

The immunological and biochemical principles of mRNA vaccine toxicity

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January 17, 2023

Abstract

The purpose of this document is to provide an appraisal of the mRNA vaccine technology in general. While most of the evidence of adverse effects adduced cited focuses on the experience with the two mRNA vaccines against COVID-19, we emphasize the general immunological and biochemical mechanisms which cause such adverse events. We also explain the underlying principles from which it follows that damage of this nature had to be expected even before the the mRNA vaccines against COVID-19 were rolled out. We argue that in light of these principles, and of the evidence that in the meantime has confirmed them in full, any and all approvals for mRNA vaccines must be revoked or, as the case may be, denied.

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1. Some elements of virology and immunology

The central thesis of this book is that the risks and the manifest harm which we have seen with the COVID-19 mRNA vaccines were predictable from first principles of immunology, and furthermore that similar harm must be expected with any future mRNA vaccines directed against other viruses. In order to properly make our case, we will first discuss how viruses multiply, and how the immune system combats and ultimately overcomes viral infections. The discussion offered in this chapter will not be comprehensive; rather, it will present, in a simplified manner, only those elements which are crucial and indispensable for evaluating this book's thesis. For a fuller exposition, we must refer the reader to appropriate standard works [14, 15].

1.1 The life cycle of a virus

You may be aware that viruses differ from other life forms by not being able to propagate independently, since virus particles are not cells and therefore lack the cellular machinery for energy metabolism and for protein synthesis. Viruses therefore use the cells of other organisms for their own propagation. To this end, the virus particles, or *virions*, must enter the cells of their host organisms and then direct those cells to manufacture offspring virions. This involves, at a minimum, the following steps (Figure 1.1):

1. A virion, which consists of proteins that enwrap a nucleic acid genome (RNA or DNA), binds to a protein receptor on the surface of the host cell. This triggers the virion's uptake into the cell.
2. The virion undergoes *uncoating*. This releases the viral nucleic acid genome, which can now direct the synthesis of new copies of the viral proteins.
3. Some, but not all viral proteins will appear on the cell surface and be incorporated into the daughter virions. Those which do not appear in the virions are referred to as *non-structural proteins*; they exist only within the infected cell and serve various purposes in viral multiplication, such as creating copies of the viral genome. Those proteins which *are* incorporated into viral particles are referred to as *structural proteins*.
4. New copies of the virus assemble at the cell surface, or sometimes within an intracellular compartment, and are then released from the cell. These daughter virions can then infect other body cells.

1.1.1 Cellular vs. viral genome structure and protein expression. Figure 1.1 was deliberately vague on the nature of the nucleic acid contained in the viral particles. There is in fact a great deal of variability—viral nucleic acids may be DNA or RNA, and they may be single-stranded or double stranded. The implications of this variability are

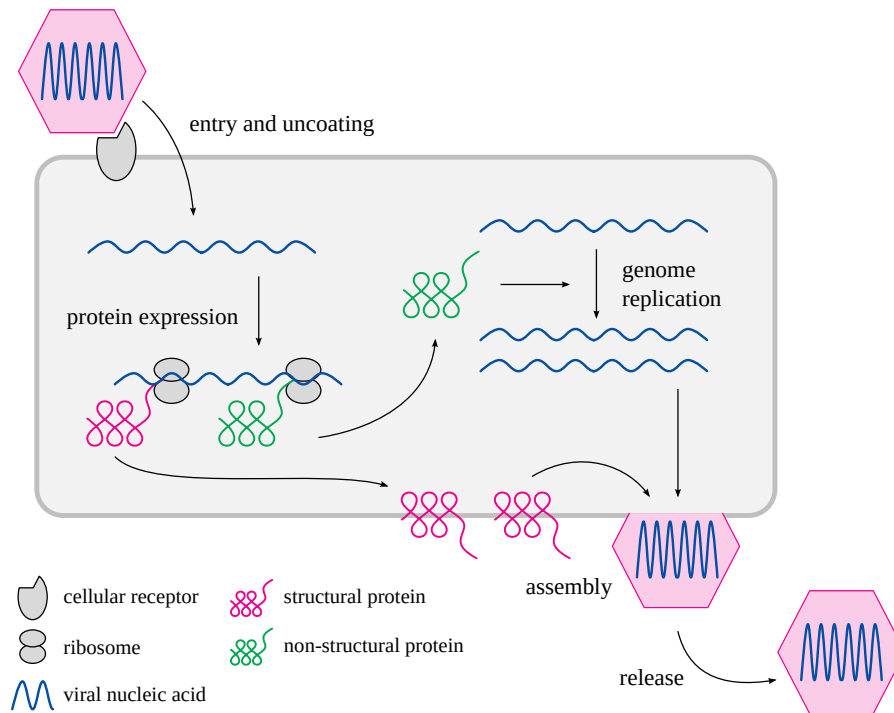


Figure 1.1 Overview of viral multiplication and protein expression (simplified). A viral particle consists of a nucleic acid genome (DNA or RNA, blue) that is enclosed by viral proteins (magenta). These protect the nucleic acid and also mediate attachment to a host cell receptor, which facilitates entry into host cells. Once inside the cell, the nucleic acid undergoes uncoating and then directs the synthesis of new copies of the viral proteins. Non-structural viral proteins exist only at the intracellular stage and serve functions such as the replication of the viral nucleic acid; these new genome copies, together with the structural proteins, will assemble into new virions, which will be released from the cell and infect additional cells.

quite interesting, but this is not the place to discuss them at length. Instead, we will just note that RNA viruses tend to have higher mutation rates than DNA viruses, and viruses with single-stranded genomes higher mutation rates than those with double-stranded ones. Thus, single-stranded RNA viruses, including coronaviruses or polio virus, tend to have the highest mutation rates. This compounds the difficulties of vaccine development, because circulating viruses may evade vaccine-induced immunity by mutating to alter or lose some of the molecular features against which that immunity is directed.¹

Figure 1.2 contrasts the mode of function of a cell's own genes to the genes of a coronavirus, which is shown here only as an example. The expression of cellular genes follows the regular pattern of transcription from the genomic DNA to messenger

¹Whether or not a virus will be prone to such immunological escape will depend not only on its mutation rate but also on its degree of adaptation to the human host. For example, both influenza and measles viruses are single-stranded RNA viruses with high mutation rates, but of the two only influenza is prone to rapid “antigenic drift” by mutation, whereas the measles virus is virtually perfectly adapted to humans already, so that mutations will offer it no selective advantage and therefore not persist. SARS-CoV-2 seems to follow the influenza paradigm, however, as had to be expected from its recent manufacture in the laboratory, which did not allow for thorough evolutionary adaptation to the human host. (With influenza virus, there is another source of genetic variation known as “antigenic shift.” It is of major importance in principle, but not in this context.)

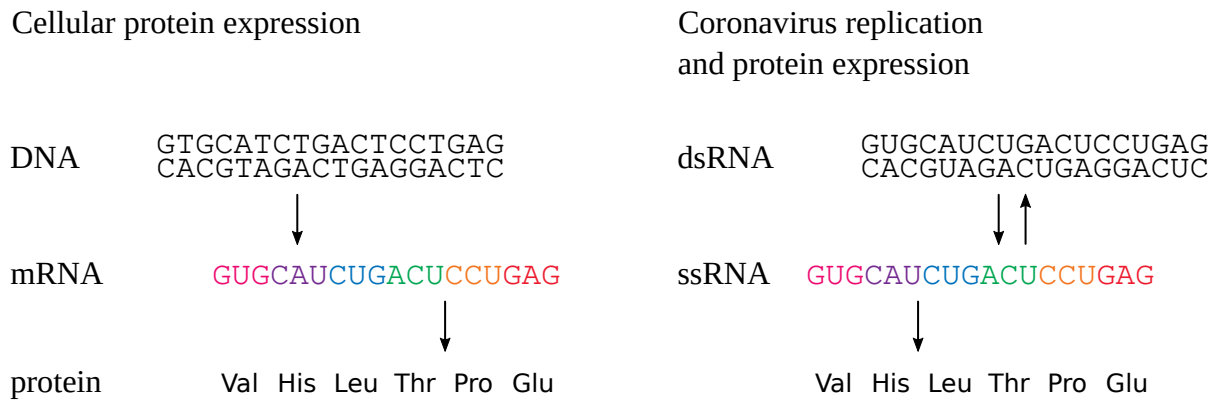


Figure 1.2 The function of the coronavirus RNA genome, compared to cellular mRNA. Left: cellular genes are expressed by transcription of DNA to mRNA, which is then translated to proteins. Right: the single-stranded RNA contained in coronavirus particles serves drives protein synthesis, too, but at the same time also serves as the template for its own replication, which involves a double-stranded RNA intermediate.

RNA (mRNA), followed by translation to protein. In contrast, coronaviruses contain a single-stranded RNA genome, which serves as the template both for protein expression and for its own replication. The replication involves a double-stranded RNA (dsRNA) intermediate, which exists only within the host cell but is never packaged into the viral particles. The RNA-dependent RNA polymerase that carries out these steps is encoded by one of the non-structural genes within the coronavirus genome.

As the figure suggests, dsRNA molecules have no role in cellular gene expression. Their presence inside a cell therefore indicates viral infection and ongoing virus replication. Remarkably, our body cells possess receptors which detect the presence of dsRNA and then activate both non-specific and adaptive immune responses to the virus in question (see Section 1.2.2.1).

1.1.2 The role of cellular receptor proteins. We just saw that the first step in viral entry and multiplication consists in binding of the virion to a cellular receptor protein. Of course, these cellular proteins do not exist for the purpose of facilitating viral entry; instead, they serve various purposes in physiology of the cell or the organism. For example, angiotensin-converting enzyme 2 (ACE2), one of several cellular proteins which facilitate the entry of SARS-CoV-2, serves to degrade angiotensin II, a peptide mediator which increases blood pressure. The binding of a virus to its receptor may interfere with this receptor’s physiological function and thus cause some of the clinical manifestations of the infection; this is indeed the case with SARS-CoV-2 [10].

The requirement of the virus for specific cell surface molecules in order to infect those cells restricts the host cell range of most viruses. This tends to mitigate the severity of those viral infections.

1.1.3 Some viruses are surrounded by a membrane envelope. In Figure 1.1, we drew the virus particle as consisting only of a nucleic acid and a protein shell (the *capsid*). While many viruses (e.g. poliovirus and adenoviruses) indeed contain only these two elements, others are additionally surrounded by an *envelope*, whose composition is similar to that of a cell membrane, i.e. it consists of lipids (fat-like molecules) and

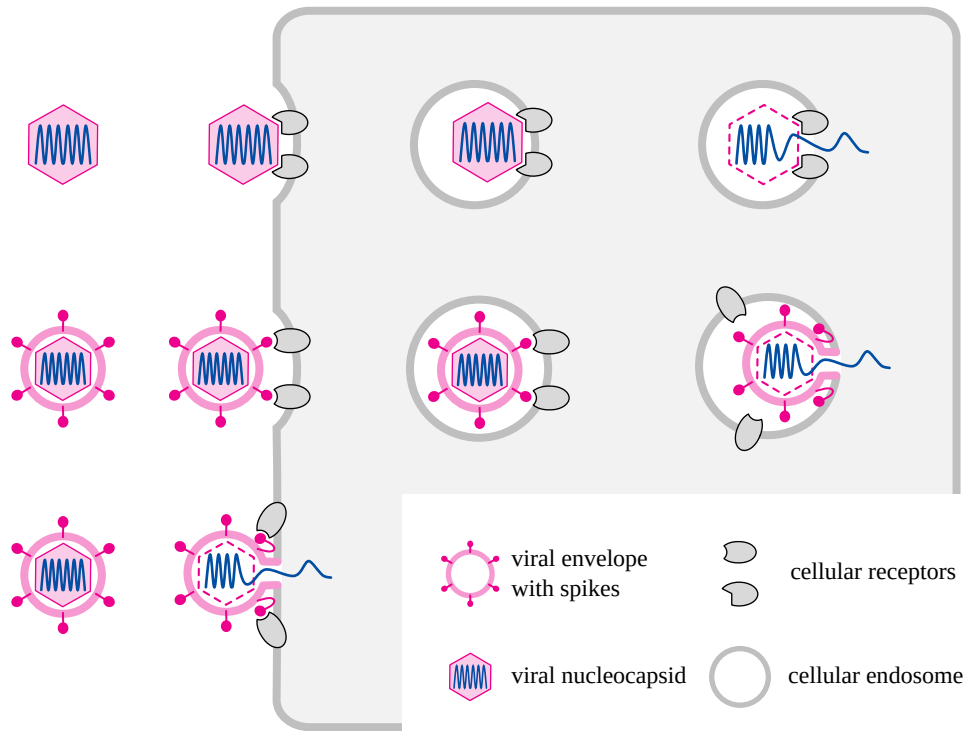


Figure 1.3 Cellular entry and uncoating of non-enveloped and enveloped viruses. **A:** many non-enveloped viruses (e.g. adenoviruses) are taken up by endocytosis. Acidification of the endosome (i.e., accumulation of H^+ within it) triggers uncoating of the viral genome and its transfer to the cytosol. **B:** enveloped viruses (e.g. influenza virus) also follow the endosomal pathway. Transfer of the genome to the cytosol involves the fusion of the viral envelope to the endosome membrane. This fusion step is triggered by a change in the molecular shape of the viral spike proteins, usually also driven by acidification. **C:** some enveloped viruses can fuse directly at the cell surface. Both of the pathways B and C have been suggested to occur with coronaviruses [14].

embedded membrane proteins. In this case, it is these membrane proteins which bind to the cellular receptors. They are often referred to as *spikes* or *spike proteins*.

In addition to engaging the cell's surface receptors, the spikes also mediate the fusion of the viral envelope to the cellular membrane, which can occur after endocytosis or directly at the cell surface. This fusion is an essential step in the transfer of the viral nucleic acid from the virus particle to the cytosol (the main compartment of the cell). Very commonly, this step is driven by the acidification of an endosome containing the virus particle (see pathway B in Figure 1.3).

Coronaviruses fall into the category of enveloped viruses. The much talked-about spike protein, which also constitutes the antigen encoded by the gene-based vaccines, mediates both receptor binding and membrane fusion. In order to bring about membrane fusion, the spike protein must undergo a change in molecular shape ("conformation").

We note in passing that the well-known drugs chloroquine and hydroxychloroquine inhibit the acidification of endosomes. It is therefore not surprising that hydroxychloroquine is clinically effective against COVID-19 [16], as it is indeed with many other viral infections [14].

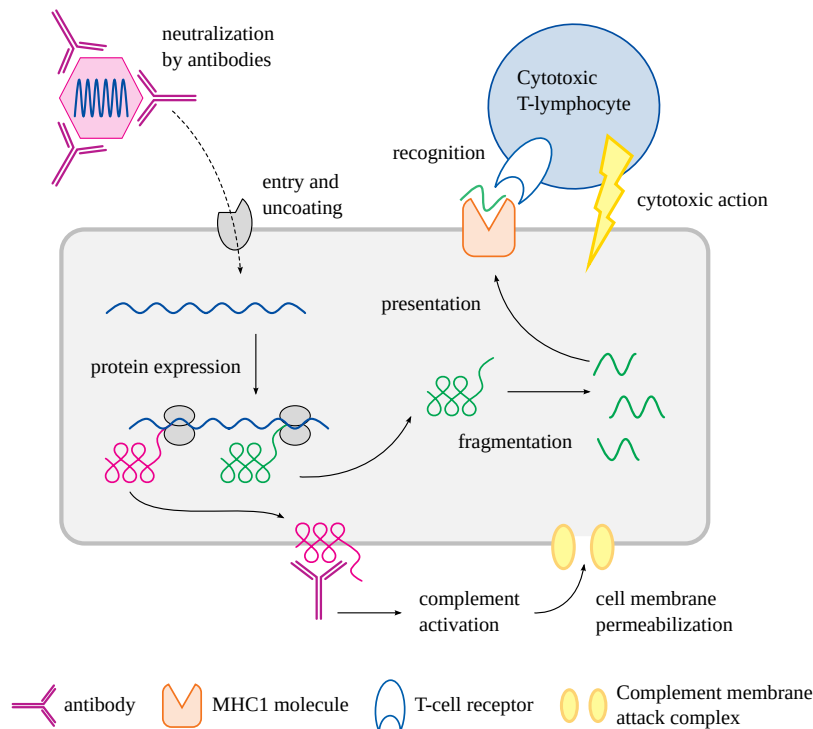


Figure 1.4 Antiviral immune effector mechanisms. This cartoon illustrates three of the mechanisms by which our immune system combats and eradicates a virus infection. Antibodies can bind to viral particles and neutralize them, i.e. prevent them from binding to and entering our body cells. They can also bind to viral proteins that appear at the cell surface and then activate *complement*, a cascade of extracellular proteins that causes the formation of transmembrane pores in the virus-infected cells. Viral proteins which remain inside the cell can be fragmented and then exposed on the cell surface, bound to a special helper protein (MHC1). Recognition of the MHC1-bound fragments by T-killer lymphocytes will activate these and cause them to unleash several cytotoxic proteins onto the virus-infected cell.

1.2 Immunity to viruses

Our immune system has a large arsenal of weapons, many of which are specifically tailored to bacteria, viruses, or other particular types of pathogens. Here, we will focus on those defense mechanisms which pertain to viral infections. These are also the most relevant for understanding the effects of mRNA vaccines—and not only antiviral vaccines such as those directed against COVID-19, but also possible future mRNA vaccines supposed provide protection against tuberculosis, malaria or other non-viral infections.

We can examine antiviral immunity by posing two key questions:

1. What are the effector mechanisms which the immune system deploys in order to check and clear an ongoing virus infection?
2. The immune system learns from experience, such that in many cases we fall ill with the same virus only once and then remain immune to it for the rest of our lives. How does this learning take place?

1.2.1 Antiviral immune effector mechanisms. Our immune system combats virus infections using two key strategies:

1. it intercepts viral particles before they can infect our body cells, and

2. it destroys those body cells which have already been infected and are currently manufacturing progeny virions.

Both of these strategies involve molecules and cells which specifically recognize and bind the antigens (proteins) of the virus in question (Figure 1.4). The killing of infected cells is largely brought about by cytotoxic T-lymphocytes, also known as T-killer cells. Figure 1.4 illustrates how these are activated. The infected cell expresses viral proteins as instructed by the viral genome, but in the process it chops some of these protein molecules into small fragments. It then exposes these protein fragments (peptides) on the cell surface, bound to a specific carrier protein (MHC1).

Cytotoxic T-lymphocytes possess specific surface proteins of their own, the *T-cell receptors*, which specifically recognize individual virus-derived peptides if these are presented by MHC1 molecules. It is important to understand that there is a very large repertoire of T-cells with different T-cell receptors, out of which only one or a few, or possibly none at all, will bind to any given virus-derived peptide. A cytotoxic T-cell whose T-cell receptors do match and bind such a peptide will be thereby induced to attack the cell that presents it. The recognition event will also stimulate the cytotoxic T-cell to divide and multiply (more on this below).

Binding and interception of virus particles—*neutralization*—is mediated by antibodies, which are extracellular proteins synthesized and secreted by *plasma cells*. These cells are descended from B-lymphocytes, which also are induced to proliferate and mature to plasma cells by encountering their cognate viral antigens (see Figure 1.7). As is the case with T-cells, there is a very large reservoir of B-cells with different surface receptors, out of which only a small subset will recognize any given antigen and then undergo activation.

Antibodies also contribute to the killing of virus-infected cells in various ways. One such mechanism is also illustrated in the Figure. It involves the *complement system*, which comprises a number of plasma proteins. The complement system is a self-amplifying cascade of proteases (protein-cleaving enzymes). It is activated by antibodies that have recognized and bound to their cognate antigens, which may be located on the surfaces of microbial cells or, with virus infections, on our own body cells. Complement activation culminates in the generation of a *membrane attack complex*, which is a large, ring-shaped structure, composed of multiple protein molecules, which quite simply punches a hole into the cell membrane.

Figure 1.5, which is taken from a seminal paper on the action mode of the complement system [17], illustrates that the complement system is perfectly capable of utterly destroying a cell. As you can see, the cells, which were exposed to antibodies and complement, are riddled with holes. The holes will break down the barrier function of the cell membrane, and the cell will die.

Membrane permeabilization is also one of the effector mechanisms deployed by cytotoxic T-cells. The pore-forming protein in question, *perforin*, is structurally similar to the main component of the complement pore (C9). This effect is augmented by the release of destructive enzymes from the T-cell, which can then enter the infected target cell through the perforin pore. In addition, the cytotoxic T-cells release mediators which induce the target cell to enter *apoptosis*—an innate program of cell suicide.

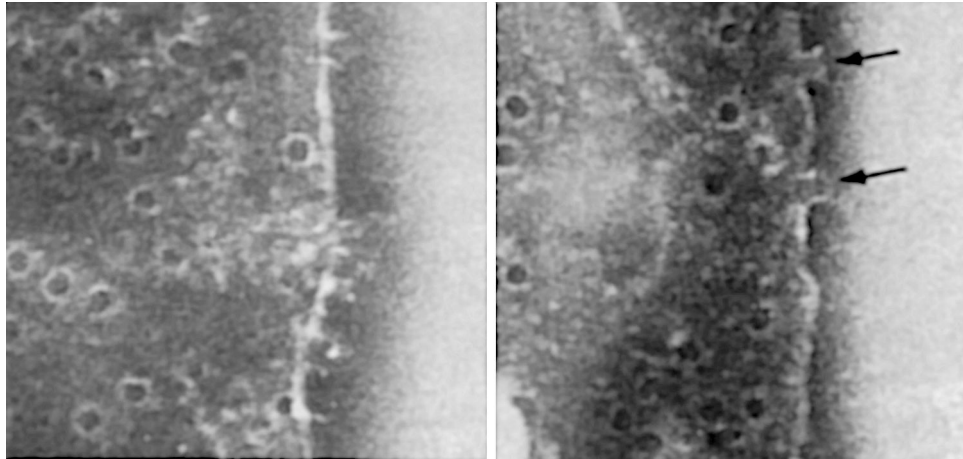


Figure 1.5 Complement membrane attack complexes forming pores on red blood cells. Antibodies against sheep red blood cells were allowed to bind to such cells in the presence of human serum, which provided the complement proteins. [18]. Most membrane attack complexes are viewed from the top. Arrows highlight individual complexes which sit on the edge of the cell; they are pictured sideways and can be seen to protrude from the cell surface.

Antibodies and T-cell receptors share structural similarities, and as noted both are capable of specific antigen recognition. However, we should note the following differences between them:

1. antibodies recognize intact antigen molecules, whereas T-cell receptors recognize them only as fragments;
2. antibodies require only the antigen itself for binding, whereas T-cell receptors will recognize their cognate peptides (protein fragments) only when they are presented by MHC molecules.

Since antibodies are themselves extracellular proteins, they will encounter their antigens only if these are present either on cell surfaces or in the extracellular space. With such antigens, antibodies can be very effective. On the other hand, the fragmentation and MHC1-dependent presentation mechanism illustrated in Figure 1.4 enables the cytotoxic T-cells to respond effectively to intracellular antigens. Thus, antibodies and cytotoxic T-cells clearly have complementary functions.

1.2.2 The activation of an antiviral immune response. We had noted above that both cytotoxic T-cells and B-cells are activated and induced to proliferate by contact with their cognate antigens, and that the T- and B-cells in question are drawn from a large preexisting pool of cells with different antigen specificities. While recognition of the specific antigen is indeed necessary for T- and B-cell activation, it is not the whole story: any specific immune response requires and begins with the activation of innate, non-specific elements of our immune system.

1.2.2.1 Specific immune responses are initiated by the non-specific immune system. You likely know from experience that a contaminated wound can become inflamed—red, swollen, and painful—rather quickly. This prompt reaction is not yet due to a specific immune response. Instead, the infecting microbes, which in this scenario are mostly bacteria, will initially activate our non-specific or innate immune system. This happens in two ways:

1. the microbial cells themselves will serve as triggers;
2. the toxic or invasive properties of the bacteria will kill some of our body cells. Some of the molecules released by decaying body cells will promote inflammation.

The complement system can be activated by bacterial cell surfaces even without the help of antibodies. Complement activation will not only permeabilize those bacterial cells, but also mark them for destruction by our *macrophages* and *neutrophil granulocytes*. These two cell types specialize in *phagocytosis*, that is, they professionally eat and kill microbes. A third phagocytic cell type are the *dendritic cells*. They are related to macrophages, but in contrast to the latter they function mostly as “messengers” rather than as “fighters”; they are crucial for triggering antibody responses to the pathogens they ingest (see Section 1.2.2.3).

Molecules released from killed bacterial cells—prominently cell wall components, but also bacterial DNA and others—will be recognized by various *pattern recognition receptors* (PRRs) within our own body cells. These PRRs are a large and structurally diverse group of proteins; a well-known subclass that you may have come across are the Toll-like receptors (TLRs). Activation of these various PRRs will induce the release of many different inflammatory mediators, collectively known as *cytokines* and *chemokines*. Some important effects of these mediators are

1. increased vascular permeability. This floods the infected tissue with plasma proteins, including antibodies and complement;
2. attraction and activation of phagocytic cells and other immune cells toward the focus of infection; and
3. activation of the subsequent specific T-cell and B-cell response to the microbial antigens encountered at the site of the infection.

Viral infections activate their own appropriate PRRs. Some of these receptors respond to double-stranded RNA, which does not normally occur in human cells and therefore signals infection with an RNA virus.² Double-stranded DNA does of course occur in human cells, but not normally in the cytosol. Its presence in that cellular compartment therefore signals infection with a DNA virus; and accordingly it, too, is detected by a suitable PRR.

Yet other types of PRRs respond to molecules which are normally present only within healthy body cells but which may be released from decaying dead cells. In the context of microbial infection, such “hidden self” signals are useful for amplifying the immune response. On the other hand, they can also contribute to autoimmune disease: once autoimmunity has passed a threshold beyond which it can destroy our own body cells, the hidden self signals released by those destroyed cells will further incite the autoimmune aggression.

1.2.2.2 Activation of cytotoxic T-cells. Once the non-specific response to an infection has set the stage, the specific immune response will begin. We will now consider

²Some PRRs will detect single-stranded RNA within endosomes, through which infecting viruses often gain entry (see Figure 1.3). Since mRNA vaccines are taken up via the endosomal route as well, they, too, may potentially activate these receptors. This effect can be suppressed by methyl-pseudouridine modification of the RNA [19], which is used by both the Moderna and the Pfizer COVID-19 vaccines (see Section 1.8.3.2).

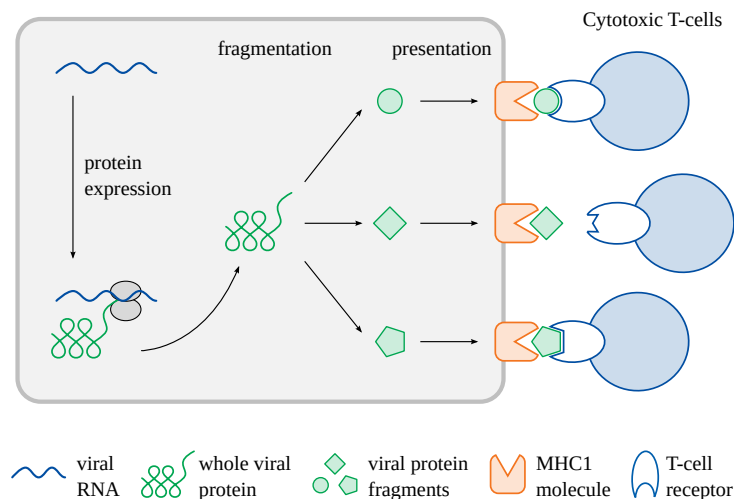


Figure 1.6 Lock and key interaction of MHC1-bound protein fragments and T-cell receptors of cytotoxic T-cells. The T-cell receptors on our body’s T-lymphocytes cover, collectively, a very large spectrum of antigen specificities, but all the receptor molecules on an individual T-cell are identical and bind to the same antigen. Only those T-cells which bind one of the protein fragments presented by a MHC1 molecule on a cell surface will be able to bind and be activated.

how the appropriate antigen-specific T-cell and B-cell clones are selectively activated, beginning with the cytotoxic T-cells.

We had seen that, whenever a cell produces a protein, a sample of those protein molecules will be chopped up into small fragments that are transported to the surface of the cell, where they become amenable for interaction with and recognition by cytotoxic T-cells. Envisage the interaction between a cytotoxic T-cell and a presented protein fragment as lock and key (Figure 1.6). Our reservoir of cytotoxic T-cells contains myriad different locks (T-cell receptors), which can fit a virtually limitless variety of possible keys (fragments). Yet, the proteins of any given virus will only give rise to a limited number of keys, which will bind and activate only a correspondingly limited subset of all available cytotoxic T-cells.

It is imperative to note that any viral protein will give rise to many fragments, which will be recognized by many different cytotoxic T-cell clones—the number of activated T-cells is small only relative to the entire reservoir of available antigen specificities, yet it is still considerable in absolute terms. A new virus mutant may generate one or a few novel protein fragments, but the majority of other fragments will remain unchanged and therefore continue to be recognized by our T-lymphocytes. Analogously, some degree of cytotoxic T-cell-based cross-reactivity and cross-protection usually exists between different members of a given virus family (see also Section 1.5). Thus, the narrative that the emergence of SARS-CoV-2 mutations must be countered, and every “variant of concern” be hunted down, by the development of customized vaccines has been ridiculous from the start.

1.2.2.3 Activation of antibody production. As noted earlier, antibodies are extracellular proteins secreted by plasma cells, which are derived from B-lymphocytes, or B-cells for short. Like T-cells, the B-cells carry surface receptors whose antigen specificity will be very diverse among B-cells, but will be the same for all receptors of a single B-cell. Unlike T-cell receptors, however, the B-cell receptors are actually antibodies. If a

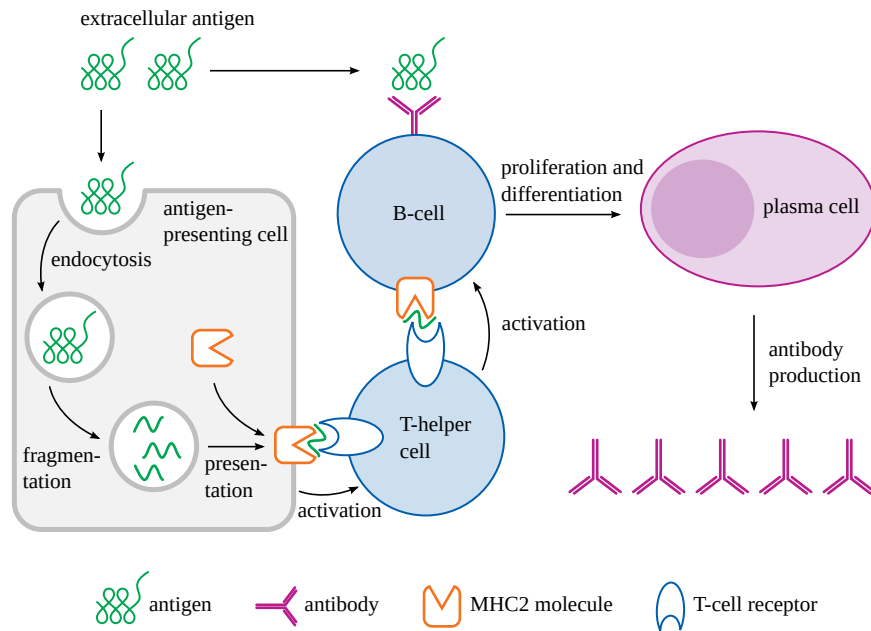


Figure 1.7 Activation of antibody production. An extracellular antigen binds to an antibody on the surface of a B-cell, and also to an antigen-presenting cell (APC; usually a dendritic cell). Within the APC, the antigen is fragmented and then presented on the cell surface bound to a MHC class 2 molecule. This complex is recognized by a T-helper cell that is thereby activated and in turn activates the B-cell, which carries out the same antigen processing and presentation steps as the APC. In response to the dual activation by the intact antigen and the T-helper cell, the B-cell will start dividing. The descendant cells will turn into plasma cells, which synthesize and secrete antibodies with the same antigen specificity as the original B-cell.

B-cells come across a suitable antigen and bind to it via their receptor antibodies. Then this B-cell will be activated: it will start dividing, and the daughter cells will eventually turn into plasma cells and start churning out antibodies. The amount of antibodies produced collectively by the plasma cells in our bodies is rather large, even when no infection is present. For illustration: our blood plasma contains some 10-12 grams of antibodies per liter, and half of this amount will be replaced about every three weeks.

While with some B-cell subtypes the binding to antigen alone is sufficient for activation, most B-cells require additional stimulation by *T-helper* lymphocytes. The entire process is outlined in Figure 1.7. It begins with the uptake of the antigen in question binds by an *antigen-presenting cell* (APC), which can be a dendritic cell or a macrophage. Inside the APC, the antigen is fragmented and then presented on the cell surface. The process resembles the presentation of intracellular antigens on other body cells (see Figure 1.4); but note that antigen-presenting cells use a distinct type of MHC molecule. While the presentation of intracellular antigens to cytotoxic T-cells involves MHC class I molecules (MHC1), the presentation of originally extracellular antigens by specialized antigen-presenting cells involves class II molecules (MHC2). These MHC2 molecules interact selectively with T-helper cells rather than with cytotoxic T-cells.

A B-cell that has captured an antigen will recruit a T-helper cell by processing that antigen the same way an APCs does. Thus, the B-cell will generate the same complexes of MHC2 with antigen-derived peptides as an APC, which will enable it to interact with the same T-cell receptors. Once a T-helper has bound to a B-cell that presents a matching antigenic peptide, it will complete the activation of that B-cell. In summary, there-

fore, the activation of B-cells requires “permission” from both antigen-presenting cells and from T-helper cells; this somewhat complex arrangement serves to prevent premature and excessive antibody responses, particularly also against self antigens. These safeguards may fail, however, which may then result in autoimmune disease.

Looking back once more at Figure 1.4, we note that it shows antibodies binding to a viral protein which is located on the surface of a cell, but not extracellularly located. How might such a cell surface protein enter the MHC2 pathway of antigen presentation? This occurs downstream of cell destruction, for example after a cytotoxic T-cell has killed the virus-infected cell in question. The remnants of that cell will then be dispersed and cleared away by macrophages and other antigen-presenting cells.

It is noteworthy that a newly formed plasma cell will initially produce a particular class of antibody called immunoglobulin M (IgM); after some weeks, it will switch over to another antibody class, most commonly IgG or IgA. The transient nature of IgM production is diagnostically useful: if an antibody response to a given antigen consists mostly of IgM, then it must be a primary response which began only recently; on the other hand, if it is mostly *not* IgM, then it has been going on for a while and may well be a secondary or “memory” response (see Section 1.4).

Note that the class switch does not change the antigen specificity of the antibodies; thus, the IgG or IgA will continue to bind the same antigen as the initially formed IgM.³

1.3 How do the highly diverse T-cell and B-cell reservoirs originate?

Above, we likened the reservoir of T-cells and their receptors to a myriad of “locks”, which between them will fit just about any antigenic “key”; and the same applies to our B-lymphocytes as well. It is now known that the truly incredible diversity of locks arises already during fetal development. How does this happen? Are locks molded in response to protein fragments (keys) as these appear during development? But then, the T-cells would be equipped with receptors exclusively recognizing “self” protein fragments, because the fetus in the womb is usually protected from infections, which means that no peptides derived from any infectious agents are available to train the developing T-cells. This could hardly serve a useful purpose. If, on the other hand, the diversity of locks should arise spontaneously and randomly, without requirement for any instructing key or template, then billions of lymphocytes might be generated that recognize “non-self” antigens, that is, those derived from extraneous agents including virus proteins.

Intriguingly, the latter is now known to be the case. However, the random nature of T-cell receptor generation also means that many T-cells will recognize “self” antigens—those derived from proteins encoded by our own DNA. Wondrously, these lymphocytes recognizing “self” are silenced or held in check throughout life (Figure 1.8). Mishaps occasionally occur in this control mechanism that can lead to autoimmune disease. Come T-cells out of cover that are reactive against antigens expressed in liver cells—come autoimmune hepatitis. Come T-cells out of cover that are reactive against insulin-producing cells in the pancreas—come autoimmune diabetes.

³While the antigen specificity of a maturing B-cell remains unchanged in principle, the binding affinity of its antibodies for their antigen *does* increase with time. This “affinity maturation” is driven by genetic point mutations.

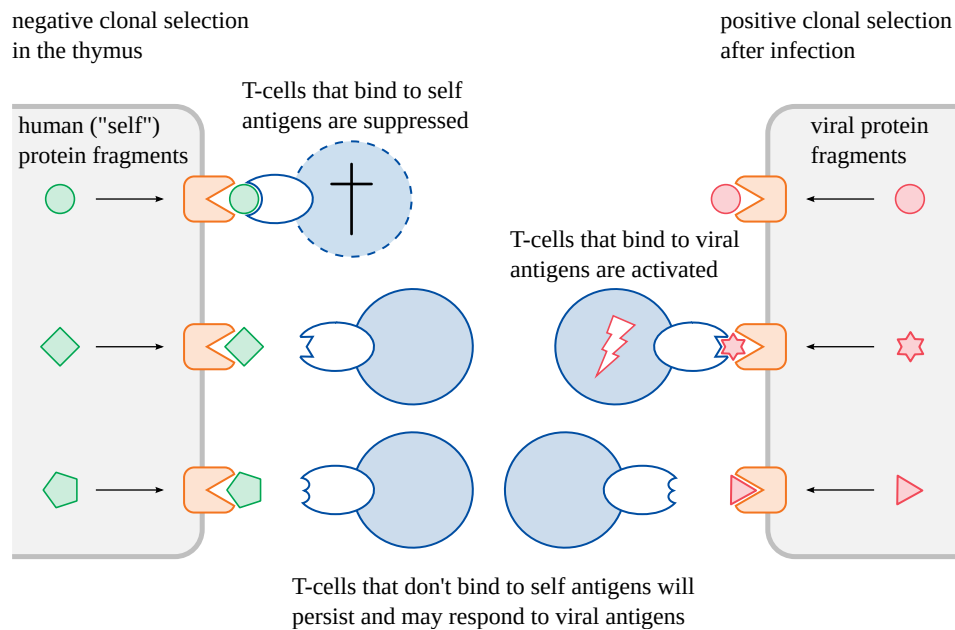


Figure 1.8 Clonal selection of T-lymphocytes. The diversity of T-cell receptors is initially generated at random, which means that many T-cells will carry receptors that bind to self antigens. In the thymus, such T-cells are “baited” by cells that express those antigens and then destroyed or suppressed. Those T-cells which do not bind self antigens persist and may at a later time be activated and induced to multiply in response to a virus infection.

But on the other hand, immune cells reactive against essentially all non-self proteins are present at birth and are ready to spring into action whenever a challenge is issued. It is for this very reason that conventional vaccinations can successfully be performed in early infancy, and also that even newborns are already able to withstand and overcome virus infections. Thus, when a Coronavirus comes around, up rises the anti-Corona team of T-cells; when flu comes around, up rises the anti-influenza team, etc. Each bout of training—each reinfection with the same, or more commonly a related viral strain—strengthens the team, enabling the virus to be more rapidly constrained and the infection terminated with increasing effectiveness.

1.4 Immunological memory

An immune response to an acute infection is transient; once the infection is overcome, most of the inflammatory cells that were activated, including the T-cells, B-cells, and plasma cells discussed above, are no longer required and thus will be removed. This will also cause the level of circulating antibodies against the germ in question to decline with time. However, a certain number of T-cells and B-cells persist as so-called *memory cells*, often for life, and they can mount a rapid and robust secondary immune response upon renewed exposure to the same pathogen.

The difference between a primary antibody response and a secondary one is illustrated in Figure 1.9. The depicted experiment was carried out with a calf which had been raised without colostrum, i.e. it had not received any maternal antibodies; thus, any antibodies observed were produced by the calf's own, initially naive immune system. The calf was deliberately infected with the same virus twice. The initial infection causes a somewhat delayed rise of antibodies. Initially, all of these antibodies are of

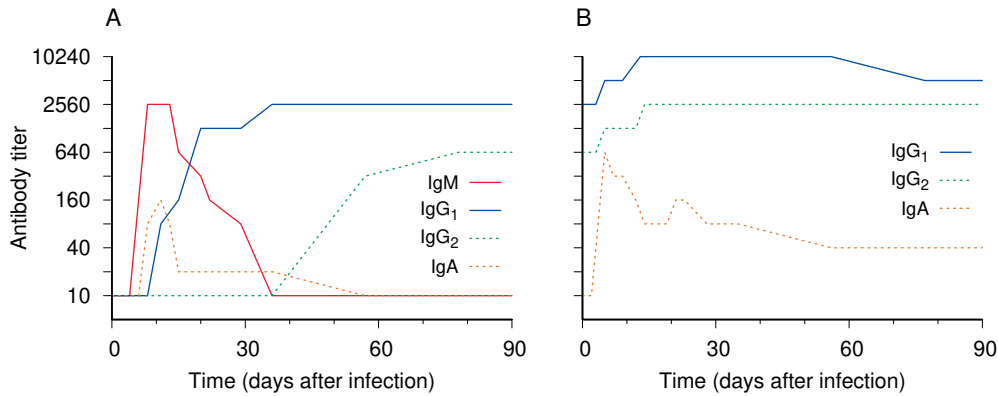


Figure 1.9 Serum antibody responses to primary and secondary virus infection. A calf was infected twice with the same virus (bovine respiratory syncytial virus), and the concentrations of different classes of serum antibodies were measured over time. The first infection causes a transient rise of IgM antibodies, which is then supplanted by IgG. Reinfection causes a rapid further rise in IgG, but IgM does not reappear. IgA rises transiently after the first infection but higher and more persistently after the second. Note the logarithmic y -axis. Adapted from Figure 1 in [20].

the IgM class. IgM is then replaced with IgG antibodies, which remain persistently high on the time scale of this experiment, but after some more months would be expected to gradually decline also. A minor, transient IgA response is also apparent.

The second infection gives rise, after a shortened initial lag phase, to a further rise of IgG. Notably, IgM antibodies do not appear at all this time. The absence of IgM from the response to the second infection proves that no new B-cell clones were activated; instead, the antibody response was entirely driven by the multiplication of memory B-cells, which had already undergone the class switch from IgM to IgG or to IgA earlier.

Secondary T-cell responses, too, are more rapid and more forceful than primary ones. The clinical correlate of a secondary immune response is usually immunity—a renewed infection with the same virus will be contained before it becomes clinically manifest. The best examples of this are of course classical childhood diseases such as measles and rubella. Smallpox could once be considered a childhood disease as well, and it, too, used to leave lifelong immunity.

The increased effectiveness of secondary immune responses is of course the whole rationale of vaccination: the less effective primary response is elicited with an (ideally) harmless derivative of the pathogenic germ, so that the pathogen itself will meet with the secondary response even on first contact. While practically lifelong persistence of memory B- and T-cells has been reported after smallpox vaccination [21], vaccine-induced immunity may be less durable with other viruses; see for example [22, 23].

1.5 Cross-immunity

A very powerful feature of our adaptive immune system is *cross-immunity*: if we are infected by a virus which is new to us, yet related to a previously encountered one, then our immune system can recognize molecular features in the new virus that are familiar from the old one and mount a secondary response against these. At the same time, it will also mount a primary response against those features which are unique to the new virus and therefore novel. This explains findings such as those illustrated

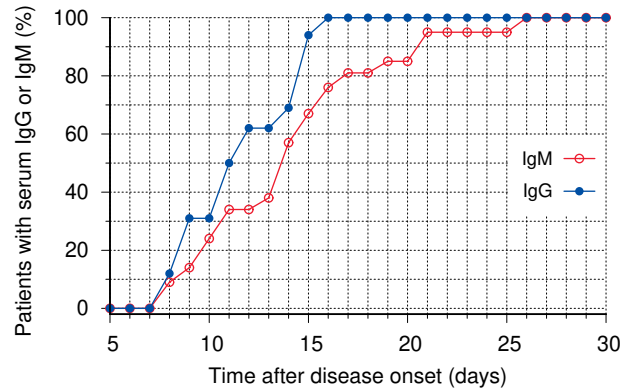


Figure 1.10 SARS-CoV-2 antibodies in the serum of COVID-19 patients. IgG and IgM were separately measured in daily blood samples of COVID-19 patients. All patients eventually develop IgM antibodies as with a primary immune response, but IgG rises before IgM, indicating that the immune response is in part secondary in nature, due to cross-immunity. Data from Figure 1A and B in [24].

in Figure 1.10. The graph tracks the development of antibodies against SARS-CoV-2 in a group of COVID-19 patients who had initially tested negative for such antibodies. Both IgM and IgG rise up, but remarkably IgG rises faster. This rapid rise is typical of a response from memory. On the other hand, all individuals eventually develop IgM as well, which indicates that a primary response is taking place. Thus, the early rise of IgG results from cross-immunity, whereas the subsequent rise of IgM represents the primary response to the novel and unique antigenic features of SARS-CoV-2.

The specific viruses most likely to have laid groundwork for the memory-type reaction to SARS-CoV-2 infection are evident from the data in Figure 1.11. In this study, serum samples from COVID-19 patients were tested for antibodies that would cross-react with the spike proteins of four other human coronaviruses, namely, SARS-CoV-1, MERS, HKU1, and OC43. In each case, SARS-CoV-2 infection significantly increased antibody levels relative to those observed in a control group of individuals not infected with SARS-CoV-2. What is more, however, with the endemic virus strains HKU1 and OC43, even the negative control group displayed fairly high antibody levels, which indicates widespread previous infection with and immunity to these strains. If someone with such immunity is infected with SARS-CoV-2, then cross-reactive memory B-cells induced by HKU1 or OC43 will be reactivated to again produce antibody. It is noteworthy that the presence of such cross-reactive antibodies correlates with reduced clinical severity of COVID-19 [26].

With SARS-CoV-1 (the original SARS virus) and with MERS, which never were endemic in the human population, antibody levels were low among the control group. In these cases, the strong increase in the level of cross-reactive antibodies among COVID-19 patients must have been induced by SARS-CoV-2 itself. We can therefore expect that recovered COVID-19 patients would enjoy a measure of cross-protection from SARS or MERS, should either virus stage a comeback, for example by eloping from another “high-security” bioweapons laboratory.

Cross-immunity between SARS-CoV-2 and other coronaviruses has also been documented with respect to T-lymphocytes [27, 28]. Most likely, widespread preexist-

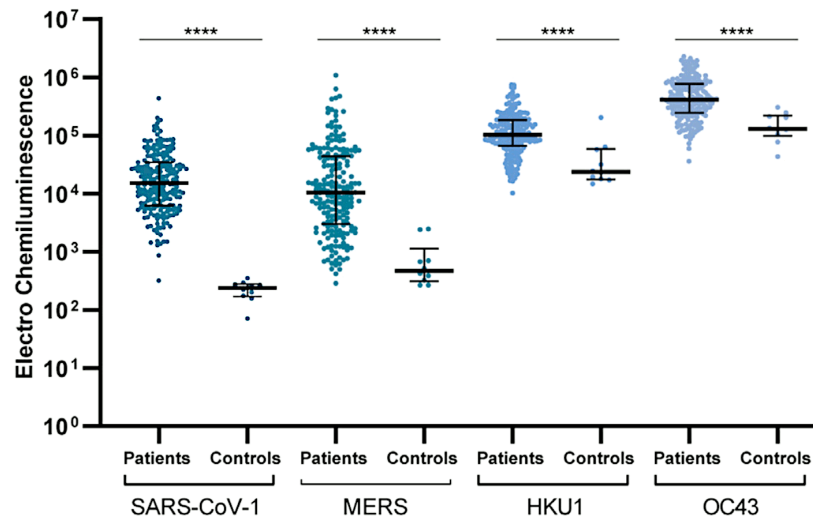


Figure 1.11 Cross-reactive IgG antibodies induced by SARS-CoV-2 infection. Serum samples from 203 individuals with evidence of SARS-CoV-2 infection and from a negative control group were assayed for the levels antibodies to the spike proteins of human coronaviruses SARS-CoV-1, MERS, HKU1, and OC43. With all four antigens, antibody titers were higher in infected patients than in controls, indicating that antibodies to the SARS-CoV-2 spike cross-react with those of the other coronaviruses. Figure adapted from [25].

ing T-cell and B-cell cross-immunity accounts for the rather benign clinical course of COVID-19 in most patients.

1.6 Who really controls viral infections: antibodies, or cytotoxic T-cells?

We have seen that virus infections elicit both antibody formation and a T-cell response. What is the respective importance of each in controlling and overcoming the virus infection? The answer is: it depends.

1.6.1 Primary vs. secondary immune response. In the first infection with a given virus (and in the absence of cross-immunity), there are no antibodies which could bind and neutralize the virus particles before entering our body cells. Therefore, by the time an immune response has been mounted, a considerable number of cells may have been infected, which then have to be eliminated. This task falls primarily to the cytotoxic T-cells, although antibody-dependent cytotoxic mechanisms also contribute (see Figure 1.4). On the other hand, if we had encountered the infecting virus before, and antibody levels are still sufficient or can be raised on short notice, then these antibodies can effectively limit the spread of the virus and therefore have a dominant role [15, p. 358].

1.6.2 Antibody-dependent enhancement. The answer also depends on the identity of the virus. While all viruses will induce specific antibodies, some viruses will not be effectively neutralized by them. This can occur because certain cells of the immune system are supposed to take up antibody-antigen complexes and destroy them. If a virus particle to which antibodies have bound is taken up by such a cell, but manages to evade destruction, then it may instead start to multiply within that immune cell. Overall, instead of protecting our cells from the virus, the antibodies will then promote the replication of the virus and worsen the disease. This effect is called *antibody-dependent*

enhancement (ADE). Clinically, ADE can cause a hyperinflammatory response (a “cytokine storm”) that will amplify the damage to our lungs, liver and other organs of our body.

Dengue fever is a natural virus infection that is often complicated by antibody-dependent enhancement; this will cause recurrent infections to be more severe than primary ones. ADE has also been observed with vaccines directed against dengue virus, respiratory syncytial virus (RSV), and measles. Vaccine-elicited ADE was observed as well with the original SARS virus (SARS-CoV-1), the MERS virus, and feline coronavirus, all of which are closely related to SARS-CoV-2 [29, 30]. SARS-CoV-1 and SARS-CoV-2 in particular are highly homologous, with 82% sequence identity at the genome level, and the viral receptor on host cells for both is ACE2. The risk of antibody-dependent enhancement in connection with COVID-19 infection and vaccination was explicitly recognized in the literature before the gene-based COVID-19 vaccines were rolled out [31–34], yet it was not rigorously evaluated during the very short clinical trials.

1.6.3 Viral evasion of T-cell cytotoxicity. While ADE permits some viruses to evade antibody-mediated neutralization, other viruses prevent the activation of cytotoxic T-cells by interfering with the MHC1-dependent antigen processing and presentation pathway outlined in Figure 1.4. Well-known examples are members of the Herpes and Poxvirus family [35].

Our immune system has an answer—the *natural killer* (NK) cells. These are lymphocytes with a peculiar set of surface receptors, which can detect the *lack* of MHC1 molecules on other cells in our body. The NK cell will thereby be activated to kill the target cell. NK cells will also be activated by antibodies bound to viral proteins on the surface of infected cells.⁴

In summary, cytotoxic T-cells will be most important in primary infections and with those viruses that induce ADE, whereas antibodies will have a dominant role in secondary infections and with those viruses that can evade the action of cytotoxic T-cells.

1.7 Immunity to respiratory viruses: systemic versus mucosal immunity

Many vaccines, including the COVID-19 ones, are aimed at viruses that infect primarily the mucous membranes of the respiratory tract before possibly spreading through the bloodstream to other organs of the body. In this context, we must note that the cells of the immune system which reside within and beneath the mucous membranes of the respiratory tract (and also of the digestive and genitourinary tracts) function somewhat independently from those immune cells which protect the interior of the body.

One key feature of the functional distinction between mucosal and systemic immunity are the two major categories of antibodies which are present in the body. Antibodies in the first category are produced by plasma cells which are located within a mucous membrane, directly beneath its uppermost cell layer (the *epithelium*). These

⁴The combined effect of antibodies and NK cells is referred to as ‘antibody-dependent, cell-mediated cytotoxicity’ (ADCC). Furthermore, NK cells are also endowed with pattern-recognition receptors for viral nucleic acids and some viral proteins. This permits them to combat a viral infection even before a full-fledged adaptive immune response sets in—they participate in both innate and adaptive immune responses.

antibodies—secretory immunoglobulin A (sIgA)—are secreted to the surface of the mucous membrane. They are thus on site to meet air-borne (or food-borne) viruses, and they may be able to prevent viral binding and infection of the cells within the mucous membrane.

The antibodies in the second category—IgG and circulating IgA—occur in the bloodstream. These antibodies can potentially counteract the spread of viruses via the bloodstream, for example when mucosal immunity fails to repel an infection of the airways or to confine it to cells of the mucous membranes alone.

Crucially, vaccines that are injected into the muscle—i.e., the interior of the body—will only induce IgG and circulating IgA, *but not secretory IgA*. The antibodies induced by such vaccines therefore cannot and will not effectively protect cells of the respiratory tract against infection by air-borne viruses [36, 37]. This realization is neither contentious nor particularly new. Even 30 years ago, McGhee et al. [37] concluded:

It is surprising that despite our current level of understanding of the common mucosal immune system, almost all current vaccines are given to humans by the parenteral route [i.e. by injection]. Systemic immunization is essentially ineffective for induction of mucosal immune responses. Since the majority of infectious microorganisms are encountered through mucosal surface areas, it is logical to consider the induction of protective antibodies and T cell responses in mucosal tissues.

The failure of intramuscular injection to induce secretory IgA was confirmed yet again in a recent study on Middle East Respiratory Syndrome (MERS) [38], which like COVID-19 is caused by a coronavirus of dubious origin. The experimental vaccine used in this study was gene-based, like the major vaccines currently deployed against COVID-19. With Pfizer’s COVID-19 vaccine, only feeble and short-lived induction of mucosal antibodies has been detected [39, 40]. With little or no secretory IgA, there is no reason to expect that vaccination will effectively inhibit replication of the virus within the mucous membranes. One therefore had to expect the failure, meanwhile manifest [41, 42], of the vaccines to prevent upper respiratory tract infection with the SARS-CoV-2 coronavirus, and thereby the spread of the virus.

The only thing that will effectively induce secretory IgA antibodies (sIgA) are naturally occurring airway infections, or possibly intranasally applied vaccines, which however so far are experimental [38].⁵ The mucous membranes of healthy individuals are consequently coated with antibodies directed against common respiratory viruses. However, the capacity of these antibodies to prevent infections is limited, which is why infections with air-borne viruses occur repeatedly throughout life.

The subordinate role of secretory IgA in combating systemic viral infections is highlighted by the fact that individuals with a very common genetic defect (selective sIgA deficiency) who are unable to produce sIgA do not suffer from dramatically increased susceptibility toward severe respiratory infections. Severe infections that spread beyond the respiratory mucous membranes will encounter the systemic part of the immune system, which protects the interior of the body, and which remains intact in

⁵One vaccine that was delivered in a biologically appropriate manner was the Sabin live vaccine against polio: it was given orally, which mimics the route of infection with the natural poliovirus. However, due to serious safety concerns (see below), this vaccine is now obsolete.

patients with the above gene defect. This part includes the antibodies found in the bloodstream, i.e. IgG and circulating IgA.

1.8 Vaccination strategies

We will now consider the different types of antiviral vaccines, beginning with the conventional ones. While these are not the focus of this book, discussing them briefly will give us some useful background for evaluating the mRNA vaccines.

Among the conventional antiviral vaccines, a key distinction is that between infectious or “live” virus vaccines on one hand, and non-infectious or “dead” ones on the other. Both types are widely used and have their respective strengths and weaknesses.

1.8.1 “Dead” vaccines. These vaccines consist of virus-derived antigens that are incapable of replicating. The traditional method for preparing such vaccines consists in chemical inactivation—the virus in question is grown in eggs or in a suitable cell culture and then treated with some chemical which will react with the viral particles and thereby destroy their ability to infect cells and replicate. A suitable procedure is described in a recent report on the development of an inactivated COVID-19 vaccine [43]. The vaccine now marketed by the Chinese company Sinovac is of this kind. Another important example is the Salk vaccine against poliomyelitis, which has reclaimed its leading place from the Sabin live polio vaccine due to the severe safety deficits of the latter (see Section 1.8.2.3).

A potential risk of traditional dead vaccines is that some infectious particles might survive the chemical inactivation process. This risk is absent with *subunit vaccines*, which have become feasible with the advent of recombinant DNA technology. A good example is the hepatitis B vaccine. Its only antigenic component is the surface antigen of the virus particle, which is recombinantly expressed *in vitro*; no intact viral genome, and therefore no infectious particles, are present at any stage of the production process.

While both chemical inactivation and recombinant subunit expression may reduce or even abolish not only the infectiousness of a virus but also the toxic activities of its viral proteins, the latter is not a given. We note specifically that the “Novavax” subunit vaccine, which contains the SARS-CoV-2 spike protein as the only antigen, has been linked to cases of myocarditis [44], as have of course the gene-based COVID-19 vaccines [45, 46].

How does the immune system respond to these dead vaccines? It will process them as extracellular antigens, that is, they will be taken up and processed by antigen-presenting cells and then induce the activation of cognate T-helper and B-cells, leading to antibody production (see Section 1.2.2.3). In contrast, no or very little activation of cytotoxic T-cells will take place. Moreover, since these vaccines are injected subcutaneously or intramuscularly, induction of mucosal immunity will be weak or absent.⁶

⁶Partial protection from infection by mucosal immunity has been reported for example with an inactivated polio vaccine [47]. Some degree of cytotoxic T-cell activation is possible through *cross-presentation*, i.e. through “spillover” of antigens from the MHC2 pathway into the MHC1 pathway of antigen presentation and T-cell activation [48, 49]. It should be noted, however, that with polio the main goal is not to inhibit mucosal infection but rather the spread of the infection through the bloodstream to the central nervous system (see Section 1.8.4). This is indeed readily achieved by the Salk vaccine.

1.8.2 Live virus vaccines. These vaccines are actual viruses that are either *attenuated* versions of the pathogenic virus in question, or they are natural viruses distinct from the pathogen but related to it. This latter case is best illustrated by Edward Jenner's invention of using the natural cowpox virus for vaccinating against smallpox. This procedure is also a good illustration of cross-immunity (see Section 1.5). The Vaccinia virus strains which were used for smallpox vaccination in the twentieth century are derived from other natural poxviruses of somewhat unclear origin [50].

In contrast, the Sabin polio vaccine and the measles vaccine are live vaccines that were derived in the laboratory through serial passage in non-human cell cultures. The principle of attenuation is simply to “encourage” the virus to adapt to its non-human host cell environment. At least some of the spontaneous mutations that help the virus grow better in non-human cells will reduce its ability to propagate in human hosts. Thus, if the virus is introduced into humans afterwards, it will tend to cause only mild infections, which however will still suffice to induce a protective immune response.

Since live virus vaccines are actual viruses, they tend to induce both antibody and cytotoxic T-cell responses; that is, the immune response more closely resembles that to the original pathogen, and therefore it can be expected to be more reliable and enduring. While this consideration favors live over dead vaccines, the live vaccines also have their own specific drawbacks.

1.8.2.1 Atypically severe infection in susceptible individuals. The virulence of the vaccine virus may be sufficiently low for healthy recipients, but those with predisposing conditions, such as immune disorders or skin diseases, may suffer severe disease after inoculation. For example, smallpox vaccination is contraindicated in persons with atopic eczema (neurodermatitis), since in them the vaccine virus may cause a systemic skin disease known as *eczema vaccinatum* [51]. Even in recipients without recognizable predisposition, smallpox vaccination has caused myocarditis and encephalitis, i.e. infection of the heart and the brain, with often severe and sometimes fatal consequences.

1.8.2.2 Transmission of the vaccine virus in the human population. Since the vaccine is a live virus, it may spread from vaccinated individuals to bystanders, and possibly onward from the latter throughout the human population. While superficial examination might suggest such transmission to be a good way for increasing the effectiveness of live vaccines [52, 53], it poses unacceptable risks, for the following reasons: the vaccine might be transmitted to persons who are at risk of severe disease from it (see above), and the virus might even revert to full virulence while spreading in the human population. Unfortunately, the latter risk is not merely hypothetical.

1.8.2.3 Reversion of the attenuated virus strain to full virulence for humans. We noted above that the process of attenuation relies on the serial passage of the virus in non-human cells, which will select random mutations that enhance growth in these cell cultures, but at the same time decrease virulence for humans. Conversely, if such an attenuated virus is inoculated into humans, then this will initiate a serial passage in human cells, which will select for mutations that revert or compensate the attenuating ones. This effect will be magnified if the virus can be transmitted from vaccinated to non-vaccinated individuals.

The occurrence of such vaccine-derived revertants is well documented with oral poliomyelitis vaccines, and some of these revertants have caused large outbreaks in

the human population. A detailed study on a cluster of such outbreaks, which had occurred in Nigeria, documented 403 cases of paralytic disease and an estimated 700,000 total infections. Furthermore, it suggested that revertant virus strains emerged multiple times during these outbreaks [54]. This example should suffice to illustrate the seriousness of the problem, which is the reason that the world has switched back to the safer dead polio vaccine.

1.8.3 Gene-based vaccines. You are likely aware that two different types of gene-based vaccines are being used against COVID-19, namely, the adenovirus-based ones produced by AstraZeneca and Johnson & Johnson, and the mRNA vaccines produced by Pfizer and Moderna. We will limit the discussion to these two types, even though there are other experimental variations on the theme.

1.8.3.1 Adenovirus-based vaccines. Adenovirus particles contain double-stranded DNA genomes, which they release within their host cells. An infected cell first transcribes the viral genome to mRNA, from which it then translates the viral proteins. In adenovirus-based vaccines, several genes of the natural adenovirus genome have been replaced with the gene encoding the vaccine antigen in question. In case of the adenovirus-based COVID-19 vaccines, this is the gene encoding the SARS-CoV-2 spike protein.

It is noteworthy that a cell infected with such a recombinant adenovirus particle will produce both the SARS-CoV-2 spike protein and the proteins of the adenovirus carrier (“vector”) whose genes remain part of the recombinant genome. Accordingly, an immune response will be elicited against all of these proteins. Some of the antibodies raised against the adenoviral proteins after the first injection can neutralize the recombinant virus particles, and they will therefore reduce the effectiveness of booster injections.

We further note that the deletion of some of the naturally occurring adenovirus genes from the recombinant genome leaves this vaccine virus “crippled”—it is able to infect human cells and to induce protein synthesis within them, but it is unable to replicate and to generate any progeny virions. This means that the entire amount of virus particles required to stimulate an immune response must be injected at once, instead of building gradually *in vivo* as would be the case with a natural virus infection or a conventional live virus vaccine. The injection of such a large dose of viral material may aggravate adverse events.

1.8.3.2 mRNA vaccines. An mRNA vaccine particle contains a synthetic mRNA, which is encased in a shell composed of various fat-like molecules or lipids, a *lipid nanoparticle* (LNP). The lipids protect the RNA in the extracellular space, and they also facilitate its uptake into the host cell. This uptake is essentially not limited by cell type—any cell can take up these mRNA/lipid nanoparticles, even though the cells of certain organs—e.g., liver, spleen, and ovaries—accumulate particularly high amounts, for reasons that will be explained in Section 4.2.1.

Once inside the cell, the synthetic mRNA sheds its lipid shell and then functions like a natural mRNA to induce the synthesis of the protein it encodes. With the COVID-19 mRNA vaccines, this is again the SARS-CoV-2 spike protein. Note, however, that with both the Pfizer and the Moderna COVID-19 vaccines, the synthetic mRNA carries a peculiar modification: one of the four nucleosides contained in natural mRNA, namely

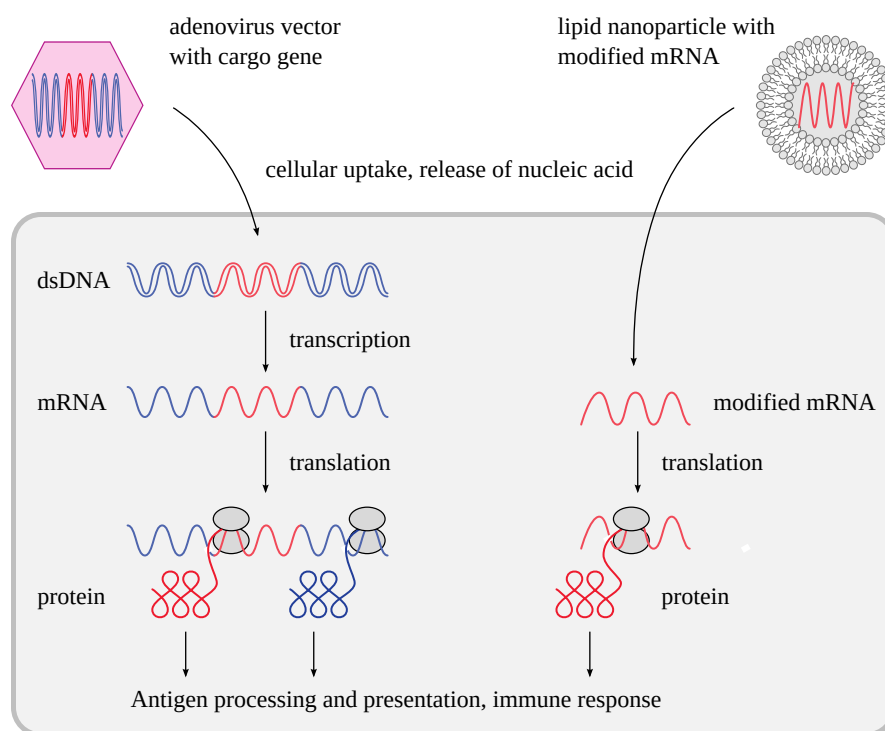


Figure 1.12 Action mechanisms of gene-based vaccines. Left: adenovirus-based vaccines contain a cargo gene (red) within their recombinant double-stranded DNA genome, which is expressed within the cell much like a cellular gene. Right: mRNA vaccines consist of a modified mRNA that is encased in a shell of lipids, which facilitate the uptake of the mRNA into host cells. It is then directly translated into antigenic proteins. Antigen processing and presentation then proceed as illustrated in Figures 1.4 and 1.7.

uridine, has been artificially replaced with 1-methyl-pseudouridine.⁷ This causes a very substantial increase in the level of translation—much more spike protein will be produced than would be the case with a natural uridine-containing mRNA [55, 56].

The synthetic mRNA encodes no other protein than spike—in contrast to the adenovirus-based vaccines, no other viral proteins are involved in the function of mRNA vaccines. Since the mRNA does not replicate inside the host cell,⁸ the full amount of nucleic acid required to produce the necessary quantity of protein antigen must again be injected at once.

1.8.3.3 The immune response induced by gene-based vaccines. Both forms of gene-based vaccines induce the intracellular production of antigenic protein; therefore, they should in principle lend themselves to the MHC1-mediated induction of a robust cytotoxic T-cell response (see Figure 1.4). However, since the spike protein encoded by all

⁷The mRNAs in the Pfizer and the Moderna vaccines carry two additional modifications: their nucleotide sequences are *codon-optimized* for maximal expression in human cells, and they carry two strategic point mutations which stabilize its *pre-fusion conformation*, i.e. they inhibit the change in the molecular shape of the spike protein that normally accompanies the fusion of the viral envelope with the cellular membrane (see Figure 1.3).

⁸This applies, at least officially, to the COVID-19 vaccines supplied to the public. However, Pfizer has developed and conducted clinical test with self-amplifying mRNA vaccines, which do encode additional viral genes. Such vaccines have not yet been deployed outside limited clinical trials.

gene-based COVID-19 vaccines is transported to the cell surface, it ends up mostly in the MHC2 pathway of antigen presentation.⁹

One would therefore expect a preferential activation of T-helper cells and a strong antibody response, but a rather feeble induction of cytotoxic T-cells. According to the limited evidence available, this is indeed the case [57].

While the gene-based vaccines may superficially resemble natural viruses or live virus vaccines, the devil is in the details—the apparently minor differences in the action modes have profound implications for the likelihood and distribution of adverse events. We will revisit this question in Section 2.3.

1.8.4 Degrees of vaccine-induced immunity, and rationales for vaccination. The ideal outcome of vaccination would be *sterilizing immunity*, that is, the virus in question will no longer be able to infect the recipients of the vaccine. The vaccinees will thereby not only be protected from clinical disease, but will also deny the virus any opportunity to propagate. If a high enough proportion of the population has received such a vaccine, then the result may be *herd immunity*: the likelihood of each case of infection to spawn another case—the *basic reproductive number*—will drop below 1, which means that the infection will peter out rather than tear through the entire population. In theory, herd immunity is also possible with a vaccine which merely reduces but does not entirely abolish infection in vaccinated people; however, it is difficult to come up with compelling real-world examples.

A vaccine that does not suppress infection may still protect from significant clinical disease. For example, poliovirus initially infects the mucous membranes of the gut, and it is from there that the virus is shed and propagated. However, this intestinal infection amounts to no more than an episode of diarrhea. The characteristic paralytic disease occurs only if the virus spreads from this initial site of propagation first into the bloodstream and then to the central nervous system. As noted in Section 1.7, intramuscularly administered vaccines will not effectively induce mucosal immunity, and indeed poliovirus can still propagate in many of the vaccine recipients [47]. However, the intramuscularly injected dead polio vaccine *will* effectively induce antibodies that circulate in the bloodstream, and these will reliably neutralize the virus before it can infect the central nervous system and induce paralytic disease.

A vaccine that does not prevent severe disease might nevertheless mitigate it; however, again it is difficult to find realistic examples, at least from the sphere of viruses. For a bacterial disease, a valid example may be the original tuberculosis vaccine, which is an attenuated live vaccine.

An intriguing benefit of herd immunity is that it protects not only the vaccine recipients, but also the non-recipients, including those in whom vaccination is inadvisable, because they are predisposed to adverse reaction to the vaccine. However, it is self-evident that only when herd immunity is actually feasible can a case be made to impose mandatory vaccination on the healthy majority for the sake of protecting the vulnerable few. The COVID-19 vaccines, which were foisted on the public with relentless coercion, have never come close to meeting this requirement.

⁹For an apparent example to the contrary, see Section 3.4.6, which discusses a clinical case in which cytotoxic T-lymphocytes against spike, but not spike protein itself, were detected within the liver.

2. Immunological mechanisms of harm by mRNA vaccines

We had seen in the preceding chapter that cells which express “non-self” antigens will be attacked and destroyed by our immune system. In viral infections, this is a necessary evil, because it leads to elimination of the befallen cells. A mitigating circumstance is that most viruses target a limited spectrum of tissues and cell types, and most tissues can regenerate, so that wounds can heal thereafter.

Proponents of mRNA vaccines commonly argue that these agents do nothing more than mimic what happens in actual virus infections. Expression of the alien protein is thereby claimed to be short-lived and confined mainly to the site of intramuscular injection. Serious adverse reactions are therefore not to be expected. Nothing, however, could be more misleading and further from the truth.

2.1 mRNA vaccines are distributed throughout the body and prominently affect the blood vessels

The assertion that the mRNA/lipid nanoparticles remain at the site of injection is now widely known to be a blatant untruth. The “vaccines” rapidly spread from the site of injection to regional lymph nodes and to the blood circulation (see Section 4.2.1). Moreover, in contrast to most viruses, mRNA vaccine nanoparticles can be taken up by any cell type, including the *endothelia*, which form of the innermost cell layer of the blood vessels.

The involvement of the endothelia immediately distinguishes mRNA “vaccination” from most naturally occurring infections. In Section 1.1, we noted that viruses depend on specific receptor molecules on the surfaces of their host cells, which limits the scope of cells and tissues they can infect. Very few viruses target endothelial cells, but those that do can cause dangerous hemorrhagic fevers; the Dengue, Ebola and Marburg viruses are examples. Intracellular bacteria that infect vascular endothelia also cause life-threatening disease (e.g. typhus and Rocky Mountain spotted fever). The clinical diseases caused by these pathogens are characterized by bleeding, often compounded by thromboembolic complications, which strikingly resembles some of the major acute adverse reactions to the mRNA vaccines.

With both the infectious hemorrhagic fevers and mRNA vaccines, the damage mechanism is quite straightforward: endothelial cells that express “non-self” antigens will come under attack by the immune system (Figure 2.1). As discussed earlier, this immune attack can involve antibody-mediated complement activation, cytotoxic T-cells, and other effector mechanisms in varying proportion. Blood clots forming in the wake of endothelial injury will result in circulatory disturbances, with sometimes grave and irreversible consequences like heart attack and stroke. The evidence on this point is unequivocal—the expression of the spike protein in the cells of the blood vessels, the

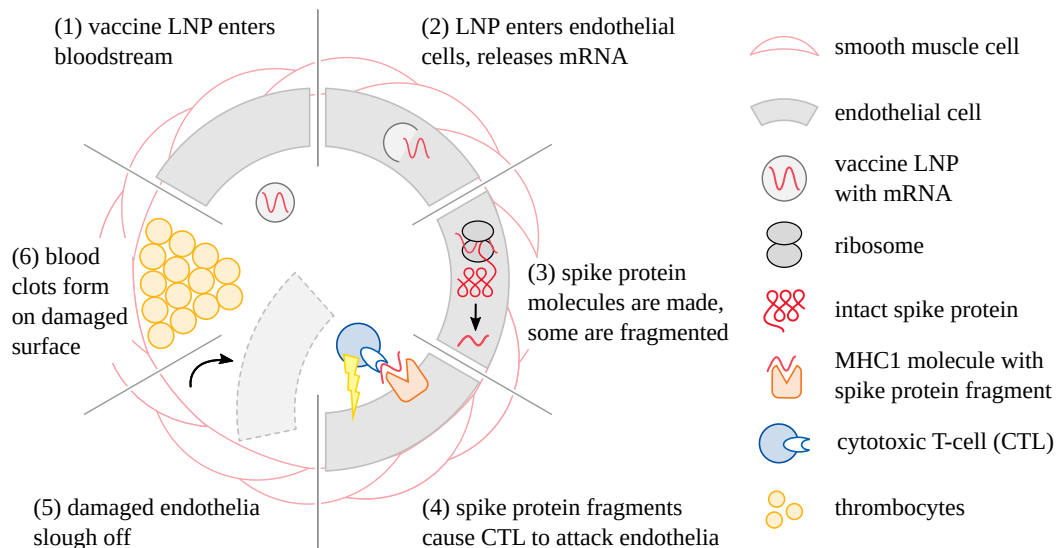


Figure 2.1 How mRNA vaccines damage blood vessels and cause clotting. After the vaccine lipid nanoparticles have entered the circulation (1), they are taken up by the endothelial cells, and the mRNA is released (2). The antigenic protein is then expressed (3) and transported to the cell surface, where it induces immune attack against the cells by antibodies and complement or by cytotoxic T-cells (4). Damaged endothelial cells slough off (5), which permits leakage of vaccine particles into the adjacent tissues. It also exposes the deeper layers of the vessel wall to the blood, which triggers thrombocyte aggregation (6) and blood clotting.

ensuing immune attack on these cells, and the induction of blood clots are all clearly visible in tissue samples from biopsies and autopsies (see Section 3.3).

2.2 The expression of spike protein in the body is widespread and long-lasting

Studies on a model mRNA vaccine have shown that the lipid nanoparticles, after intramuscular injection, rapidly enter the bloodstream. They subsequently accumulate preferentially in certain organs including the liver, the spleen, and the ovaries. The factors which determine the accumulation of the vaccine particles in different organs will be discussed later (see Section 4.1). However, at least the blood vessels themselves are exposed to the vaccine in every organ and every tissue, from which we have to expect widespread expression of the foreign antigen. With the COVID-19 mRNA vaccines, such widespread expression has indeed been directly demonstrated; some of the evidence will be presented Chapter 3.

Another important consideration is how soon the antigen is expressed, and how long this expression lasts. Ogata et al. [58] have detected expression of the SARS-CoV-2 spike protein in blood samples even on the day of the injection. The amount detectable in their samples peaked within the first week and then rapidly dropped. That short apparent duration, however, was likely due to the concomitant rise in the level of circulating antibodies. These antibodies would have bound to the antigen and thereby interfered with the detection method, which itself relied on capture of the antigen with specific antibodies.

Bansal et al. [59] reported another study on the time course of spike protein detectable in blood samples. In contrast to Ogata et al., they detected a rise only at two weeks after the initial vaccine injection. The highest levels were found at two weeks

after the second injection. Even at four months after that second injection, however, Bansal et al. still detected considerable levels—similar to those detected after the initial two weeks. These authors’ findings deviate from those by Ogata et al. in two respects: firstly, the antigen was detected after much longer time periods than reported by Ogata et al.; and secondly, Bansal et al. did not see Ogata’s early peak.

These two discrepancies may be explained by the different sampling and assay methods used in the two studies. Ogata et al. applied their antibody capture assay to regular serum samples that had not undergone any prior processing. In contrast, Bansal et al. first isolated so-called *exosomes*—cell-derived membrane vesicles—from the serum, which they then subjected to *Western blot*, i.e., separation of proteins by SDS gel electrophoresis, followed by identification of the spike protein with antibodies.

With respect to the early expression of spike protein, there is reason to prefer the data reported by Ogata et al., since they did not discard the fraction of spike protein which was *not* bound to exosomes. On the other hand, with regard to the late expression, the study by Bansal et al. is preferable, since their use of SDS gel electrophoresis should have removed the interference of serum antibodies with the detection of spike protein.

The upshot is that both the early expression reported by Ogata et al. and the late expression reported by Bansal et al. are likely correct. For a more extensive discussion of both studies, see [60]. A fairly long-lasting expression of spike after mRNA vaccination was also reported by Röltgen et al. [61], who still detected the spike protein in lymph nodes 60 days after the second injection, and at this same time point also showed the continued presence of mRNA encoding the spike. Similarly, Magen et al. [62] detected strong spike protein expression and continued presence of the RNA At one month after vaccination. Their study concerned a patient with vaccine-induced myositis, and their sample was muscle tissue located distantly from the injection site.

Such long-lasting persistence of the mRNA, and therefore of antigen expression, must be assumed to be unrelated to the identity of the encoded antigen. Instead, it is most likely a property of the delivery technology in general. The calamitous consequences of this long-lasting antigen expression will be considered below.

2.3 The mRNA vaccine LNPs fly under the radar of the immune system

Another crucial difference between real viruses and mRNA vaccines is that the particles of the former, but not the latter, are decorated with copies of the protein molecules encoded by the genome they contain. The consequences of this difference are illustrated in Figure 2.2.

We noted earlier that viruses typically cause significant disease only once, namely, when we are first infected with them; this is because we have no antibodies or other specific immune mechanisms yet which could prevent the virus from entering and multiplying within our body cells. However, after our first infection, we will have memory B-cells, which can meet any repeated infection with a rapid antibody response; the antibodies will then bind and neutralize the virus particles.

For this antibody-mediated neutralization to work, the particles of the virus must contain and expose at least some of the antigens encoded by it. That is indeed the case with all actual viruses. In contrast, the particles of an mRNA vaccine are encased

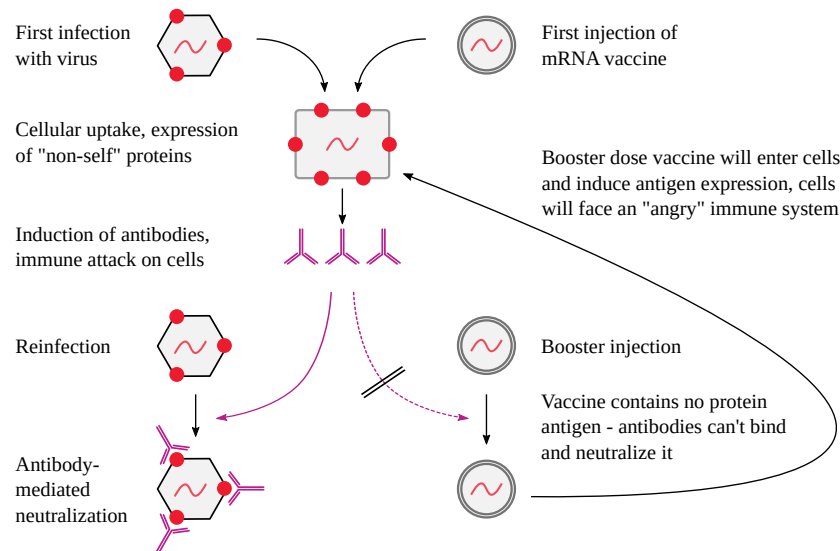


Figure 2.2 mRNA vaccines fly under the immune system's radar. Left: the particles of a proper virus are decorated with some of the proteins which are encoded by the viral genome. As a consequence, the virus will efficiently enter cells only when we are first infected with it, whereas on subsequent encounters, antibodies induced after the first infection will neutralize the virus. Right: in contrast, mRNA vaccine particles don't contain any protein antigen; therefore, antibodies against the encoded protein antigen can't prevent the particles from entering our body cells and exposing them to immune attack.

with a shell of lipid molecules only, which are not effective antigens.¹ Therefore, even though the first injection with the vaccine will induce antibodies against the encoded antigen, those antibodies will be unable to recognize and neutralize the vaccine particles when another dose is injected. The vaccine will therefore enter our body cells with undiminished efficiency. Only when the antigen is expressed and appears on the surface of those cells will the antibodies recognize it; and they will now direct the full destructive power of the immune system against those cells.

The above assumes that the antigen does appear on the cell surface in intact form. This is indeed the case for the COVID-19 spike protein, but it may not apply with some future mRNA vaccine that encodes a different antigen which remains inside the cell. In this case, however, we must expect such an antigen to be processed and presented in the form of MHC1-associated peptides; these would then attract the attention of cytotoxic T-cells. Thus, regardless of whether B-cells or T-cells dominate the memory response—the upshot is that prior immunity to the antigen encoded by the mRNA vaccine will *aggravate* the damage caused by repeated exposure to the agent.

In a nutshell, therefore, while specific immunity mitigates the harm done by repeated virus infections, it will worsen the harm done by repeated injection of an mRNA vaccine. It bears mention that such prior immunity need not have been induced by a preceding vaccine injection; the effect will be much the same when someone who has previously been infected with the virus in question receives his first vaccine injection. Thus, in the context of the COVID-19 vaccinations, the authorities' refusal to exempt

¹Some individuals actually do have preexisting antibodies against some of the lipids, particular the ones which contain polyethyleneglycol (PEG). Such antibodies can cause allergic reactions to the vaccines [63–65].

those with such natural immunity from their vaccine mandates has likely increased the number of severe adverse events substantially.

We also note that the effect discussed here might well mitigate the harm done by adenovirus vector-based genetic vaccines. While with such vaccines, too, the antigen of interest is not part of the infectious particles, the antibody response triggered against the adenoviral proteins will tend to neutralize the vaccine virus particles upon repeated injection. This is, of course, not to be understood as an endorsement of the adenovirus vector technology; the virus-based vaccines against COVID-19 have caused severe adverse events on the same scale as the mRNA vaccines [6].

2.4 Induction of autoimmune disease

2.4.1 Background. We noted in the preceding chapter that autoimmune disease is caused by the emergence and proliferation of T- and B-lymphocytes which specifically recognize “self” antigens. Autoimmune diseases usually involve various degrees of cell and tissue destruction, which are brought about by the same effector mechanisms that exist for the sake of eliminating virus-infected cells. However, in some cases, the autoantibodies may cause more subtle functional disruption, such as the inhibition of signal transmission from nerve to muscle cells in myasthenia gravis, or the excessive activation of thyroid gland growth and hormone production in Graves’ disease. In yet another paradigm, an autoimmune disease that is transient (though possibly protracted) nevertheless irreversibly damages organ function. A good example is the autoimmune aggression against the insulin-producing beta-cells of the pancreatic islets, which results in type 1 diabetes, a lifelong condition.

As the above examples suggest, the self antigens to which an autoimmune disease reacts are often organ-specific. Another illustration is the protein thyroglobulin, which occurs only in the thyroid gland, and which is a key self antigen involved in this organ’s destruction by an autoimmune disease known as Hashimoto’s thyroiditis. Blood cells, too, can be targeted by autoimmune disease. For example, some autoantibodies may destroy the thrombocytes (blood platelets), which are essential for blood clotting. The result will be “thrombocytopenic purpura”, that is, spontaneous bleeding beneath the skin and in other places. Other autoantibodies may activate the thrombocytes, in which case blood clots will be observed. Their excessive activation, too, will deplete the thrombocytes, so that the clinical picture may be a combination of clotting and bleeding. The latter has been observed after COVID-19 vaccination and termed “vaccine-induced thrombotic thrombocytopenia” (VITT).

In other cases, the autoantigens are found throughout the body, which means that an autoimmune attack on them will afflict many different organs. A good example are the anti-DNA and anti-phospholipid antibodies in systemic lupus erythematosus (SLE). As one might expect from the involvement of multiple organs, SLE is a serious disease.

2.4.2 Autoimmune disease induced by infections. Most autoimmune diseases have a strong genetic component, but on the other hand almost all of them require some additional trigger to become manifest. Such triggers can be infectious agents. One example are group A streptococci, which can cause acute rheumatic fever. The acute autoimmune disease is again transient, but it can cause irreversible damage to the heart.

With rheumatic fever and several other autoimmune diseases, the central mechanism is believed to be *molecular mimicry* [66, 67]. In this mechanism, a non-self antigen of the infectious agent closely resembles one of the body's self antigens, so that T-cell or B-cell clones whose receptors recognize one of the two will also recognize the other. Such cross-reactive lymphocyte clones are already present before the infection strikes. However, at this stage, they are not active—instead, they are in a dormant state that was imposed on them by other, regulatory T-lymphocytes in order to safeguard the body cells that express the self antigen.²

This somewhat precarious state of self-tolerance may break down when the infectious agent appears on the scene, and with it the cross-reactive microbial antigen. The infection will cause inflammation, which will provide the non-specific impetus for initiating an immune response (cf. Section 1.2.2.1). Among the many different T- and B-cell clones that will be recruited and activated by this response are the dormant ones which recognized the cross-reactive microbial antigen. They will then attack not only the microbe but also the body cells which express the corresponding self antigen. Because of the delay inherent in any adaptive immune response, the autoimmune disease will typically flare up several weeks after the infection. For example, acute rheumatic fever may be diagnosed some 1-5 weeks after the usually trivial streptococcal infection that triggered it.

Molecular mimicry is also widely believed to occur in the pathogenesis of type 1 diabetes. Several viruses have been implicated, including Coxsackie viruses, cytomegalovirus, and rotaviruses. However, other mechanisms of causation, including a persistent infection of the pancreatic islet cells with the virus in question also remain under consideration [69].

Various autoimmune phenomena and diseases have been reported in connection with COVID-19 infections and after vaccination against the disease [70, 71], and molecular mimicry has been suggested as a key mechanism [70, 72]. While this causation is conceivable, our own analysis (unpublished) shows that the count of amino acid sequence motifs which are shared between the SARS-CoV-2 spike protein and the proteins of human cells is very similar to the counts obtained with the spike proteins of other coronaviruses. Thus, if SARS-CoV-2 is indeed “the autoimmune virus”, as claimed by Halpert and Shoenfeld [70], then this must be ascribed to factors other than abundance of cross-reactive immunological determinants.

2.4.3 Deficient clearance of self antigens released from deceased cells. We discussed in Section 1.2.1 that antigens which remain inside our body cells throughout their entire life cycle will only encounter the immune system after fragmentation and presentation by MHC1 surface molecules; they will not normally encounter antibodies. Keeping these antigens away from the cells which bring about the production of anti-

²A computational study has claimed that the SARS-CoV-2 spike protein has far greater sequence similarity, and therefore greater potential for immunological cross-reaction, with human proteins than with those of animals [68]. However, these purported findings extend even to chimpanzees, which are very closely related to humans. An unpublished analysis by this chapter's author did not reproduce these findings—neither does SARS-CoV-2 spike protein contain more sequence similarity to human than to chimpanzee proteins, nor does it exceed the extent of similarity observed with the spike proteins of some other coronaviruses. Thus, any unusually high propensity of SARS-CoV-2 to trigger autoimmunity is not accounted for by the number of potentially cross-reactive epitopes.

bodies is an important aspect of self-tolerance. To maintain this separation, body cells which disintegrate must be cleared away promptly and in an orderly manner.

An important mechanism to ensure this orderly disposal of cell debris is *apoptosis*. When cells undergo programmed cell death, for example as the result of cytotoxic T-cell action, the cell fragments expose molecular markers which identify them to the scavenging phagocytes as derived from self. The phagocytes will then *not* respond as they would to the ingestion of a pathogenic microbe, and therefore will not activate T-helper cells to induce an antibody response.

If this orderly clearance mechanism is overloaded, and therefore the cellular debris is left to 'rot' before being removed, then it may no longer be recognized as derived from self. The phagocytes may then initiate the production of antibodies to the self antigens contained in the debris. These autoantibodies will further promote inflammation, which in turn will cause more destruction of cells and accumulation of cellular debris; the final result may be full-fledged autoimmune disease. In keeping with this mechanism, a number of gene defects which interfere with the clearance pathway promote the manifestation of systemic lupus erythematosus [73].

In principle, any tissue insult could potentially set in motion this pathway to autoimmunity; this includes infections, vaccinations, and apparently even physical trauma [74, 75]. In the clinical trials on the COVID-19 mRNA vaccines, many participants experienced high fever [76, 77]. Both the immunological mechanism of cell destruction and toxic activity of the lipid nanoparticles [78] may contribute to the inflammation underlying these febrile reactions. From such findings, we should expect autoimmune phenomena after vaccination to be common.

2.4.4 Autoimmune diseases induced by COVID-19 vaccines. The medical literature indeed contains numerous case reports of autoimmune diseases induced by COVID-19 mRNA vaccines. For organ-specific examples, see [79–82]; for a general overview, see [71]. The diagnoses include type 1 diabetes, thyroiditis, Guillain Barré syndrome, hepatitis, systemic lupus erythematosus, thrombocytopenic purpura (i.e. antibody-mediated blood platelet destruction), and many others. We will see some specific examples in Chapter 3.

2.5 Vaccine-induced immunosuppression

2.5.1 Manifestations of immunosuppression after COVID-19 vaccination. While autoimmune phenomena triggered by the COVID-19 vaccines have arrived in the mainstream of the medical literature, this is not yet the case with another potential consequence, namely, immunosuppression. The clearest indication of immunosuppression is provided by the numerous case reports of shingles occurring shortly after vaccination; for a large series of documented cases, see [83]. Shingles arises through the reactivation of varicella zoster virus (VZV). The initial infection with this virus causes chickenpox. While this is clinically a generalized but self-limiting disease, the virus stays behind in the sensory nerve nodes (ganglia) near the spinal cord. Most peoples' immune system manage to keep the virus in check perpetually and prevent it from ever appearing on the scene again. However, in some persons, typically middle-aged or elderly, the virus can break out into the open once more to cause shingles. The skin lesions look like those in chickenpox, but their spread is typically limited to one *dermatome*, that is, the skin area which corresponds to a single sensory nerve node. A

case of shingles may signal the presence of an underlying systemic disease that saps the immune system, and it is advisable to examine shingles patients for further signs of such a disease.

In addition to shingles, bacterial infections, often involving the digestive tract, have also been reported after COVID-19 vaccination [84–86]. Such cases, too, might be caused by immunosuppression, but blood clots and disrupted perfusion of the affected sites may well contribute; based on the published reports, it is not possible to make a clear causal attribution.

Several experienced pathologists have shared their observations on rising case numbers and increased malignancy of cancers since the beginning of the COVID-19 vaccinations (see e.g. [87]). Many such cases seem to involve the reactivation of cancers, sometimes after decades, which had been considered cured. The mechanisms of cellular immunity that keep cancer cells in check are basically the same as those which control and combat viral infections. Therefore, these reports also point to significant immunosuppression after vaccination.

2.5.2 Possible mechanisms. As noted above, immunosuppression is not yet commonly acknowledged as a significant problem caused by the COVID-19 vaccinations, and we are not aware of any published experimental research to address the question of its causation. However, several causative mechanisms are plausible (and not mutually exclusive).

2.5.2.1 Saturated bandwidth. The immune system is subject to global restraints on the extent of its activation. If its attention is focused on the sustained vaccine-induced expression of a foreign antigen in multiple tissues and organs of the body, this will divert resources from fighting actual pathogens which invade concomitantly.

2.5.2.2 Lymphocyte fratricide. We discussed earlier that body cells which express the mRNA vaccine-encoded foreign antigen will be subject to attack by cytotoxic T-cells and other cytotoxic immune effector mechanisms. Lymphocytes are not exempt; if they take up the mRNA vaccine, they too will become targets for the other lymphocytes. In this manner, the immune system would destroy itself.

2.5.2.3 Immunosuppression by lipid nanoparticles. An immunosuppressive effect of the lipid nanoparticles has been demonstrated by Qin et al. [88]. These authors measured the lymphocyte activation and the antibody response to an experimental mRNA vaccine encoding an influenza virus antigen. This experimentally induced immune response was subdued by a preceding injection of lipid nanoparticles alone (and also of another experimental mRNA vaccine). Interestingly, the immunosuppressive effect was more pronounced when both injections were applied into the same body site, suggesting that damage to the regional lymph nodes by the first injection was partly responsible. However, changes to the pattern of immune responses were also observed when the second injection was applied to another body site, and remarkably were even passed on the offspring of LNP-injected mice.

Lymphocytes are notable for their extraordinary sensitivity to apoptotic stimuli—for example, they can be driven into programmed cell death by very low doses of ionizing radiation. As we will discuss in Section 4.3.3.1, the toxicity of cationic lipids is mediated by reactive oxygen species, and the same is true of ionizing radiation. Therefore, lymphocytes might succumb to lipid nanoparticle toxicity more readily than

other cells. However, the inheritable changes of immune regulation documented by Qin et al. indicate that there is more to the LNP story than just the killing of lymphocytes.

2.6 The fundamental mechanism of damage by mRNA vaccines is completely general

Since all of the evidence of harm discussed in this chapter relates to the COVID-19 mRNA vaccines, you might wonder what we should expect from future mRNA vaccines. Should we chalk up the toxicity of the COVID-19 vaccines to the specific antigen which they encode, or is such grievous harm inherent in the mRNA technology?

In our considered opinion, the outcome with any mRNA vaccine will be much the same as it was with the COVID-19 vaccines. It is true that the spike protein itself can promote blood clotting and inflammation without any help from the immune system [89]. Nevertheless, the evidence which will be shown in Chapter 3 indicates that the grave, widespread and sustained injury to tissues and to blood vessels is mostly caused by the immune attack on spike protein-producing cells. This attack occurs simply because the spike protein is a non-self antigen; and since every other mRNA vaccine will necessarily encode its own non-self antigen, derived from whichever particular microbe it targets, we must expect that it will cause harm by the same mechanism and to a similar extent.

3. Pathological evidence of immunological harm due to mRNA vaccines

Pathologists examine the organs and tissues of deceased patients, as well as tissue specimens of live patients (biopsies), in order to establish the causes of disease. While the macroscopic examination, at autopsy, of diseased organs is important and usually sufficient to diagnose causes of death such as lung embolism or myocardial infarction, much more detail can be revealed by the use of *histopathology*, that is, the microscopic examination of tissue samples. Microscopic study can be combined with biochemical and immunological techniques for detecting the occurrence and distribution of specific molecular markers of disease. Histopathology is useful not only in post mortem studies, but also with *biopsies*, that is, tissue samples obtained from living patients.

While pathological studies on patients who had suffered or died from adverse events of the COVID-19 vaccinations were slow to appear in the medical literature, there now is substantial evidence that sheds light on the mechanisms of disease causation. As we will see, immune attack on the body's own cells and tissues is the main recurring theme.

3.1 Key techniques used in histopathology

In order to examine a tissue sample under the microscope, it first needs to be cut into delicate slices of uniform thickness. In preparation for this step, the tissue sample is typically first treated with a *fixative*, often formaldehyde, and then embedded in paraffin. The fixative prevents chemical and structural degradation of the sample, and the paraffin firms it up for sectioning.

3.1.1 Chemical staining. Another important consideration is visual contrast. Most cells and subcellular structures are colorless and not easily discernible under the microscope. To enhance contrast, the tissue samples are commonly stained with a mixture of chemical dyes. Based on their ionic charges and other properties, these dye molecules will bind preferentially to different subcellular structures.

The widely used HE staining method uses the two dyes hematoxylin and eosin. The former is bluish and binds preferentially to nucleic acids and other negatively charged molecules, whereas the latter is red and preferentially binds to proteins. The usual result is that cell nuclei, which contain large amounts of DNA, appear blue or purple, whereas most of the remaining structures will be stained predominantly red (Figure 3.1). Deposits or droplets of fat remain unstained. While the HE method is useful for routine histopathology, there are a number of interesting special-purpose chemical stains which better highlight particular physiological or pathological cell and tissue structures.

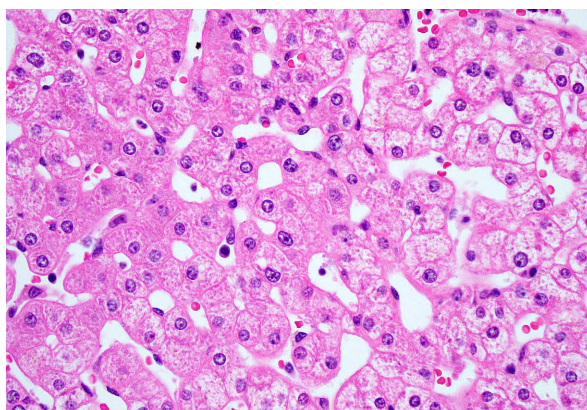


Figure 3.1 Normal liver tissue (HE-stain). Cell nuclei are purple, whereas the remainder of the cell (the cytoplasm) is pink. In this image, we can see the outlines of most cells. That is not always possible, but one can always see the nuclei. The scattered little bright-red dots are red blood cells. They are located within empty spaces (the liver *sinusoids*). In life, the sinusoids are entirely blood-filled; in this sample, however, most of the blood has been flushed out.

3.1.2 Immunohistochemistry. An important technique that very substantially enhances the power of histopathology, and of which we will see several examples, is *immunohistochemistry*. It harnesses the specificity of antibodies for selectively staining cells which contain a particular molecule of interest. For example, while all lymphocytes look alike in the HE stain, immunohistochemical detection of the CD3 cell surface antigen can be used to identify T-lymphocytes. Detection of CD4 and CD8, respectively, can be used to further distinguish T-helper from cytotoxic T-lymphocytes. And, as we will see, the expression of viral antigens such as the SARS-CoV-2 spike protein can be observed as well.

The essential steps of the method are illustrated in Figure 3.2. The tissue slice is first exposed to an antibody which specifically recognizes the molecule of interest. After allowing some time for binding to occur, the unbound surplus of antibody is washed off. A secondary antibody is then added which recognizes the first one, allowed to bind, and the unbound residue again washed off. This secondary antibody has been chemically coupled to an enzyme (a catalytic protein) which can convert a colorless, soluble precursor molecule (often diaminobenzidine) to an insoluble pigment which is deposited in situ.¹ This enzyme reaction serves as an amplification step—a single enzyme molecule can generate a comparatively very large amount of pigment, so that even a small number of molecules of interest can be readily detected.

3.2 Sources of evidence

In the following, we will for the most part rely on case reports and reviews from the peer-reviewed medical literature. In addition, we will repeatedly reference a series of autopsy examinations carried out by Arne Burkhardt, MD, emeritus professor of

¹One might wonder why the enzyme is chemically coupled to a secondary antibody rather than directly to the antigen-specific first antibody. This would indeed be possible in principle, but it is more convenient to couple the enzyme to a secondary antibody instead, since such a conjugate can be used with very many different antigen-specific primary antibodies, which need not themselves be chemically modified. For example, to detect cytotoxic T-cells rather than T-helper cells, we would simply replace the CD4-specific primary antibody with one that recognizes CD8; all other steps would remain unchanged.

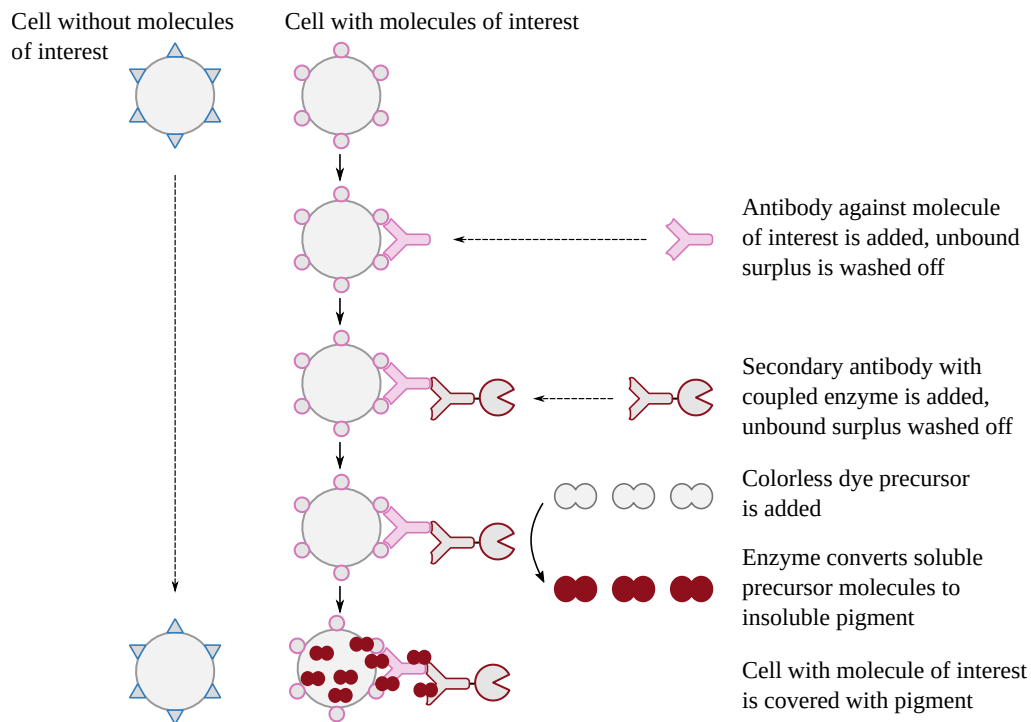


Figure 3.2 Schematic illustration of immunohistochemistry, a method for selectively detecting specific molecules of interest in tissue samples using specific antibodies. See text for details.

pathology, with the assistance of several colleagues. While Burkhardt's results have not yet been published in the form of peer-reviewed journal articles, they have been demonstrated to and vetted by other pathologists and medical doctors, and they were available to the author of this chapter.

While most of Burkhardt's findings are qualitatively confirmed by those described in peer-reviewed articles, his work does add some valuable quantitative perspective. As of this writing, Burkhardt has evaluated autopsy materials from 43 patients who died after receiving one or more COVID-19 vaccine injections. In all of these cases, the diagnosis on the death certificate had *not* made reference to those vaccines, but the bereaved families had sought a second opinion from Burkhardt. His thorough investigation led Burkhardt to conclude that causation by the vaccine was certain or likely in 22 cases, and possible in 7 more cases. He ruled out causation in only 3 cases, whereas in the remaining 11 cases a conclusive determination could not or not yet be made.

Out of all 43 deceased patients, 29 were known to have received one or more injections of mRNA vaccines, but no others. Within this subset, Burkhardt deemed causation of death by vaccination certain or likely in 14 cases. Such figures should give pause to those who have thus far accepted the mainstream narrative that severe adverse events of "extremely rare."

3.3 Vasculitis induced by mRNA vaccination

In Section 2.1, we had discussed that the blood vessels will be prominently affected by vaccine damage, since the vaccines will initially be distributed with the bloodstream; the cells of the vascular endothelium (the innermost layer of the vessel wall) will then

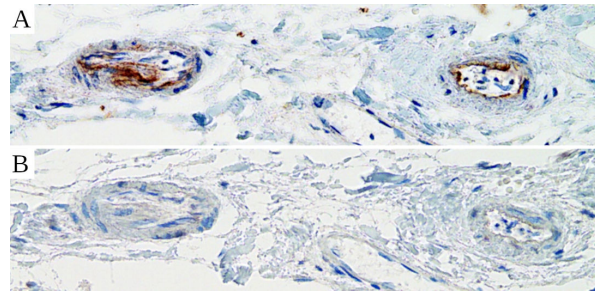


Figure 3.3 Cross section of two small blood vessels located within the wall of a larger one (a coronary artery). Immunohistochemistry for SARS-CoV-2 spike protein (A) and nucleocapsid (B). Only the spike protein can be detected, indicating that its expression was caused by the vaccine rather than by an infection with the virus. Courtesy of Michael Moerz, MD.

take up the vaccine lipid nanoparticles and start expressing the spike protein. In this section, we will consider some supporting evidence.

3.3.1 Vaccine-induced expression of spike protein in vascular endothelia. Figure 3.3 shows the expression of spike protein within the endothelium of two small blood vessels, which are located within the wall of a larger one (a coronary artery). The brown pigment seen in panel A of the figure represents the spike protein. In panel B, immunohistochemistry was used in an attempt to detect the nucleocapsid of the SARS-CoV-2 virus. The absence of brown pigment indicates that the nucleocapsid is absent.

In an infection with the virus, spike protein and nucleocapsid should be expressed and detected together. On the other hand, the gene-based COVID-19 vaccines encode only the spike protein. The detection of spike protein alone therefore confirms that its expression was caused by vaccination rather than by an undiagnosed infection with the virus.

3.3.2 Vasculitis, blood clots, and dissection: example autopsy findings. Figure 3.4 shows HE-stained tissue sections from small and large blood vessels of people who died after COVID-19 vaccination. Panel A shows a cross-section through a normal artery. We see a sturdy, compact muscular layer, which displays a more intense red color than the surrounding connective tissue. In the adjacent panel B, we see a wall section of a somewhat larger artery afflicted by vasculitis. Some muscle tissue remains intact at the bottom left, but most of the tissue has been infiltrated by inflammatory cells, including lymphocytes, and is disintegrating. Panel C shows a small blood vessel similarly affected; the higher magnification shows infiltration by lymphocytes and also granulocytes and histiocytes. Panel D shows another large vessel with vasculitis; the destruction of the wall is less advanced than in panel B, but it has caused the formation of a large blood clot, which entirely obstructs the lumen.

Panel E shows a wall section from the aorta of a vaccinated person. The image was taken at low magnification, and accordingly the infiltrating lymphocytes appear here as clouds of tiny blue specks. We see a crack running across the inflamed tissue. A crack is also visible macroscopically in panel F of the figure. The dark-colored material seen within in the crack is coagulated blood. This clinical picture is known as *aortic dissection*.

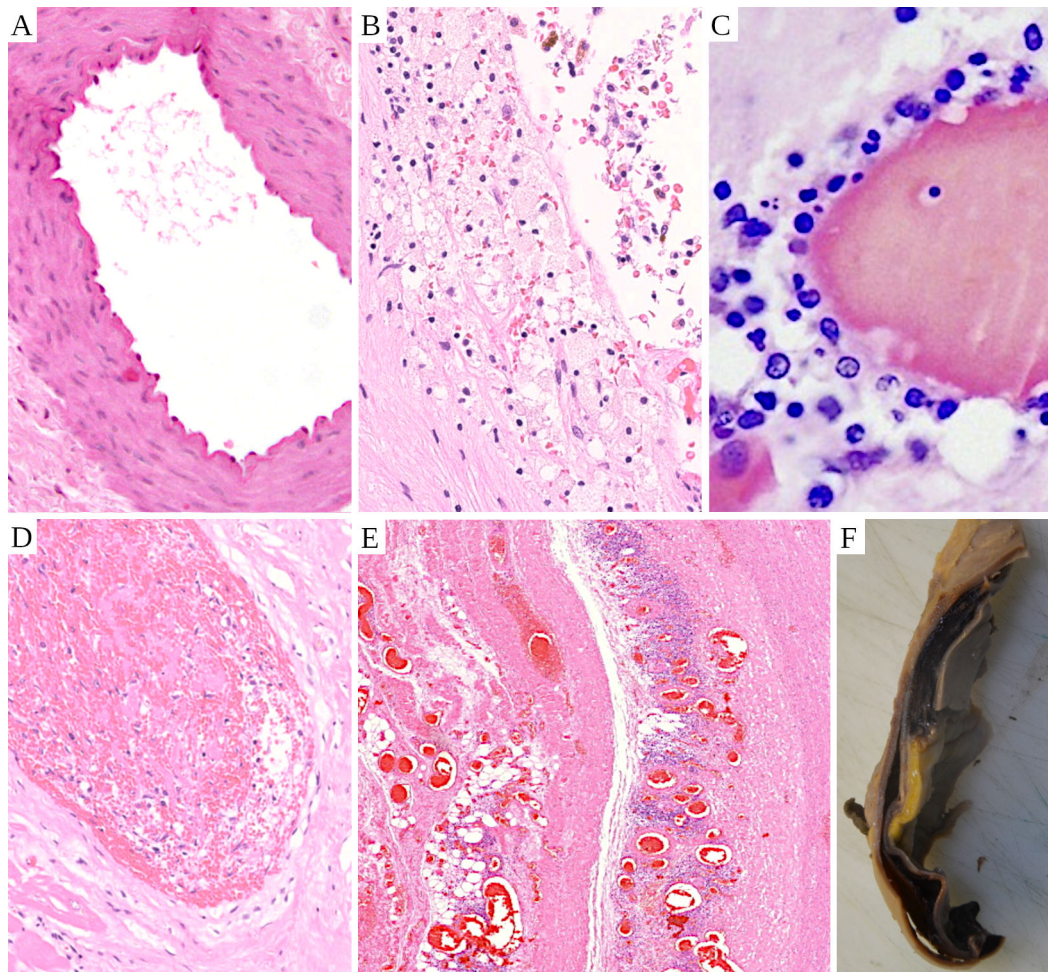


Figure 3.4 Vasculitis of small and large blood vessels. Cross sections of a normal blood vessel (A), and manifestations of vasculitis after COVID-19 vaccination in small (C) and large (B, D, E, F) blood vessels. All microscopic section were HE-stained. **A:** a normal artery with a compact and regular muscular layer. The inner surface is unbroken and clearly defined; its wavy shape is a post-mortem artifact. **B:** the wall of an artery with vasculitis. The tissue is loosened up and “moth-eaten”; it has been invaded by lymphocytes (dark round dots) and macrophages. **C:** vasculitis of a smaller vessel (pictured at higher magnification). The vessel wall is infiltrated by both lymphocytes and granulocytes. **D:** vasculitis of a larger vessel has caused a blood clot, which fills the lumen. **E:** cross section of an aortic wall, shown at low magnification. Infiltrating lymphocytes appear as clouds of tiny blue specks. To the left of the largest blue cloud, a vertical crack runs through the tissue. **F:** a crack is also visible macroscopically in this excised specimen of aortic wall from a patient with *aortic dissection*. The dark material within the crack is coagulated blood. See text for further explanations. Image credits: panel A is from [90], B and D from Dr. Ute Krüger, C from Dr. Michael Möerz, and E and F from Dr. Arne Burkhardt.

3.3.3 Aortic dissection and rupture. While dissection can occur in other arteries as well, it often affects the aorta, which is the largest blood vessel of the body. The aorta receives the highly pressurized blood ejected by the most powerful heart chamber (the left ventricle), and it therefore is subject to intense mechanical stress. If the wall of the aorta is weakened by inflammation, then it may fail under this strain. The failure begins with a rupture of the vessel’s inner layer (the *intima*). The pressurized blood will force its way into the crack and from there into the underlying muscular layer, the *media*. As it pushes on, the blood splits the vessel wall into two separate sleeves. This

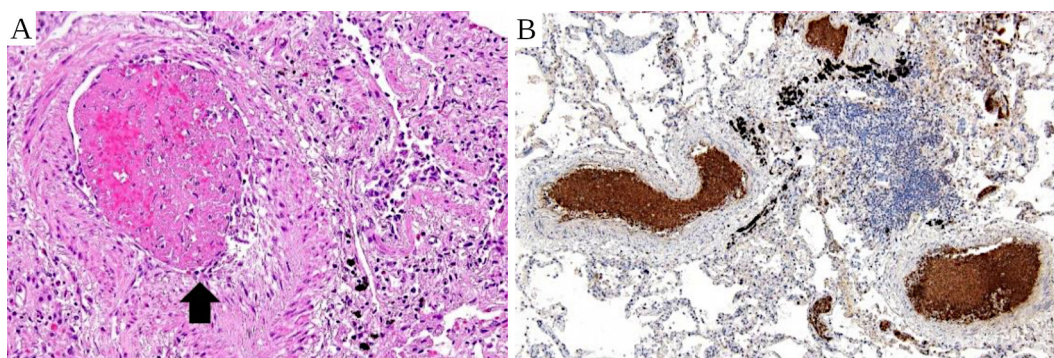


Figure 3.5 Blood clots in lung tissue. **A:** a blood clot obstructs a small artery in the lung. The wall of the vessel shows signs of vasculitis. **B:** Several lung vessels obstructed by thrombi (immunohistochemistry). The brown pigment highlights platelet factor 4, indicating that the clots are rich in platelets. The blue cloud to the right of the center is a large lymphocyte infiltrate. Figure adapted from Roncati et al. [100].

zone of separation may spread along the entire length of the aorta and even beyond into its branches. If the outer sleeve of the damaged vessel holds, then prompt surgical treatment may save the patient, but if it bursts, then the ensuing internal bleeding will be immediately fatal.

Aortic dissection has previously been reported in connection with other forms of vasculitis [91, 92], and more recently also with COVID-19 infection [93, 94]. Aortic dissection and rupture are normally quite rare, but Prof. Burkhardt found three such cases in a total of 29 patients who had died after receiving an mRNA vaccine. (These three deaths occurred between 7 and 25 days after the most recent injection.) One of these cases was also studied by immunohistochemistry, and spike protein was detected within the dissected segment of aortic wall.

The dissection and rupture of smaller arteries, sometimes facilitated by preexisting vascular malformations, has also been reported in multiple patients who had received a COVID-19 mRNA vaccine [95–99]. Prof. Burkhardt, too, found several such cases in his series of autopsies.

3.3.4 Blood clots. Vasculitis induced by mRNA vaccines has been found to affect all kinds of vessels, large and small; and so it is with blood clots induced by it. Figure 3.4D showed a blood clot in a larger vessel; several clots in smaller vessels are seen in Figure 3.5, which is taken from a case report by Roncati et al. [100] and shows tissue sections of the lung. In the right panel of the figure, we also see a large cluster of lymphocytes within the lung tissue itself. Similar observations were also made by Prof. Burkhardt.

Aye et al. [101] surveyed 35 cases of myocardial infarction after COVID-19 vaccination; of these, 31 had received an mRNA vaccine. Most of these cases had occurred within 24 hours of the injection. The same is true of two cases reported by Sung et al. [102]; both patients had received the Moderna vaccine. Kawamura et al. [103] reports another case in connection with the Pfizer vaccine. Early manifestation is also apparent in the data collected by the VAERS database [5]; to what extent this is due to preferential reporting of such early cases is presently unknown. Myocardial infarction, most often in connection with underlying inflammation of the coronary arteries, was also a common observation in the autopsies reviewed by Prof. Burkhardt.

Kolahchi et al. [104] have published a review on acute ischemic stroke—i.e., stroke due to occlusion of a brain artery—in connection with COVID-19 vaccination. While the majority of the 43 patients included in their report had received an adenovirus-vector vaccine, there were eight patients who had been given an mRNA vaccine. Notably, five of these eight developed stroke already after their first vaccine injection, quite possible due to preexisting natural immunity (cf. Section 2.3).

Another common clotting-related brain disorder is venous sinus thrombosis; here, a large vein rather than artery is obstructed by a thrombus. Like ischemic stroke, this disease is more commonly observed with the viral vector vaccines, but again there have been case reports after mRNA vaccination as well [105–108].

Arterial and venous occlusion have also been reported in many other anatomical locations; for example, Ahn et al. [109] reported a case of thrombosis of the inferior vena cava with lung embolism in a young patient who had received the Moderna mRNA vaccine. An elderly but otherwise healthy woman who developed similar manifestations after receiving the Pfizer vaccine was described by Scendoni et al. [110]. A dramatic, ultimately fatal case of multiple arterial occlusions within the gastrointestinal tract was reported by Lee et al. [111]. Multiple cases of arterial and venous occlusion with severe consequences were also found by Prof. Burkhardt in his series of autopsies.

3.3.5 Variability of vasculitis. In the foregoing, we saw examples of inflammation affecting the inner layer of blood vessels, which will be particularly likely to cause clots, as well as to the muscular middle layer (the *media*) of major arteries. In other cases, the inflammation may primarily focus on the outermost layer of a blood vessel (the *adventitia*). All three vascular layers may be affected at different sites in one patient. Burkhardt found vasculitis in one or more vascular layers in 24 deceased patients out of 29 overall who had been injected with mRNA vaccines exclusively, and in 37 out of 43 genetically vaccinated patients overall.

The underlying pathogenetic mechanism which induces vasculitis is also somewhat variable. The immune attack may be carried out primarily by lymphocytes, or antibodies and complement may dominate; in the latter case, one may also see pronounced infiltration with neutrophil or eosinophil granulocytes and with macrophages (histiocytes). Mixed infiltrates including all of these cell types are not uncommon.

Another possible variation is IgA vasculitis. This is a peculiar form of autoimmune disease, in which immunoglobulin A, one of the major antibody variants (see Section 1.7), functions as the autoantigen. In individuals genetically predisposed to the disease, formation of the autoantibodies directed against IgA may be triggered by microbial infections or by vaccinations [112]. Circulating immune complexes consisting of IgA and autoantibodies to it may be deposited in the kidneys, and more especially inside the kidney *glomeruli*, which carry out lateral flow filtration of the blood plasma as the first step of urine production. The result will be *IgA nephropathy*. Abnormal cell proliferation will be seen within the normally fluid-filled space that surrounds the glomeruli (see Figure 3.6), and functional damage to the filtration apparatus may cause blood or of plasma proteins to appear in the urine.

Another manifestation of IgA vasculitis, which may occur alone or together with the nephropathy, are characteristic skin rashes, with blood seeping from damaged small vessels into the connective tissue layer of the skin. Two such cases which occurred after mRNA vaccination were reported by Nakatani et al. [113] and Sugita et al. [114].

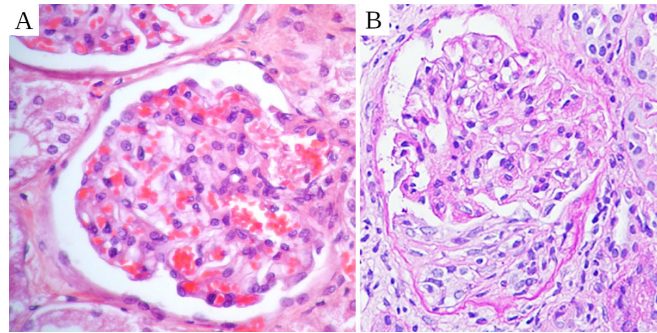


Figure 3.6 IgA nephropathy after mRNA vaccination. **A:** a normal glomerulus [90]. It consists of a coiled arteriole, whose walls function as an ultrafiltration membrane. The filtrate is captured within the surrounding empty space, which is delimited by *Bowman's capsule*. **B:** a glomerulus in IgA nephropathy after mRNA vaccination [113]. The lower third of Bowman's capsule is filled with proliferating cells as a result of inflammation.

3.3.6 The role of spike protein toxicity in vasculitis and clotting. We have so far focused our discussion of the pathogenesis on the immune response to spike protein as a foreign antigen. Additionally, however, the spike protein is endowed with intrinsic toxicity. A remarkable variety of toxic activities have been described, including for example injury to the blood-brain barrier [115, 116] and inhibition of DNA repair [117].² However, in the context of vascular damage, the main concern is the binding of spike protein to the ACE2 receptor, which occurs on many cell types, including both endothelial cells and blood platelets. Such binding will inhibit the enzymatic activity of ACE2 itself, which will promote blood clotting and possibly also inflammation [89].

The Spike protein S1 fragment can be detected circulating in the bloodstream for a few days after mRNA vaccination; levels then drop quickly as antibodies to the protein appear [58, 121]. Presumably, those antibodies will inhibit not only the detection of the circulating spike protein but also its activity. Thus, a causal contribution of direct spike protein toxicity is the most likely in adverse events which occur within a few days after vaccination, especially in those patients who received their first vaccine injection and who had no preexisting natural immunity. Heart attacks and stroke are particularly common in this period. Adverse events which become manifest after the immune response to the spike protein has set in are more likely to be caused mainly by this immune response.

3.4 Immune attack on organ-specific cells and tissues

While vasculitis and clotting can cause damage to any and all organs, there is also evidence of more direct damage to organ-specific cells. In some cases, this has been linked to the expression of spike protein in such cells; examples are muscle cells in heart and skeletal muscle, lymphocytes in the spleen, and glia cells in the brain. However, so far only very few published case reports have attempted to detect the spike protein within tissue samples from mRNA vaccine-injured patients. Accordingly, with

²On the website of the journal *Viruses* that had published it, the cited study by Jiang and Mei [117] is flagged as “retracted.” However, the scientific reasons given for this “retraction” are unconvincing; it came about most likely through political pressure behind the scenes. There have been several similar instances of scientifically baseless “retractions” of COVID-related articles [118–120].

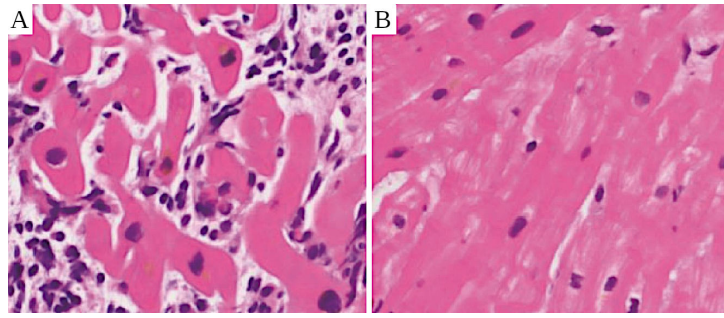


Figure 3.7 Heart muscle biopsies from a case of myocarditis after mRNA vaccination. **A:** in the acute stage (8 days after vaccination), lymphocytes and other inflammatory cells are seen between the heart muscle cells. **B:** 58 days after vaccination, the inflammation has receded. Images adapted from Koiwaya et al. [122].

most organs it is currently unknown to what extent the organ-specific cells may express spike protein. As with vasculitis, true autoimmunity that is triggered by some degree of vaccine-induced inflammation is an alternate or contributing mechanism of organ damage.

In the following, we will discuss several significant and instructive pathological studies on organs whose involvement has been repeatedly observed, without however striving for completeness.

3.4.1 Myocarditis. Expression of spike protein in heart muscle cells after COVID-19 vaccination has been documented in heart biopsies of myocarditis patients by Baumeier et al. [123]. Both mRNA and adenovirus-based vaccines were represented among the reported cases. More widespread and apparently stronger expression than reported by Baumeier et al. was detected by Burkhardt and colleagues in tissue samples from an as yet unpublished fatal case of myocarditis. Here, nucleocapsid expression was also examined but found to be negative, confirming that the expression of spike had been caused by vaccination.

As with vasculitis, the histopathological picture of myocarditis is fairly varied. The inflammatory cells invading the muscle tissue typically comprise multiple forms, but in some cases lymphocytes predominate (see Figure 3.7), whereas other cases show mainly granulocytes and histiocytes (see Figure 3.8). Several cases with a strong presence of eosinophil granulocytes were reported as well [124, 125].

The lymphocytes, where present, are predominantly T-cells; among these, cytotoxic T-cells were predominant in at least one case, as apparent from the expression of the CD8 cell surface antigen typical for these cells [46]. Inflammatory infiltrates that show predominantly granulocytes and histiocytes are compatible with an immune response that is driven primarily by antibodies and complement, both of which provide chemotactic (i.e. attracting) signals to these inflammatory cells. In keeping with this interpretation, the case reported by Choi et al. [126] showed not only inflammatory infiltrates rich in neutrophil granulocytes and histiocytes but also the activation and deposition of complement proteins on the surface of damaged heart muscle cells (Figure 3.8C).

The most straightforward explanation for this finding is that these cells had expressed the spike protein; antibodies binding to the spike molecules than triggered complement activation. In this context, it is noteworthy that the pore formed by the complement membrane attack complex will admit extracellular calcium into the cell.

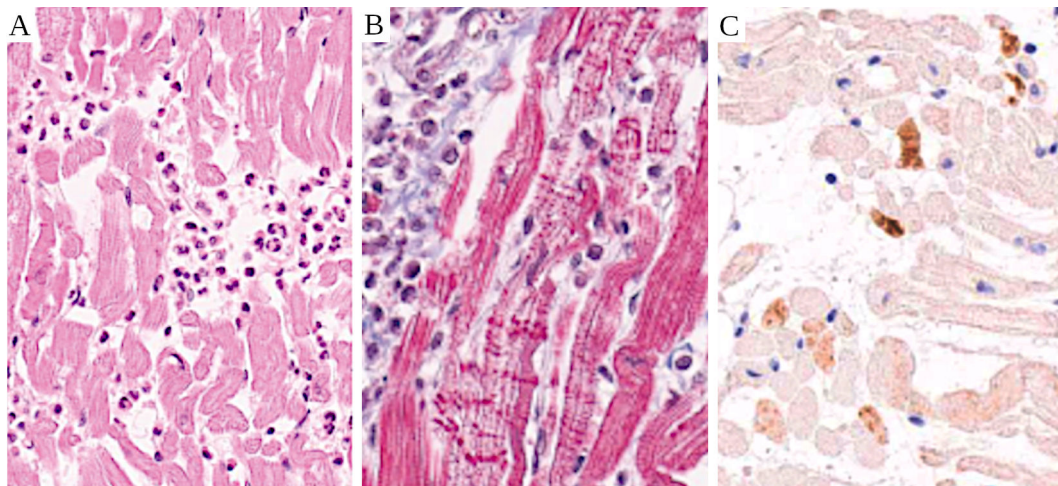


Figure 3.8 A case of rapidly fatal myocarditis after mRNA vaccination (histopathology after autopsy). **A:** neutrophil granulocytes and histiocytes (macrophages) infiltrating the heart muscle tissue. **B:** horizontal red stripes indicate cell death of heart muscle cells (contraction band necrosis). Masson's trichrome stain. **C:** complement factor C4 on heart muscle cells (immunohistochemistry). All images adapted from Choi et al. [126].

Intracellular calcium excess is an acknowledged cause of contraction band necrosis, which was a prominent feature in the histopathology presented by Choi et al. (see Figure 3.8B). We must note, however, that Choi et al. did not attempt to demonstrate this mechanism, nor did they comment on the question of how complement activation had occurred.

A similar pattern of inflammation was reported by Gill et al. [127] in two fatal cases of myocarditis after mRNA vaccination. These authors suggest that their findings “resemble catecholamine injury” to the heart. The term “catecholamines” comprises epinephrine, norepinephrine, and dopamine. Disease states with excessive catecholamine release—in particular, tumors of the adrenal glands which produce epinephrine and norepinephrine—may indeed cause damage to the heart, but the connection suggested by Gill et al. is tenuous, considering the fatal outcome in these two previously healthy young men. We submit that the pathological findings reported by Gill et al. are more readily explained by antibody-mediated immune attack on spike-expressing heart muscle cells. This question deserves to be more thoroughly elucidated in future histopathological studies.

In a recently reported case that exhibited both encephalitis and myocarditis, inflammatory changes in the heart were mostly centered on the small blood vessels, which were also shown to express spike protein [128]. However, even where these small vessels had not been obstructed, damaged muscle cells with contraction bands (cf. Figure 3.8B) were also seen. This illustrates that vasculitis and direct inflammatory damage to organ-specific cells are not mutually exclusive.

In conclusion, the histopathological picture of vaccine-induced myocarditis shows considerable variation. Lymphocytic inflammation most resembles myocarditis caused by viruses, which before the arrival of gene-based vaccines were the predominant cause of this disease. Inflammation which involves infiltration by granulocytes and other types of cells that are attracted by complement activation is compatible with a primarily antibody-mediated immune response to spike protein expression. The evidence

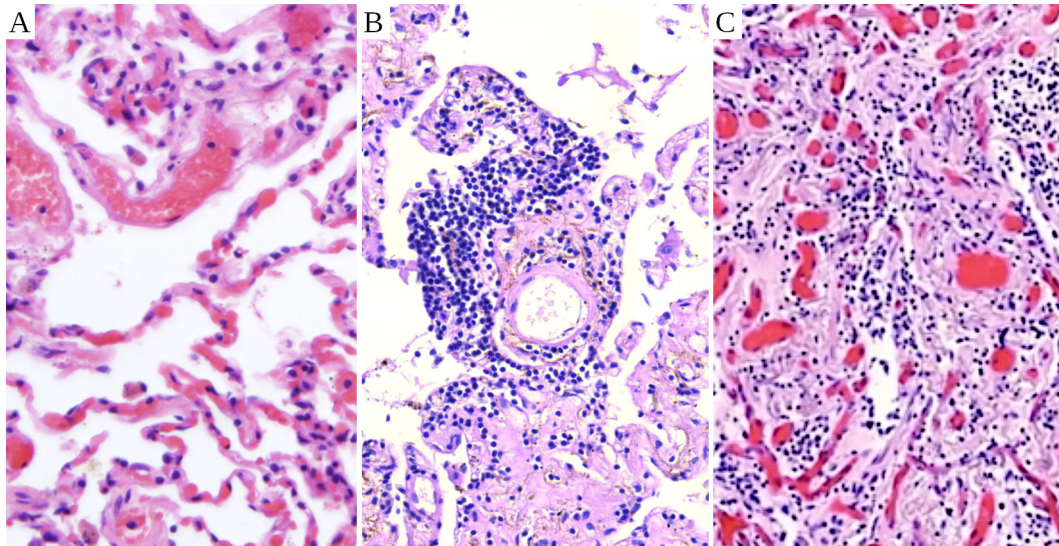


Figure 3.9 Normal lung tissue (A), and lung alveolitis (B, C) after mRNA vaccination (Moderna). In A, we see air-filled spaces (the alveoli), delimited by delicate alveolar septa with embedded, blood-filled capillaries. We also see several somewhat larger blood vessels. In B, we see dense lymphocyte infiltrates. The septa are thickened by fibrosis (scar tissue). Fibrosis is even more advanced in panel C, where air-filled spaces have almost entirely disappeared. Panel A from [90]; panels B and C courtesy of Prof. Burkhardt.

of cell and organ damage available so far seems consistent with the major immune effector mechanisms outlined already in Section 1.2.1; however, more in-depth investigations are needed to fully elucidate the immunological mechanisms underlying the varying patterns of inflammation.

3.4.2 Lung inflammation (pneumonitis). The lungs are prominently affected not only in severe cases of COVID-19 [10], but also by adverse events after vaccination. The former is unsurprising, since SARS-CoV-2 is a respiratory virus. With vaccination, one reason for their frequent involvement may be that the lungs constitute the first capillary bed which the vaccine particles will encounter after entering the bloodstream. Moreover, thrombi that form within large veins in the periphery and then become detached will be carried through the bloodstream to the lungs, where they will get stuck; this is what we refer to as lung embolism.

Burkhardt noted some form of lung involvement in 17 mRNA-vaccinated patients out of 29 overall. While some of these cases were indeed caused by embolism or the local manifestations of vasculitis, infiltration by lymphocytes and inflammation of the lung tissue itself was noted in eleven cases. Inflammatory lung disease that is not caused by infectious agents is referred to as *pneumonitis*; if the inflammation centers on the alveoli, then the term *alveolitis* is also used.

Figure 3.5B above already showed an example of lung tissue infiltrated by lymphocytes. One of Burkhardt's cases is illustrated in Figure 3.9. This patient was a 80 years old woman, who had received the second of two doses of the Moderna vaccine 40 days before her death. In addition to the inflammation in the lungs, this woman was also suffering from myocarditis; both were most likely the leading causes of her death. In the figure, we see abundant infiltration of the lungs with lymphocytes. We also see *fibrosis*, i.e. the formation of scar tissue induced by inflammation, which has thick-

ened the septa between the alveoli to such a degree that little air-filled space remains between them.

A case of mRNA vaccine-induced pneumonitis with similar, but somewhat less severe histopathological findings in a lung biopsy was reported by So et al. [129]. Importantly, their patient survived and recovered after treatment with corticosteroids. Shimizu et al. [130] have described three clinically similar cases, but performed no biopsies; their report presents only radiological images.

A peculiar form of lung involvement that has been reported several times after mRNA vaccination [131–133] is known as *radiation recall pneumonitis*. This is a rare condition that may befall patients who have previously received radiation treatment of the lungs. Irradiation itself, in high doses, is sufficient to trigger pneumonitis, but this will typically heal, often with some degree of fibrosis. When such patients subsequently receive certain drugs, then the inflammation may flare up again in the previously irradiated area.

The drugs that have so far been known to evoke this condition are mostly anti-cancer drugs. A novel variation on the theme is the occurrence after use of certain monoclonal antibodies that are used therapeutically to enhance immune responses to cancer cells [134]. While the mechanism by which the COVID-19 mRNA vaccines cause this surprising reaction remains to be elucidated, the effect hints at interactions of these vaccines with the immune system whose nature is not yet understood.

3.4.3 Brain inflammation (encephalitis). Brain tissue includes two major cell types, the *neurons* (nerve cells) and the *glia cells*. The nerve cells are of course at the heart of brain function, but the glia cells—a heterogeneous bunch—serve in many indispensable supporting functions. One of these is the formation of the *blood-brain barrier* (BBB), which they do jointly with the vascular endothelia. The BBB protects the brain from many poisons carried by the bloodstream. It is, however, probably not of equally great importance in the context of mRNA vaccine nanoparticles; this will be discussed in more detail in Section 4.1.3.

The forms of damage to the brain observed after COVID-19 vaccination resemble those also seen with other organs: vascular inflammation and occlusion, direct immune attack, and autoimmune disease. We will here focus on the latter two pathogenetic mechanisms.

3.4.3.1 Encephalitis due to an immune reaction against spike protein. If vaccine particles manage to leave the blood vessels and be taken up by cells in the surrounding brain tissue, then we must expect the immune system to attack and destroy those cells. How might it be proven that this has occurred in a given case of encephalitis? The following criteria would make such a diagnosis at least highly likely:

1. clinical manifestation within days to a few weeks of the vaccine injection;
2. detection of lymphocytes and other inflammatory cells within brain tissue;
3. detection of spike protein within the foci of inflammation.

It should be noted that criteria 2 and 3 can only be satisfied by histopathological examinations. With the brain, these are usually performed only after autopsy, since biopsies on this organ are of course particularly precarious.

While this mechanism may very well be of great importance, the supporting evidence so far is scant, because pathologists have not been looking for it. However, a

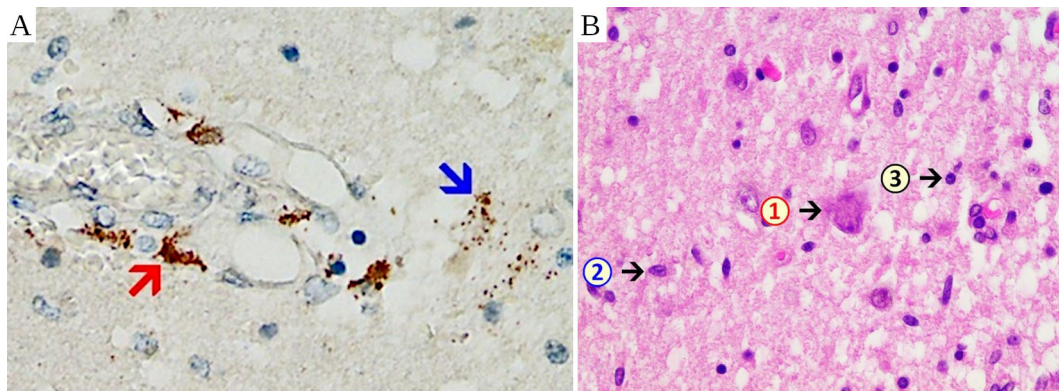


Figure 3.10 Histopathology of encephalitis. **A:** Detection of SARS-CoV-2 spike protein by immunohistochemistry, within the wall of a small blood vessel (red arrow) and within several glia cells of the surrounding brain tissue (blue arrow). **B:** an encephalitic focus (HE staining). 1: a necrotic nerve cell; the cell nucleus has vanished. 2: a microglia cell; this cell type is more prevalent than usual. 3: a lymphocyte. Images adapted from a case report by Mörz [128].

first case report that fulfills all of the above criteria has recently been published [128]. Some of the findings are reproduced here in Figure 3.10. This very meticulous study also ruled out that the detected expression of spike protein was caused by infection with the virus itself rather than by vaccination.

The patient in question had initially received a single injection of AstraZeneca’s adenovirus-based vaccine, followed by two injections of Pfizer’s mRNA vaccine. The last injection had been given seven months after the first and three weeks before the time of death. Marked expression of the spike protein, likely caused in the main by the most recent dose of mRNA vaccine, was detected in the brain capillaries and also in some of the surrounding glia cells. It must be noted that, even though neurons underwent cell death in numbers, they were not shown directly to express the spike. There seem to be three possible explanations:

1. the neurons did express the spike protein and therefore were directly attacked by the immune system, but their death interfered with the detection of the spike;
2. the neurons expressed the spike protein, but antigen expression on the surface was mostly in the form of MHC1-associated processed peptides; or
3. the neurons did not express the spike protein and were not directly attacked, but rather were killed as bystanders in the general mêlée of the inflammation.

The second alternative may seem contrived, but it has been substantiated in principle by a study on liver tissue (see Section 3.4.6 below). It would seem worthwhile to resolve this question through further studies.

3.4.3.2 Autoimmune encephalitis. In this pathogenetic mechanism, the connection between encephalitis and vaccination is less immediate: the vaccine first triggers an inflammation, which might not even have to directly affect the brain; and in the context of this inflammation an immune response is triggered not only against the spike protein but also against one or more of the body’s own proteins or other biomolecules (autoantigens; see Section 2.4). The immune system may then attack these same autoantigens within initially unaffected target organs, including the brain, and trigger inflammation here as well.

The clinical symptoms, and also the autopsy findings obtained with routine methods, will likely be very similar as with a direct immune reaction to the spike. Therefore, how might one decide whether the encephalitis is triggered by the spike protein or rather by an autoantigen? In a true autoimmune encephalitis, one should expect the following findings:

1. autoantibodies to the autoantigens in question should be detectable in blood samples;
2. the spike protein should *not be* detectable in the inflammatory lesions;
3. the temporal connection to the vaccination might be less close, because autoantigens are produced in the body perpetually.

Jarius et al. [135] reported a case of autoantibody-positive encephalitis in a patient who had initially received two doses of AstraZeneca's adenovirus-based vaccine, followed by one dose of Pfizer's mRNA vaccine. In this patient, the autoantigen was a protein expressed in the brain—*myelin oligodendrocyte glycoprotein* (MOG). These authors also provided an overview of twenty previously reported cases that involved the same autoantigen. In three of these cases, an mRNA vaccine had been used, whereas the remaining seventeen cases were associated with the AstraZeneca vaccine. Since none of these cases were fatal, no positive or negative histopathological evidence of spike protein expression in the inflammatory brain lesions was obtained.

Asioli et al. [136] reported four cases of encephalitis in which autoantibodies against the LGI1 protein were detected. Three of these cases, all from the same Italian city (Bologna), occurred after injection of mRNA vaccines. A particularly striking case that involved brain inflammation was reported by Poli et al. [137]. This patient developed three different autoimmune diseases simultaneously—demyelinating encephalitis, myasthenia, and thyroiditis. However, no specific autoantibodies were detected that could account for the encephalitis in this case.

3.4.3.3 Antibody-negative autoimmune encephalitis. This diagnosis was made in several case reports of encephalitis after injection of mRNA vaccines [138–140]. It is certainly reasonable to assume that some such cases may have been caused by unidentified autoantigens. On the other hand, without histopathology, it will often be impossible to decide whether a given case was caused by an immune reaction against an unknown autoantigen or against the vaccine-encoded spike protein.

Overall, while both direct immune response to spike protein and true autoimmunity have been substantiated as causes of post-vaccination encephalitis, their respective contributions to the overall incidence of the disease cannot be discerned from the limited evidence available.

3.4.4 Liver inflammation (hepatitis). Compared to most other interior organs, the liver is quite frequently affected by inflammation, which may be due to infectious or non-infectious causes. A brief overview of the various forms will provide useful background for judging the evidence of hepatitis induced by mRNA vaccines.

3.4.4.1 Viral hepatitis. There are several hepatitis viruses, transmitted either through the oral route (most commonly hepatitis A virus) or through contaminated blood or needles (hepatitis B and C viruses). Hepatitis A is typically acute and self-limiting. Hepatitis B and C may be transient, too, but in some patients they take a chronic course, which may progress all the way to liver cirrhosis and to organ failure.

3.4.4.2 Toxic hepatitis. The liver has a central role in the metabolic degradation of drugs and poisons. The intermediates which arise along these degradation pathways can be chemically quite reactive and give rise to toxic hepatitis. The most common case in practice is toxic hepatitis induced by alcohol, whose degradation gives rise to acetaldehyde as the reactive intermediate. In the early stages, toxic hepatitis is usually reversible upon withdrawal of the causative chemical agent.

3.4.4.3 Autoimmune hepatitis. This form of hepatitis is caused by an immune reaction to autoantigens which occur in liver tissue. Usually, multiple autoantigens are involved, and antibodies to these autoantigens are found in the blood. Most of the autoantigens in question occur not only in the liver but also in other tissues. Nevertheless, the disease typically affects the liver only, which must be due to some additional factors, either genetic or extrinsic in nature.

A hallmark of true autoimmune hepatitis is its protracted clinical course—since the inflammation is not driven by a virus that may be cleared, nor by a drug that may be withdrawn, the disease tends to linger and relapse.

3.4.4.4 Autoimmunity in viral and toxic hepatitis. While in theory the above forms of hepatitis can be neatly classified according to the cause, in practice there is considerable overlap. This is well illustrated by several studies which appeared shortly after the discovery of the hepatitis C virus (HCV): a sizable proportion of patients who had previously been diagnosed with autoimmune hepatitis were now found to harbor HCV, which was in many cases deemed causative for the disease [141–143].

We already discussed earlier how infectious pathogens can promote autoimmune disease both through tissue damage and through cross-reacting antigens (Section 2.4.2). Tissue damage will occur in viral hepatitis. As noted above, toxic hepatitis is caused by reactive drug degradation intermediates, which also will inflict cell and tissue damage. Moreover, they can attach themselves to self antigens, which are thereby altered and made to look like non-self to the immune system. This may then lead to an immune response which is directed against the chemically altered antigen, an which may also extend to its unmodified self antigen precursor. Thus, in many cases of viral and of toxic hepatitis, autoantibodies of some sort are also present; but these are considered a *consequence* rather than the cause of the observed inflammation.

It follows that detection of autoantibodies alone cannot reliably tell true autoimmune hepatitis from viral or from drug-induced forms of the disease. Furthermore, immune attack on liver cells will produce similar histopathological effects regardless of whether it is triggered by self, modified self, or genuine non-self antigens.

3.4.5 What effects on the liver should we expect with mRNA vaccines? In Chapter 4, we will discuss how mRNA vaccines, after intramuscular injection, may distribute within the body. For now, we simply note that, among all organs, the liver accumulates the most vaccine particles per unit weight of tissue, aside from only the injection site itself. At these high tissue concentrations, the synthetic cationic lipids contained in the vaccine nanoparticles are likely to cause some cell and tissue damage. Liver cell damage was indeed observed in animal trials by both Pfizer [144, p. 55] and Moderna [145, p. 49]; and according to the report by the European Medicines Agency [144], Pfizer’s own experts attributed it explicitly to the company’s proprietary and previously untested cationic lipid.

We had seen above that triggering an effective immune response requires both a non-specific “danger” signal and a specific antigen (see Section 1.2.2.1). The cytotoxic effects of the cationic lipids can provide the non-specific signal [78]. Translation of the mRNA into the spike protein would, of course, provide an effective target antigen. With these two stimuli, the stage is set for a vigorous immune response that will attack the liver cells. The ensuing inflammation will amplify the tissue damage and promote secondary immune responses to self antigens, i.e. autoimmunity. Thus, we might expect autoantibodies in at least some of the clinical cases.

That leaves the question of disease duration. While the manufacturers’ and regulators’ assurances of vaccine mRNA expression lasting only for days were overly optimistic (see Section 2.2), expression should indeed be transient. Thus, much like a case of toxic hepatitis, which will abate upon withdrawal of the drug that caused it, vaccine-induced inflammation should wane as expression of the mRNA subsides. Furthermore, we may expect that the inflammation will respond to immunosuppressive treatment with corticosteroids, as is the case with toxic hepatitis, and also with some reported cases of vaccine-induced encephalitis and pneumonitis (see above).

3.4.6 Evidence of vaccine mRNA and its expression in post-vaccination hepatitis.

The number of published case reports on hepatitis after vaccination is rather high, but most of these studies do not provide molecular detail from which one could infer the pathogenetic mechanism. Two case reports stand out in this regard. The first one, published by Martin-Navarro et al. [146], describes the detection of vaccine mRNA in a liver biopsy through in situ hybridization. The mRNA is found in abundance throughout the entire tissue specimen that was examined. The study did not attempt to measure translation of the detected RNA into spike protein.

The second study [147] continues where the first one left off—it demonstrates the expression of spike protein in these liver cells, but indirectly and with an interesting twist: it shows the presence in the liver tissue not of spike itself, but rather of cytotoxic T-lymphocytes (CTL) specific for this protein; or more precisely, specific for a certain small peptide that will arise from the spike protein’s intracellular fragmentation (see Section 1.2.2.2). The authors also tried to detect the presence of intact spike protein by immunohistochemistry, but the result was negative. A similar, not formally published finding was also shared previously in a presentation by Prof. Burkhardt, who had observed an at best weakly positive signal of the spike’s expression within liver cells. Taken together, these findings suggest that liver cells don’t express the intact spike protein at high levels, but that the fragments of the expressed amount which is expressed suffice to attract and activate specific CTLs. The key mechanism of vaccine-induced immunological cell and tissue damage by mRNA vaccines put forth by this book is therefore supported by this evidence.

3.4.7 Clinical case reports on mRNA vaccine-induced hepatitis. The number of case reports on hepatitis after COVID-19 vaccination is very large; for reviews of such cases, see [149–152]. Many of these reports show histopathological findings, which overall are fairly regular and similar. Infiltrating inflammatory cells include lymphocytes, plasma cells, and sometimes eosinophils, and they are usually concentrated around the branches of the portal vein, which drains blood from the intestines toward the liver. A representative example is shown in Figure 3.11.

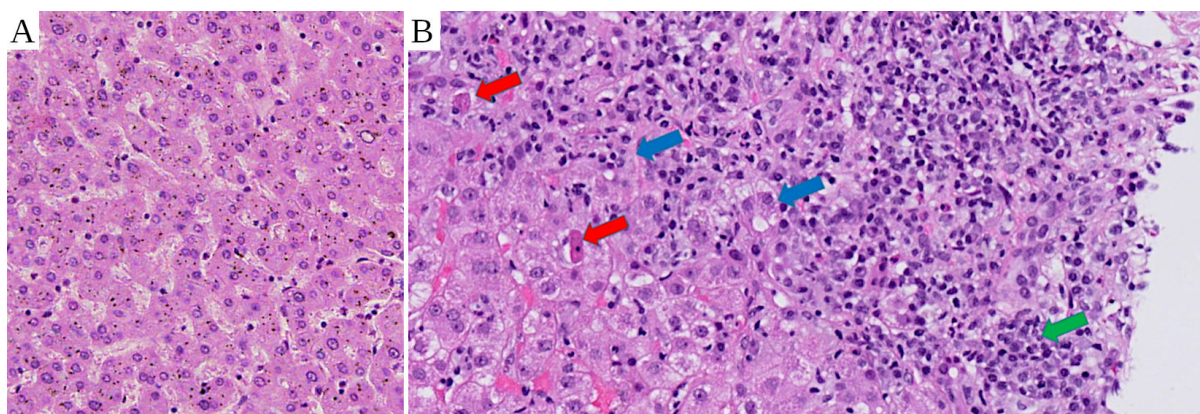


Figure 3.11 Autoimmune-like hepatitis after mRNA vaccination. **A:** section of normal liver tissue, for reference (adapted from [90]). **B:** vaccine-induced hepatitis. Lymphocytes and plasma cells abound near the top and the right. Red arrows: liver cells undergoing cell death (apoptosis). Green arrow: plasma cell (example). Blue arrows: liver cell rosettes (a morphological marker of inflammation). Image adapted from Vuille-Lessard et al. [148].

Most reports chalk up their findings to “autoimmune hepatitis”, but in many of these cases there is little or no evidence of autoantibodies, without which this diagnosis is not viable. For example, Izagirre et al. [151] report five cases from a single hospital, but in only one of them found any autoantibodies at all. Fimiano et al. [153] report a single case with very high levels of antibodies against SARS-CoV-2, but with no autoantibodies other than against thyroglobulin, a protein found only in the thyroid but not the liver. While their tentative diagnosis is autoimmune hepatitis, possibly drug-induced, the most likely cause is not autoimmunity but rather immune attack against spike protein expressed by liver cells. We posit that, in the absence of evidence to the contrary, this explanation applies to most other cases of autoantibody-negative hepatitis as well, and probably also to many cases that do show only a narrow spectrum of autoantibodies.

Efe et al. [154] provided an overview of 87 cases of hepatitis after COVID-19 vaccination from multiple clinical centers. Among these, 34 did not exhibit any autoantibodies. The clinical course in these cases was somewhat milder than in those with evidence of autoimmunity, but otherwise the spectrum of clinical and pathological findings was similar. The authors find good response to corticosteroid treatment and good long-term outcomes; this is also the general tenor of other reports. It bears mention that most of the cases reported by Efe et al. were caused by mRNA vaccines, but 23% were due to the adenovirus-based vaccine produced by AstraZeneca.

Even though the discussion of the pathogenetic mechanism remains vague in general, most reports acknowledge a connection to vaccination, even in those cases that do exhibit autoantibodies. In some cases, causation by the vaccines is supported by recurring attacks of hepatitis after repeated injections; see for example Mahalingham et al. [155] and Zin Tun et al. [156]. In summary, therefore, the evidence from the available case reports on vaccine-induced liver disease aligns closely with the expectations which were spelled out above, and which flow from nothing more than the accepted action mechanism of the mRNA vaccines, together with their known strong accumulation in liver cells.

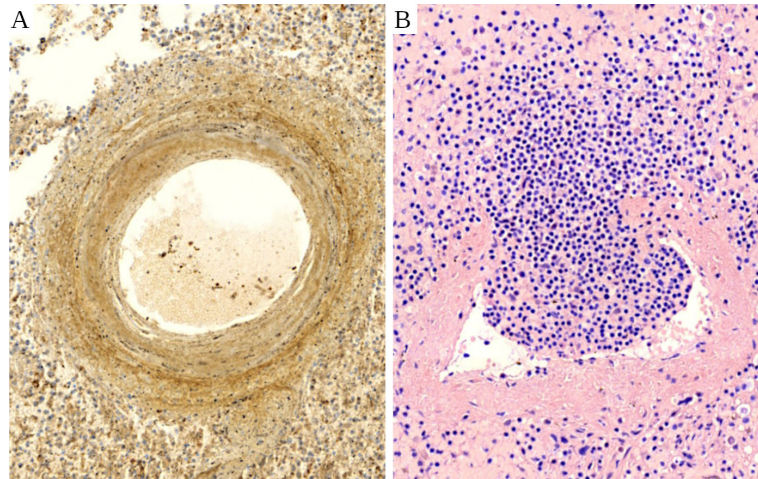


Figure 3.12 Vaccine-induced vasculitis of the spleen. Cross sections of a spleen artery. **A:** immunohistochemistry for spike protein. Strong expression is observed, with some variation between concentric layers of the vessel wall, which thereby form an “onion skin” pattern. Strong expression is also observed in the surrounding lymphatic tissue. **B:** HE stain. A large lymphocytic infiltrate is seen breaking through the wall of an artery and obstructing the lumen.

3.4.8 Kidney disease. Figure 3.6 illustrated a case of IgA nephropathy, which is one form of *glomerulonephritis*, i.e. inflammation that centers on the kidney glomeruli and is caused by autoimmunity. The second major form of kidney inflammation is interstitial nephritis, of which Tan et al. [157] present one case which occurred after the AstraZeneca adenovirus vaccine, and Mira et al. [158] one in connection with the Pfizer vaccine.

Fenoglio et al. [159] reported seventeen cases of biopsy-proven cases of glomerulonephritis, interstitial nephritis, and other forms of nephropathy after COVID-19 vaccination. Thirteen of these occurred in patients who had received an mRNA vaccine. The study also provides references to many other case reports of kidney disease. A series of six cases from another clinical center was reported by Schaubsluger et al. [160]. Such case series suggest that kidney disease after vaccination is not rare.

3.4.9 Involvement of the spleen. As of this writing, PubMed finds only on a single case report on splenic infarction after vaccination [161], as well as several reports of severe hemolytic anemia or thrombocytopenia which necessitated the removal of the spleen, but no reports on inflammatory disease of the spleen itself. However, Prof. Burkhardt has found several cases with similar and very striking manifestations of vasculitis in the spleen, one of which is illustrated in Figure 3.12. The question therefore arises in how many autopsies of vaccine-related deaths the spleen was even examined in sufficient detail at all.

3.4.10 Skin manifestations. Various afflictions of the skin have been reported after injection of COVID-19 mRNA vaccines. A comprehensive review of clinical observations, but without histopathological data, was provided by Kroumpouzou et al. [162]. Studies which include histopathology found several variants of vasculitis [113, 163], but also inflammatory infiltration of the skin’s uppermost layer, the *epidermis*, and of the *dermis*, which is the skin’s supporting layer of connective tissue [164–166].

Several reports described cases of *pemphigoid* [162, 167], an autoimmune reaction against crucial proteins which fasten the epidermis to the dermis, and whose disruption causes blisters to spring up. Pemphigoid is often triggered by drugs, presumably through a similar mechanism as was described above for toxic hepatitis (see Section 3.4.4.2).

While most of the reported skin manifestations were transient and not severe, they nevertheless merit diagnostic attention. Biopsies can be obtained from the skin with minimal risk and effort. Detection in such samples of spike protein expression by immunohistochemistry, and of vasculitis by conventional staining, should influence diagnostic considerations pertaining to any other organs adversely affected by the vaccine. For example, the skin is usually involved in systemic lupus erythematosus (SLE), which has been observed repeatedly after injection of mRNA vaccines and also of adenovirus vector vaccines [168–170]. SLE commonly causes glomerulonephritis but can involve organs other than the kidneys as well.

3.4.11 Other organs. Histopathological studies on organs other than those discussed above are comparatively rare. This does not mean that these organs may not be frequently affected; for example, Chee et al. [171] reported twelve cases of Graves disease, an autoimmune affliction of the thyroid, from a single clinic in Singapore; all of these occurred in patients who had received an mRNA vaccine. Caron [80] reviewed a sizable number of case reports on thyroid disease.

4. Pharmacokinetics and lipid toxicity of mRNA vaccines

In the preceding chapters, we have focused on the immunological mechanism by which mRNA vaccines induce disease. This mechanism is essentially the same in different organs; and because the blood vessels are prominently affected, it is clear that disease can strike in any organ. Nevertheless, for a better understanding of vaccine toxicity, it is important to consider where in the body the vaccine particles will accumulate to the highest levels, and for how long they will stay there. Such questions are the subject of *pharmacokinetics*, which we will consider in this chapter. In addition, we will also look at additional mechanisms of mRNA vaccine toxicity which arise from factors other than the expression of mRNA.

Both the pharmacokinetics of the mRNA vaccines and their chemical toxicity are intimately related to the properties of the lipid nanoparticles. Therefore, this is where we will begin our exploration.

4.1 Structure and function of lipid nanoparticles

The composition of an mRNA vaccine lipid nanoparticle is illustrated in Figure 4.1. Such a particle contains four different lipid components, two natural ones (cholesterol and phosphatidylcholine) and two synthetic ones (see Figure 4.2). The least abundant lipid is a synthetic lipid which is coupled to a water-soluble polymer, polyethylene-glycol (PEG), and which decorates the particle surface. The other three lipids are found in the particle interior. Cholesterol and phosphatidylcholine serve to stabilize the particle. The second synthetic lipid is *ionizable*, which means that it can occur in two states of electrical charge. At near neutral pH, which prevails in the extracellular space and in the cytosol, it will mostly be uncharged. On the other hand, inside an acidic environment, these lipid molecules will bind hydrogen ions (H^+) and thereby become positively charged. This effect will cause the lipid nanoparticle to disintegrate and the mRNA to be released into the cell (see later).¹

4.1.1 The biomolecular corona. One important characteristic of the vaccine lipid nanoparticles is the acquisition of a “biomolecular corona”, which consists of some of the body’s own proteins [172]. The process is facilitated by the PEG-coupled synthetic lipid molecules, which initially cover the surface of the particles. This lipid species is more water-soluble than the others and can detach from the particles, which will expose patches of more *hydrophobic* lipids—i.e., more “greasy” or water-repellent ones. Such a hydrophobic patch will then attract protein molecules which likewise have some hydrophobic surface features (Figure 4.3).

¹Those molecules of ionizable lipid which interact directly with the negatively charged mRNA inside the lipid particle are most likely positively charged even at neutral pH.

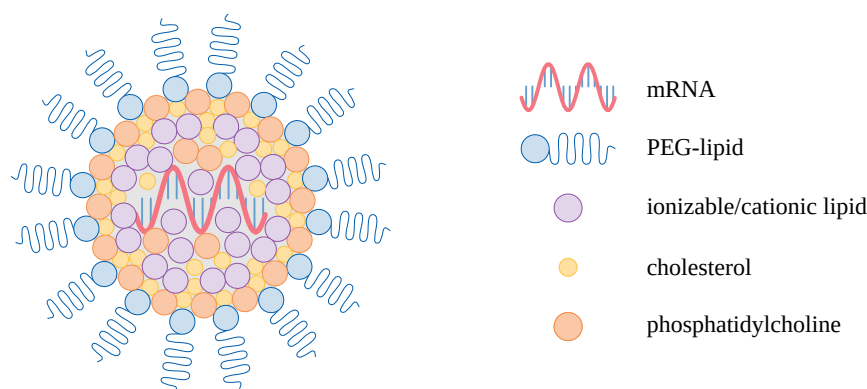


Figure 4.1 Structure of an mRNA lipid nanoparticle. The surface of the particle is covered with a synthetic lipid which is linked to the hydrophilic (water-soluble) polymer polyethyleneglycol (PEG). The negatively charged mRNA interacts mainly with the second synthetic lipid, which when ionized (protonated) carries a positive charge. Cholesterol and phosphatidylcholine are naturally occurring lipids which are added for stability.

A natural fit for this situation are the *apolipoproteins*. These are normally found on the surfaces of the body's own lipid transport particles, the *lipoproteins* (Figure 4.4A). However, other plasma proteins such as albumin, antibodies, and complement factor C3 have also been found on the surfaces of artificial liposomes and lipid nanoparticles [172].

The adsorption of apolipoproteins and of plasma proteins to the vaccine lipid nanoparticles is no mere curiosity. The physiological function of the apolipoproteins is to serve as the lipoprotein particles' "address tags"—they direct the transport of lipoproteins into cells and across cellular barriers such as the endothelia of the blood vessels. Accordingly, when the vaccine lipid nanoparticles bind such address tags, they will be recognized and transported by the cells much like the body's own natural lipoproteins.

4.1.2 Receptor-mediated endocytosis and transcytosis of lipoproteins. The purpose of the natural lipoproteins is to supply the tissues and cells with fat and cholesterol. Cells which require fat or cholesterol will take up those lipoprotein particles by way of *receptor-mediated endocytosis* and then break them down entirely (Figure 4.4B). Fat and cholesterol are used according to the cell's needs; the apolipoproteins are broken down to amino acids, which can be reused for the synthesis of new proteins.

Figure 4.4 also shows that particles that have been taken up by endocytosis may alternatively be released again by *exocytosis*. If endocytosis and exocytosis occur on opposite sides of the cell, the effect is *transcytosis*. This is the mechanism by which lipoprotein particles can cross vascular endothelial cells and thereby move between the circulation and the extravascular compartment of our tissues and organs. It appears that this is not limited to the capillaries but can also occur in arteries [173–175].

4.1.3 Traversal of vascular barriers by lipid nanoparticles. The same behavior is observed with nanoparticles that carry apolipoproteins on their surface. Kucharz et al. [176] reported that lipid nanoparticles were able to cross the walls of blood vessels

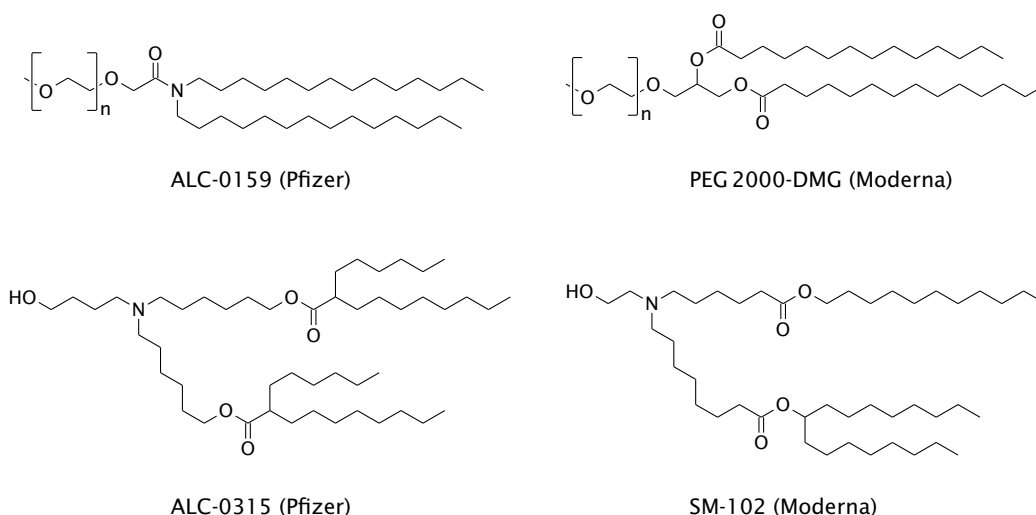


Figure 4.2 Molecular structures of the synthetic lipids contained in the Pfizer and Moderna COVID-19 vaccines. Each unmarked corner denotes a carbon atom saturated with hydrogen; the large number of such atoms gives these molecules their “greasy” character. Top: the PEG-conjugated lipids. PEG consists of polymeric ethyleneglycol moieties. One such moiety is shown within brackets; the letter *n* denotes the repetition of approximately 45 such units. Bottom: the cationic lipids. The nitrogen (N) atoms can bind a proton (H^+) and thereby acquire a positive charge.

in the brain, ending up within the brain tissue.² In their study, maximal translocation was detected in *venules*, that is, small veins, rather than capillaries or arteries. Similarly, Hartl et al. [177] reported that polymeric nanoparticles whose surface had been covalently coupled to one specific apolipoprotein (ApoE) were also able to exit from the circulation into the brain tissue.

Observations such as those reported by Kucharz et al. and Hartl et al. are rather remarkable, considering that the blood vessels of the brain are generally less permissive to solutes and particles than are those of other organs. The anatomical and biochemical features which restrict substance transport from the blood vessels to brain tissue are collectively referred to as the *blood-brain barrier* [178, 179]. The delivery of drugs across the blood-brain barrier is a preferred focus of experimental research on lipid nanoparticle behavior in vivo; transport of such particles into the tissues of other organs receives much less attention. However, without evidence of the opposite, it can be assumed that transport of such particles across vascular barriers within most other organs of the body will be at least as effective as within the brain. This may very well also include the barrier between the maternal and the fetal circulation within the placenta, but this question has yet to be properly addressed experimentally.

4.1.4 Intracellular release of the mRNA. While the biomolecular corona of a vaccine lipid nanoparticle facilitates its receptor-mediated uptake by a cell, this alone does not guarantee that the mRNA molecules contained within will be successfully released and expressed. Schlich et al. [180] have reviewed several experimental studies which indicate that only a small percentage of all mRNA molecules manage to escape from the endosomal compartment and then be translated into protein.

²While Kucharz et al. did not examine the role of apolipoproteins, the particles used were of a composition that in vivo would induce the acquisition a biomolecular corona.

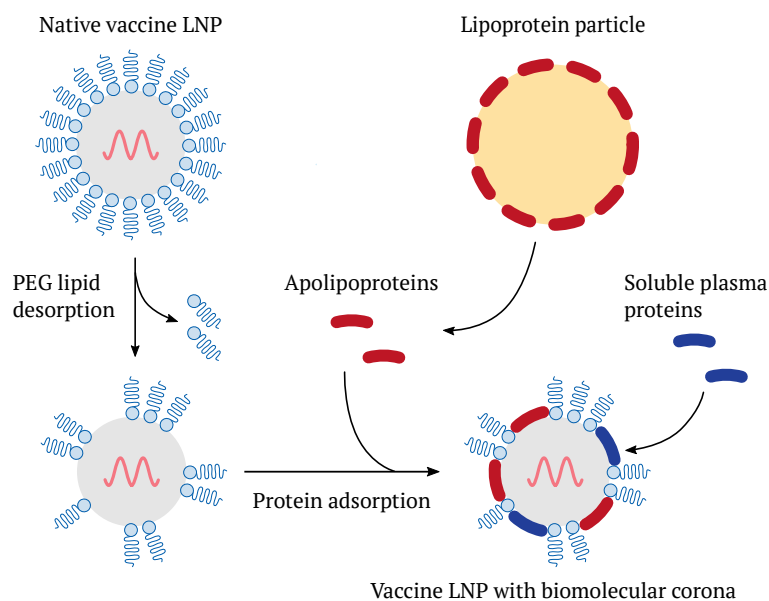


Figure 4.3 How vaccine lipid nanoparticles acquire their “biomolecular corona.” The superficially located PEG lipid can become desorbed from the particles. This exposes other lipid molecules, which may then bind various proteins found in the blood plasma. Prominent among these are *apolipoproteins*, which are normally associated with the body’s own lipid transport particles, the *lipoproteins*.

The various alternate fates of the vaccine mRNA are illustrated in Figure 4.5. The escape of the mRNA from the compartment that initially encloses it—the *endosome*—is triggered by *acidification*. The cell pumps acid into the endosome, much in the same way that certain cells within the gastric mucous membrane pump acid into the stomach. The protons (hydrogen ions) of the acid then bind to the lipid nanoparticle’s ionizable lipid molecules, which will thereby become positively charged. This will cause these lipids to disperse and to mingle with the lipid membrane which encloses the endosome, creating an escape route for the mRNA into the cytosol (Figure 4.6). On the other hand, the acid will also promote the degradation of both the lipids and the mRNA within the endosome; degradation will compete with release.

Even those mRNA molecules that have managed to escape from the endosome intact may yet be diverted by being packaged into *exosomes*, which may be released from the cell. This might occur before or after the mRNA has been translated within the cell; and furthermore, exosomes may merge with other cells and deliver the mRNA to them. Exosomes therefore may promote the persistence and the spread of the mRNA within the body even after the lipids of the LNPs have been dispersed, degraded, or excreted; they may well be important in the observed long-lasting expression of spike protein in persons who received COVID-19 mRNA vaccines.³

³We had noted earlier that the level of protein expression is greatly increased by the replacement of uridine in the mRNA with methylpseudouridine (see Section 1.8.3.2). While this is generally explained in terms of resistance to degradation, the observed kinetics of the expression [55, 56] suggest another explanation, namely, that the methylpseudouridine-modified mRNA escapes more efficiently from the endosomes into the cytosol.

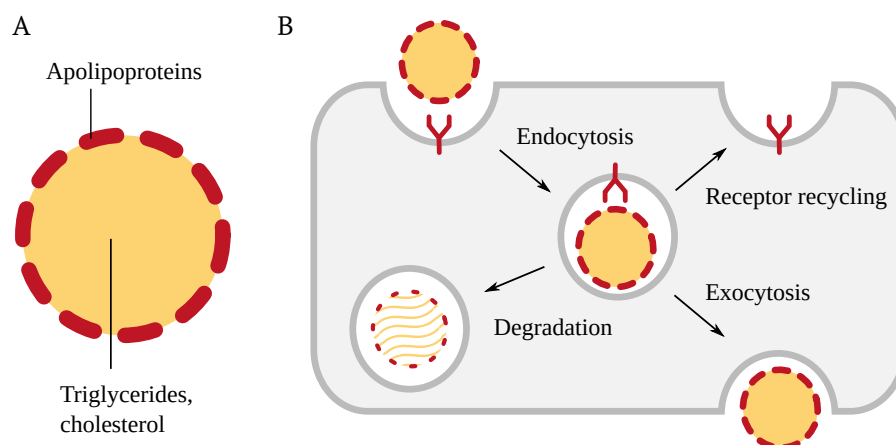


Figure 4.4 Receptor-mediated endocytosis of lipoproteins. **A:** structure of a lipoprotein particle. The core is a fat droplet which contains triacylglycerol, cholesterol and some other lipids in varying proportion. The surface is decorated with various apolipoproteins. **B:** The apolipoproteins are recognized by receptor molecules on cell surfaces. This recognition will cause the cell to engulf and ingest the particle, which may then be broken down or released again by exocytosis.

4.2 Pharmacokinetics of mRNA vaccines

The properties of the lipid nanoparticles which we considered above exert a strong influence on their transport and their fate within the human body.

4.2.1 Organ distribution of model mRNA vaccines. Above, we saw that the transport of vaccine lipid nanoparticles may resemble that of lipoproteins, which supply our cells with fat and cholesterol. All cells require some cholesterol, and most cell types can burn fat. Nevertheless, the amount of lipoprotein particles taken up and turned over varies greatly between the cells of different organs. The following organs take up particularly large amounts:

1. The liver has a central place in lipoprotein metabolism. It synthesizes a large share of all the body's lipoproteins, and it also recycles surplus lipoprotein particles.
2. Endocrine glands which produce steroid hormones use cholesterol as a precursor for hormone synthesis. These include the testes, the ovaries, and the adrenal glands.
3. The placenta requires lipoprotein both for supplying the fetus and for its own production of progestin hormones, which are necessary to sustain pregnancy.
4. The lactating breast glands acquire fat and cholesterol from lipoproteins and repack-
age them for release into the breast milk.

With this in mind, we can understand some of the observations on the distribution mRNA vaccines within the body. The data available on this question are rather sparse, but there is one topical animal study which was performed by Pfizer and submitted to health authorities in various countries.⁴ In this study, rats were injected intramuscularly with a model mRNA vaccine which encoded luciferase, a protein enzyme, rather

⁴The Japanese and Australian regulators subsequently released some of these data to the public [182–184]. The FDA and the EMA did not, but from their assessment reports on the Pfizer vaccine [144, 185] it is clear that they, too, had seen the results of this study.

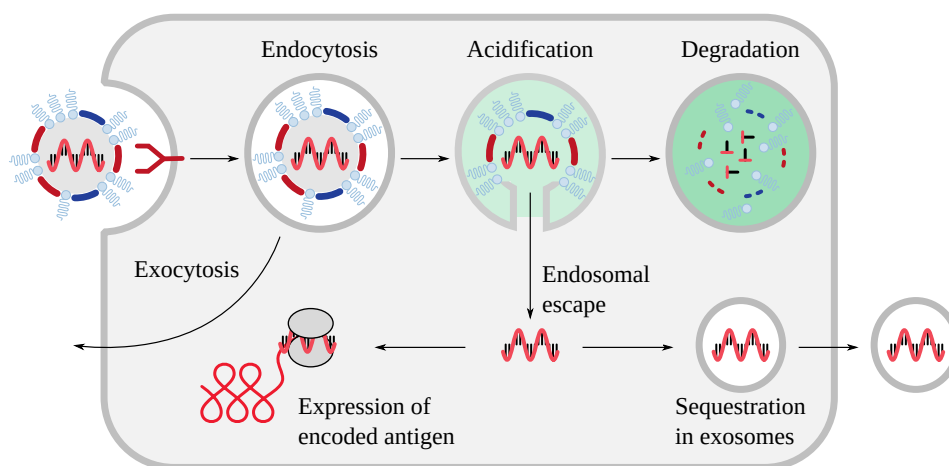


Figure 4.5 Intracellular fates of mRNA vaccine particles. A vaccine particle that has been taken up by a cell may be released again by exocytosis, or it may remain trapped in the endosome and undergo complete degradation; both processes will compete with the release of intact mRNA from the endosome. mRNA molecules that do escape intact may induce expression of the protein antigen, or they may be packaged into exosomes and released from the cell. Such endosomes may be taken up by other cells, which may then in turn express the antigen.

than the SARS-CoV-2 spike protein. For tracking the movements of this vaccine within the body, the cholesterol contained in the lipid nanoparticles had been made radioactive. The animals were sacrificed at various time points after the injection, and the amount of vaccine in the blood plasma and within different organs was determined by measuring this radioactivity.

Figure 4.7 summarizes the most important findings from this study. As early as fifteen minutes after the injection, the vaccine is detected in the bloodstream. The blood level rises for the first two hours and then drops. Concomitantly, the vaccine accumulates in various organs. We note that in most organs this accumulation reaches its highest level at 48 hours after the injection, which is also the latest data point; we therefore don't know how high it might have risen if measurements had continued for several more days.

Among the organs with the highest tissue levels, we recognize the liver, the adrenal glands, and the ovaries as ones with a high lipoprotein turnover. The testes show a notably lower level of accumulation; one likely reason is that the hormone-producing Leydig cells of the testes account only for a minor fraction of the organ tissue.

On the other hand, the high tissue levels in the spleen are not readily explained by any prominent role of this organ in lipoprotein metabolism. Most likely, elements of the LNP biomolecular corona other than apolipoproteins are responsible for this observation. Spleen tissue is very rich in immune cells, including both macrophages and lymphocytes. Many of these cells possess receptors for antibodies and for proteins of the complement system. These receptors enable the immune cells to ingest antigenic proteins, virus particles or microbial cells to which these antibodies and complement factors have bound. We already noted above that antibodies and complement factors may indeed bind to LNPs, which agrees with this interpretation.

Moderna, according to the EMA's report on this vaccine [57], also submitted some animal data on a model vaccine. This model vaccine contained six different mRNAs which encoded antigens unrelated to SARS-CoV-2. In this study, the levels of mRNA

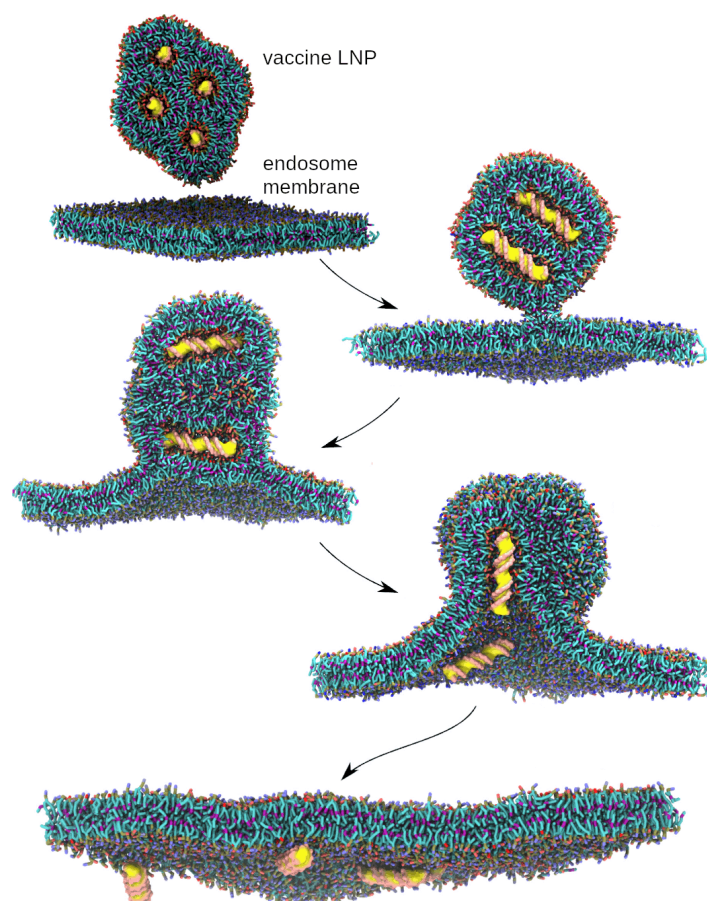


Figure 4.6 Fusion of a lipid nanoparticle with the endosome membrane, driven by electrostatic forces between lipid molecules (computer simulation). The positively charged lipids on the vaccine LNP (red head groups) repel each other but are attracted to the negatively charged lipids of the endosome membrane (blue head groups). Figure adapted from Bruininks et al. [181].

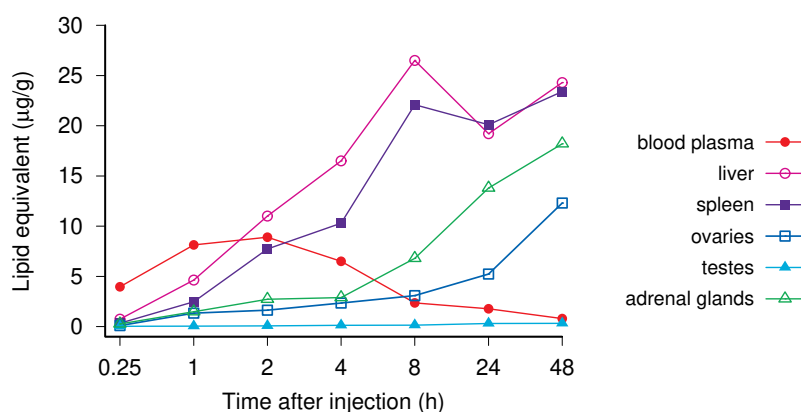


Figure 4.7 Organ distribution in rats of a model mRNA vaccine with the same lipid composition as the Pfizer/BioNTech vaccine. Plot generated from Table 2.6.5.5B in [182]. The blood plasma level rises soon after injection and then drops as the vaccine accumulates in various organs. The vaccine was measured using a radioactively labeled cholesterol derivative (unlabeled cholesterol is a regular ingredient of the vaccine lipid nanoparticles). The data represent vaccine content in micrograms of vaccine lipid per gram of tissue or milliliter of blood plasma. Note the high concentrations in liver, spleen, adrenal glands, and ovaries.

rather than of the lipids were measured. The results of Moderna's study are incompletely described in the report, but on page 47 we read:

Increased mRNA concentrations (compared to plasma levels) were found in the spleen and eye. ... Low levels of mRNA could be detected in all examined tissues except the kidney. This included heart, lung, testis and also brain tissues ... liver distribution of mRNA-1647 is also evident in this study, consistent with the literature reports that liver is a common target organ of LNPs.

The observed accumulation in spleen and liver agrees with the Pfizer study. While no specific mention is made of ovaries and adrenal glands, the wording suggests that these tissues did not accumulate Moderna's model vaccine to the same degree as Pfizer's.

We note that, regardless of the tissue levels in any specific organ, at least the blood vessels and their endothelia will be exposed to the vaccine particles in each and every organ. Accordingly, vasculitis and thromboembolic events are somewhat likely to occur in all organs. Additional tissue-specific pathology might be expected to focus on organs with high levels of accumulation. However, as we will see presently, the findings of these animal studies likely do not give a complete picture of mRNA vaccine distribution in practice.

4.2.2 Correlation of model vaccine organ distribution with histopathological findings. Among the organs with the highest accumulation of either model mRNA vaccine, only the liver has been extensively studied with histopathological methods; and as we have seen in Section 3.4.7, the literature contains numerous case reports of vaccine-induced hepatitis. Several cases of spleen involvement were reported by Prof. Burkhardt (see Section 3.4.9), but neither ovaries nor adrenal glands appear to have received much scrutiny. Histopathological case reports on the placenta in cases of vaccine-related miscarriage or stillbirth are missing from the literature thus far as well.

On the other hand, we have seen evidence of inflammation and of vaccine-induced spike protein expression in heart muscle (Section 3.4.1) and the brain (Section 3.4.3), even though these organs accumulated only comparatively low or moderate levels of the model vaccine in Pfizer's and Moderna's animal experiments. The observed inflammation is particularly remarkable with respect to the brain, which is supposed to be protected by the blood-brain barrier. In this context, we must note two important caveats:

1. The blood-brain barrier breaks down when the brain tissue is afflicted by inflammation. Accordingly, vasculitis within the brain that was induced by the first injection of an mRNA vaccine might soften up the blood-brain barrier and facilitate the entry of vaccine particles delivered with a subsequent booster injection. It would therefore have been important to examine the organ distribution of the vaccine not only after the first injection, but also after one or more repeat injections. However, this was not done in Pfizer's and Moderna's animal studies.
2. The SARS-CoV-2 spike protein has been shown in several studies to compromise the integrity of the blood-brain barrier [115, 116, 186, 187]. Spike protein which may be expressed elsewhere but reaches the brain through the bloodstream may facilitate penetration of vaccine particles into the brain. In contrast, Pfizer's model vaccine encoded luciferase, which is presumably inert in this regard. Moderna's

model vaccine encodes several proteins of Cytomegalovirus; there seems to be no information on any direct effects of these proteins on blood-brain barrier integrity.

These considerations, in combination with histopathological findings, strongly suggest that mRNA vaccines distribute more widely and effectively than Pfizer's and Moderna's very limited animal studies on model vaccines would indicate.

4.2.3 Time course of elimination and duration of activity. We had seen in Section 4.1.4 that the mRNA can become separated from the lipids after the cellular uptake of the vaccine nanoparticles. The elimination of both ingredients must therefore be considered separately.

4.2.3.1 Time course of mRNA elimination. It appears that Pfizer did not provide any data at all on the elimination of the mRNA contained in the company's COVID-19 vaccine, or even on a model mRNA vaccine. The only pertinent data in their animal study [183] consist of measurements of luminescence, which is induced by firefly luciferase, the protein encoded by that model vaccine. Reportedly, luminescence within the liver subsided within two days after injection, whereas the muscle tissue at the injection site showed detectable luminescence for nine days. This suggests, but does not prove that the mRNA itself was inactivated within a similar time frame.

The summary of Moderna's model vaccine study given in the EMA report [57] states that the *half-life* of elimination—that is, the time interval required for the level of the mRNA to drop by half—varied between 15 hours at the injection site and 63 hours in the spleen. It also states that the mixture of model mRNAs was rapidly cleared from the blood plasma, with a half-life of approximately three hours.

While these findings suggest a fairly rapid clearance of the synthetic mRNAs overall, it must be stressed that none of these studies used the mRNA deployed in the COVID-19 vaccines, and furthermore that all studies were carried out in rodents. These results can therefore not be directly applied to the current crop of mRNA vaccines and their use in human patients. As noted in Section 2.2, COVID-19 vaccine mRNA has been detected at 60 days after injection in lymph nodes [61], and at 30 days within muscle tissue of a limb other than the one which had been injected [62]. Long-lasting persistence of the vaccine mRNA in blood plasma samples of injected patients was recently reported by Fertig et al. [188]. According to these authors, all patients still tested positive on day 15 after the injection, which seems to have been the latest time point to be included. Collectively, these studies on humans show that the vaccine mRNAs may persist much longer than Pfizer's and Moderna's animal studies would suggest.

4.2.3.2 Time course of lipid elimination. The Pfizer vaccine contains two lipids which occur naturally in the human body, as well as two synthetic ones; only the latter will be considered here. According to Pfizer's own data [183], 60% of their proprietary cationic lipid (ALC-0315) will accumulate in the liver after intravenous injection. The level stays remarkably high even at two weeks after the injection, indicating very slow degradation (Figure 4.8). Their PEG-modified lipid (ALC-0159) accumulates in the liver to a lesser degree, which probably reflects its release from the lipid nanoparticles within the circulation, before these particles reach the liver; and this lipid is also more rapidly cleared from the liver tissue.

The report states that both lipids were undetectable in the urine. However, half of the PEG-lipid was excreted in the feces in unchanged form, which is most likely due to

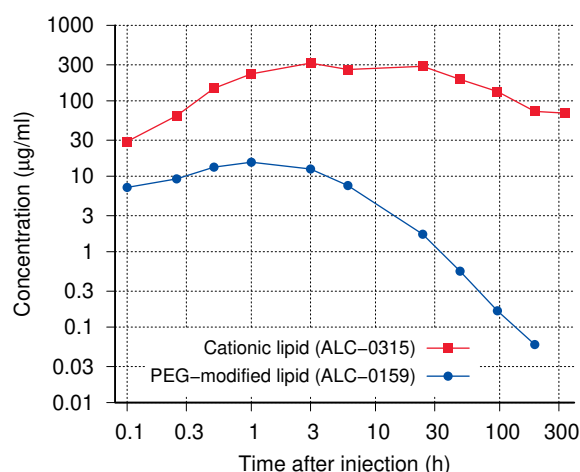


Figure 4.8 Time course of liver tissue levels of the two synthetic lipids contained in Pfizer’s COVID-19 vaccine after intravenous injection. Data from [183]. Note that both the x -axis and the y -axis are logarithmic.

its secretion into the bile by the liver cells. In contrast, only 1% of the cationic lipid was found in the feces. Therefore, about half of the PEG-lipid and most of the cationic lipid most likely undergo metabolic degradation. Some lipid metabolites were indeed characterized by in-vitro experiments, but no in vivo studies seem to be available.

According to the EMA report [57], Moderna submitted no data on the elimination of the two synthetic lipids contained in their COVID-19 mRNA vaccine. The EMA report briefly summarizes findings on a “close structural analogue” of SM-102, Moderna’s proprietary cationic lipid, stating that no persistence of this analogue beyond one week after the injection was observed in animal experiments. Considering the structure of Moderna’s PEG-conjugated lipid, fairly rapid degradation appears likely, but no evidence was provided.

While EMA reassures us that accumulation of the lipids within the body is unlikely, we must note that firstly the information provided is entirely insufficient by the usual standards of drug development and approval, and secondly that absence of lipid accumulation does not imply absence of cumulative toxicity. This is explained below in Section 4.3.3.2.

4.3 Lipid nanoparticle toxicity

We will again limit this discussion to the two synthetic lipid species. The PEG-conjugated lipids are the less abundant of the two, and the only mechanism of harm on record consists in allergic reactions to these lipids. In contrast, the cationic lipids account for almost half of the total lipid in the vaccine LNPs, and they can exert toxicity outright, without any “assistance” from the adaptive immune system.

4.3.1 Allergic reactions caused by PEG-conjugated lipids. Polyethyleneglycol (PEG)-conjugated lipids are not known to cause significant toxicity through outright chemical reactivity or disruption of cellular structures. However, they may trigger allergic reactions in some individuals whose blood plasma contains antibodies against PEG. Such antibodies may arise in response to the initial injection with an mRNA vaccine, and the allergy might then become clinically manifest after a subsequent injection with the same or another mRNA vaccine. However, antibodies to PEG have also been found

blood samples of patients who had never received any injections with an mRNA vaccine, nor with any other PEG-containing medicine [189]. In such patients, the antibodies may have been induced by laxatives or cosmetics containing PEG, but immunological cross-reaction with other chemicals also seems possible.

PEG allergy manifests itself clinically as *anaphylaxis*, i.e. it sets in shortly and acutely after the injection. It induces welts on the skin, and in some patients also circulatory failure (anaphylactic shock; [190]). This is analogous to bee or wasp sting allergy, which is most dangerous if the poison is perchance injected directly into the bloodstream. Anaphylactic shock in response to an mRNA vaccine may well also involve accidental intravenous injection.

Anaphylaxis is caused by the release of specific inflammatory mediators—histamine, platelet-aggregating factor, and leukotrienes—from inflammatory cells, particularly *mast cells*. The most straightforward trigger for this release is antigen-specific immunoglobulin E (IgE). However, other mechanisms can contribute, in particular complement activation, which may be triggered by the more common and abundant IgG and IgM antibodies. Only IgG and IgM seem to have been documented in clinical cases of PEG allergy [191]; whether PEG-specific IgE also occurs in such cases has apparently not yet been determined.

The binding of antibodies to PEG-conjugated medicines and the subsequent activation of complement will also accelerate the removal of these medicines from the circulation by phagocytes [192]. In the case of the mRNA vaccines, such accelerated clearance might modify the immune response to the encoded antigen.

4.3.2 Inflammatory signaling by cationic lipids. Several experimental studies have shown that cationic lipids similar to those used in the Pfizer and Moderna COVID-19 vaccines induce strong inflammatory reactions. The spectrum of cellular signaling pathways involved is rather broad and somewhat variable between different lipid species Loney et al. [193]. A recent study by Ndeupen et al. [78] demonstrated strong inflammatory responses to synthetic lipid nanoparticles with or without RNA. The cationic lipid used in this study was proprietary, and its chemical structure was not specified, but it was most likely similar to the two cationic lipids used in the COVID-19 vaccines (see Figure 4.2). This agrees with the frequent observation of local and also systemic inflammatory reactions among COVID-19 vaccine recipients; however, from such clinical observations alone it is not possible to discern the respective contributions of mRNA and of lipids to that inflammation.

We had seen in Section 1.2.2 that the induction of a specific immune response requires the activation of non-specific defense mechanisms, which may come about either by outright tissue damage or by the stimulation of various pattern recognition receptors. The protein antigens contained in conventional vaccines will not usually themselves provide either kind of stimulus. Such vaccines are therefore supplemented with so-called *adjuvants*, that is, natural or synthetic substances which provide the missing non-specific immune activation. In keeping with their proinflammatory effect, cationic lipids have been shown to act as adjuvants [194, 195]. It is likely that the cationic lipids contained in the COVID-19 mRNA vaccines also function in this manner, in addition to their essential role in the intracellular release of the mRNA.

4.3.3 Chemical toxicity of cationic lipids. The ability of cationic lipids to release the vaccine mRNA from the endosomal compartment depends crucially on their positive charge. The natural lipids which form the cell's membranes are all either neutral or negatively charged (anionic). Cationic molecules of different kinds will be strongly attracted to these negatively charge cell membranes, and they will tend to destabilize and disrupt them (cf. Figure 4.6). There are many variations on this theme. For example, our own phagocytes produce cationic peptides, which they use to disrupt the cell membranes of pathogenic microbes [196]; proteins may contain positively charged peptide motifs that facilitate their translocation across membranes [197]; and cationic detergents tend to be effective disinfectants [198].

The ionizable lipids such as those used in the current COVID-19 vaccines will only be partially charged at the concentration of H^+ ions (or the pH value) that prevails within the cytosol, i.e. within the cell at large, outside the endosome. This is an improvement over previous generations of cationic lipids that will carry a positive charge at all times, regardless of pH. Nevertheless, even the ionizable lipids will remain charged within the cytosol to some degree, and therefore able to disrupt cell membranes.

4.3.3.1 Cationic lipids induce reactive oxygen species. A key effect that occurs downstream of the membrane disruption by cationic lipids is the production of *reactive oxygen species* (ROS). There are several membrane-associated enzyme systems likely to be involved in producing these ROS, including NADPH oxidase and the mitochondrial electron transport chain [199]. Regardless of the exact mechanism of their generation, these ROS will attack various sensitive targets within the cell, including both membrane lipids and DNA [200]. Membrane damage to the mitochondria is likely to amplify the production of ROS. Damage to mitochondria or to the cell's DNA will trigger apoptosis.

In this connection, we must note that of all cell types in the body the lymphocytes are far and away the most susceptible to apoptotic stimuli.⁵ While Filion and Phillips [202] found macrophages to be more susceptible to the cytotoxic effects of a cationic lipid, it must be noted that they employed a rather different lipid mixture, and the susceptibility profile might be different with the lipids contained in the COVID-19 vaccines. Immunohistochemistry has shown COVID-19 mRNA vaccines to induce expression of spike protein in lymphocytes, which suggests that these may be subject to chemical toxicity from the lipid nanoparticles as well. Since the lymphocytes are the backbone of the adaptive immune system, we must expect that cationic lipid toxicity will cause immunosuppression.

Reactive oxygen species also arise within normal cell metabolism, and accordingly our body cells have some capacity to scavenge them and to mitigate the damage. An important scavenger for ROS and their various toxic conversion products is the thiol compound glutathione (G-SH). It is noteworthy that cellular glutathione levels vary greatly between different tissues; for example, Hazelton and Lang [203] reported that in rats G-SH levels were three times higher in the kidney than in the heart, and three times higher again in the liver. Thus, while the liver tends to strongly accumulate lipid

⁵See in particular the example of adenosine deaminase deficiency, a metabolic disease that causes genotoxic stress to all body cells yet selectively eradicates the lymphocytes. This causes severe combined immunodeficiency (SCID) [201].

nanoparticles, it also has the largest metabolic reserve for coping with lipid toxicity. Other organs with lower G-SH reserve might suffer more severe damage than the liver in spite of lower LNP tissue levels. This is one of the many questions that should have been addressed in preclinical safety testing of the COVID-19 vaccines, but were not.

4.3.3.2 DNA damage is cumulative. Broadly speaking, drug effects may be reversible or irreversible. A good example of a drug that can have both reversible and irreversible effects is alcohol: the effect on mood and vigilance subsides when the drug is inactivated by metabolism, whereas inflammation of the liver will fester and may turn into cirrhosis, which is permanent even after complete withdrawal of the drug.

Reversible drug effects will give rise to cumulative toxicity only if the drug itself accumulates within the body, that is, if repeated applications occur before previous doses have been completely eliminated. However, as the example of liver cirrhosis illustrates, the same is not true of irreversible drug effects. DNA damage is by its very nature irreversible, even though some lesions are successfully reverted by the cell's DNA repair systems. Since ROS induced by cationic lipids induce such DNA damage, we must assume that these lipids pose a problem of cumulative toxicity regardless of their own accumulation as such.

4.3.3.3 Toxicity of experimental or approved LNP drugs and vaccines. The most favorable reports on the toxicity of any LNP-based drug concern the single such drug that has passed a regular approval process. The RNA contained in this drug (patisiran, Onpattro®) is not an mRNA—it is designed not to induce the expression of a foreign antigen, but rather to reduce (“silence”) the expression of a “self” protein. This protein, *transthyretin*, is produced in the liver, and accordingly the lipid nanoparticles have been optimized for accumulation in this organ.⁶

The composition of the LNPs employed in this drug is rather similar to those used in Moderna's and Pfizer's COVID-19 vaccines. Here, one must note that patisiran is applied at far higher doses than are the COVID-19 vaccines; the uniformly favorable reviews on its safety [204–206] are therefore quite remarkable. Considering this ostensibly positive experience, we might ask why the same lipid nanoparticle system was not used by Moderna in their attempts to treat another metabolic disease concerning the liver, namely, Crigler Najjar syndrome; while “proof of concept” studies in animals have been presented [207], insurmountable toxicity problems reportedly were the reason behind the company's decision to abandon this effort and turn to vaccines instead [208, 209].

Preclinical data on the toxicity of the cationic lipids contained in Pfizer's and Moderna's COVID-19 vaccines are too sparse to permit any definitive conclusions as the absence or presence of toxicity. However, some results which are briefly summarized in the EMA report on the Moderna vaccine, and which point to measurable levels of DNA damage, will be discussed in Chapter 5.

⁶Transthyretin circulates in the blood plasma and transports the major thyroid hormone (thyroxine, T₄). In some rare patients, aberrantly folded transthyretin may form deposits (“amyloid”), which can damage the function of the heart and the peripheral nerves. Reducing the expression of the protein using patisiran reportedly improves clinical outcomes [204].

5. Genotoxicity of mRNA vaccines

Genotoxicity means toxic damage to our genes, that is, to our DNA. It may affect the germline cells, that is, the oocytes in the ovaries and the sperm-producing cells in the testes, or the *somatic* cells, which comprise all cells of the body which are not part of the germline. Genotoxicity is sometimes used for therapeutic purposes; the effects of ionizing radiation and of cytotoxic anticancer drugs are almost completely due to DNA damage. The purpose of such treatment is to drive cancer cells into apoptosis. It is of course fraught with side effects: apoptosis will not be limited to cancer cells alone, and surviving cells may acquire mutations, which may in the long term enhance the growth of the cancer or induce new, secondary malignancies.

At lower intensity, DNA damage will not trigger outright cell death, and therefore no acute clinical symptoms; however, the risk of mutations and therefore of inducing cancer still applies. A major discovery in radiation biology and medicine, and one which was initially greeted with much skepticism, was that prenatal exposure to even the low doses of radiation which are used in X-ray diagnostics will cause a measurable increase in the incidence of childhood cancer and leukemia. First reported in 1956 by Stewart et al. [210],¹ this finding was later confirmed in two independent large-scale studies in the UK [211] and the U.S. [212]. While the risk's exact magnitude remains under debate, it is generally considered similarly high as in the first decade after birth, which is the most sensitive period of extra-uterine life [213]. Even though the dose-adjusted cancer risk of ionizing radiation declines with increasing age, it will not drop to zero.

The same must be assumed of DNA damage caused by chemical agents. We had seen in Section 4.3.3 that cationic lipids may induce reactive oxygen species (ROS), which in turn may cause DNA damage. We thus should ask if there is any evidence of DNA damage from the lipids contained in the COVID-19 mRNA vaccines.

According to the EMA assessment report on the Pfizer/BioNTech vaccine [144], this manufacturer did not provide any experimental data on the potential cytotoxicity of their lipid mixture (and the EMA committed a grave error in letting them get away with it). In contrast, Moderna, in its own application to the EMA, did supply some experimental data.

5.1 Genotoxicity studies on the cationic lipid contained in Moderna's mRNA vaccine

In the animal experiments reported by Moderna, *polychromatic* erythrocytes (red blood cells, RBC) were counted, as were those with *micronuclei*.

¹The X-ray doses used in diagnostic imaging at the time were considerably higher than those in use today, yet nevertheless far lower than those required then and now in therapeutic irradiation.

5.1.1 Increased abundance of polychromatic red blood cells. Polychromatic RBC are those which have only just finished their differentiation inside the bone marrow and disposed of their nuclei. At this stage, they still retain their ribosomal RNA, which causes them to appear bluish rather than red in the Giemsa stain, which is a routine method used for differentiating blood cells.

Changes in the percentage of RBC with this characteristic indicate changes in erythrocyte maturation kinetics. Genotoxic agents may decrease [214] or increase [215] this parameter. Differences between sexes are expected to be small. Using a luciferase-encoding mRNA packaged into a lipid mixture which contained SM-102, the cationic lipid, Moderna found a significantly decreased level of erythrocyte polychromasia. However, this effect was observed only in male rats. The reported gender difference casts doubt on the statistical power of Moderna's study.

5.1.2 Increased abundance of micronuclei. Using a different model mRNA but again the same lipid mixture containing SM-102, Moderna found "statistically significant increases in micronucleated erythrocytes ... in both sexes." A so-called micronucleus is a chromosome fragment which resulted from chromosome damage to an erythrocyte precursor cell [215, 216] and then left behind in the cytoplasm when the main nucleus was expelled in the final step of that cell's maturation. The micronucleus assay is widely used to detect genotoxicity in vivo [216].

The EMA report on the Moderna vaccine [57] quotes a study submitted by the company to the effect that the observed increase of micronucleated RBC might have been due not to genotoxicity, but rather to the impeded clearance of these cells from the bloodstream as a consequence of the vaccine's spleen toxicity. However, no proof of this contention is shown; and the EMA report further states that "a strong increase in *Molecular initiating events* ... was observed 48 hours after the final administration in the highest dose group in male rats." While no details are given as to the exact nature of the event which was observed, the phrase "increase in molecular initiating events" clearly suggests an actual rise in the rate of formation of genetically damaged cells, rather than merely a decrease in their clearance.

5.1.3 Summary. In conclusion, while the available description of Moderna's experimental findings is rather incomplete, it strongly suggests that the SM-102 lipid contained in the company's COVID-19 vaccine is indeed genotoxic. This agrees with prior observations of genotoxicity associated with liposomes containing similar cationic lipids, reviewed for example by Inglut et al. [217]. Unless proof positive to the opposite is provided, we must assume the same regarding Pfizer's structurally similar ALC-0315 lipid.

We stress again that any form of genotoxicity, at any dose, implies a certain risk of cancer and leukemia. Thus, the prospect of frequently repeated COVID "booster shots," as well as of extending mRNA technology to vaccines against other pathogens or non-infectious diseases, conjures up a very grave public health risk.

5.2 Insertion of mRNA vaccine sequences into the host cell genome

Aside from the cationic lipids' chemically mediated genotoxicity, there is a second major risk of damage to the cell's genome that arises from the mRNA component itself. In connection with the emergency use authorizations for the COVID-19 mRNA

vaccines, this risk was altogether disregarded by the EMA and other regulators. It will become clear in the following that this cavalier approach was scientifically unjustified.

5.2.1 The genotoxicity risks of the COVID-19 mRNA vaccines were dismissed based on outdated science. In the EMA assessment report on the Pfizer/BioNTech vaccine, we find the following succinct statement [144, p. 50]:

No genotoxicity studies have been provided. This is acceptable as the components of the vaccine formulation are lipids and RNA that are not expected to have genotoxic potential.

Apparently, EMA's experts were assuming that RNA in general will not affect the integrity of the host cell genome. The first exception to this rule has been known since 1970, when oncogenic retroviruses were found to carry a *reverse transcriptase* activity. This enzyme will copy the viral RNA genome into DNA, which then inserts into the host cell genome [218, 219]. The realization that eukaryotic cells themselves have similar reverse transcriptase activities came several years later [220], but it could hardly be considered a novelty in 2020.

5.2.1.1 Genomic insertion of RNA viruses through cellular reverse transcriptase activities. The first studies to demonstrate the existence of mammalian DNA sequences that were derived from an RNA virus which was *not* a retrovirus were reported by Klennerman et al. [221] in 1997. The virus in question was Lymphocytic Choriomeningitis Virus, which infects mice. Since this virus does not itself encode a reverse transcriptase enzyme, it followed that the observed partial DNA copies of the viral RNA genome had to have been created through reverse transcription by cellular enzymes. The molecular mechanism was later elucidated in detail by scientists from the same laboratory [222]. It turned out that a *retrotransposon* had accomplished both the reverse transcription of the viral RNA and the insertion of the DNA copy into the cellular genome.

5.2.1.2 The biological role of cellular retrotransposons. Retrotransposons are mobile genetic elements in the cellular genome that encode the complete protein apparatus for generating additional copies of themselves. Most of the time, it is the mRNA of the retrotransposon itself that ends up being copied back into DNA and inserted. However, the retrotransposon proteins may occasionally undergo a *template switch*—they lose their own mRNA template and pick up another RNA molecule instead, which will then undergo reverse transcription into DNA and be inserted into the cellular genome (Figure 5.1).

There are several homologous families of retrotransposons, of which in humans the most active and important one is the LINE-1 family [223–225]. Since the location of new insertions within the genome is largely random [226], the biological outcomes are quite varied. If the insertion occurs within a functional gene, that gene may be disrupted; if insertion occurs in the vicinity of a functional gene, then the activity of the latter may be regulated upward or downward. Depending on the specific role of the affected gene, the behaviour of the cell may be changed, and cancer or other diseases may result [227, 228].

While retrotransposon activity differs between the types and functional states of our body cells, it is noteworthy that retrotransposons are active in both dividing and non-dividing cells [229] and also in oocytes [230]. We must therefore expect that viral or other foreign RNAs may be inserted by retrotransposons not only into somatic

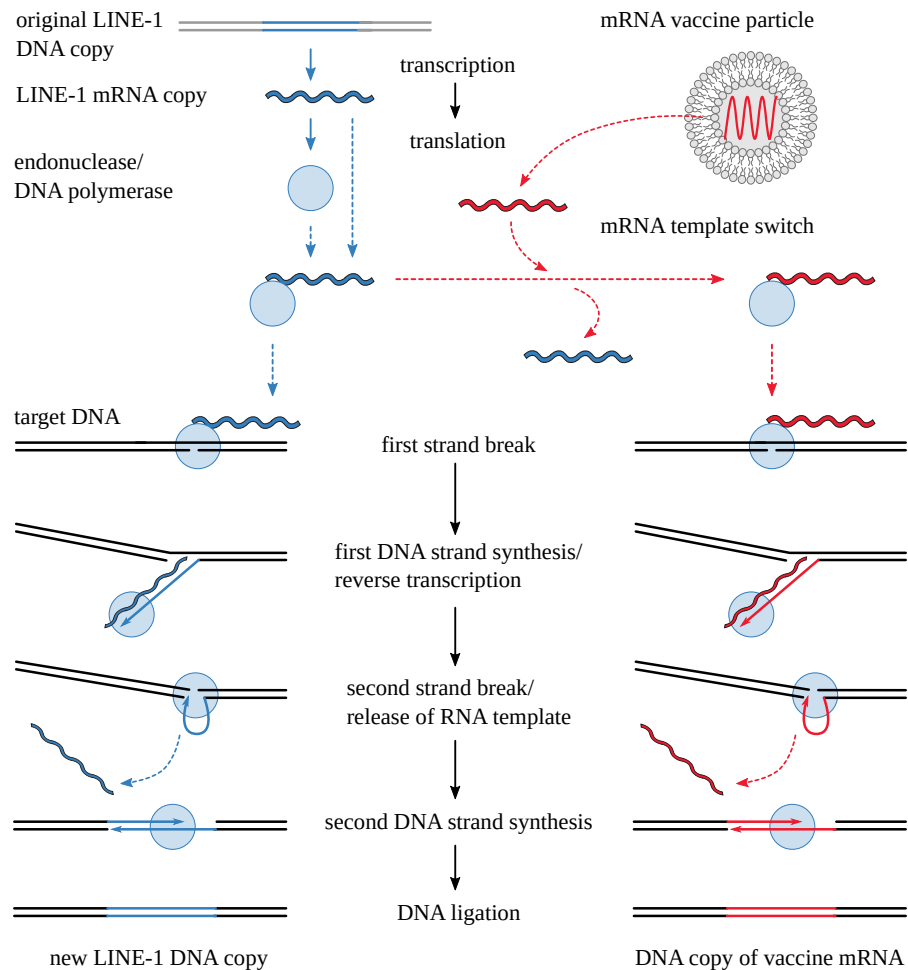


Figure 5.1 How the LINE-1 retrotransposon may copy a vaccine mRNA into DNA and insert into into the host cell genome. The process begins with the transcription of an existing LINE-1 instance into an mRNA copy. Translation of this mRNA produces two proteins, one of which is a bifunctional endonuclease/DNA polymerase, i.e. it can both cut DNA and synthesize it. This molecule binds to the LINE-1 mRNA and then finds a new DNA target site. It cleaves the first DNA strand. Through reverse transcription, it then extends one of the free ends with a DNA copy of the mRNA. Once this step is complete, the second strand of the target DNA is cleaved, and the second strand of the new LINE-1 copy is synthesized along the first. The process can be usurped early on by another mRNA molecule, such as a vaccine mRNA, by dislodging the LINE-1 mRNA from its endonuclease/polymerase. Such a template switch will produce an inserted DNA copy of the substitute RNA.

cells, and thereby potentially cause cancer, but also into germline cells, and therefore propagate within the human population.

5.2.1.3 Genomic DNA sequences derived from non-retroviral RNA viruses. A multitude of RNA viruses other than retroviruses have given rise to partial copies found in the genomes of mammals and other vertebrates [231–234]. Similar findings have been made in other eukaryotic organisms such as fungi, plants and protozoa [235–237]. All of these virus-derived sequences must have arisen through some kind of retrotransposition mechanism, which clearly substantiates the above point that retrotransposition can occur in the germline cells of all these species.

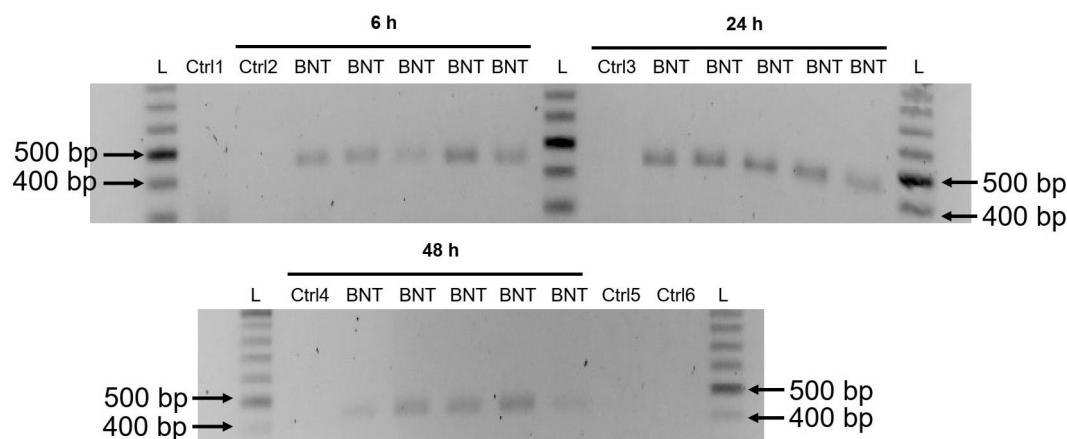


Figure 5.2 Detection of copies of the Pfizer vaccine mRNA within the cellular DNA of a human liver cell line (taken from Figure 5 in [239]). The cells were exposed to the vaccine for the lengths of time indicated. Cellular DNA was then isolated, and DNA copies of the vaccine mRNA detected by PCR amplification of a fragment 444 base pairs (bp) in length. All samples labelled with ‘BNT’ had been treated with the vaccine. Each of them shows a PCR product of the expected length, as is evident from comparison to a DNA fragment length standard (‘L’). Samples labelled with ‘Ctrl *n*’ were controls: Ctrl 1–4 contained DNA from cells not incubated with vaccine, Ctrl5 contained RNA (not DNA) from vaccine-treated cells, and Ctrl6 the same but additionally treated with RNase, which step was also performed in the purification of DNA samples. As expected, none of the control samples yield the PCR product.

While all of the observations cited here pertain to sequences derived from RNA viruses, retrotransposition by LINE-1 is not sequence-specific [238], and there never was any reason to exclude the possibility that other RNA sequences, such as for example those of the Pfizer or Moderna mRNA vaccines, would be subject to the same mechanism.

5.2.1.4 Summary. Even though this had not yet been experimentally demonstrated when the COVID-19 mRNA vaccines were given emergency approval, there was ample precedent to suggest the *strong possibility* that DNA copies of the vaccine mRNA would arise and be inserted into the cellular genome. Rather than waving away this risk as they did, EMA and other regulators should have obligated Pfizer and Moderna to carry out the necessary studies for excluding this risk *before* green-lighting authorization.

5.2.2 Host cells generate DNA copies of the vaccine mRNA and insert them into their own genome. In the time that has passed since the emergency use authorizations for the COVID-19 vaccines, substantial new evidence has accrued regarding the genetic risks posed by the COVID-19 mRNA vaccines.

Already in 2021, it was demonstrated that partial DNA copies of the genomic RNA of the SARS-CoV-2 virus can insert into the cellular DNA of infected cells [240]. Even though this does not directly relate to the mRNA vaccines, it does show that SARS-CoV-2-derived RNA sequences are not exempt from the general mechanism. Moreover, this study demonstrated that the insertion was mediated by LINE-1 retrotransposons.

Of even greater and more immediate relevance is the recent demonstration by Aldén et al. [239] that the mRNA contained in the Pfizer vaccine itself is reversely transcribed within the cells of a human-derived liver cell line (see Figure 5.2). The findings reported in this initial study suggest but do not rigorously prove the participation of

LINE-1 in this retrotransposition event. In this context, we must note that all of the active retrotransposons within the human genome belong to the so-called *non-LTR* class, with which the reverse transcription of the RNA into DNA is inextricably linked to its insertion into the DNA, as is illustrated for LINE-1 in Figure 5.1. Thus, while we can't be absolutely certain that DNA copy of the vaccine sequence was indeed generated by LINE-1, this question is not crucial—we must assume nevertheless that the DNA copy became inserted into the cellular genome.

5.3 Known and plausible risks that arise from the recently established genomic insertion of Pfizer/BioNTech vaccine

The results reported by Aldén et al. [239], even though preliminary in some respects, pose some very serious questions that can no longer be ignored by the regulatory authorities.

5.3.1 Likelihood of DNA insertion occurring in vivo. One remarkable feature of Figure 5.2 is that the PCR product which signals genomic insertion is observed in each of the DNA samples isolated from vaccine-treated cells. This indicates that one or more insertion events have occurred in each experiment. As noted earlier, the Pfizer/BioNTech vaccine mRNA is modified with 1-methylpseudouridine, which will protect the mRNA from certain degradative pathways [241–244]. It is quite conceivable that such protection would increase the likelihood of reverse transcription and insertion. This question has apparently not been experimentally elucidated; not having compelled the manufacturers of the COVID-19 mRNA vaccines to carry out such experiments is another glaring oversight committed by the regulators.

In the experiments depicted in the Figure, the concentration of vaccine was higher than that which can be expected to occur in vivo. However, in the absence of evidence to the contrary, it is reasonable to surmise that the likelihood of insertion will be the same for each individual mRNA molecule and independent of the number of such molecules within a given cell. Thus, the number of insertion events in vivo would be proportional to the total amount of mRNA injected, which exceeds the total amount used in all of the samples shown in Figure 5.2. While we do not yet know how the efficiency of genomic insertion compares between the particular human cell line used by Aldén et al. and the various cell types found in the human body, we must expect, until proof positive of the opposite is obtained, that some insertion events will occur in many, if not all vaccinated persons.

Retrotransposition is particularly likely to occur in actively dividing cells, because during cell division the membrane barrier which separates the nucleus from the cytoplasm transiently breaks down; this facilitates access of the mRNA, bound to the retrotransposon-encoded proteins, to the genomic DNA. While most tissues inside the body have lower proliferation rates than cell cultures in vitro, some proliferate at comparable rates; this includes in particular the bone marrow and the intestinal mucous membranes. Moreover, we reiterate that retrotransposition (i.e., genomic insertion) events may occur in non-dividing cells also [229].

5.3.2 Biological consequences of DNA insertion. With the LINE-1 retrotransposon at least, DNA insertions are apparently distributed in a random fashion [226], but they will occur preferentially within or near transcriptionally active genes, since the DNA of

inactive genes will be tightly packed into complexes with histone proteins and therefore poorly accessible. The genotoxic effect of an insertion on an active gene can be manifested in several ways.

5.3.2.1 Gene inactivation. Insertion may occur within a gene and disrupt it. This can lead to the loss of important cellular gene products (i.e., proteins) and thus, potentially, to the development of disease including cancer [227, 228]. Insertion may be accompanied by the deletion of large gene fragments [245].

5.3.2.2 Gene regulation. Transcriptional and epigenetic regulation mechanisms may be affected, thus modulating protein expression levels upward or downward with unpredictable and undesirable results. Indirect regulatory effects may affect even distant genes located on other chromosomes.

5.3.2.3 Activation of oncogenes. This is a special case of the preceding point, but it is important enough to be highlighted separately. The occurrence of malignancies through DNA integration and activation of cancer-promoting genes (oncogenes) has been demonstrated in clinical trials with a retroviral vector for the genetic treatment of children with SCID-X1 (severe combined immune deficiency) [246]. These malignancies will typically become manifest only several years after the completion of treatment [247]. Therefore, thorough long-term investigations concerning possible genotoxic effects of chromosomal integration are absolutely necessary, in both the pre-clinical and the clinical trial stages, for a valid benefit-risk analysis. This does not apply just with retroviral vectors, but with any recombinant nucleic acid that can end up inserting into the chromosomes of the cell. With both the adenovector- and the mRNA-based COVID-19 vaccines, the risk of insertion into the chromosomal DNA must be taken seriously [248].

5.3.2.4 Autoimmune-like disease. Integration of the spike protein gene into the host cell could lead to permanent expression of this antigen and thus induce chronic autoimmune-like disease.

5.3.2.5 Germline integration. We noticed above that Pfizer's own experiments indicate a high level of vaccine accumulation in the ovaries (see Section 4.2.1). Furthermore, LINE-1 and other retrotransposons are active and cause genomic insertion events in human oocytes [230]. In combination, these findings indicate that the mRNA gene sequences may be integrated into the DNA of oocytes, and hence into the human germline. Insertion into male germline cells cannot be ruled out either, even though in the cited animal study the tissue levels of the model mRNA vaccine in the testes was significantly lower than in the ovaries.

Should this indeed come to pass—should the germline cells of vaccinated individuals be rendered transgenic—then the risk of spawning or conceiving transgenic children will not be limited to these individuals only, but it will necessarily be shared by their current or future spouses. In effect, an entire generation of future parents will be exposed to this risk.

5.3.3 Summary. Integration of the mRNA sequences into somatic cells is likely and implies a risk of cancer and of autoimmune disease. Moreover, the risk of germline integration, resulting in transgenic offspring, cannot be denied. These risks must ur-

gently be addressed through in-depth animal studies. Meanwhile, the authorizations of any and all mRNA vaccines in current use must urgently be revoked.

Signatures

SIGNED AT Waterloo, Ontario, Canada, on January 17, 2023



Dr. Michael Palmer

SIGNED AT Martinsrade, Schleswig-Holstein, Germany on January 17, 2023



Prof. Dr. Sucharit Bhakdi

SIGNED AT Warder, Schleswig-Holstein, Germany on January 17, 2023



Dr. Wolfgang Wodarg

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Michael Palmer, MD, was until March 2022 an Associate Professor of Biochemistry in the Department of Chemistry at the University of Waterloo, Ontario, Canada. He obtained a board certification in Medical Microbiology and Infectious Disease Epidemiology from the German province of Rhenania-Palatinate while working with Dr. Sucharit Bhakdi at the University of Mainz, Germany. His research has focused on bacterial toxins and lipopeptide antibiotics, and his teaching experience includes medical microbiology, metabolism, and pharmacology.

Wolfgang Wodarg, MD, is a specialist in pulmonary and bronchial internal medicine, hygiene and environmental medicine, epidemiology, and public health; Honorary Member of the Parliamentary Assembly of the Council of Europe and former Head of the Health Committee of the Parliamentary Assembly of the Council of Europe; former Member of the German federal parliament (the Bundestag); initiator and spokesman for the study commission 'Ethics and Law in Modern Medicine;' author and university lecturer.

Bibliography

- [1] T. Binder: *The Prevailing Corona Nonsense Narrative, Debunked in 10 or 26 Minutes*. 2021. URL: <https://doctors4covidethics.org/the-prevailing-corona-nonsense-narrative-debunked-in-10-or-26-minutes/>.
- [2] L. Yan et al.: Unusual Features of the SARS-CoV-2 Genome Suggesting Sophisticated Laboratory Modification Rather Than Natural Evolution and Delineation of Its Probable Synthetic Route. *Preprint* (2020). DOI: [10.5281/zenodo.4028830](https://doi.org/10.5281/zenodo.4028830).
- [3] L. Yan et al.: SARS-CoV-2 Is an Unrestricted Bioweapon: A Truth Revealed through Uncovering a Large-Scale, Organized Scientific Fraud. *Preprint* (2020). DOI: [10.5281/zenodo.4073131](https://doi.org/10.5281/zenodo.4073131).
- [4] K. Schwab and T. Malleret: *COVID-19: The Great Reset*. Forum Publishing, 2020.
- [5] Anonymous: *OpenVAERS COVID Vaccine Data*. 2021. URL: <https://www.openvaers.com/covid-data>.
- [6] B. Shilhavy: *43,898 Dead, 4,190,493 Injured Following COVID Vaccines in European Database of Adverse Reactions*. 2022. URL: <https://healthimpactnews.com/2022/43898-dead-4190493-injured-following-covid-19-vaccines-in-european-database-of-adverse-reactions/>.
- [7] J. P. A. Ioannidis: Infection fatality rate of COVID-19 inferred from seroprevalence data. *Bull. World Health Organ.* (2020), BLT.20.265892. URL: https://www.who.int/bulletin/online_first/BLT.20.265892.pdf.
- [8] J. P. A. Ioannidis: Reconciling estimates of global spread and infection fatality rates of COVID-19: An overview of systematic evaluations. *Eur. J. Clin. Invest.* 5 (2021), e133554. PMID: [33768536](https://pubmed.ncbi.nlm.nih.gov/33768536/).
- [9] P. A. McCullough et al.: Multifaceted highly targeted sequential multidrug treatment of early ambulatory high-risk SARS-CoV-2 infection (COVID-19). *Reviews in cardiovascular medicine* 21 (2020), 517–530. PMID: [33387997](https://pubmed.ncbi.nlm.nih.gov/33387997/).
- [10] P. E. Marik et al.: A scoping review of the pathophysiology of COVID-19. *Int. J. Immunopathol. Pharmacol.* 35 (2021), 20587384211048026. PMID: [34569339](https://pubmed.ncbi.nlm.nih.gov/34569339/).
- [11] Anonymous: *WHO advises that ivermectin only be used to treat COVID-19 within clinical trials*. 2021. URL: <https://www.who.int/news-room/feature-stories/detail/who-advises-that-ivermectin-only-be-used-to-treat-covid-19-within-clinical-trials>.
- [12] M. Palmer and S. Bhaki: *Vascular and organ damage induced by mRNA vaccines: irrefutable proof of causality*. 2022. URL: <https://doctors4covidethics.org/vascular-and-organ-damage-induced-by-mrna-vaccines-irrefutable-proof-of-causality/>.
- [13] U.S. National Library of Medicine: *ClinicalTrials.gov*. 2022. URL: [ClinicalTrials.gov](https://clinicaltrials.gov).
- [14] D. M. Knipe and P. M. Howley: *Fields Virology*. Wolters Kluwer, 2013.
- [15] P. J. Delves et al.: *Roitt's Essential Immunology*. Wiley Blackwell, 2017.
- [16] P. Marik: *An overview of the MATH+, I-MASK+ and I-RECOVER Protocols (A Guide to the Management of COVID-19)*. 2021. URL: <https://covid19criticalcare.com/wp-content/uploads/2020/12/FLCCC-Protocols-%E2%80%93-A-Guide-to-the-Management-of-COVID-19.pdf>.
- [17] S. Bhakdi and J. Tranum-Jensen: Molecular nature of the complement lesion. *Proc. Natl. Acad. Sci. U. S. A.* 75 (1978), 5655–5659. PMID: [281714](https://pubmed.ncbi.nlm.nih.gov/281714/).

- [18] J. Tranum-Jensen et al.: Complement lysis: the ultrastructure and orientation of the C5b-9 complex on target sheep erythrocyte membranes. *Scandinavian journal of immunology* 7 (1978), 45–6. PMID: [635472](#).
- [19] K. Karikó et al.: Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 23 (2005), 165–75. PMID: [16111635](#).
- [20] T. G. Kimman et al.: Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. *Journal of clinical microbiology* 25 (1987), 1097–106. PMID: [2954996](#).
- [21] S. Crotty et al.: Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* 171 (2003), 4969–73. PMID: [14607890](#).
- [22] L. Yang et al.: Waning immunity and re-emergence of measles and mumps in the vaccine era. *Curr. Opin. Virol.* 40 (2020), 48–54. PMID: [32634672](#).
- [23] S. Vygen et al.: Waning immunity against mumps in vaccinated young adults, France 2013. *Euro Surveill.* 21 (2016), 30156. PMID: [26987576](#).
- [24] J. Qu et al.: Profile of Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin. Infect. Dis.* 71 (2020), 2255–2258. PMID: [32337590](#).
- [25] S. S. Nielsen et al.: SARS-CoV-2 elicits robust adaptive immune responses regardless of disease severity. *EBioMedicine* 68 (2021), 103410. PMID: [34098342](#).
- [26] M. Dugas et al.: Less severe course of COVID-19 is associated with elevated levels of antibodies against seasonal human coronaviruses OC43 and HKU1 (HCoV OC43, HCoV HKU1). *Int. J. Infect. Dis.* 105 (2021), 304–306. PMID: [33636357](#).
- [27] J. Mateus et al.: Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 370 (2020), 89–94. PMID: [32753554](#).
- [28] A. Grifoni et al.: Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* 181 (2020), 1489–1501.e15. PMID: [32473127](#).
- [29] C.-T. Tseng et al.: Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PLoS One* 7 (2012), e35421. PMID: [22536382](#).
- [30] C. Y. Yong et al.: Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. *Front. Microbiol.* 10 (2019), 1781. PMID: [31428074](#).
- [31] M. Cloutier et al.: ADE and hyperinflammation in SARS-CoV2 infection—comparison with dengue hemorrhagic fever and feline infectious peritonitis. *Cytokine* 136 (2020), 155256. PMID: [32866898](#).
- [32] W. S. Lee et al.: Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. *Nat. Microbiol.* 5 (2020), 1185–1191. PMID: [32908214](#).
- [33] J. A. Tetro: Is COVID-19 receiving ADE from other coronaviruses? *Microbes and infection* 22 (2020), 72–73. PMID: [32092539](#).
- [34] H. Ulrich et al.: Dengue Fever, COVID-19 (SARS-CoV-2), and Antibody-Dependent Enhancement (ADE): A Perspective. *Cytometry A* 97 (2020), 662–667. PMID: [32506725](#).
- [35] A. B. Schuren et al.: Recent advances in viral evasion of the MHC Class I processing pathway. *Curr. Opin. Immunol.* 40 (2016), 43–50. PMID: [27065088](#).
- [36] Y. Kurono: The mucosal immune system of the upper respiratory tract and recent progress in mucosal vaccines. *Auris nasus larynx* (2021). DOI: [10.1016/j.anl.2021.07.003](#).
- [37] J. R. McGhee et al.: The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine* 10 (1992), 75–88. DOI: [10.1016/0264-410x\(92\)90021-b](#).
- [38] M. H. Kim et al.: Superior immune responses induced by intranasal immunization with recombinant adenovirus-based vaccine expressing full-length Spike protein of Middle East respiratory syndrome coronavirus. *PLoS One* 14 (2019), e0220196. PMID: [31329652](#).

- [39] L. Meyer-Arndt et al.: Cutting Edge: Serum but Not Mucosal Antibody Responses Are Associated with Pre-Existing SARS-CoV-2 Spike Cross-Reactive CD4⁺ T Cells following BNT162b2 Vaccination in the Elderly. *J. Immunol.* 208 (2022), 1001–1005. PMID: [35121642](#).
- [40] E. Martinuzzi et al.: A Single Dose of BNT162b2 mRNA Vaccine Induces Airway Immunity in SARS-CoV-2 Naïve and recovered COVID-19 subjects. *Clin. Infect. Dis.* (2022). PMID: [35579991](#).
- [41] N. V. V. Chau et al.: *Transmission of SARS-CoV-2 Delta Variant Among Vaccinated Healthcare Workers, Vietnam*. 2021. DOI: [10.2139/ssrn.3897733](#).
- [42] A. Singanayagam et al.: Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *Lancet Infect. Dis.* (2021). DOI: [10.1016/s1473-3099\(21\)00648-4](#).
- [43] H. Wang et al.: Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with Potent Protection against SARS-CoV-2. *Cell* 182 (2020), 713–721.e9. PMID: [32778225](#).
- [44] E. Twentyman et al.: Interim Recommendation of the Advisory Committee on Immunization Practices for Use of the Novavax COVID-19 Vaccine in Persons Aged ≥18 years—United States, July 2022. *MMWR* 71 (2022), 988–992. DOI: [10.15585/mmwr.mm7131a2](#).
- [45] D.-H. Kang et al.: Fulminant Giant Cell Myocarditis following Heterologous Vaccination of ChAdOx1 nCoV-19 and Pfizer-BioNTech COVID-19. *Medicina* 58 (2022). PMID: [35334625](#).
- [46] S. Kazama et al.: Biopsy-Proven Fulminant Myocarditis Requiring Mechanical Circulatory Support Following COVID-19 mRNA Vaccination. *CJC Open* 4 (2022), 501–505. PMID: [35187464](#).
- [47] H. Jafari et al.: Polio eradication. Efficacy of inactivated poliovirus vaccine in India. *Science* 345 (2014), 922–5. PMID: [25146288](#).
- [48] P. Guernonprez and S. Amigorena: Pathways for antigen cross presentation. *Springer Semin. Immunopathol.* 26 (2005), 257–71. PMID: [15592842](#).
- [49] D. Sengupta et al.: Proteasomal degradation within endocytic organelles mediates antigen cross-presentation. *EMBO J.* 38 (2019), e99266. PMID: [31271236](#).
- [50] L. Qin et al.: Evolution of and evolutionary relationships between extant vaccinia virus strains. *J. Virol.* 89 (2015), 1809–24. PMID: [25410873](#).
- [51] M. J. Babic: Eczema vaccinatum: a reaction to the smallpox vaccine. A report of a rare but potentially lethal consequence. *Am. J. Nurs.* 107 (2007), 30–1. PMID: [17667384](#).
- [52] S. L. Nuismer et al.: Eradicating infectious disease using weakly transmissible vaccines. *Proc. Biol. Sci.* 283 (2016). PMID: [27798311](#).
- [53] A. J. Basinski et al.: Evaluating the promise of recombinant transmissible vaccines. *Vaccine* 36 (2018), 675–682. PMID: [29279283](#).
- [54] C. C. Burns et al.: Multiple Independent Emergences of Type 2 Vaccine-Derived Polioviruses during a Large Outbreak in Northern Nigeria. *J. Virol.* 87 (2013), 4907–4922. PMID: [23408630](#).
- [55] O. Andries et al.: N¹-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J. Control. Release* 217 (2015), 337–344. DOI: [10.1016/j.jconrel.2015.08.051](#).
- [56] N. Pardi et al.: Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. *J. Exp. Med.* 215 (2018), 1571–1588. PMID: [29739835](#).
- [57] Anonymous: *EMA Assessment report: COVID-19 Vaccine Moderna*. 2021. URL: https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf.
- [58] A. F. Ogata et al.: Circulating SARS-CoV-2 Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients. *Clin. Infect. Dis.* 74 (2022), 715–718. PMID: [34015087](#).
- [59] S. Bansal et al.: Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines. *J. Immunol.* 207 (2021), 2405–2410. PMID: [34654691](#).

- [60] M. Palmer and S. Bhakdi: *Long-term persistence of the SARS-CoV-2 spike protein: evidence and implications*. 2021. URL: <https://doctors4covidethics.org/long-term-persistence-of-the-sars-cov-2-spike-protein-evidence-and-implications-2/>.
- [61] K. Röltgen et al.: Immune imprinting, breadth of variant recognition and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell* (2022). PMID: [35148837](#).
- [62] E. Magen et al.: Clinical and Molecular Characterization of a Rare Case of BNT162b2 mRNA COVID-19 Vaccine-Associated Myositis. *Vaccines* 10 (2022). PMID: [35891299](#).
- [63] M. A. Bruusgaard-Mouritsen et al.: Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. *Clin. Exp. Allerg.* (2021). PMID: [33394522](#).
- [64] L. Klimek et al.: Allergenic components of the mRNA-1273 vaccine for COVID-19: possible involvement of polyethylene glycol and IgG-mediated complement activation. *Allergy* (2021). PMID: [33657648](#).
- [65] A. Troelnikov et al.: Basophil reactivity to BNT162b2 is mediated by PEGylated lipid nanoparticles in PEG allergic patients. *J. Allerg. Clin. Immunol.* (2021). PMID: [33991580](#).
- [66] Y. Wang et al.: Cross-reaction of SARS-CoV antigen with autoantibodies in autoimmune diseases. *Cell. Mol. Immunol.* 1 (2004), 304–7. PMID: [16225774](#).
- [67] M. F. Cusick et al.: Molecular mimicry as a mechanism of autoimmune disease. *Clin. Rev. Allergy Immunol.* 42 (2012), 102–11. PMID: [22095454](#).
- [68] D. Kanduc and Y. Shoenfeld: Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine. *Immunol. Res.* 68 (2020), 310–313. PMID: [32946016](#).
- [69] K. T. Coppieters et al.: Viral infections and molecular mimicry in type 1 diabetes. *APMIS* 120 (2012), 941–9. PMID: [23051179](#).
- [70] G. Halpert and Y. Shoenfeld: SARS-CoV-2, the autoimmune virus. *Autoimmun. Rev.* 19 (2020), 102695. PMID: [33130000](#).
- [71] Y. Chen et al.: New-onset autoimmune phenomena post-COVID-19 vaccination. *Immunology* 165 (2022), 386–401. PMID: [34957554](#).
- [72] J. Lyons-Weiler: Pathogenic priming likely contributes to serious and critical illness and mortality in COVID-19 via autoimmunity. *Journal of translational autoimmunity* 3 (2020), 100051. PMID: [32292901](#).
- [73] L. E. Muñoz et al.: Autoimmunity and chronic inflammation—two clearance-related steps in the etiopathogenesis of SLE. *Autoimmun. Rev.* 10 (2010), 38–42. PMID: [20817127](#).
- [74] C. A. Cañas et al.: Patients with relapsing polychondritis and previous cartilage trauma present more autoimmunity phenomena. *Rheumatol. Int.* 32 (2012), 541–3. PMID: [21267573](#).
- [75] F. Tanriverdi et al.: Investigation of antihypothalamus and antipituitary antibodies in amateur boxers: is chronic repetitive head trauma-induced pituitary dysfunction associated with autoimmunity? *Eur. J. Endocrinol.* 162 (2010), 861–7. PMID: [20176736](#).
- [76] R. W. Frenck et al.: Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *N. Engl. J. Med.* (2021). PMID: [34043894](#).
- [77] K. Ali et al.: Evaluation of mRNA-1273 SARS-CoV-2 Vaccine in Adolescents. *N. Engl. J. Med.* (2021). PMID: [34379915](#).
- [78] S. Ndeupen et al.: The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. *iScience* 24 (2021), 103479. PMID: [34841223](#).
- [79] F. Bril et al.: Autoimmune hepatitis developing after coronavirus disease 2019 (COVID-19) vaccine: Causality or casualty? *J. Hepatol.* 75 (2021), 222–224. PMID: [33862041](#).
- [80] P. Caron: Autoimmune and inflammatory thyroid diseases following vaccination with SARS-CoV-2 vaccines: from etiopathogenesis to clinical management. *Endocrine* (2022). PMID: [35763241](#).
- [81] M. E. Mingot-Castellano et al.: COVID-19 Vaccines and Autoimmune Hematologic Disorders. *Vaccines* 10 (2022). PMID: [35746569](#).

- [82] A. Pujol et al.: Thyroid as a target of adjuvant autoimmunity/inflammatory syndrome due to mRNA-based SARS-CoV2 vaccination: from Graves' disease to silent thyroiditis. *J. Endocrinol. Invest.* 45 (2022), 875–882. PMID: [34792795](#).
- [83] A. Català et al.: Cutaneous reactions after SARS-COV-2 vaccination: A cross-sectional Spanish nationwide study of 405 cases. *Br. J. Dermatol.* (2021). PMID: [34254291](#).
- [84] K. Ajmera et al.: Gastrointestinal Complications of COVID-19 Vaccines. *Cureus* 14 (2022), e24070. PMID: [35573556](#).
- [85] Y.-T. Hung et al.: Haemorrhagic bullous pyoderma gangrenosum following COVID-19 vaccination. *J. Eur. Acad. Dermatol. Venereol.* (2022). PMID: [35398933](#).
- [86] J. Mitchell and Q.-Y. Yue: Appendicitis as a possible safety signal for the COVID-19 vaccines. *Vaccine X* 9 (2021), 100122. PMID: [34746743](#).
- [87] U. Krüger: *COVID vaccination and turbo cancer: pathological evidence*. 2022. URL: <https://doctors4covidethics.org/covid-vaccination-and-turbo-cancer-pathological-evidence/>.
- [88] Z. Qin et al.: Pre-exposure to mRNA-LNP inhibits adaptive immune responses and alters innate immune fitness in an inheritable fashion. *PLoS Pathog.* 18 (2022), e1010830. PMID: [36054264](#).
- [89] A. V. Letarov et al.: Free SARS-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection. *Biochemistry Mosc* 86 (2021), 257–261. PMID: [33838638](#).
- [90] S. Belham: *Histology World*. 2022. URL: <http://www.histology-world.com/>.
- [91] T. Iino et al.: Polyarteritis nodosa with aortic dissection: necrotizing vasculitis of the vasa vasorum. *J. Rheumatol.* 19 (1992), 1632–6. PMID: [1361205](#).
- [92] B. M. Voorzaat et al.: Alpha-1 Antitrypsin Deficiency Presenting with MPO-ANCA Associated Vasculitis and Aortic Dissection. *Case Rep. Med.* 2017 (2017), 8140641. PMID: [28367219](#).
- [93] A. Ramandi et al.: Aortic dissection and Covid-19; a comprehensive systematic review. *Current problems in cardiology* (2022), 101129. PMID: [35139402](#).
- [94] M. A. Tuncer et al.: Causality Association Between COVID-19 Infection and Aortic Dissection. *Anatol. J. Cardiol.* 26 (2022), 338–339. PMID: [35435848](#).
- [95] K. Chida et al.: Rupture of Vertebral Artery Dissecting Aneurysm after mRNA Anti-COVID-19 Vaccination: A Report of Two Cases. *NMC Case Rep. J.* 9 (2022), 95–100. PMID: [35646499](#).
- [96] K. M. Chue et al.: Spontaneous rare visceral pseudoaneurysm presenting with rupture after COVID-19 vaccination. *ANZ J. Surg.* 92 (2022), 915–917. PMID: [34480824](#).
- [97] B. H. Kim and M. C. Yoo: Intracranial Hemorrhage Due to Potential Rupture of an Arteriovenous Malformation after BNT162b2 COVID-19 mRNA Vaccination in a Young Korean Woman: Case Report. *Vaccines* 10 (2022). PMID: [35334996](#).
- [98] S. Oshida et al.: Intracranial aneurysm rupture within three days after receiving mRNA anti-COVID-19 vaccination: Three case reports. *Surg. Neurol. Int.* 13 (2022), 117. PMID: [35509565](#).
- [99] R. Takeyama et al.: Intracerebral hemorrhage due to vasculitis following COVID-19 vaccination: a case report. *Acta Neurochir. Wien* 164 (2022), 543–547. PMID: [34783899](#).
- [100] L. Roncati et al.: A Three-Case Series of Thrombotic Deaths in Patients over 50 with Comorbidities Temporally after modRNA COVID-19 Vaccination. *Pathogens* 11 (2022). PMID: [35456110](#).
- [101] Y. N. Aye et al.: Acute Myocardial Infarction and Myocarditis following COVID-19 Vaccination. *QJM* (2021). PMID: [34586408](#).
- [102] J. G. Sung et al.: Acute Myocardial Infarction Within 24 Hours After COVID-19 Vaccination. *Am. J. Cardiol.* 156 (2021), 129–131. PMID: [34364657](#).
- [103] Y. Kawamura et al.: A case of ST segment elevation myocardial infarction within 24 h of a third dose of COVID-19 mRNA vaccine. *Cardiovasc. Revasc. Med.* (2022). PMID: [35718694](#).
- [104] Z. Kolahchi et al.: Acute ischemic stroke and vaccine-induced immune thrombotic thrombocytopenia post COVID-19 vaccination; a systematic review. *J. Neurol. Sci.* 439 (2022), 120327. PMID: [35752132](#).

- [105] A. Alhashim et al.: Extensive Cerebral Venous Sinus Thrombosis (CVST) After the First Dose of Pfizer-BioNTech BNT162b2 mRNA COVID-19 Vaccine without Thrombotic Thrombocytopenia Syndrome (TTS) in a Healthy Woman. *Am. J. Case Rep.* 23 (2022), e934744. PMID: [35136010](#).
- [106] J. Finsterer and S. Nics: Venous sinus thrombosis after the second jab of an mRNA-based SARS-CoV-2 vaccine. *Brain Hemorrhages* 3 (2022), 36–38. PMID: [34901793](#).
- [107] M. I. A. Qureshi et al.: Venous sinus thrombosis after the first dose of Pfizer BioNTech vaccine. *BMJ Case Rep.* 15 (2022). PMID: [35606039](#).
- [108] L. Dias et al.: Cerebral Venous Thrombosis after BNT162b2 mRNA SARS-CoV-2 vaccine. *J. Stroke Cerebrovasc. Dis.* 30 (2021), 105906. PMID: [34111775](#).
- [109] E. Y. Ahn et al.: Pulmonary embolism and inferior vena cava thrombosis in a young male patient after mRNA-1273 (Moderna) immunization: A case report. *Tuberc. Respir. Dis. Seoul* (2022). PMID: [35822319](#).
- [110] R. Scendon et al.: Multiple sites of thrombosis without thrombocytopenia after a second dose of Pfizer-BioNTech COVID-19 vaccine. *Int. J. Immunopathol. Pharmacol.* 36 (2022), 3946320221128534. PMID: [36123789](#).
- [111] T.-G. Lee et al.: Acute gastric and non-mesenteric colonic infarction following mRNA COVID-19 vaccination. *Asian J. Surg.* 45 (2022), 1469–1470. PMID: [35618584](#).
- [112] H. Suzuki et al.: The pathophysiology of IgA nephropathy. *J. Am. Soc. Nephrol.* 22 (2011), 1795–803. PMID: [21949093](#).
- [113] S. Nakatani et al.: New-onset kidney biopsy-proven IgA vasculitis after receiving mRNA-1273 COVID-19 vaccine: case report. *CEN Case Rep.* (2022). PMID: [35075622](#).
- [114] K. Sugita et al.: Development of IgA vasculitis with severe glomerulonephritis after COVID-19 vaccination: a case report and literature review. *CEN Case Rep.* (2022). PMID: [35275366](#).
- [115] B. J. DeOre et al.: SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation. *J. Neuroimmune Pharmacol.* 16 (2021), 722–728. PMID: [34687399](#).
- [116] S. Raghavan et al.: SARS-CoV-2 Spike Protein Induces Degradation of Junctional Proteins That Maintain Endothelial Barrier Integrity. *Frontiers Cardiovasc. Med.* 8 (2021). PMID: [34179146](#).
- [117] H. Jiang and Y.-F. Mei: SARS-CoV-2 Spike Impairs DNA Damage Repair and Inhibits V(D)J Recombination In Vitro. *Viruses* 13 (2021), 2056. PMID: [34696485](#).
- [118] R. N. Kostoff et al.: Why are we vaccinating children against COVID-19? *Toxicol. Rep.* 8 (2021), 1665–1684. PMID: [34540594](#).
- [119] J. Rose and P. A. McCullough: A Report on Myocarditis Adverse Events in the U.S. Vaccine Adverse Events Reporting System (VAERS) in Association with COVID-19 Injectable Biological Products. *Current problems in cardiology* (2021), 101011. URL: <https://tinyurl.com/Rose-McCullough-Myocarditis>.
- [120] H. Walach et al.: Experimental Assessment of Carbon Dioxide Content in Inhaled Air With or Without Face Masks in Healthy Children: A Randomized Clinical Trial. *JAMA pediatrics* (2021). PMID: [34190984](#).
- [121] A. F. Ogata et al.: Ultra-Sensitive Serial Profiling of SARS-CoV-2 Antigens and Antibodies in Plasma to Understand Disease Progression in COVID-19 Patients with Severe Disease. *Clin. Chem.* 66 (2020), 1562–1572. PMID: [32897389](#).
- [122] H. Koiwaya et al.: Serial histopathologic assessment of fulminant myocarditis after the first mRNA COVID-19 vaccine dose. *Eur. Heart J.* 43 (2022), 1995. PMID: [35178573](#).
- [123] C. Baumeier et al.: Intramyocardial Inflammation after COVID-19 Vaccination: An Endomyocardial Biopsy-Proven Case Series. *Int. J. Mol. Sci.* 23 (2022), 6940. PMID: [35805941](#).
- [124] R. Ameratunga et al.: First Identified Case of Fatal Fulminant Necrotizing Eosinophilic Myocarditis Following the Initial Dose of the Pfizer-BioNTech mRNA COVID-19 Vaccine (BNT162b2, Comirnaty): an Extremely Rare Idiosyncratic Hypersensitivity Reaction. *J. Clin. Immunol.* (2022). PMID: [34978002](#).

- [125] A. K. Verma et al.: Myocarditis after Covid-19 mRNA Vaccination. *N. Engl. J. Med.* 385 (2021), 1332–1334. PMID: [34407340](#).
- [126] S. Choi et al.: Myocarditis-induced Sudden Death after BNT162b2 mRNA COVID-19 Vaccination in Korea: Case Report Focusing on Histopathological Findings. *J. Korean Med. Sci.* 36 (2021), e286. PMID: [34664804](#).
- [127] J. R. Gill et al.: Autopsy Histopathologic Cardiac Findings in Two Adolescents Following the Second COVID-19 Vaccine Dose. *Arch. Pathol. Lab. Med.* (2022). PMID: [35157759](#).
- [128] M. Mörz: A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against Covid-19. *Vaccines* 10 (2022), 2022060308. DOI: [10.3390/vaccines10101651](#).
- [129] C. So et al.: COVID-19 mRNA vaccine-related interstitial lung disease: Two case reports and literature review. *Respirol. Case Rep.* 10 (2022), e0938. PMID: [35355663](#).
- [130] T. Shimizu et al.: Interstitial pneumonitis after COVID-19 vaccination: A report of three cases. *Allergol. Int.* 71 (2022), 251–253. PMID: [34772608](#).
- [131] N. M. Hughes et al.: Radiation Recall Pneumonitis on FDG PET/CT Triggered by COVID-19 Vaccination. *Clin. Nucl. Med.* 47 (2022), e281–e283. PMID: [34739397](#).
- [132] K. Shinada et al.: Radiation recall pneumonitis after COVID-19 vaccination. *Thorac. Cancer* 13 (2022), 144–145. PMID: [34791816](#).
- [133] C. R. Steber et al.: Rapid Development of Clinically Symptomatic Radiation Recall Pneumonitis Immediately Following COVID-19 Vaccination. *Cureus* 13 (2021), e14303. PMID: [33968515](#).
- [134] K. McGovern et al.: Radiation recall pneumonitis in the setting of immunotherapy and radiation: a focused review. *Future Sci. OA* 5 (2019), FSO378. PMID: [31245041](#).
- [135] S. Jarius et al.: MOG encephalomyelitis after vaccination against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2): case report and comprehensive review of the literature. *J. Neurol.* (2022). PMID: [35737110](#).
- [136] G. M. Asioli et al.: Anti-LGI1 encephalitis following COVID-19 vaccination: a case series. *J. Neurol.* (2022). PMID: [35751687](#).
- [137] K. Poli et al.: Multiple Autoimmune Syndromes Including Acute Disseminated Encephalomyelitis, Myasthenia Gravis, and Thyroiditis Following Messenger Ribonucleic Acid-Based COVID-19 Vaccination: A Case Report. *Front. Neurol.* 13 (2022), 913515. PMID: [35711270](#).
- [138] A. Vogrig et al.: Acute disseminated encephalomyelitis after SARS-CoV-2 vaccination. *Clin. Neurol. Neurosurg.* 208 (2021), 106839. PMID: [34325334](#).
- [139] J. Werner et al.: New-onset refractory status epilepticus due to autoimmune encephalitis after vaccination against SARS-CoV-2: First case report. *Front. Neurol.* 13 (2022), 946644. PMID: [36051224](#).
- [140] J.-J. Gao et al.: Acute encephalitis after COVID-19 vaccination: A case report and literature review. *Hum. Vaccin. Immunother.* (2022), 2082206. PMID: [35700455](#).
- [141] K. H. Meyer zum Büschenfelde et al.: Hepatitis C virus (HCV) and autoimmune liver diseases. *Arch. Virol. Suppl.* 4 (1992), 201–4. PMID: [1333322](#).
- [142] F. B. Bianchi: Autoimmune hepatitis: the lesson of the discovery of hepatitis C virus. *J. Hepatol.* 18 (1993), 273–5. PMID: [8228118](#).
- [143] A. Jurado et al.: Autoimmune hepatitis type 2 and hepatitis C virus infection: study of HLA antigens. *J. Hepatol.* 26 (1997), 983–91. PMID: [9186828](#).
- [144] Anonymous: *EMA Assessment report: Comirnaty*. 2021. URL: https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf.
- [145] Anonymous: *FDA briefing document: Moderna mRNA-1273*. 2020. URL: <https://www.fda.gov/media/144452/download>.
- [146] L. Martin-Navarro et al.: In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID19 vaccine-related hepatitis. *J. Hepatol.* (2022). PMID: [36116717](#).

- [147] T. Boettler et al.: SARS-CoV-2 vaccination can elicit a CD8 T-cell dominant hepatitis. *J. Hepatol.* 77 (2022), 653–659. PMID: [35461912](#).
- [148] E. Vuille-Lessard et al.: Autoimmune hepatitis triggered by SARS-CoV-2 vaccination. *J. Autoimmun.* 123 (2021), 102710. PMID: [34332438](#).
- [149] H. Shroff et al.: Liver injury following SARS-CoV-2 vaccination: A multicenter case series. *J. Hepatol.* 76 (2022), 211–214. PMID: [34339763](#).
- [150] J. M. Pinazo-Bandera et al.: Acute hepatitis with autoimmune features after COVID-19 vaccine: coincidence or vaccine-induced phenomenon? *Gastroenterol. Rep. Oxf* 10 (2022), goac014. PMID: [35498817](#).
- [151] A. Izagirre et al.: Autoimmune hepatitis following COVID-19 vaccination. *J. Autoimmun.* 132 (2022), 102874. PMID: [35985054](#).
- [152] L. Camacho-Domínguez et al.: COVID-19 vaccine and autoimmunity. A new case of autoimmune hepatitis and review of the literature. *Journal of translational autoimmunity* 5 (2022), 100140. PMID: [35013724](#).
- [153] F. Fimiano et al.: Autoimmune hepatitis or drug-induced autoimmune hepatitis following Covid-19 vaccination? *Liver Int.* 42 (2022), 1204–1205. PMID: [35230737](#).
- [154] C. Efe et al.: Liver injury after SARS-CoV-2 vaccination: Features of immune-mediated hepatitis, role of corticosteroid therapy and outcome. *Hepatology* (2022). PMID: [35567545](#).
- [155] A. Mahalingham et al.: First report of post-transplant autoimmune hepatitis recurrence following SARS-CoV-2 mRNA vaccination. *Transpl. Immunol.* 72 (2022), 101600. PMID: [35390478](#).
- [156] G. S. Zin Tun et al.: Immune-mediated hepatitis with the Moderna vaccine, no longer a coincidence but confirmed. *J. Hepatol.* 76 (2022), 747–749. PMID: [34619252](#).
- [157] F. S. Tan et al.: Acute interstitial nephritis after COVID-19 vaccination. *BMJ Case Rep.* 15 (2022). PMID: [35589264](#).
- [158] F. S. Mira et al.: A Case of Acute Interstitial Nephritis After Two Doses of the BNT162b2 SARS-CoV-2 Vaccine. *Int. J. Nephrol. Renovasc. Dis.* 14 (2021), 421–426. PMID: [34887676](#).
- [159] R. Fenoglio et al.: New Onset Biopsy-Proven Nephropathies after COVID Vaccination. *Am. J. Nephrol.* 53 (2022), 325–330. PMID: [35354140](#).
- [160] T. Schaubsluger et al.: De novo or recurrent glomerulonephritis and acute tubulointerstitial nephritis after COVID-19 vaccination: A report of six cases from a single center. *Clin. Nephrol.* 97 (2022), 289–297. PMID: [35142282](#).
- [161] A. Anderson et al.: Post-COVID-19 vaccination occurrence of splenic infarction due to arterial thrombosis. *BMJ Case Rep.* 14 (2021). PMID: [34876440](#).
- [162] G. Kroumpouzos et al.: Cutaneous Complications of mRNA and AZD1222 COVID-19 Vaccines: A Worldwide Review. *Microorganisms* 10 (2022). PMID: [35336199](#).
- [163] N. Nishimura et al.: IgA Vasculitis Following COVID-19 Vaccination. *Mod. Rheumatol. Case Rep.* (2022). PMID: [35253880](#).
- [164] D. Revilla-Nebreda et al.: New-onset acral lesions on hands after administration of mRNA-1273 vaccine against SARS-CoV-2: clinical images and histopathological study of three cases. *J. Eur. Acad. Dermatol. Venereol.* 35 (2021), e747–e749. PMID: [34310777](#).
- [165] T. Grieco et al.: COVID-19 infection and BNT162b2 vaccine triggering sarcoid-like lesions in the same patient. Response to: Sarcoid-like reaction in a patient recovering from COVID-19 pneumonia. *JAAD case reports* 23 (2022), 162–163. PMID: [35079616](#).
- [166] S. Chopra et al.: Cutaneous skin manifestation following messenger RNA Moderna SARS-CoV-2 vaccine with dermal hypersensitivity reaction histopathology. *JAAD case reports* 16 (2021), 24–25. PMID: [34414254](#).
- [167] V. Larson et al.: Clinical and histopathological spectrum of delayed adverse cutaneous reactions following COVID-19 vaccination. *J. Cutan. Pathol.* 49 (2021), 34–41. PMID: [34292611](#).

- [168] M. N. Alrashdi et al.: Systemic lupus erythematosus with acute pancreatitis and vasculitic rash following COVID-19 vaccine: a case report and literature review. *Clin. Rheumatol.* 41 (2022), 1577–1582. PMID: [35175446](#).
- [169] A. Kreuter et al.: Induction and exacerbation of subacute cutaneous lupus erythematosus following mRNA-based or adenoviral vector-based SARS-CoV-2 vaccination. *Clin. Exp. Dermatol.* 47 (2022), 161–163. PMID: [34291477](#).
- [170] I. Sagy et al.: New-onset systemic lupus erythematosus following BNT162b2 mRNA COVID-19 vaccine: a case series and literature review. *Rheumatol. Int.* 42 (2022), 2261–2266. PMID: [36098769](#).
- [171] Y. J. Chee et al.: SARS-CoV-2 mRNA Vaccination and Graves' Disease: A Report of 12 Cases and Review of the Literature. *J. Clin. Endocrinol. Metab.* 107 (2022), e2324–e2330. PMID: [35235663](#).
- [172] V. Francia et al.: The Biomolecular Corona of Lipid Nanoparticles for Gene Therapy. *Bioconjug. Chem.* 31 (2020), 2046–2059. PMID: [32786370](#).
- [173] C. H. Kao et al.: Visualization of the transport pathways of low density lipoproteins across the endothelial cells in the branched regions of rat arteries. *Atherosclerosis* 116 (1995), 27–41. PMID: [7488331](#).
- [174] I. Snelting-Havinga et al.: Immunoelectron microscopic visualization of the transcytosis of low density lipoproteins in perfused rat arteries. *Eur. J. Cell Biol.* 48 (1989), 27–36. PMID: [2743992](#).
- [175] E. Vasile et al.: Visualization of the binding, endocytosis, and transcytosis of low-density lipoprotein in the arterial endothelium in situ. *J. Cell Biol.* 96 (1983), 1677–89. PMID: [6853599](#).
- [176] K. Kucharz et al.: Post-capillary venules are the key locus for transcytosis-mediated brain delivery of therapeutic nanoparticles. *Nat. Commun.* 12 (2021), 4121. PMID: [34226541](#).
- [177] N. Hartl et al.: From adsorption to covalent bonding: Apolipoprotein E functionalization of polymeric nanoparticles for drug delivery across the blood-brain barrier. *Adv. Ther. Wein* 4 (2021). PMID: [33542947](#).
- [178] B. Obermeier et al.: Development, maintenance and disruption of the blood-brain barrier. *Nat. Med.* 19 (2013), 1584–96. PMID: [24309662](#).
- [179] N. R. Saunders et al.: The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. *Front. Neurosci.* 8 (2014), 404. PMID: [25565938](#).
- [180] M. Schlich et al.: Cytosolic delivery of nucleic acids: The case of ionizable lipid nanoparticles. *Bioeng. Transl. Med.* 6 (2021), e10213. PMID: [33786376](#).
- [181] B. M. Bruininks et al.: A molecular view on the escape of lipoplexed DNA from the endosome. *Elife* 9 (2020). PMID: [32297853](#).
- [182] Anonymous: SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048) 2.6.4 [Summary statement of the pharmacokinetic study] (Japanese). 2020. URL: https://www.pmda.go.jp/drugs/2021/P20210212001/672212000_30300AMX00231_I100_1.pdf.
- [183] Anonymous: SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048) 2.6.4 Summary statement of the pharmacokinetic study [English translation]. 2020. URL: <https://archive.org/details/pfizer-confidential-translated>.
- [184] Anonymous: Nonclinical Evaluation Report BNT162b2 [mRNA] COVID-19 vaccine (COMIR-NATY™). 2021. URL: <https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf>.
- [185] Anonymous: FDA briefing document: Pfizer-BioNTech COVID-19 Vaccine. 2020. URL: <https://www.fda.gov/media/144245/download>.
- [186] T. P. Buzhdygan et al.: The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood-brain barrier. *Neurobiol. Dis.* 146 (2020), 105131. PMID: [33053430](#).
- [187] D. Petrovski et al.: Penetration of the SARS-CoV-2 Spike Protein across the Blood-Brain Barrier, as Revealed by a Combination of a Human Cell Culture Model System and Optical Biosensing. *Biomedicines* 10 (2022). PMID: [35052867](#).

- [188] T. E. Fertig et al.: Vaccine mRNA Can Be Detected in Blood at 15 Days Post-Vaccination. *Biomedicines* 10 (2022), 1538. DOI: [10.3390/biomedicines10071538](https://doi.org/10.3390/biomedicines10071538).
- [189] C. Lubich et al.: The Mystery of Antibodies Against Polyethylene Glycol (PEG) - What do we Know? *Pharm. Res.* 33 (2016), 2239–49. PMID: [27271335](https://pubmed.ncbi.nlm.nih.gov/27271335/).
- [190] P. Sellaturay et al.: Polyethylene Glycol-Induced Systemic Allergic Reactions (Anaphylaxis). *J. Allerg. Clin. Immunol.* 9 (2021), 670–675. PMID: [33011299](https://pubmed.ncbi.nlm.nih.gov/33011299/).
- [191] G. T. Kozma et al.: Anti-PEG antibodies: Properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals. *Adv. Drug Deliv. Rev.* 154–155 (2020), 163–175. PMID: [32745496](https://pubmed.ncbi.nlm.nih.gov/32745496/).
- [192] T. L. Cheng et al.: Accelerated clearance of polyethylene glycol-modified proteins by anti-polyethylene glycol IgM. *Bioconjug. Chem.* 10 (1999), 520–8. PMID: [10346886](https://pubmed.ncbi.nlm.nih.gov/10346886/).
- [193] C. Loney et al.: Cationic lipids activate intracellular signaling pathways. *Adv. Drug Deliv. Rev.* 64 (2012), 1749–58. PMID: [22634161](https://pubmed.ncbi.nlm.nih.gov/22634161/).
- [194] D. Christensen et al.: Cationic liposomes as vaccine adjuvants. *Expert review of vaccines* 10 (2011), 513–21. PMID: [21506648](https://pubmed.ncbi.nlm.nih.gov/21506648/).
- [195] M.-G. Alameh et al.: Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity* 54 (2021), 2877–2892.e7. PMID: [34852217](https://pubmed.ncbi.nlm.nih.gov/34852217/).
- [196] J. Jarczak et al.: Defensins: natural component of human innate immunity. *Hum. Immunol.* 74 (2013), 1069–1079. PMID: [23756165](https://pubmed.ncbi.nlm.nih.gov/23756165/).
- [197] F. Milletti: Cell-penetrating peptides: classes, origin, and current landscape. *Drug Discov. Today* 17 (2012), 850–60. PMID: [22465171](https://pubmed.ncbi.nlm.nih.gov/22465171/).
- [198] S. Rajagopal et al.: Eight gram-negative bacteria are 10,000 times more sensitive to cationic detergents than to anionic detergents. *Can. J. Microbiol.* 49 (2003), 775–9. PMID: [15162202](https://pubmed.ncbi.nlm.nih.gov/15162202/).
- [199] J. Yu et al.: Integrative proteomics and metabolomics analysis reveals the toxicity of cationic liposomes to human normal hepatocyte cell line L02. *Molecular omics* 14 (2018), 362–372. PMID: [30247494](https://pubmed.ncbi.nlm.nih.gov/30247494/).
- [200] C. C. Winterbourn: Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* 4 (2008), 278–86. PMID: [18421291](https://pubmed.ncbi.nlm.nih.gov/18421291/).
- [201] W. L. Nyhan: Disorders of purine and pyrimidine metabolism. *Mol. Genet. Metab.* 86 (2005), 25–33. PMID: [16176880](https://pubmed.ncbi.nlm.nih.gov/16176880/).
- [202] M. C. Fillion and N. C. Phillips: Toxicity and immunomodulatory activity of liposomal vectors formulated with cationic lipids toward immune effector cells. *Biochim. Biophys. Acta* 1329 (1997), 345–56. PMID: [9371426](https://pubmed.ncbi.nlm.nih.gov/9371426/).
- [203] G. A. Hazelton and C. A. Lang: Glutathione contents of tissues in the aging mouse. *Biochem. J.* 188 (1980), 25–30. PMID: [7406884](https://pubmed.ncbi.nlm.nih.gov/7406884/).
- [204] I. Urits et al.: A Review of Patisiran (ONPATRO®) for the Treatment of Polyneuropathy in People with Hereditary Transthyretin Amyloidosis. *Neurol. Ther.* 9 (2020), 301–315. PMID: [32785879](https://pubmed.ncbi.nlm.nih.gov/32785879/).
- [205] D. Adams et al.: Long-term safety and efficacy of patisiran for hereditary transthyretin-mediated amyloidosis with polyneuropathy: 12-month results of an open-label extension study. *Lancet Neurol.* 20 (2021), 49–59. PMID: [33212063](https://pubmed.ncbi.nlm.nih.gov/33212063/).
- [206] H. Lin et al.: Experience of patisiran with transthyretin stabilizers in patients with hereditary transthyretin-mediated amyloidosis. *Neurodegener. Dis. Manag.* 10 (2020), 289–300. PMID: [32519928](https://pubmed.ncbi.nlm.nih.gov/32519928/).
- [207] J. F. Apgar et al.: Quantitative Systems Pharmacology Model of hUGT1A1-modRNA Encoding for the UGT1A1 Enzyme to Treat Crigler-Najjar Syndrome Type 1. *CPT Pharmacometrics Syst. Pharmacol.* 7 (2018), 404–412. PMID: [29637732](https://pubmed.ncbi.nlm.nih.gov/29637732/).
- [208] D. Garde: *Lavishly funded Moderna hits safety problems in bold bid to revolutionize medicine.* 2017. URL: <https://www.statnews.com/2017/01/10/moderna-trouble-mrna/>.

- [209] W. Webb: Moderna's "Hail Mary". 2021. URL: <https://unlimitedhangout.com/modernas-hail-mary/>.
- [210] A. Stewart et al.: Malignant disease in childhood and diagnostic irradiation in utero. *Lancet* 271 (1956), 447. PMID: [13358242](#).
- [211] A. Stewart and G. W. Kneale: Radiation dose effects in relation to obstetric x-rays and childhood cancers. *Lancet* 1 (1970), 1185–8. PMID: [4192374](#).
- [212] E. B. Harvey et al.: Prenatal x-ray exposure and childhood cancer in twins. *N. Engl. J. Med.* 312 (1985), 541–5. PMID: [3969117](#).
- [213] R. Wakeford: Childhood leukaemia following medical diagnostic exposure to ionizing radiation in utero or after birth. *Radiat. Prot. Dosimetry* 132 (2008), 166–74. PMID: [18922822](#).
- [214] Y. Suzuki et al.: The micronucleus test and erythropoiesis. Effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P/N ratio). *Mutagenesis* 4 (1989), 420–4. PMID: [2516221](#).
- [215] J. A. Heddle et al.: The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 123 (1983), 61–118. PMID: [6888413](#).
- [216] S. Sommer et al.: Micronucleus Assay: The State of Art, and Future Directions. *Int. J. Mol. Sci.* 21 (2020). PMID: [32102335](#).
- [217] C. T. Inglut et al.: Immunological and Toxicological Considerations for the Design of Liposomes. *Nanomaterials* 10 (2020). PMID: [31978968](#).
- [218] D. Baltimore: RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 226 (1970), 1209–11. PMID: [4316300](#).
- [219] S. Spiegelman et al.: Characterization of the products of RNA-directed DNA polymerases in oncogenic RNA viruses. *Nature* 227 (1970), 563–7. PMID: [4317039](#).
- [220] Y. Sakaki et al.: The LINE-1 family of primates may encode a reverse transcriptase-like protein. *Cold Spring Harb. Symp. Quant. Biol.* 51 Pt 1 (1986), 465–9. PMID: [2438081](#).
- [221] P. Klennerman et al.: A non-retroviral RNA virus persists in DNA form. *Nature* 390 (1997), 298–301. PMID: [9384383](#).
- [222] M. B. Geuking et al.: Recombination of retrotransposon and exogenous RNA virus results in nonretroviral cDNA integration. *Science* 323 (2009), 393–6. PMID: [19150848](#).
- [223] C. Esnault et al.: Human LINE retrotransposons generate processed pseudogenes. *Nat. Genet.* 24 (2000), 363–7. PMID: [10742098](#).
- [224] R. Cordaux and M. A. Batzer: The impact of retrotransposons on human genome evolution. *Nature reviews. Genetics* 10 (2009), 691–703. PMID: [19763152](#).
- [225] W. Ding et al.: L1 elements, processed pseudogenes and retrogenes in mammalian genomes. *IUBMB Life* 58 (2006), 677–85. PMID: [17424906](#).
- [226] I. Ovchinnikov et al.: Genomic characterization of recent human LINE-1 insertions: evidence supporting random insertion. *Genome Res.* 11 (2001), 2050–8. PMID: [11731495](#).
- [227] C. R. Beck et al.: LINE-1 Elements in Structural Variation and Disease. *Annu. Rev. Genomics Hum. Genet.* 12 (2011), 187–215. PMID: [21801021](#).
- [228] J. R. Kemp and M. S. Longworth: Crossing the LINE Toward Genomic Instability: LINE-1 Retrotransposition in Cancer. *Front. Chem.* 3 (2015), 68. PMID: [26734601](#).
- [229] S. Kubo et al.: L1 retrotransposition in nondividing and primary human somatic cells. *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006), 8036–41. PMID: [16698926](#).
- [230] I. Georgiou et al.: Retrotransposon RNA expression and evidence for retrotransposition events in human oocytes. *Hum. Mol. Genet.* 18 (2009), 1221–8. PMID: [19147684](#).
- [231] V. A. Belyi et al.: Unexpected inheritance: multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes. *PLoS Pathog.* 6 (2010), e1001030. PMID: [20686665](#).

- [232] M. Horie et al.: Endogenous non-retroviral RNA virus elements in mammalian genomes. *Nature* 463 (2010), 84–7. PMID: [20054395](#).
- [233] M. Horie and K. Tomonaga: Non-retroviral fossils in vertebrate genomes. *Viruses* 3 (2011), 1836–48. PMID: [22069518](#).
- [234] A. Katzourakis and R. J. Gifford: Endogenous viral elements in animal genomes. *PLoS Genet.* 6 (2010), e1001191. PMID: [21124940](#).
- [235] S. Chiba et al.: Widespread endogenization of genome sequences of non-retroviral RNA viruses into plant genomes. *PLoS Pathog.* 7 (2011), e1002146. PMID: [21779172](#).
- [236] E. V. Koonin: Taming of the shrewd: novel eukaryotic genes from RNA viruses. *BMC Biol.* 8 (2010), 2. PMID: [20067611](#).
- [237] H. Liu et al.: Widespread horizontal gene transfer from double-stranded RNA viruses to eukaryotic nuclear genomes. *J. Virol.* 84 (2010), 11876–87. PMID: [20810725](#).
- [238] O. Dhellin et al.: Functional differences between the human LINE retrotransposon and retroviral reverse transcriptases for in vivo mRNA reverse transcription. *EMBO J.* 16 (1997), 6590–602. PMID: [9351839](#).
- [239] M. Aldén et al.: Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr. Issues Mol. Biol.* 44 (2022), 1115–1126. PMID: [35723296](#).
- [240] L. Zhang et al.: Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proc. Natl. Acad. Sci. U. S. A.* 118 (2021). PMID: [33958444](#).
- [241] U. Sahin et al.: mRNA-based therapeutics—developing a new class of drugs. *Nat. Rev. Drug Discov.* 13 (2014), 759–780. PMID: [25233993](#).
- [242] B. R. Anderson et al.: Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. *Nucleic Acids Res.* 38 (2010), 5884–92. PMID: [20457754](#).
- [243] B. R. Anderson et al.: Nucleoside modifications in RNA limit activation of 2'-5'-oligoadenylate synthetase and increase resistance to cleavage by RNase L. *Nucleic Acids Res.* 39 (2011), 9329–9338. DOI: [10.1093/nar/gkr586](#).
- [244] K. Karikó et al.: Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol. Ther.* 16 (2008), 1833–40. PMID: [18797453](#).
- [245] N. Van de Water et al.: A 20.7 kb deletion within the factor VIII gene associated with LINE-1 element insertion. *Thromb. Haemost.* 79 (1998), 938–42. PMID: [9609225](#).
- [246] F. J. T. Staal et al.: Sola dosis facit venenum. Leukemia in gene therapy trials: a question of vectors, inserts and dosage? *Leukemia* 22 (2008), 1849–1852. PMID: [18769449](#).
- [247] S. Hacein-Bey-Abina et al.: Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* 118 (2008), 3132–42. PMID: [18688285](#).
- [248] W. Doerfler: Adenoviral Vector DNA- and SARS-CoV-2 mRNA-Based Covid-19 Vaccines: Possible Integration into the Human Genome—Are Adenoviral Genes Expressed in Vector-based Vaccines? *Virus Res.* 302 (2021), 198466. PMID: [34087261](#).