred to as COVID-19. It's a fortuitous coincidence that this book is being written during an infectious pandemic and published as the pandemic slowly (hopefully) regresses. Along with the associated bioscience, epidemiology, and public health issues regarding COVID-19, its most intimate relationship with science is with immunology. It is without question the most relevant science addressing such a pernicious disease and the biomedical, emotional, and mental trauma it effectuates.

It's sad that any book (or chapter) related to something as calamitous as a worldwide, infectious pandemic needs be written at all. The coincidence, however, of such a book chapter written at a time that can enlighten readers to the profound relationship immunology brings to this human tragedy can't be overstated. It must be regarded as an opportunity to provide humankind with yet a further understanding of "the paradox of the immune system." Its negative effects as "an enemy and villain" produces clinical dilemmas, yet its applications as "our friend" gives us a greater understanding of the virus and leads to the development of vaccines to provide remedies and hope. Indeed, immunology will help us restore our personal well-being and worldwide public health.

A reasonable understanding of a ubiquitous and "novel" viral infectious disease, the nature of COVID-19 requires first a brief historical background on contagious infections and pandemics. Then we will address the pathogenesis of viral infections and specifically, the immunologic and immunogenic mechanisms and theories of SARS-CoV-2, followed by the clinical diagnostic and therapeutic considerations of coronavirus infections. Finally, we will present and address the profound epidemiologic and public health implications associated with the COVID-19 global pandemic. Notwithstanding it being a fraction of the body of literature already published on the topic, throughout this Chapter and at the end, we will include reviews and research from the ever-growing body of literature that is helping us better understand and hopefully, eventually control this viral infectious pandemic and those that inevitably will follow.

Much of the discussion in this chapter will have direct relationships to the immunology and genetic information presented in previous chapters in this book. The 2 greatest strengths I feel this Chapter brings to the reader as compared to other COVID-19 literature are: (1) it provides an organized summary of all the important and valuable information (to date) you need to know regarding the immunology of the coronavirus, infectious pandemics, and COVID-19 specifically; and (2) and perhaps its greatest value is that the collective chapters of the book provide immediate access to the most valuable, relevant immunological information regarding COVID-19 for quick reference. This relevant information (and its companion glossary of terms, not readily available in many books or in most articles) provides a fuller understanding of this critical subject for readers with backgrounds at any level of science, immunology, or medical experience.

Finally, I dedicated this book with my deepest sympathy to those souls lost during this COVID-19 pandemic, to their families, and to all those in health care and their families who have sacrificed so much. Sometimes words of appreciation fall short when there is so much suffering. But all I can hope is that, in some small way, personally or professionally, this literary contribution might help some of the heroes from this pandemic who made such unselfish sacrifices. I sincerely thank them all.

2. Background considerations

2.1 Definitions

An endemic level of disease can be defined as that level of observable disease found in a community and considered a baseline or expected level. Occasionally, the expected level of disease may rise, often suddenly, in a defined geographic area and is termed an "outbreak." If the rise in the cases is

grouped in a specific place, it is considered a "cluster," but if they are broadly distributed, it is considered an "epidemic." Finally, a pandemic refers to an epidemic that has spread over several countries or continents, usually affecting large numbers of people (sound familiar?) [1].

Epidemics and pandemics occur when an infectious agent (e.g., a virus) is sufficiently virulent and contagious enough to be conveyed to a large number of susceptible hosts (humans). These conditions may result from:

- A recent increase in amount or virulence of the agent;
- The recent introduction of the agent into a setting where it has not been before;
- An enhanced mode of transmission so that more susceptible persons are exposed;
- A change in the susceptibility of the host response to the agent; and/or
- Factors that increase host exposure or involve introduction through new portals of entry.

2.2 History of pandemics

2.2.1 Historical overview

Outbreaks of infectious disease have shaped the economic, political, and social aspects of human civilization, their effects often lasting for centuries. These outbreaks have defined some of the basic tenets of modern medicine with the development of the principles of epidemiology, prevention, immunization, and the field of public health. Throughout history, pandemic outbreaks have decimated societies, determined outcomes of wars, and wiped out entire populations. Yet paradoxically, they have ushered in new innovations, created and advanced sciences including medicine, immunology, genetics, and public health, as well as fields of economics and political science systems.

The best-known examples of recorded plagues are those referred to in religious writings starting with the Old Testament. The Athenian plague is an historically documented event that occurred in 430−26 B.C. during the Peloponnesian War. This plague affected a majority of the inhabitants of the overcrowded city-state and claimed lives of more than 25% of the population. Subsequent plagues over the centuries affected the Roman Empire (the Antonine plague), the Justinian plague, and forward to 13th century and the Black Plague, a global outbreak of the bubonic plague that originated in China in 1334, arrived in Europe in 1347, and over the following 50 years it reduced the global population from 450 million to possibly below 300 million. Some estimates claim that the Black Death claimed up to 60% of lives in Europe at that time [2].

2.2.2 Recent history

Three influenza pandemics occurred at intervals of several decades during the 20th century, the most severe of which was the so-called "Spanish Flu" (caused by an A[H1N1] virus), estimated to have caused 20 to 50 million deaths in 1918—19. Milder pandemics occurred subsequently in 1957—58 (the "Asian Flu" caused by an A[H2N2] virus) and in 1968 (the "Hong Kong Flu" also caused by an A[H3N2] virus), that were estimated to have caused one to four million deaths each.

Polio (classified as an epidemic) occurred in the United States from 1916 to its peak in 1952. Of the 57,628 reported cases, there were 3145 deaths. Dr. Jonas Salk developed a vaccine and in 1962, the average number of cases dropped to 910. The Centers for Disease Control and Prevention (CDC) Trusted Source reports that the United States has been polio-free since 1979. Unfortunately, there have been recent reports of new cases of polio developing in industrialized and developing countries [3].

The first influenza pandemic of the 21st century occurred in 2009—10 and was caused by an influenza A(H1N1) virus. This H1N1 pandemic was a reprise of the "Spanish flu" pandemic from 1918, but with far fewer devastating consequences. Suspected as a reassortment of bird, swine, and human flu viruses, it was coined the "swine flu." For the first time, a pandemic vaccine was developed, produced, and deployed in multiple countries during the first year of the pandemic. While most cases of pandemic H1N1 were mild, globally it is estimated that this 2009 pandemic caused between 100,000 and 400,000 deaths in the first year alone. Other prominent epidemics and pandemics that occurred in the early 21st century included Ebola, Lassa fever, Middle East respiratory syndrome coronavirus (MERS-CoV), Nipah and henipa viral diseases, Zika, and others.

The first outbreak of SARS was at the start of the 21st century. It was caused by the SARS Corona virus (SARS-CoV-1) and started in China. It affected fewer than 10,000 individuals, mainly in China and Hong Kong, but also in other countries, including 251 cases in Canada (Toronto). The severity of respiratory symptoms and mortality rate of about 10% caused a global public health concern. Through the vigilance of public health systems worldwide, the outbreak was contained by mid-2003. This certainly is a sad statement when considering the virtually uncontrolled evolution and spread of the SARS-CoV-2 pandemic being experienced during the second decade of the 21st century. How can we have let it happen? The novel coronavirus (SARS-CoV-2), being more contagious than the SARS-CoV-1, was allowed to spread uncontrolled because of inadequate attention (personal responsibility and political accountability—*aka* "total insanity!") to the simplest cardinal rules of public health to controlling infectious disease, that is, testing, quarantine, social distancing, copious hygiene (hand-washing), wearing

masks, and contact tracing. Such a sad statement has resulted in otherwise avoidable, unimaginable human suffering [4].

2.3 HIV and AIDs [5]

Among the most serious pandemics of the 20th and 21st century is (note the use of the present tense) the human immunosuppressive virus (HIV) first documented in 1981. The pandemic first appeared to be a rare lung infection originating in Africa. Now it is known that it damages the body's immune system and compromises its ability to fight off infections. Acquired immune deficiency syndrome (AIDS) is the final stage of HIV and the sixth leading cause of death in the United States among people 25—44 years old. While no cure currently exists, treatments (antiretroviral therapy [ART]) have been developed and the number of deaths has fallen to 19% since 2005.

Second only to COVID-19, HIV is considered one of the world's most serious health and development challenges since the first cases were reported in 1981. As of 2020, approximately 76 million people have become infected with HIV since the start of the epidemic. There are approximately 38 million people currently living with HIV, and 10s of millions of people have died of AIDS-related causes since the beginning of the epidemic. Considered an epidemic in the U.S., its worldwide distribution qualifies it as a pandemic as well. Whatever the case, stop and consider what these facts lay bare. In the year 2021, we are living with two active pandemics and no less than four in the past two decades. Biologists estimate that 380 trillion (10^{38}) viruses are living on our planet, land and sea, and inside our bodies right now—10 times the number of bacteria [6]. Given that fact, it doesn't take much imagination to realize that we will be living with infectious pandemics for a long, long time. Such a thought raises the Santayana adage that "those who do not remember the past are condemned to repeat it." We can only hope that the science skeptics (and no less the climate crisis deniers) finally wake up and help us shape a future for our children, their children, and for all humanity.

2.3.1 The human immunodeficiency virus (HIV)

HIV is a virus that attacks the T_H (or CD4) cells that help the body fight infection, making a person more vulnerable to other infections and diseases. It is spread by contact with certain bodily fluids of a person with HIV, most commonly during unprotected sex (sex without a condom or HIV medicine to prevent or treat HIV), or through sharing injection drug equipment. As with other viruses, the human body can't get rid of HIV and no effective HIV cure exists. So, once you have HIV, you have it for life. However, by taking HIV medicine like ART [7], people with HIV can live long and healthy lives and prevent transmitting HIV to their sexual partners. In addition, there are effective methods to prevent getting HIV through sex or drug use, including preexposure prophylaxis and postexposure prophylaxis.

2.3.2 Acquired immunodeficiency syndrome

If left untreated, HIV can lead to the disease AIDS (*acquired immunodeficiency syndrome*). AIDS is the late stage of HIV infection that occurs when the body's immune system is badly damaged because of the virus. In the U.S., most people with HIV do not develop AIDS because taking HIV medicine every day as prescribed stops the progression of the disease.

A person with HIV is considered to have progressed to AIDS when [8]

- the number of their CD4 cells falls below 200 cells per cubic millimeter of blood (200 cells/mm3). (In someone with a healthy immune system, CD4 counts are between 500 and 1600 cells/mm3.) or
- they develop one or more opportunistic infections regardless of their CD4 count.

Without HIV medicine, people with AIDS typically survive about 3 years. Once someone has a dangerous opportunistic illness, life expectancy without treatment falls to about 1 year. HIV medicine (ART) can still help people at this stage of HIV infection, and it can even be lifesaving. People who start ART soon after they get HIV experience more benefits, that's why HIV testing is so important.

3. Incidence and prevalence of COVID-19

Originating in the City of Wuhan, China in December 2019, the novel coronavirus spread rapidly throughout China (epidemic) and within 2 months, it had spread throughout the entire world becoming a pandemic labeled COVID-19. At the time of this writing (early 2021), this pandemic had spread to 213 countries and territories and has escalated to greater than 112 million reported cases and greater than 2.5 million deaths worldwide. In the United States, the rate of infection is greater than 28.6 million cases or 25.5% of the worldwide total and greater than 508,000 deaths or 20.3% of the world's total (statistics as of March 2021 [9,10]). Unfortunately, I anticipate that readers of this chapter will look back upon these numbers and their escalation as a regrettable measure of the lack of compliance with the necessary public health concurrence.

In the United States, COVID-19 has already become the number three leading cause of death in 2020, just behind heart disease and cancer. *(As stated in Chapter 6, during final proofing of this manuscript [late 2021], new report indicated that statistic having grown to over 700,000 deaths making it the leading cause of death in the U.S, How sad to have ever let that happen [11]).* During the pandemic, the CDC reported that life expectancy dropped by at least a full year during the first half of 2020 (men saw a drop of 1.2 to 75.1 years, while women saw a

decrease of 0.9 years to 80.5), the greatest drop since World War II and COVID-19 mortality rate more than doubled over the subsequent 12-month period. There is little doubt that when you read this book, these case numbers and mortality rates will have grown substantially, hopefully less than currently predicted. However, as improved viral controls (i.e., vaccines and antiviral drugs) become more available (a good thing), there seems to be an ominous increase in "super spreader" events (a bad thing) worldwide with large crowd gatherings in close proximity, not wearing masks, as well as some governments (state and national) disregarding necessary restrictions. Such ignorance of the need for public health preventive measures can only lead to increased disaster. (It's just so frustrating to see the politicization of such a public health crisis.)

3.1 Viral mutations

Mutations are the changes in the structure of the DNA or RNA of a gene, resulting in a variant form of the genome that may be transmitted to subsequent generations. Most microorganisms are based on a DNA genome. Some viruses, including the coronavirus, have RNA-based genomes instead. In general, viral RNA genomes are much more mutation-prone than those based of DNA. This distinction is important because RNA-based viral mutations have a greater potential for increased virulence, greater transmissibility, and worst of all, producing a resistance to drugs. So oftentimes, a vaccine developed for a particular viral genome becomes ineffective for its mutated variant. Such is the concerns with the variants developing in the SARS-CoV-2 virus.

As the prevalence of coronavirus increases across the planet, there is concern for any factors that might increase the transmissibility and severity of infection. Different variants are evolving from "mutations" of the viral genome. Evidence of this has been discovered in a number of countries (UK, South Africa, Brazil, and the U.S.) so far. The CDC described a variant strain of SARS-CoV-2 that was discovered in parts of the UK (specifically the South East), that accounted for 60% of infections in London. Studies have shown that some variants spread more quickly than others. The 614G sequence variant demonstrates a mutation in the virus's spike protein (more on this below), representing a potential risk of worsening the pandemic. Other variants have been associated with higher rates of infectivity (e.g., the delta variant and a rapidly evolving omicron variant).

Public Health of England has said there is no evidence new variants tested are resistant to the Pfizer-BioNTech vaccine, that is now being given across the country to high-priority groups such as healthcare workers. Nor is there any indication at this point of increased infection severity associated with the new variants (although this is being refuted now in more studies in multiple

countries). A new South African variant (omicron) identified in the United Kingdom is considered completely different from the UK variants and seems to spread more easily and quickly than other variants discovered so far. The CDC announced in November 2020 that it had launched the National SARS-CoV Strain Surveillance (NS3) program to help track the viruses undergoing mutation characterization. The CDC stated that there is currently no evidence that the variants discovered so far that can cause more severe illness or increased risk for death.

4. Pathogenesis, immunologic, and immunogenic considerations for SARS-CoV-2

4.1 Mechanisms

Viruses are not living cells or organisms. They are obligate parasites or nonliving organisms that lack metabolic machinery of their own to generate energy or to synthesize proteins. Rather, they require a living host to exploit or infect (enter) so they can replicate to complete their life cycle (see Fig. 7.1 and Life Cycle below). The invading virus uses either its genomic DNA or RNA to replicate in the host cell. Coronaviruses (CoV) are a family of RNA viruses that typically cause mild respiratory disease in humans. They include MERS-CoV and SARS-CoV-1, thought to be driven by the spillover of bat-adapted CoVs into an intermediate host (see below). The novel coronavirus (SARS-CoV-2) is a single positive-strand RNA virus that is the largest genome known. Thus, these viruses are poorly adapted to the human host and if transmitted to humans (e.g., SARS-CoV-2), they are generally associated with more severe clinical presentations. Also, if infection occurs (and subsequent mutations), it can be highly transmissible from person to person as SARS-CoV-2 has demonstrated [12].

Coronavirus disease leads to fast activation of innate immune cells, especially in patients developing severe disease. Innate immune activation, levels of many proinflammatory effector cytokines (e.g., TNF, IL-1b, IL-6, IL-8, G-CSF [granulocyte colony-stimulating factor] and GM-CSF [granulocyte-macrophage colony-stimulating factor]), as well as higher levels of chemokines (e.g., MCP1, IP10, and MIP1α) are also found in those who are critically ill. The levels of some T cell-derived cytokines (e.g., IL-17) are also increased. Often with these cytokines, a "cytokine storm" develops that triggers a hyperinflammatory state. As stated above and in Chapter 4 on chronic inflammation (the progenitor of *all* disease), this inflammatory clinical response leaves virtually all organ systems vulnerable to adverse effects from the novel coronavirus [13]. Of increasing concern are the cardiovascular effects resulting from perivasculitis (inflammation of the adventitia and endothelial
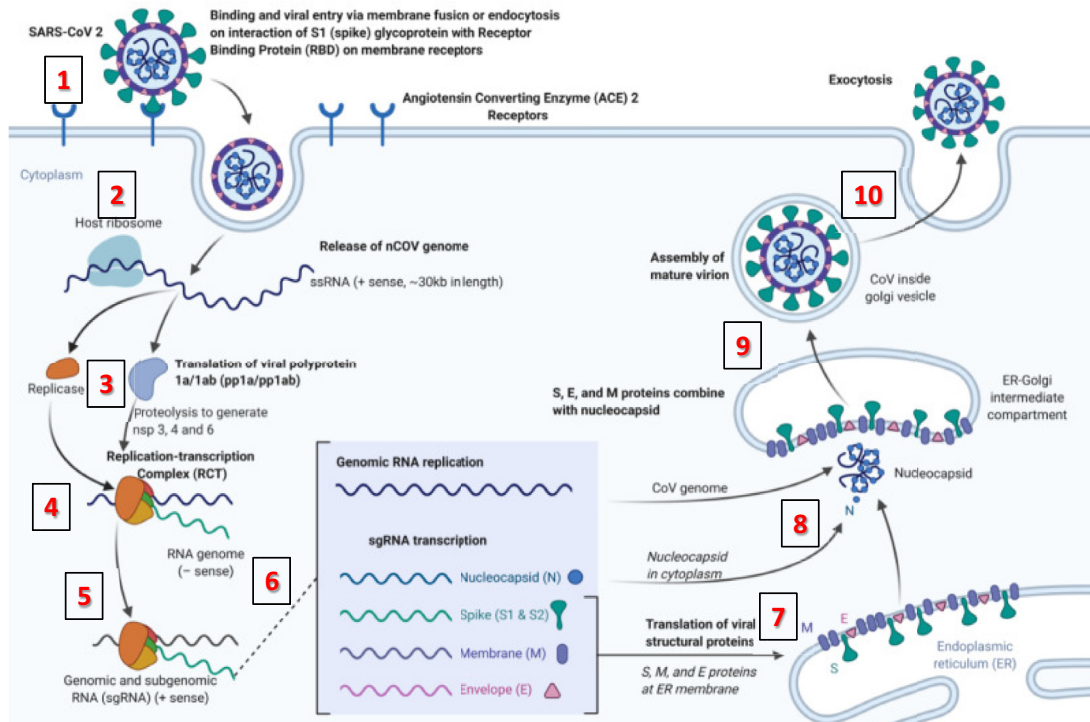
SARS-CoV-2 LIFE CYCLE

Figure 7.1 SARS-CoV-2 life cycle. The life cycle of the novel coronavirus (SARS-CoV-2) begins when its spike protein attaches to an ACE2 receptor on a cell membrane (1) and penetrates the cell wall where it replicates a genomic RNA (2–4), then produces "subgenomic RNAs" (5–6), synthesizes various spike proteins through translation (7) and new genomic RNA becomes the genome of a new virus particle (8). This combines with the strand genomic RNA, merges in the endoplasmic reticulum-Golgi apparatus into a complete virus particle within a vesicle (9), and the new viral particles are released (exocytosis) to the extracellular region (10). *Shereen MA, Khana S, Kazmi A, et al. COVID-19 infection.*

lining of blood vessel walls—see Chapter 4, page 76). Antiinflammatories (corticosteroids) and cytokine inhibitor drugs (e.g., checkpoint inhibitors, IgG, Interleukin six blockers) are being studied and beginning to show some benefits in advanced cases and late-stage disease [14].

4.2 Life cycle of SARS-CoV-2

The pathogenesis and life cycle of SARS-CoV-2 includes a complex of RNA genomic transfers and regenerations to produce the proliferation of the virus. The extracellular and intracellular (host cytoplasm) process involved is illustrated in Fig. 7.1 and traced through the following steps. I think you will be able to follow the "bouncing ball" through this life cycle and the illustration may (or may not) help. If you're completely flummoxed by the whole thing,

don't worry. Just wear your mask, get vaccinated and you'll never need to know the difference between a nonstructural protein from a ham sandwich (rich in protein, I might add).

1. When the spike protein of SARS-CoV-2 binds to the ACE-2 receptor (described below) of the host cell, the virus enters the cell;
2. Then, the fatty envelope of the virus is peeled off by the host ribosome that releases the viral genomic RNA into the cytoplasm of the cell;
3. The ORF1a and ORF1b (genes) RNAs are produced by genomic RNA and then translated into pp1a and pp1b proteins, respectively;
4. Protein pp1a and ppa1b are cleaved by protease (proteolysis) to make a total of 16 nonstructural proteins;
5. Some of the nonstructural proteins form a replication/transcription complex (RNA-dependent RNA polymerase, RdRp), that use the (+) strand genomic RNA as a template (see Chapter 3, page 51 for transcription and translation explanation);
6. The (+) strand genomic RNA produced through the replication process becomes the genome of a new viral particle;
7. Subgenomic RNAs produced through transcription are translated into structural proteins (in the diagram: S: spike protein, E: envelope protein, M: membrane protein, and N: nucleocapsid protein) that form a viral particle;
8. Spike, envelope, and membrane proteins enter the endoplasmic reticulum of the cell, and the nucleocapsid protein is combined with the (+) strand genomic RNA to become a nucleoprotein complex;
9. This complex merges into the complete virus particle in the endoplasmic reticulum-Golgi apparatus compartment; and
10. The new viral particles are released (exocytosis) to extracellular regions through the Golgi apparatus and the vesicle.

(Chapter 3 may have aided in your understanding of this step-by-step life cycle explanation and may have added to your genomic knowledge base. If you got even half of all that, you're way ahead of 99% of the world's population.)

4.3 Theories

Several studies suggest that antibodies against non-SARS-CoVs are highly prevalent in the general population including children, suggesting that many or most individuals have been infected by CoVs in the past and have potentially developed a certain degree of (protective) immune response [15]. The severity and the clinical picture in many patients could even be related to the activation of an exaggerated immune mechanism ("cytokine storm"), causing uncontrolled inflammation, akin to autoimmune disease (i.e., the immune system as "Enemy #2"). The hypothesis that SARS-CoV-1 (or other,

antigenically similar CoV-1) has silently infected a significant proportion of the population, inducing "herd immunity" (see "Treatment and management strategies" below) needs to be confirmed. Indeed, immunity against the infection, or patterns of semiimmunity (capacity of the immune system to avoid severe infection) may be due to cellular immunity rather than proinflammatory humoral immune responses [16]. Animal models suggest that the efficiency of T lymphocyte-mediated immune responses (see Chapter 2, page 31) is also pivotal for controlling SARS-CoV infections [16]. (Lots of stuff from Chapters 1 and 2 resurfacing here.)

Within 19 days after symptom onset, a total of 100% of 285 patients with COVID-19 tested positive for antiviral immunoglobulin-G (IgG). Seroconversion for IgG and IgM (transition of the test results for IgG or IgM against SARS-CoV-2 from negative to positive results in sequential samples) occurred simultaneously or sequentially. Both IgG and IgM titers plateaued within 6 days after seroconversion [17]. Thus, serological testing may be helpful for the diagnosis of suspected patients with negative reverse-transcriptase–polymerase-chain-reaction (RT–PCR) (an antigen diagnostic test—see below) results and for the identification of asymptomatic infections.

There is currently no data on the specific role of either humoral or cellular immunity or innate immunity in patients recovering from COVID-19. T lymphocytes responsible for clinically relevant antiviral immune responses have a significant chance to be locally present in, or close to, respiratory epithelia. It is very possible that the exclusive detection of humoral immunity against SARS-CoV-2 leads to an underestimation of the anti-SARS-CoV-2 immune responses. It becomes plausible that, after infection by SARS-CoV-2, a sort of race decides the course of the events. Either a cellular innate immune response rapidly clears SARS-CoV-2 without any (or mild) clinical signs of infection or the virus causes a state of immunosuppression that debilitates and sometimes overwhelms the host's (human) defense [18].

4.4 Natural pathogenesis (theorized)

Researchers have analyzed genomic data related to the overall molecular structure of the new coronavirus. Their testing has traced this novel coronavirus to a strain of Malaysian anteater (pangolin) containing genomic regions that are very closely related to the human virus. Their analysis showed that the genome resembles that of a bat coronavirus discovered after the COVID-19 pandemic began. However, in "SARS-CoV-2 testing," the binding region of the spike protein resembles the novel virus found in pangolins (anteaters). This provides additional evidence that the coronavirus that causes COVID-19 almost certainly originated in nature, most likely in bats [19] with an intermediate animal (anteater or monkey?) host and

ultimately transmitted to humans ("zoonotic spillover") [20]. This genetic information concludes that "coronaviruses clearly have the capacity to jump species boundaries and adapt to new hosts" (virus recently reported in Malaysian tigers in Bronx Zoo [21]). This information makes it predictable that more will emerge in the future. This phenomenon could be a serious ongoing treat when considering the reverse, human to animal (anthroponotic) transmission that is now being reported with increased frequency. Reports include common pets (including dogs and cats) though rare, multiple species, and familial primate species (homids) that interact with humans in zoos (gorillas, orangutans, chimpanzees, and bonobos) [22].

Most important among these findings is the receptor binding domain (spike protein) that dictates how the virus is able to attach and infect human cells (see Life cycle above). This comparative analysis of genomic data dispelled the postulate that the virus was laboratory constructed or was a "manipulated" virus. Rather, it promotes a lesson learned to reduce human exposure to wildlife and to ban the trade and consumption (e.g., "wet markets" in China) of wildlife. However, as not all of the early COVID-19 cases were wet market associated, it is possible that the emerging story is more complicated than first suspected.

4.5 Genetic and genomic considerations

The genomic data of the new coronavirus responsible for COVID-19 show that its spike protein contains some unique adaptations. One of these adaptations provides special ability of this coronavirus to bind to a specific protein on human cells called angiotensin converting enzyme (ACE-2) [23]. Human ACE-2 is expressed in epithelial cells of the lung and serves as an entry receptor site for SARS-CoV-2 spike protein. ACE-2 genetic polymorphism (occurrence of different forms in the life cycle of an individual organism) represented by diverse genetic variants in the human genome has been shown to affect virus-binding activity suggesting a possible genetic predisposition to COVID-19 infection. Thus, analysis of genetic variants and genome sequencing from asymptomatic, mild or severe COVID-19 patients should be performed to classify and predict people based on their vulnerability or resistance to potential COVID-19 infection [24]. Genome sequencing is also critical in determining new variants of the virus among the infected population.

The entire genome of the 2019-novel coronavirus is more than 80% similar to the previous human SARS-like bat CoV [25]. Thus, previously used animal models for SARS-CoV can be utilized to study the infectious pathogenicity of SARS-CoV-2. CRISPR-mediated (see CRISPR, Chapter 6, page 168 and below) genetically modified hamsters or other small animals can be utilized for the study of the pathogenicity of novel coronaviruses.

Finally, an interesting finding was made among SARS-CoV-2-infected pa-tients. Researchers found a haplotype (a group of genes inherited together from a single parent) on chromosome 12 that reduces the risk of severe Covid-19 infection. This genetic mutation is associated with about a 22% reduction in relative risk of becoming severely ill with COVID-19 when infected by SARS-CoV-2. This region encodes proteins that activate enzymes (proteases) that are important during infections with RNA viruses. The ge-netic region involved affects the body's immune response to RNA viruses such as the coronavirus. This is a mutation that has been passed down over the millennia because it is assumed to help people survive the frequent viral infections among humans [26]. It reminds me of the negative evolutionary correlate we spoke about back in Chapter 6 (page 168) regarding the "pro-cancer" programmed death protein on the T cell. That one was theo-rized as "culling the herd" while this one seems to promote phylogeny (evolutionary history of a species). Don't you wish "natural selection" and Darwinism would make up its mind?

4.6 Autoantibody rogue B-cell association with COVID-19 [27]

As mentioned in Chapter 5, late in the proofreading portion of this manu-script (late 2021), new findings were revealed regarding the association of memory B cells (MBCs), *aka* autoantibody rogue B-cells, with late COVID-19 infections. Researchers at Rockefeller University, Yale University and inter-national research teams [28] detected these autoantibodies that could neutralize and lower, relevant concentrations of interferons. Their initial studies included 3595 patients with critical COVID-19 that were confirmed among individuals admitted to an intensive-care unit with advanced COVID-19 infections. Overall, 13.6% of these patients possessed autoantibodies, with the proportion ranging from 9.6% of those below the age of 40, up to 21% of those over 80.

This cohort study indicated that around 10% of people with severe COVID-19 had autoantibodies that attack and block type 1 interferons and their critical role in fighting off viral infections. Further studies on 35,000 blood samples of healthy in patients found an increase in B-cell autoan-tibodies against type 1 interferon ranged from 9.6% in patients below age 40 up to 21% in patients over 80. Autoantibodies were also present in 18% of people who had died of the disease. This massive increased prevalence in patients over 80 largely explains the high risk of severe COVID in people in the elderly population. These studies seem to indicate that rogue B-cell autoantibodies are a cause, rather than a consequence, of critical COVID-19.

5. Clinical considerations for coronavirus (SARS-CoV-2) infection

5.1 Clinical manifestations (signs and symptoms)

Reported illnesses with the novel coronavirus have ranged from mild symptoms to severe illness and death for confirmed COVID-19 cases. The symptoms may appear 2–14 days after exposure (based on the incubation period of SARS-CoV viruses). As with any infectious disease, the array of symptoms can vary considerably, but there are eight cardinal symptoms in adults and children (and other possibilities) which are the defining complex of the clinical disease [29], including:

In adults:	In children (age 5 to 17):	Other possible symptoms:	
1. Loss of taste;	1. Loss of taste	1. Severe fatigue;	9. Sore eyes;
2. Loss of smell;	2. Loss of smell;	2. Heavy arms/legs;	10. Sneezing;
3. Fever;	3. Fever;	3. Tightness in chest;	11. Diarrhea;
4. New persistent cough;	4. Headache;	4. Hoarse voice;	12. Sore throat;
5. Shortness of breath;	5. Shortness of breath;	5. Nasal congestion;	13. Difficult sleeping;
6. Chills;	6. Chills;	6. Dizziness;	14. Abdominal pain
7. Loss of appetite;	7. Loss of appetite;	7. Chest pain;	15. Numbness/tingling
8. Muscle aches.	8. Muscle aches.	8. Nausea and vomiting	

Elderly and immune compromised patients are at greater risk for contracting the virus and for poor outcomes. However, significant numbers of young and healthy people are also being reported with severe infections, though generally with better outcomes. Spread occurs through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs [30].

Older age, obesity, and comorbidities have consistently been reported as the greatest risk factors for unfavorable prognosis or protracted disease, called "post-COVID syndrome," "long COVID," or "long haulers syndrome." [31] This syndrome however, is found in a full range of COVID-19 patients, with

symptomatology ranging from severe to nonexistent. The syndrome itself includes all sorts of problems with inflammatory responses in the brain ("brain-fog"), around the heart (myocarditis), around the nerves (neuropathies), around the muscles (myosititis), etc. It's clear that in addition to the immediate clinical effects of SARS-CoV-2, the novel coronavirus can have long-term manifestations, experts say [32]. Less clear so far has been how the number and types of comorbidities influence outcomes. An epidemiologic clarification was provided through a nationwide Chinese retrospective cohort study [33] involving 1590 PCR-confirmed (see Antigen testing below) COVID-19 cases (mean age, 49 years; 43% female) diagnosed between December 11, 2019, and January 31, 2020. The most common symptoms were fever, dry cough, and fatigue (88%, 70%, and 43%, respectively). In addition, there are reports of neurological symptoms, such as cognitive changes, or "brain fog" (suspected brain invasion of macrophages suppressing neural transmission—see also Chapter 4, page 82), headaches, paresthesia, and dysautonomia (a group of medical conditions caused by problems with the autonomic nervous system—ANS).

According to the 2019 American Thoracic Society/Infectious Disease Society of America guideline for community-acquired pneumonia criteria [34], 16% of the cases were considered severe. Reported proportions with comorbidities included 17% hypertension, 8% diabetes, 4% cardiovascular disease, 2% cerebrovascular disease, 2% chronic obstructive pulmonary disease, 1% chronic kidney disease, and 1% malignancy. At least one comorbidity was significantly more common in severe than in nonsevere cases (33% vs. 10%). Obesity puts those with COVID-19 at particularly high risk of death, more so than related risk factors such as diabetes or hypertension, according to a study of patient records by researchers from Kaiser Permanente [35]. In people with rare autoimmune diseases (RAIDs, some listed in Table 5.1, page 98), the risk of early death has risen with COVID-19. The most common RAIDs were giant-cell arteritis (22%), systemic lupus erythematosus (22%), juvenile inflammatory arthritis (13%), unspecified arteritis (12%), and polymyositis (10%).

5.2 Diagnostic testing [36]

The clarion call during the early stages of the COVID-19 pandemic was "Testing, Testing, Testing." Tracking ("contact tracing") an invisible virus is one of the most valuable ways to control it, and the most effective strategy to accomplish that goal starts with building a comprehensive system to test anyone who may be infected. Upon accomplishing that, those positive cases can be isolated and "contact traced" (identifying persons who may have come into contact with the infected person) and testing them as well and isolate all positive cases.

Other indirect biomarkers are being identified as diagnostic indicators for the degree and severity of a SARS-CoV-2 infection. In Chapter 1 (page 14), a malfunction of interferon (IFN-1) was identified as attributing to an increase of at least 3.5% of patients with life-threatening COVID-19 disease. Other studies of elevated concentrations of inflammatory cytokines and blood markers including C reactive protein, lactate dehydrogenase, and other digestive enzymes demonstrate the gut microbiome being linked with the severity of COVID-19. The gut microbiota dysbiosis after disease resolution can also contribute to persistent symptoms ("long COVID"), highlighting the diagnostic importance of gut microorganisms in inflammation and COVID-19 [37]

The definitive "diagnostic" process in diagnosing COVID-19 is conducted through two types of tests (below), one testing for the antigen (people who are currently infected) and second testing for antibodies to the antigen (people previously infected who have developed antibodies to the virus). Regrettably, contact tracing has not proven valuable (I can attest to this personally having been a volunteer tracer) due to subjects reluctance to share necessary contact information.

5.2.1 Antigen testing

An antigen test reveals if a person is actively infected with the SARS-CoV-2 virus. The test detects certain proteins that are part of the virus. Using a nasal or throat swab to get a fluid sample, antigen tests can produce results in minutes. Because these tests are faster and less expensive than molecular tests (below), some experts consider antigen tests more practical to use for large numbers of people. Once the infection has gone, the antigen disappears. A positive antigen test result is considered very accurate, but there's an increased chance of false-negative results due to testing procedures, meaning it's possible to be infected with the virus but have negative antigen test results. Therefore, antigen tests aren't as sensitive as molecular tests (below). But antigen tests are readily available in that they already exist for strep throat, influenza, tuberculosis, HIV, and other infectious diseases.

Viral (antigen) tests identify the virus in samples from your respiratory system, such as a swab from the inside of your nose. It is possible to isolate the coronavirus from respiratory secretions, blood, urine, and fecal samples for diagnostic testing. Clinically, infections can be diagnosed with respiratory viral panels that are widely commercially available.

5.2.2 Molecular genetic test (PCR test)

This test detects genetic material of the virus using a lab technique called PCR. Also called a PCR test, a healthcare worker collects fluid from a nasal or throat swab or from saliva. Results may be available in minutes if analyzed

onsite or 1–2 days if sent to an outside lab. Molecular tests are considered very accurate (kind of the gold standard) when properly performed by a healthcare professional, but the newer rapid tests appear to miss some cases. The FDA also approved certain COVID-19 at-home test kits, available only with doctor approval. It can be done with a nasal swab kit or a saliva kit. The sample is mailed to a lab for testing. The FDA warns consumers against buying unapproved home tests, because they may be inaccurate and unsafe.

5.2.3 Antibody testing

Antibody tests check a person's blood by looking for antibodies, that may (or may not) tell if the person had a past infection with the coronavirus. Antibodies are proteins that help fight off the infections and thus can provide immunity and protection against getting the infection again (see Chapter 2). Neutralizing antibodies are specific to an antigen (the virus) and thus provide protection only against the specific disease associated with the antigen (in the case of coronavirus as the antigen, the disease being COVID-19). If the person is exposed to the antigen (coronavirus) again, the antibodies produce "memory" toward the disease (remember the B_M cells and anamnestic protection from back in Chapter 1?). However, there are increasing reports of reinfection with the novel coronavirus suggesting that some coronavirus antibodies may not be neutralizing.

Except in instances in which viral testing is delayed, antibody tests should not be used to diagnose a current COVID-19 infection. An antibody test may not show if you have a current COVID-19 infection because it can take 1–3 weeks after infection for your body to make antibodies. To see if you are currently infected, you need a viral (antigen) test.

5.2.4 Genome sequencing [38]

Genome sequencing is essentially determining the order of chemical "bases" of a DNA molecule (see Chapter 3, page 55). Sequencing efforts in the early stages of COVID-19 helped determine the structure of the virus, as well as its early mutations that increased transmissibility and produced a massive pandemic. Sequencing in late 2020 and the beginning of 2021 began identifying the more virulent and transmissible variants in the UK, South Africa, and finally the highly virulent delta variant in the U.S.

In the United States, sequencing was conducted in only 0.3% of tested samples ranking it 43rd (embarrassing) in the global genome sequencing database project. Genome sequencing is essential in identifying, dealing with and tracking the spread of the newly emerging and increasingly virulent and transmissible SARS-CoV-2 variants. Such transmissibility suggests that these variants are already widespread. The CDC announced doubling of the U.S. sequencing effort that is expected to find more variants in that the virus can

mutate every time it transmits to a new host. With enough mutations, a strain may be able to evade current vaccines. If a vaccine-resistant strain is identified in the future, vaccine research will have to be directed through evidence-based data. That evidence will only come from genome sequencing. (You can tell that this prescient paragraph was written prior to the delta variant surge in 2021.)

5.3 Treatment and management strategies [39]

Care for coronavirus patients is supportive in nature and may include rest, supplemental oxygen, fluid administration, and, for critically ill patients, being managed in intensive care units and receiving rescue therapies such as extracorporeal membrane oxygenation (pulmonary ventilation). Stringent infection control is critical to preventing transmission to caretakers, healthcare workers, and other patients. Droplet precautions (e.g., personal protective equipment (PPE) including surgical or procedure masks, gown, gloves, and face shields) are indicated during the treatment of all coronavirus patients, and such protocols for droplet-spread respiratory viruses that are part of hospital infection control practices. Additional respiratory precautions may also be appropriate during aerosol-generating procedures.

At the time of the writing of this Chapter on COVID-19, treatment and management strategies continue to grow, some proving effective and some ineffective. In that this is being written at the height of the pandemic (early 2021), it must be considered a prospective view of appropriate treatment and management as recommended by the medical experts guiding us through this difficult period. It will be of interest to the readers and future planners in the months and years ahead, to evaluate retrospectively which of these treatment and management approaches proved to be of most value. Hopefully, it will be an insightful lesson to future generations in their preparedness and response to epidemics and pandemics they may face. Future readers of this book will be able to retrospectively assess the strengths and weaknesses of each.

5.3.1 General measures

5.3.1.1 Basic preventive steps

(1) Shelter-in-place or "self-isolation" (remain in your home with only absolutely necessary outdoor activities);

(2) Social distancing (separation of >6 to 10 feet between people);

(3) Avoid gatherings of more than 5 to 10 people;

(4) Wash your hands copiously and frequently;

(5) Face masks (at first CDC and surgeon general suggest for use only if infected, now it's strongly recommended for fulltime use—N90 masks preferable);

(6) If symptoms occur (fever, cough, chills, aches, and pains), get tested and if positive, selfquarantine for minimum 14 days and retest ×2 before assuming normal activities;

(7) If symptoms advance over 2–3 days, seek medical attention.

5.3.1.2 Mitigation

This process includes procedures and policies to reduce risks of infectious spread. Results of mitigation are measured by "flattening the (modeling) curve." This is an inverted bell shape curve with the x-axis representing time and the y-axis representing number of cases [40]. Not pretty during surges!

5.3.1.3 Contact tracing

Epidemiologists, or "disease detectives," start with the index patient, sometimes called "patient zero." Depending on what they already know about that patient's condition—how the disease is spread, its natural history, what symptoms it causes—interview the patient to learn about their movements and identify all close contacts (persons, places, and things). Based on the answers, public health workers contact each associated person to explain their risk, offer screening for the infection and conduct regular monitoring for symptoms of the infection. As mentioned above, this important public health measure is not progressing well due to limited "tracer personnel" and public resistance to sharing information.

5.3.1.4 Modeling

(1) Study the mechanisms by which disease is spreading (i.e., investigative epidemiologic and public health analysis);

(2) Monitor (graphically) through testing positive case volumes, death rates, and other vital statistics;
- Mortality;
- New cases (total and per 100,000 pop.);
- Hospital admissions;
- Intensive care admittance;
- extracorporeal membrane oxygenation (pulmonary ventilation)

(3) Predict the future course of an outbreak; and

(4) Evaluate strategies to control a pandemic. These strategies are developed through the modeling data described above.

5.3.2 Immunotherapeutics

It appears that SARS-CoV-2 infection has two phases. The early phase includes the infectious stage (approxiamtely 3 to 9 days) where the virus is replicating followed by the later stage (7 to 21 days) where the disease is driven by an exaggerated immune/inflammatory response to the virus. This is

the phase that leads to tissue damage, organ failures and oftentimes, the post-COVID syndrome or long haulers syndrome. Based on this understanding, and as has been shown in clinical outcomes, antiviral therapies would have the greatest effect early in the course of time and unlikely to be more beneficial in the later stages of the disease.

(You might want to re-read the therapeutics and immunotherapies discussed in Chapters 5 and 6. Other than vaccines [which in essence are themselves immunotherapeutics], these immunomodulating drugs, particularly the monoclonal antibodies [see Chapter 5, Table 5.7] are proving to be [in conjunction with proper public health measures] a significant hope for the future.)

5.3.2.1 Monoclonal antibodies

From the previous chapters, I'm sure by now, you appreciate the significant benefits monoclonal antibodies (any drug with the name suffix, "… mab") are providing to humanity (thanks to "our immune friend" and heroes like Dr. Fauci, Dr. Francis Collins [NIH Director] and others), so too are its benefits being recognized in COVID-19 therapy. As previously described, these are laboratory engineered antibodies used to mimic the immune system's own antibodies for a specific antigen (see Chapters 2 and 4).

In the early stages, usually when the patient is at home, monoclonal antibodies have proven effective in limiting the infectious stage. The most effective monoclonals (as of September 2021) have been Bamlanivimab and the combination of casirivimab plus imdevimab [41]. Based on clinical trials and their performance on early infections, the FDA issued EUAs in November 2020 [42] for use in treatment of outpatients with mild to moderate COVID-19 who are high risk for progressing to severe disease and/or hospitalization. Other monoclonal antibodies including interleukin-6 receptor antagonists (suppressing ILK-6), tocilizumab and sarilumab have also been shown to improve outcomes and survival rates, especially when used in combinations.

Regeneron, the combination of casirivimab and imdevimab (together called REGN-COV2), is directed against the spike protein of SARS-CoV-23 for use in patients with early infection. (As effective as they are, monoclonal antibodies should not be mistaken or substituted for vaccine properties which are preventative. Monoclonal antibodies are therapeutics and used asap upon infection.) An August 2021 clinical trial showed 70% improvement in outpatients who had presented within 7 days after the onset of symptoms and within 72 h after a positive result on quantitative RT-PCR testing of nasopharyngeal swab samples. Key end points were achieved including a reduction from baseline viral load from day 1 through day 7 and reduction in percentage of patients who had at least one Covid-19—related medically attended visit through day 29. One state in the U.S.

(Florida) recommends this monoclonal treatment (free) in favor of vaccination (at 95% effective). First off, it is being used broadly in place of vaccination which, as described above, is inappropriate use. Second, the cost of one Regeneron treatment (infusion plus facility costs) ranges anywhere from $1250 to $6500 versus $20 for one vaccination [43]. It's hard to believe that such a vaccine strategy alternative is not in the best interest of the public health. Unfortunately, sometimes politicians preempt science.

San Francisco-based Vir Biotechnology has identified several human neutralizing monoclonal antibody (mab) candidates against SARS-CoV-2. The antibody's ability to neutralize the SARS-CoV-2 live virus has been confirmed in two different laboratories. It binds to an epitope, the specific site on the viral antigen molecule that is also seen on the SARS-CoV-1 virus that causes SARS. This means the antigen is highly conserved and less likely to disappear should the viruses mutate or develop resistance to the antibody [44].

These results complement the findings of a trial that evaluated three doses (700 mg, 2800 mg, and 7000 mg) of a single monoclonal antibody, bamlanivimab (LY-CoV555). Reductions in nasopharyngeal RNA levels of SARS-CoV-2 were detected after 3 days of treatment in all groups with a greater decline in the combined-dose bamlanivimab group than in the placebo group. The results suggest that monoclonal antibodies, when administered early in the infectious period serve as an effective antiviral agent to reduce the viral load in the nasopharynx. The effects of monoclonal antibodies and other drugs on viral load may prove to be an important criterion for the development of agents to treat early Covid-19 [45].

5.3.2.2 Convalescent plasma (serum)
Plasma can be collected from the blood of patients who have recovered from COVID-19. The red and white blood cells are separated and put back into the donor's bloodstream while the blood plasma, rich with virus-fighting antibodies is kept aside. In one such experiment, 403 monoclonal antibodies were isolated from three convalescent COVID-19 patients. They showed that the patients had strong immune responses against the infecting viral protein, a complex that binds to receptors on the host cell. From this information, a subset of antibodies from the serum was able to neutralize the virus. Nonetheless, late 2020 results of convalescent plasma have proven equivocal [46].

5.3.2.3 Dexamethasone (and corticosteroids)
As discussed above and in detail in Chapter 4, chronic inflammatory organ diseases (e.g., heart, lungs, kidneys) may occur in severe Covid-19, with a subgroup of patients having markedly elevated levels of inflammatory

biomarkers. Several therapeutic interventions have been proposed to mitigate inflammatory organ injury in viral pneumonia including glucocorticoids (i.e., dexamethasone). Glucocorticoids have been widely used in syndromes closely related to Covid-19, including SARS, MERS, severe influenza, and community-acquired pneumonia. However, the evidence to support or discourage the use of glucocorticoids under these conditions is inconclusive. In patients hospitalized with Covid-19, the use of dexamethasone resulted in lower 28-day mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization but not among those receiving no respiratory support. Other steroids are also beginning to show some promising results during early stages of the disease.

A readily available, inexpensive corticosteroid, dexamethasone, has been found to improve survival in hospitalized patients who require supplemental oxygen, and its greatest effect observed in patients who require mechanical ventilation. Therefore, the use of dexamethasone is strongly recommended for seriously ill patients in the hospital setting but not advised for patients not on ventilation [47].

5.3.2.4 Antiviral drugs [48]

A universal vaccine effective against all viruses including SARS-CoV-2 is the holy grail of immunology and vaccinology. But short of that, current vaccines are not sufficient or even predictable in treating viruses, especially their mutations and variant infections of RNA viruses (like coronaviruses). Different from recombinant and mRNA vaccines that manipulate the body's immune response to the virus and its genetics, antiviral drugs attempt to boost the immune defense to inhibit viral development. They block receptors so viruses cannot bind to and enter healthy cells and they lower the amount of active virus in the body. Antivirals may be broad spectrum and treat a variety of viruses while others target a specific viral protein to disable the virus and remain nontoxic to the host cells. Thus, most antivirals are considered relatively harmless to the host, and thus can be used aggressively (large dosages) to treat infections (e.g., acyclovir) [51].

Among the growing list of antiviral drugs for COVID-19, Molnupiravir by Merck/Ridgeback Biotherapeutics and Paxlovid by Pfizer (see below) have proven effective in certain forms and variants of the SARS-CoV-2 virus and have been given FDA emergency use authorization approved. Other drugs, FDA approved for uses other than antiviral therapy, are being promoted as having antiviral qualities based on anecdotal evidence. Scientific-based studies have proven these drugs to be questionable or wholly ineffective in the treatment of coronaviruses and have even shown adverse effects in their use for such therapy (see below). These drugs include (but are not limited to) hydroxychloroquine (combined with the antibiotic Azithromycin) and Ivermectin (an antiparasitic drug use mostly in veterinary medicine).

5.3.2.4.1 Paxlovid (Pfizer). Paxlovid (nirmatrelvir/ritonavir) is a protease inhibitor, similar to those used against HIV. SARS-CoV-2 uses its cellular enzymes (protease) to replicate its RNA-containing "polyproteins" (see Life Cycle, page 189). By blocking the enzyme's activity, the drug prevents the production of new, functional viral particles. Paxlovid is quickly broken down in the body, so it needs a booster in the form of a second drug called ritonavir (also a protease inhibitor) to keep it active for a longer period in the host. Notwithstanding potential drug–drug interactions, these drugs have shown significant promise with a 90% reduction in hospitalization rates [49].

5.3.2.4.2 Molnupiravir (Merck/Ridgeback Biotherapeutics). Molnupiravir is known as a nucleoside analogue. It mimics one of the RNA proteins that make up SARS-CoV-2. Once inside cells, the virus uses a polymerase enzyme to attach to its RNA and assemble them into new copies of viral RNA. The virus needs a template for construction of new viral RNA and molnupiravir interrupts this template and causes the virus to continuously mutate until it virtually destroys itself with defective genetic material. Due to some side effects from the drug, it is used only in high-risk patients with advanced disease [50].

5.3.2.4.3 Remdesivir. This antiviral drug is thought to interfere with the mechanism that coronavirus uses to make copies of itself (see Fig. 7.1 and discussion on Life Cycle above). Scientists are still working out exactly how that occurs. A preliminary report published in The New England Journal of Medicine showed that the drug shortened recovery time for people with COVID-19 from an average of 15 days to about 11 days. The drug also seems to show increased benefits when used in combination therapies (e.g., with Baricitinib and monoclonal antibodies).

In the advanced stages of COVID-19, Remdesivir is the only FDA approved drug. It is recommended for use in hospitalized patients who require supplemental oxygen. However, it is not routinely recommended for patients who require mechanical ventilation because of a lack of data showing any benefit at this advanced stage of the disease. The addition of Baricitinib with remdesivir (i.e., a combination) proved to be superior to Remdesivir alone in reducing recovery time and accelerating improvement in clinical status among hospitalized Covid-19 patients [51].

5.3.2.4.4 Hydroxychloroquine (Plaquenil) combined with azithromycin (Zithromax). A small sample survey showed that hydroxychloroquine treatment (a biologic used for malaria and lupus) is associated with viral load reduction in COVID-19 patients and its effect is reinforced by azithromycin (an antibiotic). A study reported in the New England Journal of Medicine concludes that results do not support the use of hydroxychloroquine at present, outside randomized clinical trials testing its efficacy. Further work is warranted to determine if these compounds could be useful as chemoprophylaxis to prevent the transmission of the virus without significant adverse

effects. Continuing studies are proving increasingly negative and demonstrating more potential adverse effects than benefits. These clinical findings were never considered when hydroxychloroquine was enthusiastically promoted by a U.S. political figure (a president to be exact) with no medical knowledge [52].

5.3.2.4.5 Ivermectin. This drug is approved by the FDA to treat people with intestinal strongyloidiasis and onchocerciasis, two conditions caused by parasitic worms. In addition, some topical forms of ivermectin are approved to treat external parasites like head lice and for skin conditions such as rosacea. Some forms of animal ivermectin are approved to prevent heartworm disease and treat certain internal and external parasites. It is important to note that these products are different from the ones for people, and safe only when used in animals as prescribed. Currently available data do not show ivermectin is effective against COVID-19 [53].
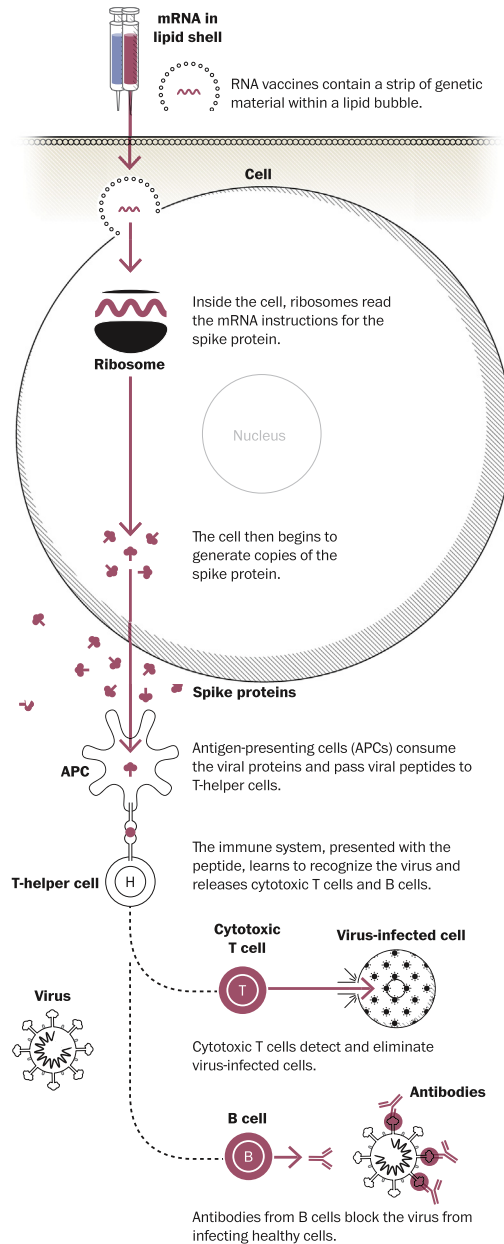
5.3.2.4.6 Other developing antiviral drugs for COVID-19. Besides continuing development of new vaccines to address SARS-CoV2 resistant variants, new antiviral drugs will continue to be developed as adjunctive therapies to vaccines and any other evolving immunotherapeutic measures to COVID-19. No doubt by the time you are reading this, beyond all of the drugs and treatment modalities listed below and mentioned in this section, there will be an array of new therapeutic measures being implemented for patient treatments and hopefully, for prevention [54].

- Interferons: antiviral cytokines under investigation;
- Lopinavir/Ritonavir and other HIV protease inhibitors
- Nitazoxanide: antiparasitic drug under investigation
- Lopinavir/Ritonavir and other HIV protease inhibitors
- Fluvoxamine: an antidepressant pill as an antiinflammatory
- Budesonide: an inhaled steroid used to prevent asthma symptoms

5.3.3 Vaccines [55]

By definition, a vaccine is a biological preparation that provides active, innate, and adaptive immunity to a particular infectious disease (e.g., measles, flu, SARS-CoV-2) by stimulating antibodies or manipulating messenger RNA (mRNA) to attack the source of the infection. The traditional approach has been to develop an agent that resembles the disease-causing microorganism made from weakened or killed (called an attenuated form or viral vector) fragments of the offending microbe, its toxins, or one of its surface proteins. This process induces a subclinical antigen stimulus that is recognized by the immune system that produces corresponding immune cells and antibodies, but without inducing clinical disease. In the novel coronavirus, the spike protein was targeted for most of the vaccine human clinical trials. Research centered on how the immune system, particularly B and T cells, responded to the spike protein and revealed the classic B cell response of producing the antibodies that recognize SARS-CoV-2, while T cells play their classic role in supporting the development of the B-cell response.

mRNA in lipid shell

RNA vaccines contain a strip of genetic material within a lipid bubble.

Cell

Inside the cell, ribosomes read the mRNA instructions for the spike protein.

Ribosome

Nucleus

The cell then begins to generate copies of the spike protein.

Spike proteins

APC

Antigen-presenting cells (APCs) consume the viral proteins and pass viral peptides to T-helper cells.

The immune system, presented with the peptide, learns to recognize the virus and releases cytotoxic T cells and B cells.

T-helper cell H

Cytotoxic T cell   **Virus-infected cell**

Virus

Cytotoxic T cells detect and eliminate virus-infected cells.

Antibodies

B cell

Antibodies from B cells block the virus from infecting healthy cells.

Sources: National Institutes of Health; Centers for Disease Control and Prevention; Nature Research; Columbia University

Figure 7.2
mRNA (messenger RNA) vaccine. An mRNA molecule imbibed with an engineered strip of genetic material to mimic the RNA of the virus. It is injected into the patient and generates copies of the spike protein which produce APCs that produce an antibody immune response that destroys the virus. *Washington Post. 2020.*

Vaccine research teams also work on the development of vaccines using the virus itself, in a weakened or inactivated form while some use viral-vectors wherein a virus such as measles or adenovirus (recombinant serotype Ad5) is genetically engineered so that it can produce or "mimic" corresponding proteins to the target microbe. The teams that showed the greatest success (as of early 2021) against the RNA novel coronavirus used genetic instructions in the form of messenger RNA (mRNA) to prompt a subclinical immune response from the virus.

5.3.3.1 mRNA vaccines [56]
The science of the mRNA vaccine is an elegant model of immunology and genetics technology (Fig. 7.2). An RNA virus (e.g., novel coronavirus) means its genetic material is encoded in RNA rather than DNA. Once the virus is inside our cells, it releases its RNA and makes long viral proteins to compromise the immune system (see page 190 and Fig. 7.1). Inside the cells, the cell ribosomes read the mRNA instructions for the spike protein and the cell begins to generate copies of it. Genomic transcription and translation (see Chapter 3, Genetics and Genomics, page 45) produce copies of the virus' surface receptors (spike proteins, in the case of novel coronavirus). Then, as the patient's immune system recognizes a "foreign invader," it initiates an APC/ T_H and T_C response (remember those from Chapter 1?) that release T_H cells that generate cytotoxic T_C and B cells (if you'd like, go back to Chapter 1, Fig. 1.3 and 1.4 to review this innate immune process). The T_C cells go to work doing their job of phagocytizing the virus while the B cells generate antibodies that bind and block the virus from infecting healthy cells [57]. Goodbye virus…at least for 6 to 12 months they are projecting at which time we will probably be looking at COVID-19 booster shots.

One of the weapons in our cells is an RNA surveillance mechanism called nonsense-mediated mRNA decay (NMD) that protects us from many genetic mutations that could cause disease. The genome of COVID-19 is a positive-sense, single-stranded RNA that can evade NMD and prevent it from degrading RNA by producing proteins that interacts with certain proteins that modify the chemical structure of RNA. With the progression of new viral strains, the mRNA vaccines can be easily genetically reprogrammed to recognize mutant viral strains (called variants) and allow for the rapid development (within weeks) of second-generation vaccines that directly target processes critical to a virus's life cycle [57].

The first vaccines approved by the FDA in early 2021 were the mRNA (messenger RNA) technology that had been in development for a number of years but not yet used or approved. Two pharmaceutical manufacturers, Pfizer and Moderna, were the first to produce a viable product for delivery, distribution, and storage. All three of those aspects of successful production proved as challenging as the science itself. Storage was the major issue in

that Pfizer's product needed storage at -94°F and Moderna -50°F. Since initial distribution, those temperatures were moderated (decreased) after extended testing. But the logistics of distribution and delivering sufficient drug and associated requirements (syringes, needles, and trained vaccinators) in most countries proved problematic, especially given the need for two shots given 3 weeks apart.

Subsequent vaccines (mRNA and viral vector technologies) requiring normal storage temperatures and only one shot have been subsequently (as of this writing date) approved and introduced. Success in this type of mRNA vaccine proved 95% effective in December 2020 and led to worldwide use of Pfizer's BNT162b2 and Moderna's ChAdOx1 mRNA vaccines since 2021. This form of mRNA vaccine has also proven effective against variants of the novel coronavirus [59].

AI and immunoinformatics (see below) play a central role in vaccines by suggesting components understanding viral protein structures, and helping medical researchers hunt through tens of thousands of relevant research papers at an unprecedented pace. AI supported preclinical studies in mice of a candidate vaccine based on this spike protein are already underway at NIH's Vaccine Research Center (VRC) [58]. But there will be many more steps after that to test safety and efficacy, and then to scale up to produce millions of doses. National Institute of Allergy and Infectious Diseases is working with numerous biotechnology companies (AstraZeneca, Pfizer, J&J, Moderna, et al.) to use the latest findings developed in vaccine research using messenger RNA (mRNA), molecules that serve as templates for making proteins. The goal is to direct the body to produce a spike protein in such a way to elicit an immune response and the production of antibodies. Other forms of vaccine candidates are also in preclinical development.

5.3.3.2 CRISPR-Cas13 and RNA screening [60]
A new Cas13 RNA screen (vs. Cas 9 from Chapter 6, page 168) has been developed to establish guide RNAs for the COVID-19 coronavirus and human RNA segments that could be used in vaccines, therapeutics, and diagnostics. Similar to CRISPR-Cas9 (see Chapter 6, page 168), a novel Cas13-based editing tool enables researchers to target mRNA (vs. DNA for the Cas9 enzyme) and knockout genes without altering the genome. Using the CRISPR-Cas13 enzyme, researchers have created a genetic screen for RNA, currently designed for use on humans, that they say could also be used on RNA containing viruses and bacteria.

The developers have used their parallel-screening technique to create optimal guide RNAs for the SARS-CoV-2 coronavirus that could be used for future detection and therapeutic applications. The platform is optimized to

run massively parallel genetic screens at the RNA level in human cells because it is based on the CRISPR-Cas13 enzyme that targets RNA instead of DNA. The data are collected by targeting thousands of different sites in human RNA transcripts to create a predictive model to expedite identification of the most effective Cas13 guide RNAs.

5.3.4 Vaccination (immunization)

Vaccination is the act of getting a vaccine, usually in the form of an injection into the arm of a person (immunization) to protect against a disease. Testing for an effective vaccine begins with giving the vaccine to animals such as mice or monkeys to see if it produces an immune response. Then Phase 1 vaccinates a small number of people to test safety and dosage as well as to confirm that it stimulates the immune system. Phase 2 includes hundreds of people split into groups (viral injected and placebo, called a double-blind study where members of the two groups are kept unknown, "blind" to the researchers), such as children and the elderly, to see if the vaccine acts differently in them as well as safety and ability to stimulate the immune system. Phase 3 injects the vaccine into thousands of people (again, two groups) to see how many become infected, compared with volunteers who received a placebo. These trials can determine any rare side effects that might be missed in earlier studies.

Finally, if the vaccine protects against the coronavirus in at least 50% of vaccinated people it is considered effective and regulators decide whether to approve the vaccine or not. During a pandemic (or any such studies), a vaccine trial may be terminated if the test group is demonstrating too negative a result. Conversely, if the test results are proving highly effective (much better result in the test group that the placebo group), the FDA may issue an EUA (emergency utilization authorization) even before getting formal approval. The mRNA vaccines (Pfizer and Moderna) proved highly effective at 95% and 94%, respectively, and received immediate EUA in January 2021 [61].

At least seven teams are developing vaccines using the virus itself (J&J just got EUA for a viral vector vaccine with 75% effectivity and AstraZeneca received UK approval), in a weakened or inactivated form. Around 25 groups say they are working on viral-vector vaccines. A virus such as measles or adenovirus (recombinant serotype Ad5) is genetically engineered so that it can produce coronavirus proteins in the body. At least 20 teams are aiming to use genetic instructions (in the form of DNA or RNA) for a coronavirus protein that prompts an immune response. Many researchers are now experimenting with injecting coronavirus proteins directly into the body to mimic the coronavirus's outer coat. When you read this Chapter, it's most likely you will know.

5.3.4.1 R naught (R^O or R0) and herd immunity

The concept of herd immunity is an epidemiological formula in which a sufficient amount of people are immunized or vaccinated against a pathogen, thus reducing the rate of infection throughout the population. The vaccination levels must produce a threshold called the "R-Naught" or R_O (The SIR ["susceptible-infectious-recovered"]) formulation, a factor that determines the transmissibility of the pathogen. It denotes the average number of secondary cases of an infectious disease that 1 case would generate in a completely susceptible population. That is, when one infected person infects greater than one other person, a potential exponential increase in infections results leading to an epidemic or pandemic. If, however, transmission on average remains below an R_O of one person, this will result in a decreasing spread in infection and eventually into a majority of the population (an estimated 70%–80% needed) to produce "herd immunity."

In the absence of a vaccine, developing herd immunity to an infectious agent requires large amounts of people actually being infected, developing antibodies to the infectious agent and thus becoming immunized against future infection. Scientists are not always certain if this immunity is permanent or for how long it might last. But even assuming that immunity is long-lasting, a very large number of people must be infected to reach the 70%–80% herd immunity threshold required. During this process, mortality of certain infections like SARS-CoV-2 could reach unacceptable levels as occurred in Sweden where herd immunity was aspired to prematurely [62].

Nor does a pathogen magically disappear when the herd immunity threshold is reached. Rather, it only means that transmission begins to slow down and that a new epidemic is unlikely to start up again. An uncontrolled pandemic could continue for months after herd immunity is reached, potentially infecting many more millions in the process. These additional infections are what epidemiologists refer to as "overshoot." [63].

5.3.4.2 Human vaccines project

Researchers are comprehensively genetically sequencing the human immune (the "immunome") system, a system billions of times larger than the human genome. The goal is to encode the genes (the antibody-encoding genes—see Chapter 1, page 16) responsible for circulating B cell receptors. This can provide potentially new antibody targets for vaccines and therapeutics that work across populations. The Human Vaccines Project seeks to define the genetic predisposition of people's ability to respond and adapt to an immense range of diseases [64].

The SARS-CoV-2 COVID-19 pandemic will certainly expedite further progress on this critical area of clinical research. The study specifically looks at one part of the adaptive immune system, the circulating B-cell receptors that are

responsible for the production of antibodies, considered the primary determinant of immunity in people. The receptors form unique sequences of nucleotides (DNA base compounds) known as receptor "clonotypes." [65] This creates a small number of genes that can lead to an incredible diversity of receptors, allowing the immune system to recognize almost any new pathogen (a little complicated, but give it a reread and you'll see what a great potential it has).

This Project marks a crucial step toward understanding how the human immune system works, setting the stage for developing next-generation health products, drugs, and vaccines through the convergence of genomics and immune monitoring technologies with machine learning and artificial intelligence (AI) [66].

5.3.4.3 A vaccine epitaph [67]

Finally, a sad chapter in the U.S. history of vaccinations lingers in the minds and hearts of people of color. It has generated a mistrust of the medical system among some Black Americans regarding vaccination and it produced a stark disparity in morbidity and mortality for those who got COVID-19 vaccinations early in the U.S. As of February 2021, more than 60% of Caucasian American were vaccinated versus roughly 6% of African Americans. This mistrust is rooted in the infamous study of a vaccine for syphilis that left Black men in Tuskegee, Ala., to suffer from the disease. In 1932, the U.S. Public Health Service recruited hundreds of Black men as human subjects for the study (399 men with syphilis and 201 without).

The researchers offered free meals and checkups, but never explained that participants would be human subjects in a study designed to withhold medical treatment. "I think a part of the challenge is that there's still considerable anxiety about the vaccine," says Amir Farooqi, director of the Central Alabama VA. Dr. Reuben Warren, director of Tuskegee University's bioethics center, notes the mistrust of the healthcare system among African Americans is "both historical and current."

6. Immunoinformatics (computational immunology) [68]

The explosion of new immunological data through increased research in understanding the immune system, particularly in infectious disease pathogenesis and the application of the knowledge from bioinformatics, has led to a better understanding of the importance of the immune system through immunoinformatics (computational immunology). Through increased knowledge of the immune system, AI research, and the cost-effective, specific, and effective approaches like *in silico* immunoinformatics (scientific experimentation and research conducted or produced by means of computer

modeling or computer simulation), the concerns for emerging and potentially resurging diseases caused by pathogenic organisms, antigenic variability/complex lifecycle of pathogens (see Fig. 7.1, life cycle, above) and the need of personalized vaccination can be combated on a molecular level. (Wow! That may have been the longest sentence in this entire manuscript. Maybe the copyeditors will rework it, cause I sure ain't going to try.)

AI and immunoinformatics are being used to better understand the structure of proteins involved in SARS-Cov-2 infection in search for potential treatments and vaccines (perfect example, the mRNA vaccine). Proteins have a three-dimensional structure, that is determined by their genetically encoded amino acid sequence (Next-gen sequencing [NGS] of genetic code), and this structure influences the role and function of the protein. An AI Google DeepMind system called AlphaFold [69] uses amino acid sequencing and protein structure to make predictions to construct a "potential of mean force" that can be used to characterize the protein's shape. This system has been applied to predict the structures of six proteins related to SARS-CoV-2.

In silico immunoinformatics depends on experimental science ("wet lab") to produce raw data for analysis. Thus, its predictions are not formal proofs of any concepts. They do not replace the traditional experimental research methods of actually testing hypotheses. The quality of immunoinformatics predictions depends on the quality of data and the sophistication of the algorithms being used (remember the good old, "garbage in – garbage out" axiom?). Sequence data from high-throughput analysis often contain errors. If the sequences are wrong, or annotations incorrectly, the results from the downstream analysis could be misleading as well (ergo, "garbage in—garbage out"). The future of immunological research will be enhanced by the ability to make discoveries in biologics (e.g., vaccines) more effectively and efficiently through combined AI and *in silico* immunoinformatics with traditional experimental research methods. Notwithstanding the credit certain narcissistic politicians like to take for the rapid development of COVID-19 vaccines, it was the combination of brilliant researchers, AI, and immunoinformatics that brought home the bacon in a desperate COVID-19 human crisis.

7. Epidemiology and public health considerations in COVID-19

7.1 Current epidemiologic considerations

The world celebrated the newly discovered vaccines in early 2021 that began the mitigation and reversal of the COVID-19 pandemic. The suffering and ill-effects of the novel coronavirus have been devastating to the world through

its toll on lives and its ever-increasing death toll, not to mention the crippling economic effects it has had on individuals and governments.

Without a doubt the first hooray must go to the teams of research scientists at companies like Pfizer, Moderna, AstraZeneca, J&J, and others in varying stages of vaccine research. The second cheer should go to the governments of most industrialized countries who prioritized vaccine development with little concerns for costs of such initiatives. Certainly, included in that cheer must be the U.S.'s Operation Warp Speed that committed its best and brightest to answer the clarion call. Whereas, such successes in vaccine development had been experienced historically, none was accomplished in months of the initial and growing spread of the infectious disease. Rather, the world had to wait years, sometimes decades, and sometimes "never."

The very existence of a fertile and brilliant body of worldwide researchers has been applauded for years by an appreciative public who have benefitted from their efforts. Also, accolades must be given to the governments who offered selfless, humanitarian support in the form of leadership, some humble and some perhaps, ego-driven. But perhaps the greatest cheer should go to the contribution made by the immunology, genetic, and AI technologies that provided the research scientists and the tools needed to accomplish the task in record time. Those achievements by the research and scientific community started many years ago with the early work of heroes in immunology and genetic research mentioned throughout this book.

Answers are available to the researchers today within minutes to seconds, thanks to new methods of rapid whole genome sequencing (WGS) [70] and AI big data analytics. Indeed, the scientific work over the past 10−20 years, plus their concentrated efforts over just 6 months are truly the backbone of the success witnessed in the development of the COVID-19 vaccines. We must all be thankful for these accomplishments that have begun to reduce the human suffering and economic devastation brought upon the world from this COVID-19 pandemic. But we must also remain scrupulously vigilant.

Today, the impact of COVID-19 and its rapidly evolving variants portend equal or more disastrous effects than the Spanish Flu of 1918−19, the Asian Flu of 1957−58, the Hong Kong Flu of 2003, and the SARS (SARS-CoV-1 coronavirus) of 2003. SARS-CoV-2 novel coronavirus is a far more contagious member of the coronaviruses (CoVs), the large family of enveloped, positive-strand RNA viruses responsible for a substantial portion of upper respiratory tract infections. Many countries (e.g., China, Singapore, Hong Kong, South Korea, Italy, Spain, and the USA) have relied on an extrapolation of classic infection-control and public-health measures similar to those used for SARS-CoV-1 to contain the COVID-19 pandemic. They range from extreme quarantine measures, "shelter-in-place," "social distancing," to painstaking detailed contact tracing with hundreds of contact tracers. However, these

measures may not be effective in the coming years for tackling the scale of COVID-19.

Vertically integrated digital and AI technologies are being introduced for monitoring, surveillance, detection, prevention of COVID-19, and to mitigate its spread and its direct and indirect impact to worldwide healthcare systems. The initial reaction in many countries to COVID-19 is for healthcare facilities to reduce or even cease many clinical services, including closure of clinics and postponement of medical appointments or elective surgeries. However, such strategies cannot be sustained indefinitely if the COVID-19 pandemic extends beyond 6 months. Healthcare systems should plan to use digital technology "virtual clinics" using telehealth consultations with imaging data uploaded from peripheral sites and interpreted remotely. This would ensure that patients continue to receive standard clinical care while reducing physical crowding of patients into hospitals. Chatbots staffed by health professionals can also provide early diagnoses as well as patient education. And blockchain technologies can coordinate hospital, clinics, and pharmacy patient information.

7.2 Public health considerations and recommendations

Undoubtedly, by the time you read this book, the public health literature and more so, programs and research in the epidemiology, bioscience considerations, clinical aspects, and immunological considerations regarding COVID-19 will have proliferated into a major body of new science and "disruptive technologies." Indeed, the reemergence of yet another more virulent SARS-CoV virus and global pandemic will emphasize the ongoing and permanent challenge that infectious diseases pose and the need for global cooperation and preparedness, even during "interim" periods.

A well-done opinion piece written by Michael Gerson in the Washington Post in late February 2021 entitled "Six takeaways from covid-19 that could shape our future," highlights the best public health advise we can "takeaway" from the COVID-19 pandemic [76]

1. We are in a brutal evolutionary struggle between humans and microbial pathogens. The pathogens evolve much faster than we adapt. A new pathogen emerges, on average, every 4—5 years. Covid-19 has been bad, but the larger danger comes from a novel influenza that has a high fatality rate and is highly transmissible before the development of symptoms. There is a 1% yearly probability of an influenza pandemic that could cause six million deaths or more.
2. About 75% of new emerging diseases are zoonotic (originating in animals). Humans amplify that threat in a variety of ways. Our appetites are insatiable for animal protein (e.g., pigs, cows, and god forbid, wet-markets). Deforestation is bringing humans into more frequent

contact with wildlife such as bats (spreading infection). It has been well documented now that popular house pets (dogs and cats) can contract and transmit the coronavirus. Scientists in the Predict program have discovered 1200 animal-borne diseases over the past several years and estimate there may be 700,000 more we don't know about.

3. The world is "a little blue marble" and spreading disease to and from our borders in places such as Brazil, South Africa, or Britain can, as we are seeing with the coronavirus, lead to genetic variants that evade immunity, vaccine, and become potentially deadly. Fighting diseases in one country is not as effective as them being fought "everywhere."

4. The U.S. government failed to provide early and adequate support for testing and contact tracing with SARS-CoV-2. It failed in a timely manner to effectively distribute medical supplies and equipment, standardize epidemic data, and enforce rational triggers for stay-at-home orders and school closings. Such errors resulted in an inadequate response to the COVID-19 pandemic (and unspeakable death). We cannot allow such errors in adherence and commitment to public health guidelines to occur again.

5. A large number (perhaps a majority) of Americans failed to take relatively minor preventive steps such as mask-wearing and social distancing. Part of this was because of a president who consistently played down and politicized the public health crisis. But the problem runs deeper than political ineptitude and resistance to commonsense measures. Epidemiology and public health dictate the necessity of demographic assessments of age, comorbidity, socioeconomic factors, and basic living conditions in a pandemic or for that matter, any health-related issues. How would the U.S. have behaviored if political and societal actions had been more closely aligned and sensitive to these epidemiologic factors and risk of death? It requires conscientious, organized, astute, and empathetic governing to assess specific and generic risks to protect humanity.

6. Finally, calibrating our responses and responsibilities to the urgencies and dangers we face as a society, will protect the public health and improve the well-being of humankind in general. Attention to climate change, pollution, farming, deforestation, wildlife, health, precision health, disease prevention, and providing adequate federal planning, resources, and funding for the next pandemic is our only hope for a better future.

And I'll add one more to this list of takeaways regarding lessons learned from the COVID-19 pandemic regarding our personal health and wellness. A conscious effort toward physical activity and weight management in our lifestyle will bolster host antiviral, immune defense, and improve the vaccine immune response. I have tried to accentuate the importance and value of weight control numerous times throughout this book, but I failed to mention the importance of physical activity as well. A 30- to 60-min regimen of

walking, running, gym workouts, or other physical sports can stimulate the ongoing exchange of important types of white blood cells between the circulation and tissues. Exercise-induced increases in antipathogenic leukocytes may also enhance immunosurveillance, reduce illness risk, and lower systemic inflammation. This pandemic should be a wake-up call to everyone that good health habits, health prevention, proper diet, and exercise are what we need to win this war.

I began this Chapter by reporting on the number of worldwide COVID-19 recorded cases and deaths to date. The number reminds me of a sad saying. "One death is a tragedy—2.5 million is a statistic." We cannot let ourselves become inured to this sad calamity. Maybe if we think of it as 2.5 million personal tragedies (and growing), we'll realize what the world and each of us as caring individuals have endured through this apocalyptic pandemic. Will things get better? Certainly, the development of vaccines and a path toward herd immunity is now our hope. But will we continue to face human tragedies, not statistics, of epic proportions in the future? As I stated at the beginning of this chapter, it is estimated that there are 380 trillion (10^{38}) viruses residing in environmental ecosystems throughout the world. Let us all hope and pray that the applications of immunology, genetic science, AI technologies, and mostly our personal and societal efforts will meet and defeat this public health challenge of infectious disease pandemics and will help humanity create a better place in which we and future generations all can live.

8. Brief research summaries on infectious diseases and COVID-19

(Reference citations for each research study presented below can be found in the corresponding footnote. Also, a listing of available scientific reference sources and databases used by the author are included in the book's Acknowledgments.)

By now, you have probably recognized my strong interest in AI, as I admitted to back in the Preface of the book. As I mentioned, I thought that the majority of readers of this will have some interest in the AI research directly related to the topics we've been discussing. AI research tends to be more practical in its applications than pure bioscience, laboratory research and thus, may be of more interest to readers of this book. I hope you have found some, if not all of the summaries of interest.

1. Forbes Magazine reported on a global AI database company, BlueDot, using an AI-powered algorithm, machine learning, and natural-language processing (NLP) to analyze information from a multitude of sources that can track over a 100 infectious diseases [72].

2. AI is playing an important role in evaluating the pathogenesis, diagnosis, and treatment of the SARS-CoV-2 virus. There is an urgent need to develop a system with AI-based machine learning capacity to analyze and integrate imaging-based, patient-based, clinician-based, and molecular measurements-based data, to fight the outbreak of COVID-19 and enable more efficient responses to unknown infections in the future [73].

3. Vaxign is a reverse vaccinology tool being used with Vaxign-ML machine learning tool to predict COVID-19 vaccine candidates. A study applied the state-of-the-art Vaxign reserve vaccinology and Vaxign-ML machine learning strategies to the entire SARS-CoV-2 proteomes including both structural and nonstructural proteins for vaccine candidate prediction. The results indicate for the first time that many nonstructural proteins could be used as potential vaccine candidates [74].

4. AI technologies are powerful tools against COVID-19 and widely used in combating this pandemic. A survey investigated the main scope and contributions of AI in combating COVID-19 from the aspects of disease detection and diagnosis, virology and pathogenesis, drug and vaccine development, and epidemic and transmission prediction. AI mainly focuses on medical image inspection, genomics, drug development, and transmission prediction, and thus still has great potential in this field [75].

5. On March 16, 2020, the White House issued a call to action for global AI researchers to develop new algorithms and data mining techniques to assist in COVID-19-related research. Within a short period of time, advanced machine learning techniques were developed and implemented to better understand the pattern of viral spread, further improve diagnostic speed and accuracy, develop novel effective therapeutic approaches, and potentially identify the most susceptible people based on personalized genetic and physiological characteristics. This is only the beginning of a permanent role AI will play in global healthcare [76].

6. One of the main challenges in medical microbiology is to develop novel experimental approaches that enable a better understanding of bacterial infections and antimicrobial resistance (especially in light of the COVID-19 pandemic). Today, the use of *in silico* experiments (research conducted by means of computer modeling or computer simulation) jointly with computational and machine learning offer an in depth understanding of systems biology, allowing us to use this knowledge for the prevention, prediction, and control of infectious disease. An in-depth knowledge of host—pathogen—protein interactions, combined with a better understanding of a host's immune response and bacterial fitness, is key determinants for halting infectious diseases and antimicrobial resistance dissemination [77].

7. IoTs (Internet of Things) are providing a platform that allows public-health agencies access to data for monitoring the COVID-19 pandemic.

For example, the "Worldometer" provides a real-time update on the actual number of people known to have COVID-19 worldwide, including daily new cases of the disease, disease distribution by countries and severity of disease (recovered, critical condition or death). Johns Hopkins University's Center for Systems Science and Engineering has also developed a real-time tracking map for following cases of COVID-19 across the world, using the data collected from US CDC, the World Health Organization (WHO), the European Center for Disease Prevention and Control, the Chinese Center for Disease Control and Prevention (China CDC), and the Chinese website DXY [78].

8. Big data are providing opportunities for performing modeling studies of viral activity and for guiding individual country healthcare policymakers to enhance preparation for the outbreak. Using three global databases, WHO International Health Regulations, the State Parties Self-Assessment Annual Reporting Tool, Joint External Evaluation reports and the Infectious Disease Vulnerability Index, health authorities are performing AI modeling studies of "nowcasting" and forecasting COVID-19 disease activity throughout the world for public-health planning and control worldwide [79].

9. When the Covid-19 pandemic enters dangerous new phases, the critical question becomes whether and when to take aggressive public health interventions to slow down the spread of COVID-19. A study was undertaken to develop AI inspired methods for real-time forecasting and evaluating intervention strategies to curb worldwide spread. A modified autoencoder for modeling the transmission dynamics of the epidemics is being developed and applied to the surveillance data of cumulative and new Covid-19 cases and deaths from WHO, as of March 16, 2020. Total peak number of cumulative cases and new cases in the world with later intervention could reach 255,392,154 by January 2021. However, the total peak number of cumulative cases in the world with one-week earlier intervention were reduced to 1,530,276. We observed that delaying intervention for 1 month caused the maximum number of cumulative cases to increase 166.89 times, and the number of deaths increase from 53,560 to 8,938,725. Disastrous consequences if immediate action to intervene are not taken [80].

10. MIT published a paper describing the needed changes in three areas if we want AI to be useful in future pandemics. First, prediction through database companies using a range of NLP algorithms to monitor news outlets and official health-care reports in different languages around the world; second, machine-learning models with large datasets for examining medical images to catch early signs of disease that human doctors miss, from eye disease to heart conditions to cancer; third, identifying cures through big data analysis of drug trials and design algorithms to

highlight biological and molecular structures matching drugs with candidates [81].

11. Advanced deep learning-based algorithms known as the convolutional neural network (CNN) exert a great effect on extracting highly essential features, mostly in terms of medical images. This technique using CT and X-ray image scans has been adopted in most of the recently published articles on the coronavirus with remarkable results. Furthermore, according to this paper, it can be noted and said that deep learning technology has potential clinical applications [82].

12. A new framework has been proposed to detect COVID-19 using built-in smartphone sensors (IoTs). The proposal provides a low-cost solution that ordinary people can use on their smartphones for the virus detection purposes. The designed AI enabled framework reads the smartphone sensors signal measurements to predict the grade of severity of the pneumonia as well as predicting the result of the disease [83].

13. AI and deep learning algorithms are being developed to enhance the detection and diagnosis of COVID-19. The need to provide access to accurate and low-cost tests for the diagnosis of COVID-19 is critical. Such AI algorithms can be used as an initial screening tool for suspected cases so that patients at higher risk could have confirmatory laboratory-based tests and be isolated if necessary. These algorithms could help healthcare providers triage patients with COVID-19 into potentially three groups: the 80% who have mild disease; the 15% who have moderate disease; and the 5% who have severe disease, including those at high risk of mortality. Finally, AI can facilitate the discovery of novel drugs with which to treat COVID-19 [84].

14. Continuing efforts are being made to develop novel diagnostic approaches to COVID-19 using machine learning algorithms. Machine learning-based screening of SARS-CoV-2 assay designs using a CRISPR-based virus detection system (see Cas13 above) is demonstrating high sensitivity and speed. Neural network classifiers have been developed for a large-scale screening of COVID-19 patients based on their distinct respiratory pattern. Iso, a deep-learning based analysis system of thoracic CT images, was constructed for automated detection and monitoring of COVID-19 patients over time. Rapid development of automated diagnostic systems based on AI and machine learning can not only contribute to increased diagnostic accuracy and speed but will also protect healthcare workers by decreasing their contacts with COVID-19 patients [85].

Chapter highlights (key points)

1. A novel coronavirus (SARS-CoV-2), probably zoonotically transferred from a bat to a monkey to a human late in 2019, has produced an infectious pandemic of epic proportions labeled COVID-19.

2. As an RNA virus, SARS-CoV-2 is prone towards mutations resulting in variants (e.g., delta, omicron—so far, as of January 2022) which can be more virulent and contagious than the originating novel coronavirus.

3. Messenger RNA (mRNA) vaccines have been developed (in record time) that seem to provide protection of greater than 95% with periodic booster enhancements.

4. An altered mRNA molecule vaccine with an engineered strip of genetic material to mimic the RNA of the virus is injected into the patient causing (actually "tricking") the spiked protein of the virus to induce neutralizing antibodies that destroy the invading coronavirus.

5. Reaching greater than 70% to 80% of the population ("herd immunity" when a sufficient amount of people are immunized ["R-Naught" $R0 < 1$]) requires comprehensive vaccination, comprehensive testing, masks, and social distancing, along with supplemental protection (boosters) against subsequent spread.

6. Diagnostic testing including antibody testing (to detect active or past infection), PCR antigen testing (to detect active infection), and the gold standard, genetic sequencing to identify the code of the invading virus or any variants.

7. Besides essential vaccination (prevention), antiviral agents to treat infections (most effective early) have been developed using monoclonal antibodies (e.g., Regeneron), convalescent plasma, and modified antiviral drugs (e.g., Remdesivir, Paxlovid, etc.). In late stages, strong corticosteroids (e.g., dexamethasone) have demonstrated some value (but limited and not adequate in some advanced cases).

8. Sadly, but not surprising, theories have been advanced by unqualified sources promoting untested medications (e.g., hydroxychloroquine), even dangerous drugs (e.g., Ivermectin) which desperate or naïve people grasp as "magic bullets."

9. A bright spot regarding COVID-19 (if not the light at the end of the tunnel) is the intense scientific research which is producing new drugs and technologies including new methods of RNA screening of viruses, new vaccines (to address potential variants), new genetic procedures (e.g., CRISPR-Cas13), as well as sophisticated AI immunoinformatics (computational immunology) and precision medicine strategies.

10. There are estimated to be over 380 trillion (10^{38}) viruses on planet earth. It doesnot take much imagination to realize that we will be living with infectious pandemics for a long time to come. Let us hope that

perfidious politicians step aside, that humans do what's right, and that we all pray for science to guide us on a safe path forward.

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