Infections, Pandemics and Immune Defenses

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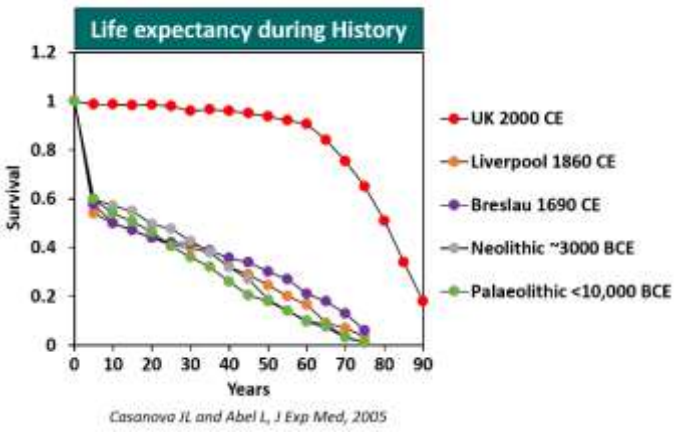
1. Historical Introduction

Life expectancy (i.e. mean duration from birth to death) of humans has hovered around 25 years for most of our presence on earth. Values recovered from skeletons in prehistoric times and at various, well studied historical periods (e.g. Egyptian, Roman, medieval societies) did not point to any significant changes and as recently as in 1860 records from parishes in Liverpool indicate that half of the population there had died off at around 25 to 30 years. Admittedly, the records show that at all times, a

Figure 1



small although significant proportion of individuals lived up to higher ages. As illustrated in Figure 1, a spectacular change in overall life expectancy occurred recently, within some 150 years, up to the values noted at present (see UK 2000, in Figure 1). The reasons of this change will be discussed below.

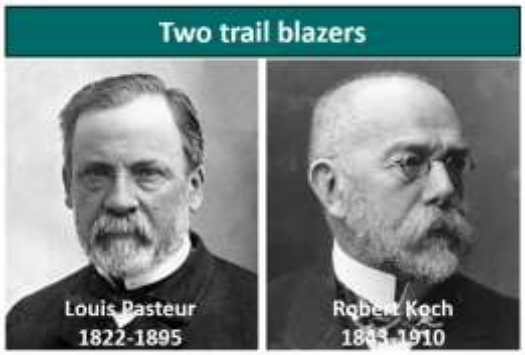
Further, although humanity repeatedly suffered from pandemics, the reasons of these terrible events were unknown and mostly attributed to divine interventions, notably to the idea of divine retribution following major missteps or misbehaviors of societies or possibly just their leaders, as illustrated in the Bible, to quote only one example. These pandemics were mostly terrifying and claimed occasionally the deaths of up to two thirds of a given population. History has recorded for us a series of such pandemics, namely the Athenian plague which devastated Athens at the beginning of the Peloponnesian war in 430 BCE eliminating one third of its population. Nearly one thousand years later, the Justinian plague affected initially the Byzantine Empire, from 542 CE on,

with extensions to the neighboring areas and occasional relapses exacting a death toll estimated at more than 25 million people and inducing a frightening economic crisis. Several hundred years later, in Europe the Black Death pandemic from 1346 on eliminated probably more than 30 million people. Subsequent pandemics were namely the Spanish Flu (estimates are in the range of >50 million deaths) at the end of the First World War (Europe, USA). A more recent pandemic is that of AIDS which claimed so far an estimated 40 million lives and which is still affecting people all over the world. And as we write these lines, a terrifying new pandemic is upsetting the whole planet and has affected, up to now, 200 million persons and induced the death of more than 4 millions.

Is there a common cause of the relatively low life expectancy of humans up to the 19th century and the many highly destructive pandemics? Beyond some common factors such as famine and wars, there is one essential common thread and that is the infection by microbial organisms. At the end of the

17th century a Dutch scientist, Antonie van Leeuwenhoek, invented the microscope which led to the discovery of the totally unknown world of microorganisms. During the second half of the 19th century, that is nearly to say: yesterday, a series of groundbreaking discoveries by physicians, biologists and chemists (see Figure 2 for the portraits of two of the trail blazers: Louis Pasteur and Robert Koch) led to the understanding that these microorganisms were responsible for individual infectious diseases and by spreading from individuals to whole populations, were at the origins of pandemics. The history of these discoveries represents one of the most brilliant episodes of biomedical research - they are at the origins of several new scientific fields, namely those of microbiology and immunology, and have been the subject of several excellent reviews, some of which are given in the Appendix to this article (*see Further*

Figure 2



Pictures taken from internet
(https://fr.wikipedia.org/wiki/Louis_Pasteur
https://en.wikipedia.org/wiki/Robert_Koch)

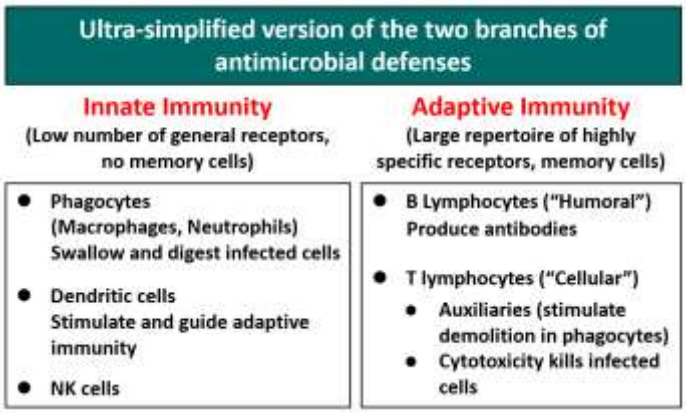
Reading 1).

These discoveries set the stage for a rational development of methods to fight infections and by extension, pandemics. A first arm which became rapidly available was that of hygiene, which in conjunction with asepsis and antisepsis, significantly reduced infections in many instances. The spectacular work of Louis Pasteur, building on some earlier studies by Jenner, a century earlier, set a rationale for vaccination, which became slowly widespread and is estimated to have so far saved the lives of two billion persons, largely of children. During and after the Second World War the use of antibiotics spectacularly reduced the mortality caused by bacteria and fungi in human (and animal) populations. Of note however, antibiotics are not active against viral infections.

In this presentation, I will focus on the human defense reactions against a virus, the SARS (for Severe Acute Respiratory Syndrome) coronavirus at the origin of the pandemic COVID-19 (for Corona Virus Induced Disease-2019). For a better

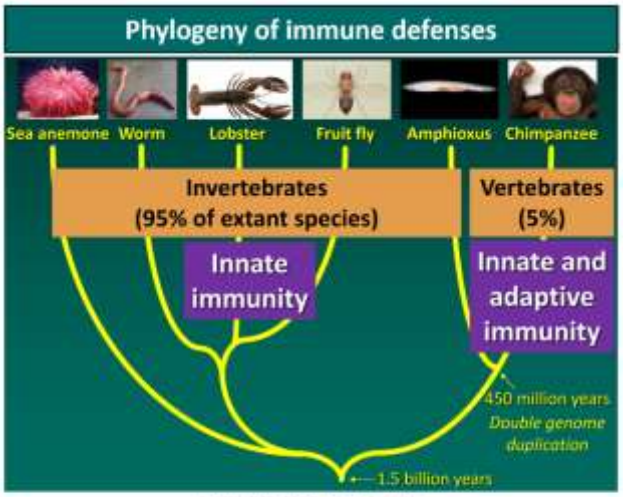
understanding of these reactions, I will first introduce a broad and oversimplified picture of the human immune defenses (Figure 3). These defenses build on two arms referred to as “Innate Immunity” and “Adaptive Immunity”. The basics of innate immunity were discovered by Elyah Metchnikoff at the end of the 19th century and consist primarily in phagocytosis (uptake followed by destruction) of microbes (or dying cells) by dedicated blood cells, such as phagocytes or neutrophils. Other cell types of innate immunity are the dendritic cells and the Natural Killer (NK) cells. Innate immunity serves primarily as a first line defense reaction, and recognizes microbes (etc.) through a restricted number of genome-encoded receptors (see Section 2, present in small numbers for each cell). Innate immunity as such is devoid of specific memory of the initial aggressor. Adaptive Immunity relies on

Figure 3



two types of blood cells, referred to as lymphocytes: the B lymphocytes, the major producers of antibodies and the T lymphocytes, which exert either a cytotoxic function allowing them to kill for instance virus-infected cells, and the so-called helper T lymphocytes, which in particular stimulate antibody production in the B cells. Lymphocytes express special types of receptors (BCR and TCR receptors) which are rearranged from genome fragments and are highly specific for a given antigen structure, with as a rule, one single type of specific receptor per cell. Both types of lymphocytes are endowed with memory of the initial aggressor which allows them to respond with a markedly increased efficiency to a second challenge of the same aggressor. However, as this response involves the proliferation of the responsive lymphocytes, it requires a few days of delay and hence the adaptive immune response is not an immediate reaction (taking normally some 5 to 7 days in humans), in contrast to the innate immune response. As we will see, the adaptive immune response is at the basis of

Figure 4



Pictures taken from internet (https://www.britannica.com/animal/sea-anemone https://www.oregonmetro.gov/tools-living/yard-and-garden/composting/worm-bin-trouble-shooting https://www.bonappetit.com/test-kitchen/cooking-tips/article/how-to-choose-the-best-lobster https://www.theatlantic.com/science/archive/2018/02/fruit-fly-drosophila/553967/ https://www.lindependant.fr/2021/05/12/littoral-mediterraneen-lamphioxus-ce-cousin-lointain-qui-est-pourtant-si-proche-9541409.php https://www.nationalgeographic.com/animals/mammals/facts/chimpanzee)

vaccination. Innate immunity, as illustrated in Figure 4, has appeared early in evolution and has been maintained in all animal species, including of course in humans. Adaptive immunity is restricted to jawed Vertebrates and appeared considerably later, probably some 450 million years ago (in now extinct Placoderms), after a double genome duplication providing considerable possibilities for evolving new structures and functions. A central question, which was already pointed at by Metchnikoff and Ehrlich when they shared the Nobel Prize for Physiology or Medicine in 1908 (Figure 5), was whether the

Figure 5

Pictures taken from internet (https://www.pasteur.fr/en/institut-pasteur/history/ilya-mechnikov-elie-metchnikoff-french-1845-1916 https://www.nobelprize.org/prizes/medicine/1908/ehrlich/biographical/)

appearance of adaptive immunity - with its fantastic repertoire of recognition receptors - had evolved to replace innate immunity or was set to dialogue with innate immunity, and if so, via which molecular mechanisms.

The answer to these questions remained tentative till the early 1990s. In contrast to the brilliant progress which the studies on the characterization of the receptors of adaptive immunity had experienced in the second half of 20th century, the receptors of innate immunity remained poorly understood at that time.

2. Innate Immune Receptors and the Activation of Adaptive Immunity

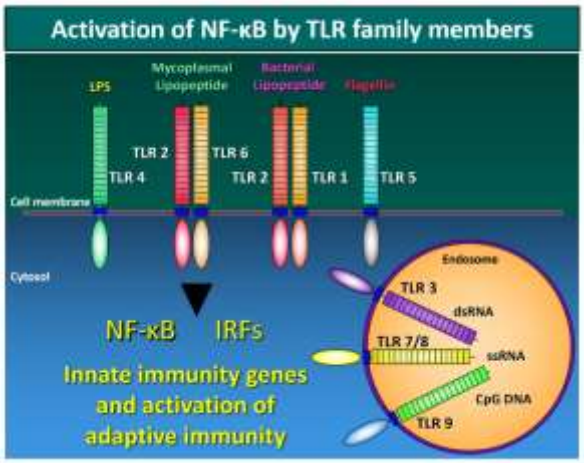
Our group in Strasbourg attacked this problem by addressing an insect model: the fly *Drosophila* (*this section is largely based on studies detailed in: Further Reading 2*). Like all invertebrates, *Drosophila* relies only on innate immunity to confront microbial pathogens. It became rapidly

apparent that in response to an experimentally induced infection, flies produced several families of antimicrobial peptides. Similar molecules have been found since in nearly all animal species which were subsequently investigated: they are primarily membrane disruptive on various sorts of microbes and are essential components of the antimicrobial first line defenses. The promotors of the genes encoding these peptides were systematically found to contain nucleotide sequences conferring inducibility to an essential immune responsive transcriptional activator, named NF-κB by Sen and Baltimore in reference to their initial discovery in the promoters of genes encoding κ light chains in immunoglobulins expressed in B lymphocytes in humans. We went on to show that the NF-κB transactivator was mandatory for the microbe-induced expression of the antimicrobial peptides of the innate immune defense of *Drosophila*. This established a first compelling molecular parallel between an innate immune response in *Drosophila* and an adaptive response in mammals. We

performed subsequently a series of experiments in flies which led to the discovery that a gene cascade was initiated by microbial cell wall components which had appeared in the blood of infected flies and led to the maturation of a precursor ligand which then bound to transmembrane receptors. These receptors were referred to as Tolls (in reference to their initial discovery by Nüsslein-Volhard in a genetic analysis of early embryonic development). Rapidly after the establishment of the role of Toll receptors in the antimicrobial defense of flies, transmembrane receptors similar to the insect immune receptors were identified in human cell line (*see Janeway and Medzhitov in Further Reading 2*) and hereafter have been referred to as TLRs for Toll-like receptors. Importantly, these studies also showed that the activation of these TLRs in the *in vitro* model induced expression of genes of the adaptive immune response. Further, it was shown (*see Poltorak et al. in Further Reading 2*) that the effect of bacterial lipo-polysaccharide on the induction of the cytokine TNF was mediated by a

member of the TLR family. We have by now learned that TLRs are a central group of innate immune receptors both in invertebrates and vertebrates and they are present in the various groups shown in Figure 4. Interestingly, their roles may be relevant for both regulation of development and activation of defense pathways depending on the species and of the state of development. In humans, TLRs are located on the cytoplasmic membrane or in endosomal vesicles (Figure 6). The leucine-rich repeat recognition domains of these transmembrane proteins scan the extracytoplasmic space or the endosomal space for the presence of microbial structures, predominantly but not exclusively lipopeptides, lipopolysaccharides, various forms of RNA and DNA, etc. In response to their binding these microbial structures, they activate signaling cascades in the cytoplasm which lead to the

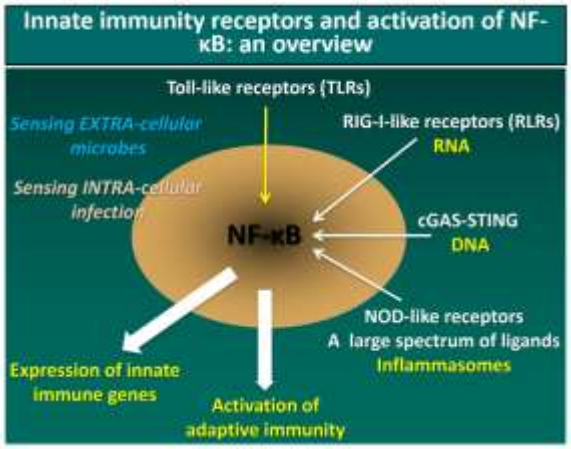
Figure 6



For details, see Takeda K and Akira S in Further Reading 2

stimulation of the transactivator NF-κB and direct the transcription of a variety of immune response genes which will concur to stabilize or to clear the infection. More recent data have unraveled several additional immune receptors (Figure 7), which also activate NF-κB in response to binding microbial patterns (molecules) within the cytoplasm, complementing the roles of TLRs which scan the extracytoplasmic field. These cytosolic receptors are the RIG-I-like receptors (RLRs) which bind to RNA and the DNA binding protein cGAS, and the NOD-like receptors (NLRs). The latter (23 members in humans, absent from *Drosophila*) have a large spectrum of ligands and can form structures called inflammasomes which have major defense activities, namely cleavage of pro-IL1 and pro-IL18 to their active forms IL-1 and IL-18, which upon secretion will contribute to inflammation.

Figure 7



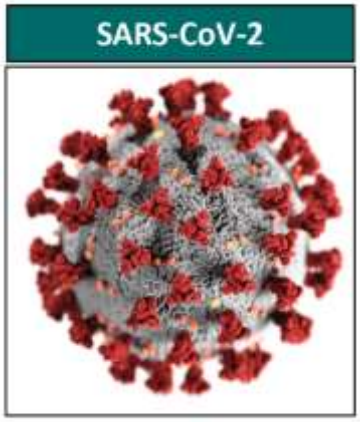
In summary, innate immunity senses the presence of microbes (and other dangers) through a limited number of receptors of microbial structures (patterns) and alerts the organisms to the presence of an infection (or danger resulting from injury, for instance). The response is poorly specific, but globally adapted to the type of aggressor (fungi, Gram-positive or Gram-negative bacteria, viruses, other insults). In vertebrates, in addition, this recognition by the first line defense will activate dendritic cells (which are part of the innate immune system), which in turn can direct the transformation of naïve lymphocytes into effector lymphocytes: the response of these lymphocytes will be highly specific for each lymphocyte towards the initial antigen presented by the dendritic cell and keep a memory of this first encounter allowing for a more intense response in case of reinfection.

3. Immune Defenses against SARS-CoV-2

At the end of 2019/beginning of 2020, a severe

infection of the human airways was detected in Wuhan (central China) and rapidly in other countries and was linked to a coronavirus, now dubbed SARS-CoV-2 (for Severe Acute Respiratory Syndrome-Coronavirus-2, Figure 8). Within a very short time, the virus was sequenced and its sequence made available by its Chinese authors to the international community in early January 2020 and OMS declared in March 2020 that the world was facing a new pandemic.

Figure 8



Picture taken from internet (https://covid-19.sciensano.be/fr)

SARS-CoV-2 is a single stranded enveloped RNA virus entering humans via the airways into the lungs and causes damage not only in the airway system and the lungs, but also, depending on the patients, to the cardiovascular system, the kidneys, the central nervous system etc. The symptoms of the disease, referred to as COVID-19, are fever, cough, myalgia, agousia, dyspnea and acute respiratory distress which can lead to death. Importantly, 40% of the infected persons are asymptomatic and are mostly

not aware that they carry the virus (although they can propagate it), 40% have mild symptoms, of whom one fifth will eventually require hospitalization namely in intense care units. About 1% to 2% of the infected population eventually will succumb to the disease.

Of note, the negative evolution is particularly observed in elderly persons presenting comorbidities (namely obesity, diabetes, cardiovascular conditions) or undergoing immunosuppression treatments. At the time of writing these lines, efficient vaccines do exist and protect efficiently against severe forms of the disease and death. However, vaccination is still relatively or strongly restricted in many countries due to insufficient availability, to financial hurdles, as well as to antivaccination movements. Current estimates are that by mid-2021, over 200 million persons have been infected by this virus resulting in some 4 million deaths; these figures are certainly an underestimate as many cases have not been reported.

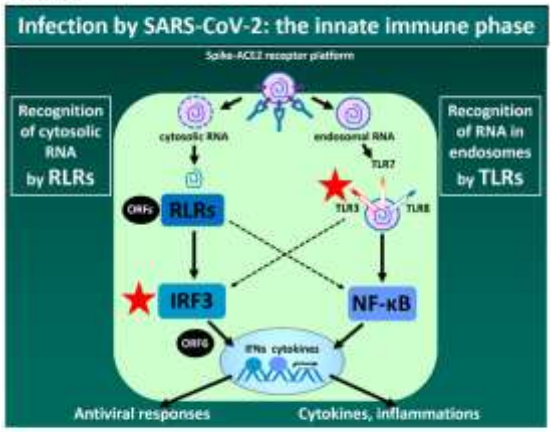
COVID-19 as a pandemic has generated in the

biomedical community worldwide a flow of studies and more than 100,000 publications or preprints have appeared over the last one and a half year. It is of course out of question that in this short article I can make a full analysis of this literature. I will restrain to summarizing here some of the outstanding results. As a caveat I wish to mention that neither our laboratory nor myself were involved in the studies reported in this section 3 of the present article, in contrast to many aspects reported in Section 2. However, as we will see below, innate immunity and innate immune receptors discussed in Section 2 have appeared as crucial players in fighting COVID-19. However, as a reminder, *Drosophila* is our laboratory model and is infected by a multitude of viruses and has developed efficient defense reactions against these viruses (*for Further Reading, see 3*). In all likelihood however, it does not serve as a host to coronaviruses, but many aspects of the innate immune responses – which evolved more than one billion years ago (see above), have been conserved and are also pertinent in higher

organisms.

The SARS-CoV-2 virus enters the cells by associating with the angiotensin converting enzyme which serves as its surface receptor on many epithelia, particularly on epithelial cells in the nasal cavity and the respiratory airways. The viral RNA is released into the cytoplasm and/or present in endosomes (Figure 9). Viral replication/ transcription occurs in dedicated complexes on double-membrane vesicles. The innate immune receptors which bind to invading RNA are receptors which I have discussed above, namely, in the cytosol, the RIG-I-like receptors (RLRs) RIG-I and MDA5 and, in the endosomes the Toll-like receptors TLR3, TLR7, and TLR8. Upon binding to cytosolic RNA, the RLRs activate an intracytoplasmic signaling cascade which leads to the phosphorylation of the transcription factors Interferon Regulatory Factor 3

Figure 9



For Legends, see text - in addition, the black circles indicate two examples of blocks in the activation pathways due to proteins corresponding to virus-encoded genes. The red stars give two examples of blocks in these pathways detected by genetic analyses.

(IRF3) and IFR7, which translocate into the nucleus where they direct the transcription of Interferons (IFNs). Viruses which have interacted within the endosomal compartment with Toll-like Receptors activate via a well-established signaling pathway the classical transactivator NF-κB, which translocates into the nucleus and controls the transcription of proinflammatory cytokines which will be secreted into the blood and determine an inflammatory state. We have to keep in mind that the RLR and TLR signaling pathways may cross-talk at some steps in their respective signaling cascades and thus exert additive effects. The present view seems to favor the proposal that the RLRs direct primarily an antiviral response and that the TLR pathway initiates essentially a proinflammatory response. The secreted Interferons direct the transcription of a large number of effector genes (the so-called Interferon Stimulated Genes, ISGs) many of which concur to block the transcription of viral genes and in this way block the infection. This evasion mechanism is dramatically helped by several of the

genes encoded by the viral genome itself which are being transcribed in the infected cells. Some of these proteins can indeed block well-defined steps in the signaling cascades which lead from the recognition of the viral RNAs by the RLRs mentioned above to an antiviral response, e.g. by suppressing recognition of viral RNA by RLRs. The current view is that if the interferon response is rapid and efficient, the virus will not be able to duplicate massively and exert deleterious effects on the cells: SARS-CoV-2 is thus kept under control and the patients are "asymptomatic" or show only a mild disease phenotype. The critical roles of the interferon arm of the anti-SARS-CoV-2 defense are further documented by the important observation that some patients with life-threatening pneumonia had inborn errors of TLR3- and IRF7- dependent type I interferon production. Some of these studies also revealed the presence of neutralizing autoantibodies against IFNs in patients with life-threatening COVID-19, further underlining the relevance of the interferon arm in the fight against the virus (*for*

details, see the data in the Zhang et al. paper and Bastard et al paper referenced in Further Reading 3). So far, we have concentrated on the early stage of the infection and underlined that the first crucial element is a rapid and efficient innate immune response triggered upon recognition of the virus through its cytosolic (and endosomal) RNA. This is of course a purely innate immune step which also triggers, via the activation of the dendritic cells, the adaptive immune response. This step requires some time (days) and will lead to the production of antibodies directed against viral structural determinants: these antibodies can bind to viruses in the blood and prevent their entry into the cells of the patients (neutralizing antibodies). If this reaction is massive, the viral threat will be overcome in cooperation with the interferon response. Further, the dendritic cells will also activate the cytotoxic 'killer" T cells which will scan for virally infected cells exposing viral structural determinants and destroy these cells. The inflammation triggered at the beginning of the infection has many effects

which initially favor the antiviral response, namely by increasing the permeability of the capillaries and allowing for the influx of phagocytes into the alveoli. However, this inflammation may get out of control when the level of released cytokines (messenger molecules of the immune system) raises to excessive values ("cytokine storm" or "cytokine release syndrome"). A variety of immunopathological effects are then triggered, which account for the severity of many forms of this disease and can lead to the death of patients. They are the subject of intense clinical research, and are beyond the scope of this review article.

4. *Conclusions and Perspectives*

Covid-19 is a zoonosis and has developed recently into a highly contagious pandemic

The disease is extremely heterogeneous: although a large proportion of infected people are asymptomatic - but transmit the virus - one

in five persons has a severe form; but the overall mortality rate is relatively low (1% to 2%) as compared to other pandemics - the long-term effects however are not yet understood

A remarkably diligent effort of the international biomedical community has established the general outlines of the infection and led to a basic understanding of the immune response, which exhibits an early innate immune facet followed by a strong adaptive immune response with a potent memory allowing for efficient vaccination; the latter has been very successfully harnessed by novel methods, based on mRNA injections coding for a specific segment of the virus (spike protein). When the innate immune responses are inadequate, and namely when the levels of cytokines become too high, immunopathological effects result in severe problems which are often life-threatening

The massive distribution of the virus worldwide

has favored the appearance of significant numbers of variants: of note, to date the variants identified differ primarily in their contagiosity but not in the severity of the diseases which they cause

The better and intimate understanding of the life cycle of the virus in vivo will hopefully lead to the development of small molecules capable of interfering specifically with the life cycle - in addition to the highly efficient vaccines already available, administration of pills containing small molecules will hopefully remove one day the threat of SARS-CoV-2 from humanity, in all areas of the world, whether rich or poor, and in all segments of societies, including the persons ideologically opposed to vaccination

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Further Reading (FR):

This invited review was destined to cover

superficially a vast array of scientific fields to an audience not particularly familiar with most of these fields. Providing a reference list for all the discoveries mentioned in this text is both impossible and not helpful anyway. I have therefore decided to propose for the readers interested in the various subfields touched upon here, a small list of recent easily accessible reviews. Of note also, all together these reviews feature more than 2,000 relevant references. The numbers given in the text are marked as FR (for further reading) and refer mostly to several reviews regarding the data/problems raised in the corresponding paragraphs of the text. Some of the data discussed in this presentation were the subject of Nobel Prize Awards and I have taken the liberty of including in the reference list access numbers to the corresponding Nobel Lectures, which provide the benefit of many historical insights. - JH

Further Reading

Articles of interest for further information and historical context of the data discussed in this general text – and for hundreds of additional references

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Section 2

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