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COVID-19: Immunology, vaccines and epidemiology

About this free course

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Contents

Introduction and guidance

Introduction

Have you ever wondered why some people are very badly affected by a particular viral infection, while others are asymptomatic? Differences in the effectiveness of their immune systems play a part, but so do the type of virus, genetic variability in the human population, and whether a person has any immunity from previous infections or vaccination.

This free badged course, *COVID-19: Immunology, vaccines and epidemiology*, will take you through some of the key science that underpinned the global efforts to control COVID-19. You will learn about virology, immunology and vaccinology. You will carry out practical online laboratory activities, in which you will learn how to detect antibodies against SARS-CoV2 using a standard antibody-detection technique called ELISA, an enzyme-linked immunosorbent assay. You will see how the detection of antibodies helped epidemiologists to track the course of the COVID-19 pandemic. You will also see how quantitation of antibodies against the spike protein was a critical measure, determining which vaccines would be most likely to protect against the disease.

The course lasts 24 hours and is comprised of eight 'weeks'. You can work through the course at your own pace, so if you have more time one week there is no problem with pushing on to complete a further study week.

There will be weekly interactive quizzes, of which Weeks 4 and 8 will provide you with an opportunity to earn a badge to demonstrate your new skills. You can read more on how to study the course and about badges in the next sections.

After completing this course, you will be able to:

- understand how the immune system protects against viral infection
- detect antibodies against COVID-19, using the ELISA technique
- distinguish individuals who have had a COVID-19 infection, from those who have had a COVID-19 vaccination
- understand how the detection of antibodies (serology) can be used to track an epidemic
- understand the different strategies developed for producing vaccines against COVID-19.

Before you begin, you should have a basic knowledge of cell biology, and an understanding of how DNA and RNA hold genetic information. If you are unfamiliar with these areas, then you may find the following OpenLearn materials on molecular and cellular biology to be a good starting point:

- DNA, RNA and protein formation
- A tour of the cell.

Moving around the course

In the ‘Summary’ at the end of each week, you will find a link to the next week. If at any time you want to return to the start of the course, click on ‘Full course description’. From here you can navigate to any part of the course.

It’s also good practice, if you access a link from within a course page (including links to the quizzes), to open it in a new window or tab. That way you can easily return to where you’ve come from without having to use the back button on your browser.

The Open University would really appreciate a few minutes of your time to tell us about yourself and your expectations for the course before you begin, in our optional start-of-course survey. Participation will be completely confidential and we will not pass on your details to others.

What is a badged course?

Digital badges are a new way of demonstrating online that you have gained a skill. Colleges and universities are working with employers and other organisations to develop open badges that help learners gain recognition for their skills, and support employers to identify the right candidate for a job.

Badges demonstrate your work and achievement on the course. You can share your achievement with friends, family and employers, and on social media. Badges are a great motivation, helping you to reach the end of the course. Gaining a badge often boosts confidence in the skills and abilities that underpin successful study. So, completing this course could encourage you to think about taking other courses.

How to get a badge

Getting a badge is straightforward! Here’s what you have to do:

- read each week of the course
- score 50% or more in the two badge quizzes in Week 4 and Week 8.

For all the quizzes, you can have three attempts at most of the questions (for true or false type questions you usually only get one attempt). If you get the answer right first time you will get more marks than for a correct answer the second or third time. Therefore, please be aware that for the two badge quizzes it is possible to get all the questions right but not score 50% and be eligible for the badge on that attempt. If one of your answers is incorrect you will often receive helpful feedback and suggestions about how to work out the correct answer.

For the badge quizzes, if you're not successful in getting 50% the first time, after 24 hours you can attempt the whole quiz, and come back as many times as you like.

We hope that as many people as possible will gain an Open University badge – so you should see getting a badge as an opportunity to reflect on what you have learned rather than as a test.

If you need more guidance on getting a badge and what you can do with it, take a look at the OpenLearn FAQs. When you gain your badge you will receive an email to notify you and you will be able to view and manage all your badges in My OpenLearn within 24 hours of completing the criteria to gain a badge.

Get started with Week 1.

Week 1: How the body recognises a viral infection

Introduction

This week, you will be introduced to a range of acute and chronic viral infections, focusing on acute viral infections. You will take a close look at influenza-A and SARS-CoV2, the causative agent of COVID-19, as key examples – not just because of the recent pandemic but also because they are very informative. The role of antibodies in protection against viral disease and reinfection is an important component of immunity. This area, and vaccine development, will be discussed later in the course, but for now you will start with an overview of viruses and then move on to see how the innate immune system recognises and responds to an acute viral infection.

Video content is not available in this format.
Video 1 Introduction to Week 1

By the end of this week, you should be able to:

- outline the range of different viral infections
- describe the structure and genomes of influenza-A and SARS-CoV2 viruses
- identify intracellular receptors of the innate system that recognise viruses
- understand how **interferons** delay the spread of an acute virus infection.

The Open University would really appreciate a few minutes of your time to tell us about yourself and your expectations for the course before you begin, in our optional

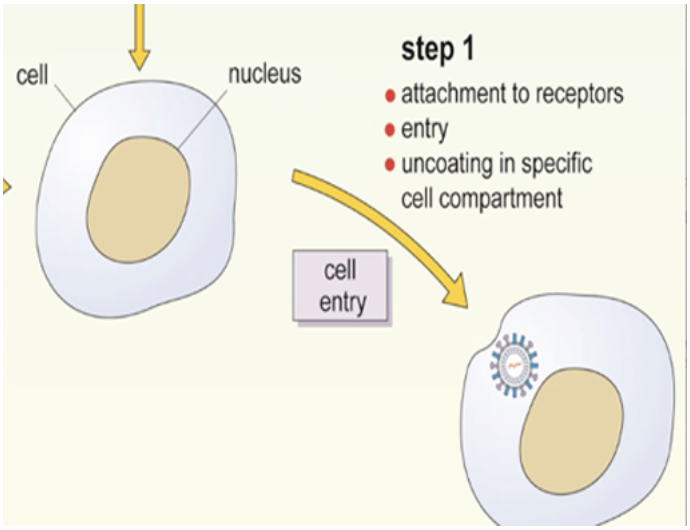
start-of-course survey. Participation will be completely confidential and we will not pass on your details to others.

1 Viral infections

All viruses are small packages containing the genetic information needed to replicate themselves, but they can only do so by infecting cells of a host organism. However, beyond this basic description, viruses are enormously variable. Often they produce damage or disease in their host, including humans, and for this reason they are a very important group of pathogens.

It would be impossible in a short course to cover the great range of viruses that cause disease in humans. However, we can look at the life-cycle of a typical virus infection in Video 2.

Video content is not available in this format.
Video 2 Life-cycle of a typical virus infection



1.1 Acute infections

The life-cycle shown earlier in Video 2 is typical of an acute virus infection, such as influenza or COVID-19. An acute infection is one where a person becomes infected, and the immune system then reacts to destroy the virus and virus-infected cells. In the end, the immune system prevails and there is no longer any of the virus left in that host – this is referred to as **sterile immunity**. Usually, an acute infection will last for a few weeks, at most.

However, some virus infections can evade the immune system and lie low within cells of the host for months or years, producing no symptoms. Such infections are said to be latent. In addition, they may reactivate or stay continuously active over this period; in this case they are responsible for a **chronic** infection.

Activity 1 Acute and chronic viral infections

Allow 10 minutes

Look at Table 1 below, which lists some viruses that produce disease in humans. From your previous knowledge or experience try to decide which of these viruses

produce an acute infection and which produce a latent or chronic infection. Write your answers in the boxes provided in the last column. Then click on ‘Reveal feedback’ to see the answers. The first box has been filled in for you.

Table 1 Viral infections

Virus	Disease	Acute or latent/chronic infection
SARS-CoV2	COVID-19	Acute
Rubella virus	German measles	*Provide your answer...*
Ebola virus	Ebola	*Provide your answer...*
Human immunodeficiency virus (HIV)	Acquired immune deficiency syndrome (AIDS)	*Provide your answer...*
Norovirus	Gastroenteritis (vomiting, diarrhoea)	*Provide your answer...*
Rhinovirus	Common cold	*Provide your answer...*
Epstein Barr virus	Glandular fever	*Provide your answer...*
Herpes simplex virus	Cold sores	*Provide your answer...*
Poliovirus	Poliomyelitis (Infantile paralysis)	*Provide your answer...*
Varicella zoster virus	Chicken pox, shingles	*Provide your answer...*
Mumps virus	Mumps	*Provide your answer...*

Here is the completed table:

Table 1 Viral infections (completed)

Virus	Disease	Acute or latent/chronic infection
SARS-CoV2	COVID-19	Acute
Rubella virus	German measles	Acute
Ebola virus	Ebola	Acute
Human immunodeficiency virus (HIV)	Acquired immune deficiency syndrome (AIDS)	Chronic
Norovirus	Gastroenteritis (vomiting, diarrhoea)	Acute
Rhinovirus	Common cold	Acute
Epstein Barr virus	Glandular fever	Acute/Chronic
Herpes simplex virus	Cold sores	Latent
Poliovirus	Poliomyelitis (Infantile paralysis)	Acute
Varicella zoster virus	Chicken pox, shingles	Acute/Latent
Mumps virus	Mumps	Acute

Rubella virus, Ebola virus, Norovirus, Rhinovirus, Poliovirus and Mumps virus cause acute infections. Notice that acute infections can still cause very serious diseases and in some cases (eg polio) the damage lasts for a lifetime, even if the viral infection is relatively short. HIV causes a persistent chronic infection. Epstein Barr virus causes glandular fever, an acute infection, but usually persists as a symptomless chronic infection for years. Herpes simplex can remain latent for many years and sporadically reactivate to produce cold sores. Varicella zoster produces chicken pox as an acute illness, but becomes latent in some people and reactivates to produce shingles. As you may have deduced, chronic and **latent** infections are persistent infections that continue after the initial acute infection with that virus.

Don’t worry if you did not get all of these. In reality, the outcome in any individual may be different from the usual course of infection. For example, in people who are immunosuppressed or immunodeficient, acute viral infections are often slower to clear and are more likely to become chronic infections.

1.2 Chronic and latent infections

So what is the key difference between acute and chronic viral infections? Essentially, they have different reproductive strategies. After infection, viruses such as SARS-CoV2 and influenza replicate quickly and then spread by aerosol droplets, which are inhaled into the nose, throat and respiratory system – they complete their life cycle within days, before the immune system has fully geared up to eliminate them.

Conversely, chronic infections such as Herpes simplex and HIV can evade the immune response for years. During this time, the virus is shed more slowly, sometimes sporadically and the method of transmission is often by direct contact between individuals.

These persistent viruses have a variety of strategies for evading the immune response. Here are some examples:

- HIV mutates continuously within an individual to avoid being recognised by antibodies.
- HIV subverts the cellular machinery which promotes recognition of virus-infected cells.
- Herpes simplex remains latent in neurons, and deploys decoy proteins that fool the immune system into recognising the cell as not-infected.
- Epstein Barr virus (EBV) secretes a signalling molecule (cytokine), which deviates the immune response away from that which eliminates EBV-infected cells.
- Human papilloma virus (HPV) (Figure 1) produces very low levels of viral proteins, so the immune system has little foreign material to recognise.

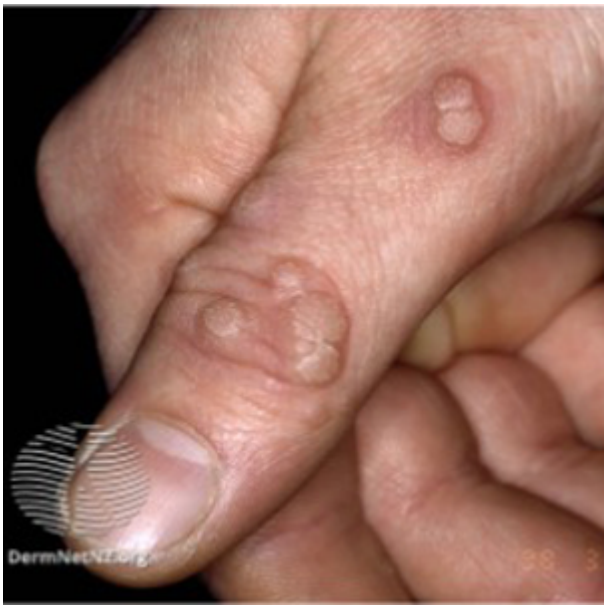


Figure 1 Warts are produced by many types of human papilloma virus (HPV)

Indeed, each of these viruses has numerous systems for evading immune responses. It is a fascinating area of research. However, to understand it requires a knowledge of how the immune system would normally eliminate a virus or a virus-infected cell. Before that you need to understand how the immune system recognises viruses and that is based on some knowledge of virus structure.

2 The anatomy of a virus

Viruses come in a variety of different shapes and sizes. The simplest consist of a protein shell called the **nucleocapsid**, which contains the viral genetic material (nucleic acid). These are called non-enveloped viruses, to distinguish them from the more complex enveloped viruses, as shown in Figure 2.

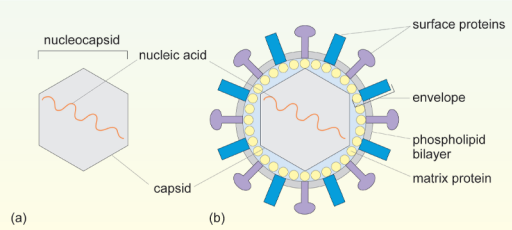


Figure 2 (a) The basic structure of a non-enveloped virus (b) and an enveloped virus

Enveloped viruses also have a nucleocapsid containing the viral genome. The nucleocapsid is contained within a **viral envelope**, a composite structure which includes a phospholipid bilayer, derived from the plasma membrane of the host cells which produced the virus. Inside the membrane, viral matrix proteins connect the nucleocapsid to the envelope. The matrix is also important in organising the assembly of new virus particles. The envelope also contains viral proteins and some residual host proteins from the infected cell.

The critical thing to notice is that some proteins are on the outside of the virus whereas others, such as the nucleocapsid and matrix proteins, are on the inside. This is important because it affects what parts of the virus can be recognised by different elements of the immune system.

As you can see in Figure 3, enveloped viruses are released from infected cells by budding from the plasma membrane. Examples of enveloped viruses are influenza-A, HIV and SARS-CoV2. The transmission electron micrographs show different stages in the assembly (1, 2) budding (3) and release (4) of HIV from the surface of an infected cell. This process illustrates step 4 in Video 2.

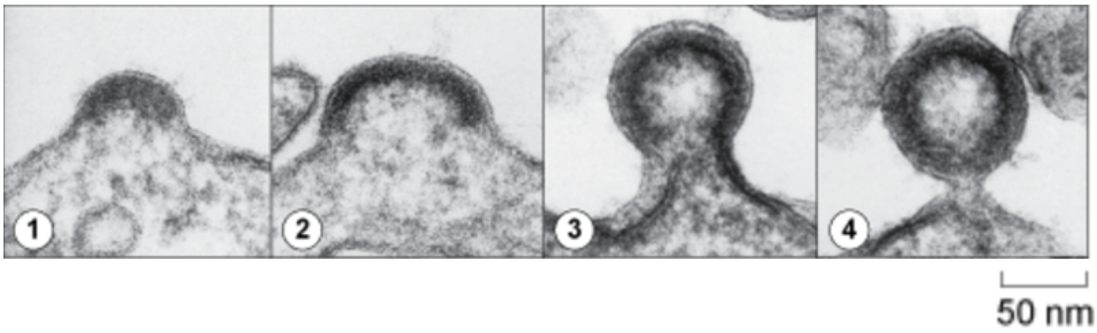


Figure 3 Transmission electron micrographs

In addition to the components described above, most viruses contain a number of enzymes and auxiliary proteins which are required to initiate infection of the cell and replication of the virus.

2.1 Types of virus

You have now considered a great variety of viruses, which differ in size, shape and life-cycle, some of which are shown in Figure 4. In this course you will focus on Influenza-A, an orthomyxovirus, and SARS-CoV2, which is a coronavirus.

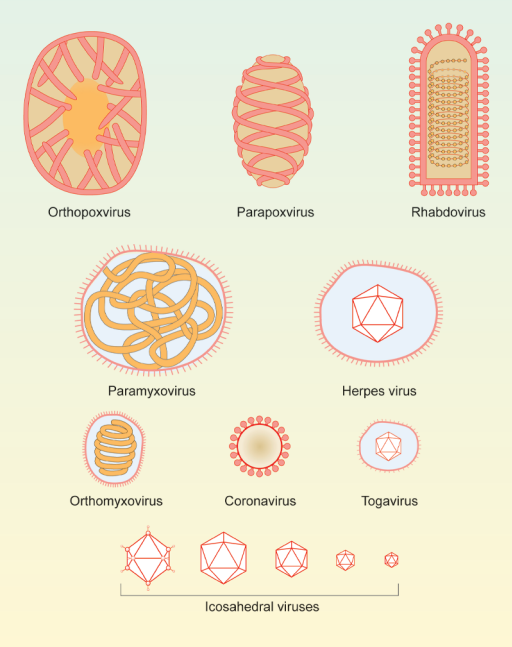


Figure 4 The morphology and approximate relative sizes of different families of virus

In addition to their obvious differences in size and shape, viruses are classified according to their genetic material and how it is replicated:

- DNA or RNA
- single-stranded (ss) or double stranded (ds)
- positive sense or negative sense; this relates to whether the genome directly encodes protein (positive) or whether it must be replicated before it can direct protein synthesis (negative).

This is referred to as the Baltimore system of classification. The details of virus replication are beyond the scope of this course. It is sufficient to know that Influenza-A has a genome with eight segments of ssRNA, which is negative-sense, whereas SARS-CoV2 has a single segment of ssRNA, which is positive sense.

One important point to note is that replication of the ssRNA genomes of these viruses involves an intermediate of dsRNA; double-stranded RNA is not a standard component of uninfected host cells.

2.2 Influenza-A and SARS-CoV2

You will now look in a bit more detail at influenza-A and SARS-CoV2, both of which cause acute respiratory infections.

Activity 2 Comparing influenza-A and SARS-CoV2

Allow 5 minutes

Take a moment to consider any other similarities. Try to identify three similarities. Write your answer in the box provided below.

Provide your answer...

They are both enveloped viruses, with a ssRNA genome. They are spread by aerosol droplets and you might also have noted that they both cause pandemics, with waves of infection as new strains arise.

Now try to identify three differences between influenza-A and SARS-CoV2. Write your answer in the box provided below.

Provide your answer...

The genome of influenza-A is segmented (eight segments) and negative-sense, whereas that of SARS-CoV2 is a single, positive-sense segment of ssRNA. You might also have spotted that the shape of the viruses is different, as shown in Figure 4.

Another difference between these viruses is in how they infect cells and exactly which cells can become infected. The first stage of infection is the binding of a viral surface protein to a protein receptor on the host cell. Since this interaction is highly specific it determines which cell types can be infected by each type of virus. As shown in Figure 5, the nucleocapsid contains viral protein and RNA (ribonucleoprotein) and the envelope of influenza-A has two external viral proteins – the haemagglutinin and neuraminidase.

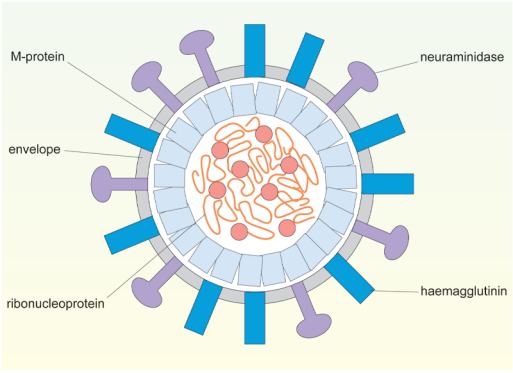


Figure 5 Structure of influenza-A virus

The haemagglutinin (H) is responsible for attachment of the virus to the target cell. It binds to carbohydrate units (sialic acid) which are attached to a number of different proteins on the host cell surface. (Proteins with bound carbohydrate units are called **glycoproteins**). Because the target glycoproteins are quite widely distributed on different cell types, influenza-A can infect several different types of cell. The neuraminidase (N) is involved in

virus release and spread. Strains of influenza-A are classified according to which haemagglutinin and neuraminidase they have, eg H3N2.

An electron-micrograph of a corona virus is shown in Figure 6. It has prominent spikes on the outside, which give it a crown-like appearance, and this was the origin of the name for this group of viruses. You can see the structure of the virus shown diagrammatically in Figure 7 below.

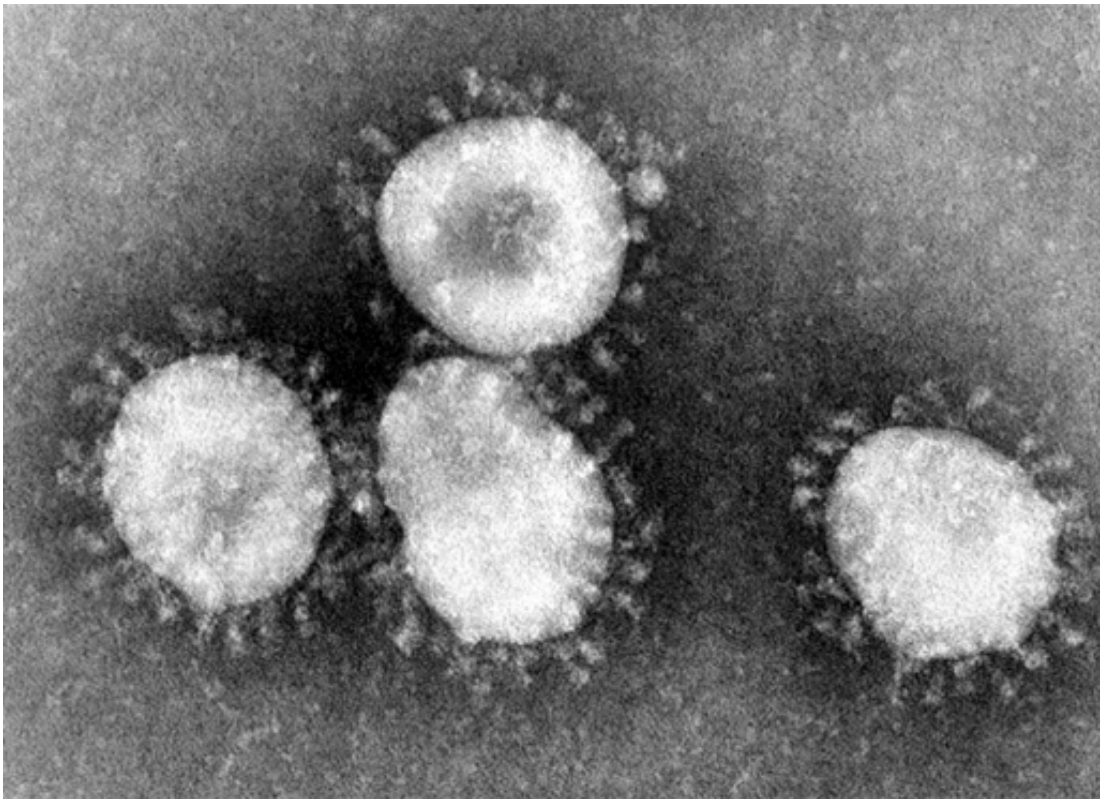


Figure 6 Electron micrograph of a coronavirus

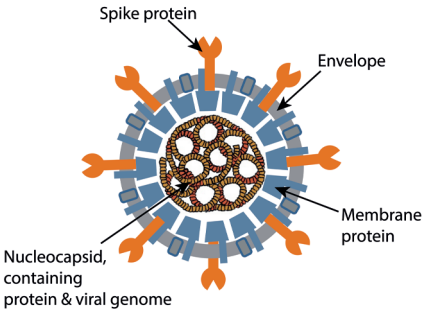


Figure 7 Structure of SARS-Cov2

The spike protein allows the virus to attach to target cells by binding to a protein called the ACE2 receptor (ACE2 = Angiotensin converting enzyme-2). The part of the spike protein which directly contacts the ACE2 receptor is the '**receptor-binding domain (RBD)**'. This

detail is very relevant when we consider what antibodies are most effective in protecting against COVID-19 – antibodies which recognise the RBD are particularly important.

- Would you expect SARS-Cov2, to infect the same cells as influenza-A? If so, why?
- SARS-Cov2 binds to cells that have the ACE2 receptor, whereas influenza-A binds to a number of surface glycoproteins that are recognised by the haemagglutinin. So we might expect SARS-CoV2 and influenza-A to infect different sets of cells.

Different strains of a virus can also selectively target distinct sets of cells and this is referred to as '**viral tropism**'.

In humans the ACE2 receptor is found in many tissues, but at particularly high levels on the upper surface of epithelial cells facing into the alveoli of the lung and the lumen of the small intestine. It is also found on the endothelial cells which line the inside of blood vessels and in the smooth muscle of arteries. This observation partly explains why the lung is particularly targeted by COVID-19. But other tissues may be affected in more severe cases, when the virus spreads through the body.

2.3 Proteins encoded by SARS-CoV2

The genome of SARS-CoV2 is large for an RNA virus. It consists of a single piece of positive sense ssRNA with 29,903 nucleotides which encode 19 proteins, as shown in Figure 8. Two genes encode 16 non-structural proteins nsp1-nsp16. The genes for the four structural proteins (Spike, Envelope, Membrane glycoprotein, Nucleocapsid) and the auxiliary proteins (3a, 6,7,8,10) are indicated. There are untranslated regions (UTR) at the 5' and 3' ends of the genome. The non-structural and auxiliary proteins are required for virus replication, assembly and release.

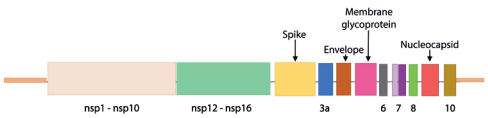


Figure 8 The genome of SARS-CoV2

New variants of SARS-CoV2 have shown mutations in many of these genes. However, it is variation in the spike protein that is of particular interest and importance, because antibodies against the spike protein are protective against infection, and the spike protein is the key component of all current vaccines against COVID-19 (Feb. 2023).

Figure 9 shows the spike protein diagrammatically. It is a trimeric glycoprotein with two subunits, which is inactive until it comes into contact with a host cell. On contact, an enzyme on the host cell surface (TMPRSS2) cleaves and activates the spike protein so that it can now bind to the ACE2 receptor.

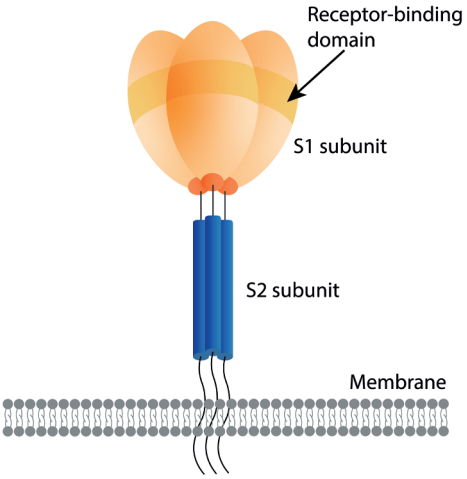


Figure 9 Diagram of SARS-CoV2 spike protein

In the next section, you will now start to look at how the immune system can recognise a viral infection.

3 Recognition of viral infection

To combat a virus infection, the immune system must first recognise the virus, and/or virus-infected cells.

- From your knowledge of the structure and life-cycle of an enveloped virus, identify three distinctive features of a virus or virus-infected cell, that could distinguish it from normal host cells.
-
  - The virus has proteins encoded by the viral genome which are different from the host proteins, for example the spike protein, haemagglutinin, M-protein or nucleocapsid.
  - If a cell is infected by an enveloped virus, then some viral proteins are inserted into the membrane of the cell, before the individual viruses assemble and bud off.
  - The genome of influenza-A and SARS-Cov2 is RNA, whereas the genome of mammalian cells is DNA. The replication of these viruses involves dsRNA, which is not a regular component of host cells.

As you will see, there are two major types of immune response – innate immune responses and adaptive immune responses. Adaptive immune responses improve with time, especially following repeated encounters with the same pathogen. In contrast, innate immune responses do not display immunological memory, and hence do not improve significantly over time.

The **adaptive immune system** primarily recognises foreign proteins, such as virus-encoded proteins. Any biological molecule that can be recognised by the adaptive immune system is called an **antigen**. We will look at antigen-recognition and adaptive immune responses against viruses in Week 2.

Adaptive immune responses take several days to become fully active. During this early period of infection, the innate immune system acts as a first line of defence. The innate immune system recognises ‘pathogen-associated molecular patterns (PAMPs)’ which are distinctive biological components of bacteria, viruses and fungi. For the rest of this week, you will consider some of these PAMPs and how they trigger innate immune responses to viruses.

3.1 Pathogen-associated molecular patterns (PAMPs)

Cells of the body have internal receptors that allow them to recognise components of viruses. They belong to a family of ten receptors, called **Toll-like receptors** (TLRs), that recognise components of pathogens. Those most relevant for detection of viral infection are listed in Table 2. They are present in cells of the immune system and epithelial cells in mucous membranes – for example, at sites of potential virus infection.

Table 2 Toll-like receptors that recognise viral infection

Receptor	Location	Recognises
TLR3	Endosome or cell surface	dsRNA

TLR7	Endosome	ssRNA
TLR8	Endosome	ssRNA

The importance of the TLRs is demonstrated by the rare individuals who lack them. For example, TLR3 deficiency is associated with susceptibility to herpes simplex infection.

Notice that these receptors face into the endosome. When viruses such as SARS-CoV2 enter a cell they are first taken into an endosome, where the viral capsid is removed, releasing the viral RNA. The released RNA can be immediately recognised by the TLRs facing into the endosome. Recognition of the viral RNA triggers activation of the infected cell via a transcription factor, NFκB, which has been described as a 'master-switch of inflammation'. One important action of NFκB is to induce the synthesis of **Type-1 interferon (IFN)**, a signalling molecule that helps control viral infection.

NFκB also induces synthesis and secretion of a variety of other signalling molecules, collectively called **cytokines** which control the development of inflammation; this is normally beneficial for controlling infection. However, excessive cytokines can damage host tissues. You may have heard the term 'cytokine storm', which refers to damage produced by excess cytokine production. In some patients, this was a particular problem with COVID-19 infection – where the virus infection was not well-controlled by the immune system, the collateral damage from the cytokines exacerbated the damage caused by the virus.

You will now look at how interferon limits the spread of virus.

3.2 Interferons and anti-viral proteins

When cells become infected, interferon (IFN) signals to neighbouring cells to induce synthesis of **anti-viral proteins**, which are normally inactive, but it puts the cell into a state of 'alert'. Should the neighbouring cells later become infected, viral components activate the antiviral proteins, to shut down mRNA production and protein synthesis. This process is shown diagrammatically in Figure 10. An infected cell (1) releases IFN, which acts on receptors on neighbouring cells (2) to induce antiviral proteins (3). If that cell later becomes infected with a virus, the antiviral proteins are activated (4) to induce a virus-resistant state where protein synthesis is inhibited (5).

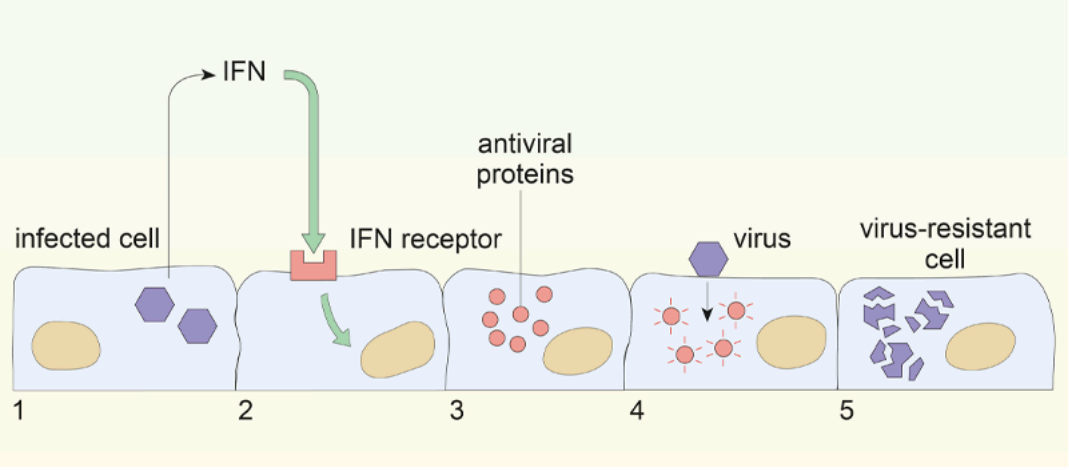


Figure 10 Anti-viral action of interferon

- What advantage is there for a cell to shut down protein synthesis? What advantage is there for the infected host?
- There is no advantage to the individual cell, since all cells must synthesise protein to survive, however it also stops virus production in that cell and thus slows virus spread within the body.

Interferons were originally identified for their role in limiting virus replication. However, they have numerous additional effects in the adaptive immune system. Specifically, they enhance the ability of all cells to present antigens to T lymphocytes (T cells), which are primarily responsible for elimination of virus-infected cells. You will hear a lot more about this in Week 2. But first we look at one more element of the innate immune system: how a replicating virus is detected.

3.3 Cytosolic receptors for viral infection

After a virus has escaped from the endosome of an infected cell, virus components are located in the cytoplasm. Receptors are also present in the cytoplasm, that recognise and limit viral replication and spread. They belong to a family of receptors called the 'Retinoic acid-inducible gene I (RIG1)-like helicases' which are understandably abbreviated to **RLHs**. Two receptors are particularly important in this context:

- RIG-1 itself which recognises short dsRNA
- MDA5 which recognises long dsRNA

- Why would recognition of cytoplasmic dsRNA, enable a cell to identify an ongoing viral infection?
- dsRNA is an intermediate produced during replication of RNA genome viruses, which is not a regular component of host cells.

After binding to dsRNA these receptors localise to mitochondria via an adapter protein IPS-1. They then activate transcription factors NFκB and IRF3 (Interferon regulatory protein-3) which translocate to the nucleus of the cell and activate transcription of a number of genes including the genes for type-1 interferon, as shown in Figure 11. You can also see that RIG-1 and MDA5 are cytoplasmic receptors for dsRNA. They lead to activation of transcription factors IRF3 and NFκB, which promote transcription of genes for type 1 interferons, and other cytokines

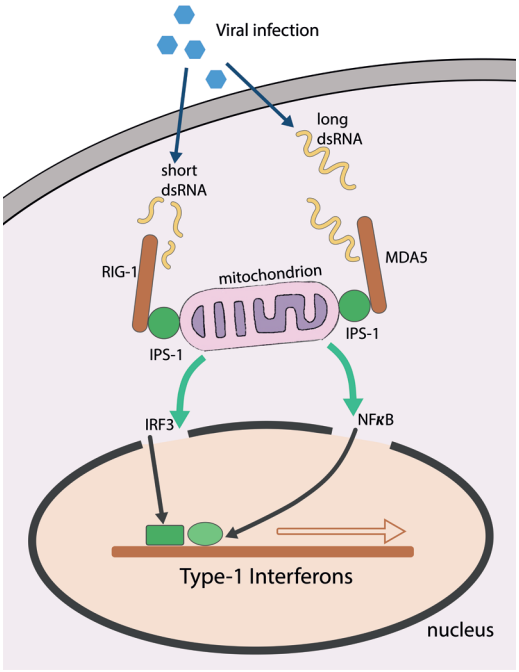


Figure 11 Recognition of viral dsRNA.

4 Week 1 quiz

Check what you have learned this week by taking the end-of-week quiz.

Week 1 practice quiz

Open the quiz in a new window or tab, then return to this week when you're done.

5 Summary

This week, we introduced the wide range of viruses that produce disease in humans, before focusing on two respiratory viruses − influenza-A and SARS-CoV2. Both are enveloped viruses with an RNA genome that produce acute infections that are spread by aerosol droplets.

Distinctive components of virus infection can be recognised by the immune system. The adaptive immune system recognises virus antigens, whereas the innate immune system recognises pathogen-associated molecular patterns. In the case of influenza-A and SARS-CoV2 the PAMPs are the ssRNA of the viral genome or the dsRNA, which is an intermediate of virus replication. TLRs recognise viral RNA in endosomes, while RLHs recognise dsRNA of replicating virus in the cytoplasm.

After recognising the virus, infected cells secrete interferon, a cytokine which signals to neighbouring cells, to slow virus spread. Other cytokines induce inflammation, which normally helps control infection, but can contribute to host tissue damage.

The actions of the innate immune system hold the line against the virus until the adaptive immune response gets into action, but that takes several days… And that is what you will learn about next week.

You should now go to Week 2.

Week 2: How the immune system combats viral infection

Introduction

During the COVID-19 pandemic, you will have heard a lot about antibodies and how vaccines induce neutralising antibodies that protect against infection. But antibodies are just one element of immune defence. Do you remember hearing about T cells and immunological memory? This week you will learn more about these and about the range of adaptive immune responses that combat viruses and deal with virus-infected cells.

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Audio 1 Introduction to Week 2

By the end of this week you should be able to:

- describe how T cells and NK cells recognise and destroy virus-infected cells
- outline how B cells recognise antigens and make antibodies
- list the major functions of different classes of antibody and their roles in combating a virus infection
- understand how innate and adaptive immune defences act in complementary ways
- understand the basis of immunological memory, which underpins vaccination.

1 Adaptive immune defences

In Week 1, you learnt about innate immune responses, and some of the ways that the body can recognise a virus or virus-infected cell. This week, you will learn about the adaptive immune response to viruses.

- What is the key difference between the innate and adaptive immune responses?
- Innate immune responses do not improve following repeated encounters with the same pathogen. Adaptive immune responses are stronger and more effective each time the same antigen contacts a person's immune system – in essence the immune system adapts.

The principal cells of the adaptive immune system are white blood cells or leukocytes, which are distributed between various lymphoid organs such as lymph nodes and the spleen. Leukocytes traffic between organs via the blood and lymphatic system and interact with other cell types in the body in immune defence. Viruses are pathogens that live inside cells of the body but move between cells via the blood, tissue fluids and extracellular spaces. In each case, the immune system has to be able to recognise the pathogen and mount an appropriate response.

The cells responsible for immune recognition are **lymphocytes**, a set of leukocytes that are found in blood and throughout the body, although they are particularly concentrated in lymphoid tissues and specialised areas of mucosal tissue.

Lymphocytes fall into two basic categories: **T cells**, which develop in the thymus and **B cells**, which develop in the bone marrow.

- What term is used for a molecule that is recognised by a lymphocyte?
- Antigen – see Week 1, Section 2.

A third group of lymphocytes which are important in antiviral defence is the Natural Killer or **NK cells**. However, unlike T cells and B cells, the defence provided by NK cells does not improve significantly following repeated encounters with the same virus. For this reason, NK cells are really part of the innate immune system, although it is easier to consider them alongside T cells, since they act in complementary ways.

Video 1 below illustrates the principal cells of the immune system and explains how they are involved in immune defence against viruses.

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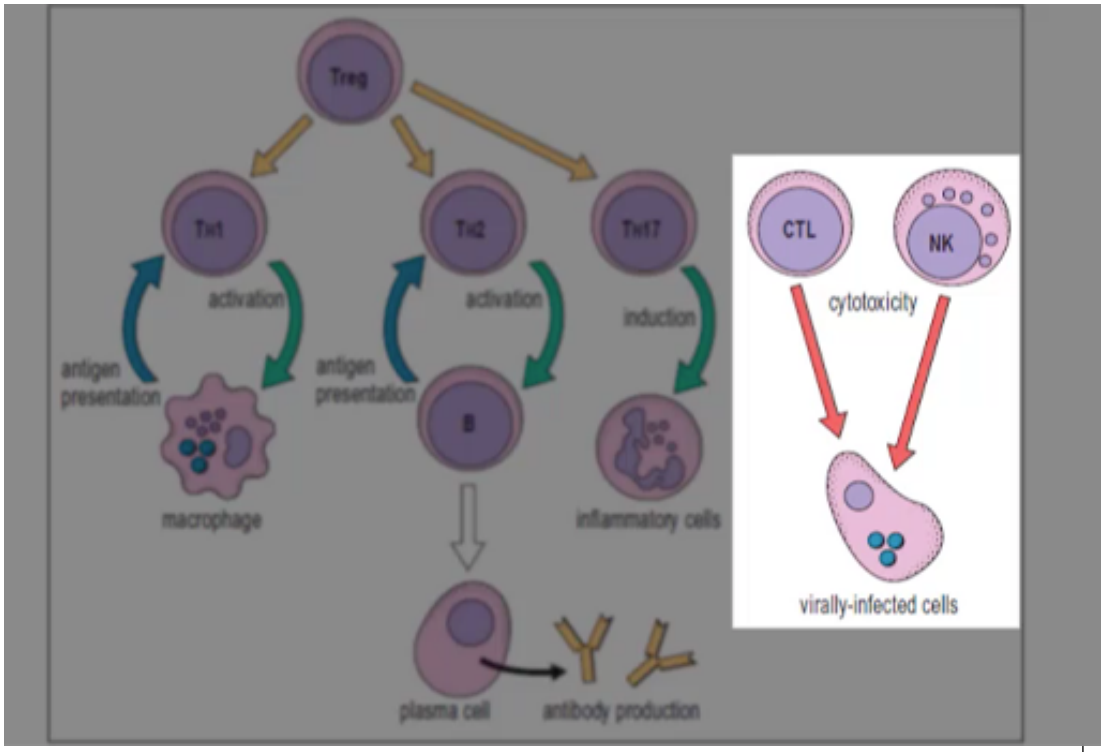
Video 1 Immune defence



If there is one key message you take away from this section, it is that T cells recognise antigens originating from inside the cells of the body, whereas B cells produce antibodies that recognise antigens in extracellular spaces and tissue fluids.

For the rest of this week, you will look at how lymphocytes and antibodies protect against virus infection, starting with the cytotoxic T lymphocytes.

1.1 Cytotoxic T cells

T cells recognise antigens through their T cell receptor (TCR). To be precise, T cells recognise antigen fragments presented to them by cell-surface molecules encoded by the major histocompatibility complex (**MHC molecules**), shown in Figure 1. There are in fact, two types of MHC molecule – class I and class 2. Here we will concentrate on MHC class I molecules. All cells of the body continuously sample their own internal proteins and present polypeptide fragments on the cell surface bound to MHC class I molecules.

Internal molecules of the cell, which can be any intracellular molecule including a viral antigen (blue hexagon) are broken down into peptide fragments. These antigenic peptides are presented by MHC molecules on the cell surface, where they may be recognised by T cells that have an appropriate T cell receptor. (Note: in Figure 1 and later diagrams, the scale of the cells and the receptor molecules will differ.)

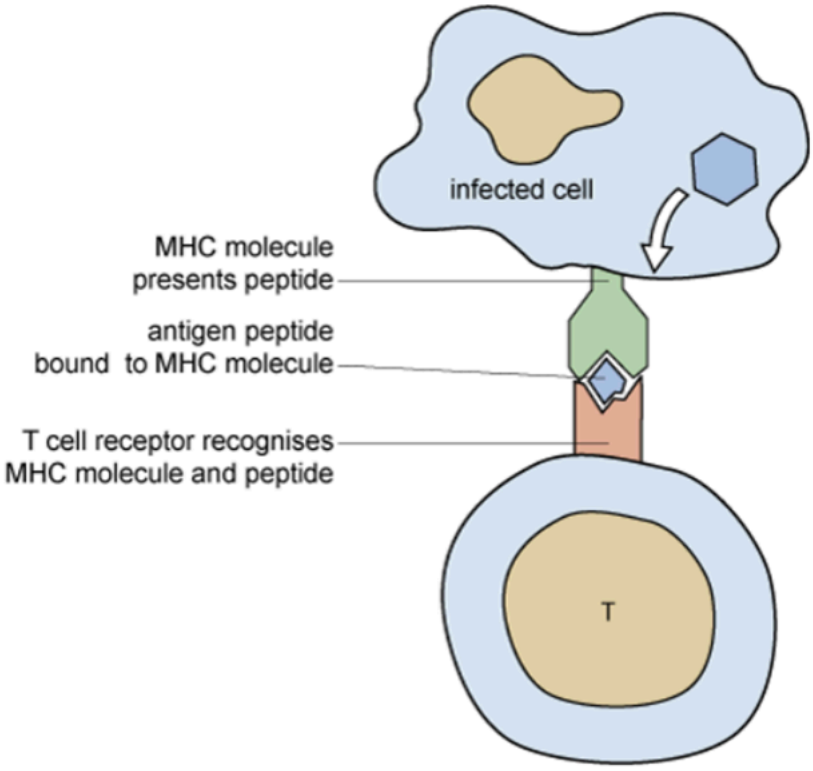


Figure 1 MHC molecules

If the cell has become infected by a virus, then polypeptide fragments of viral proteins will also be presented by the MHC class I molecules. If a cytotoxic T cell recognises the antigen fragment+MHC molecule, then it can signal to the infected cell to induce **apoptosis** – programmed cell death. You will learn more about this later, but first you will look in a bit more detail at the MHC molecules.

1.2 MHC Molecules

The MHC was originally identified because of its role in promoting rejection of foreign tissue grafts. However, this is not its true physiological function, which is presentation of a cell's internal peptides for review by cytotoxic T cells. The MHC is a gene complex that encodes several different MHC class I and class 2 molecules. The genes are highly variable between different individuals – no two people have the same set of MHC genes (except identical twins). The antigenic peptides that are bound to MHC class I molecules are 8–10 amino-acid residues in length, and they are bound non-covalently in a cleft on the outer surface of the MHC molecule, as shown in Figure 2.

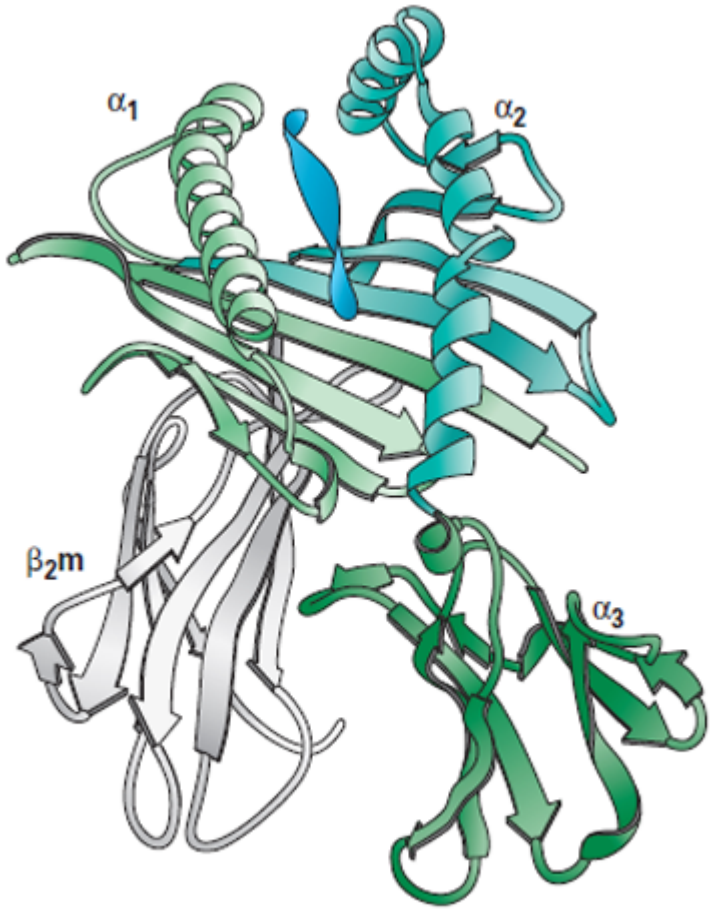


Figure 2 MHC class I

Exactly which peptides can bind to each MHC molecule depends on the amino-acid residues lining the antigen-binding cleft, which is different for each variant MHC molecule. Since everyone has different variants of the MHC molecules, the way that antigenic peptides are presented to T cells is different for each individual. Put simply, everyone's immune system is genetically unique!

In the next section, you will now look at where those antigenic peptides come from.

1.3 Antigen processing and presentation

As previously noted, a cell samples its internal proteins and presents them on MHC class I molecules. The way it does this is called '**antigen processing**' and is illustrated in Figure 3. Cells have an organelle called a proteasome, which breaks down cytosolic proteins into peptides that are transported into the endoplasmic reticulum by a transporter (TAP1/2), where they can associate with MHC class I molecules.

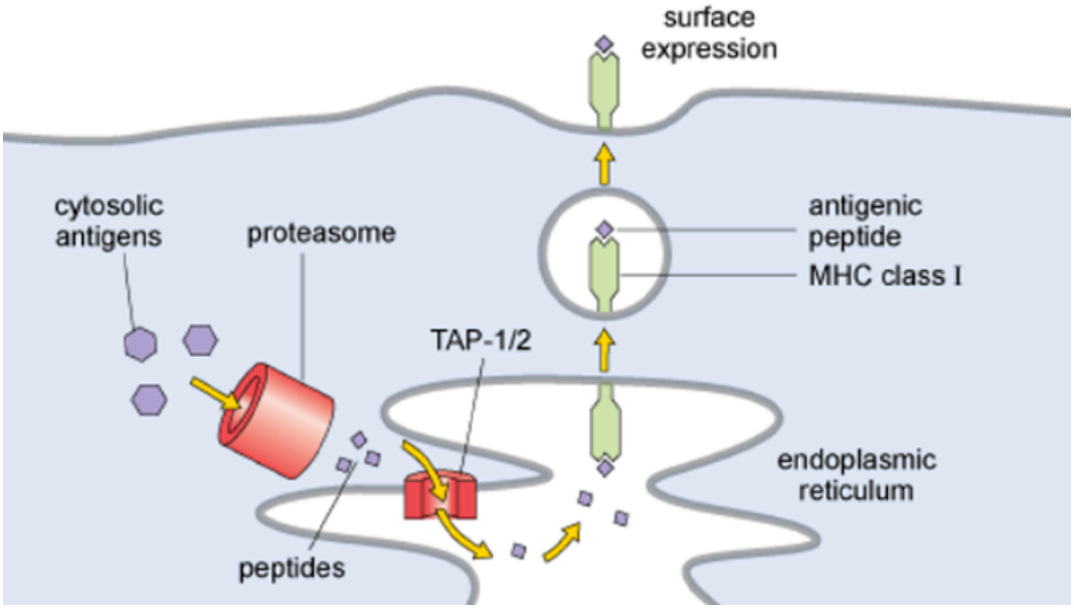


Figure 3 Antigen processing by the proteasome

- What determines whether a peptide will be able to bind to an MHC molecule?
- The amino acid residues lining the antigen-binding cleft on the MHC molecule interact with the amino acid residues in the peptide, so binding depends on both the variant of the MHC molecule and the sequence of the peptide.

The antigenic peptides are trimmed to size by enzymes in the endoplasmic reticulum before the MHC molecule is transported to the plasma membrane in order to present the antigen.

1.4 Cytotoxicity

When a cytotoxic T cell recognises a virus-infected cell, it can signal to it to induce apoptosis. Video 2 below shows a cytotoxic T cell engaging a target cell infected with the influenza virus. As you can see, a cytotoxic T cell (the smaller cell) binds to an influenza-infected target cell. The T cell has granules containing mediators that damage the membrane of the target cell and activate apoptosis. If the T cell recognises the target these granule associated mediators are released into the space between the cells. Apoptosis is seen as membrane blebbing (blobs) and condensation of the nucleus.

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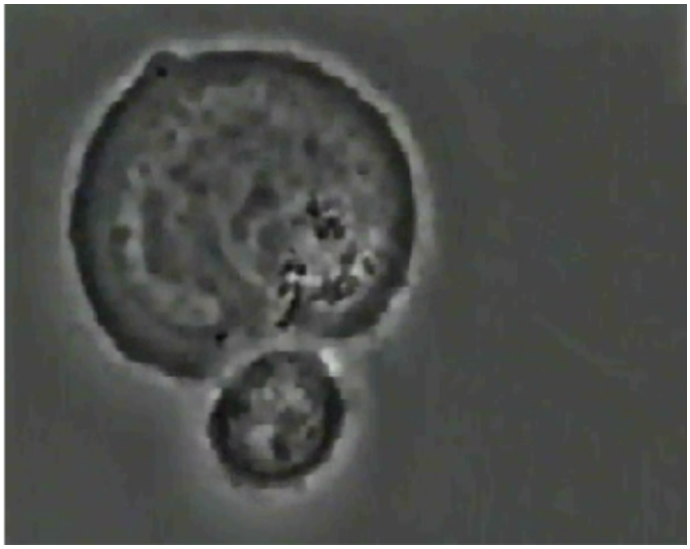
Video 2 Apoptosis



The mechanisms by which the T cell induces apoptosis are beyond the scope of this course, but can be summarised as follows:

1. Release of a molecule, perforin, which punches holes in the plasma membrane of the target cell.
2. Release of granule-associated enzymes, which enter the target cell through the holes created, and activate the cell's endogenous systems, for inducing apoptosis.
3. Release of cytokines that bind to receptors on the target cell, that activate different endogenous pathways to apoptosis.

As you can see, the T cell does not exactly kill the infected target cell, but causes it to die by suicide.

1.5 Natural Killer cells

Many viruses have developed adaptations that help them to evade immune responses. A typical strategy is to prevent expression of MHC class I molecules so that viral antigens are not presented effectively to the cytotoxic T cells. However, the immune system has an alternative system for tackling this strategy – these are the Natural Killer cells, or NK cells.

NK cells are a group of lymphocytes that normally recognise MHC molecules on cells of the body, via 'killer-inhibitory receptors' (KIR). If the NK cell recognises MHC molecules on a cell of the body, it leaves it alone. However, if a cell has lost its MHC molecules, perhaps because it has been infected by a virus trying to escape detection, then the NK cell is no longer inhibited, and it delivers a cytotoxic signal to the target cell, as illustrated in Figure 4. The mechanisms used to induce apoptosis are similar to those deployed by cytotoxic T cells.

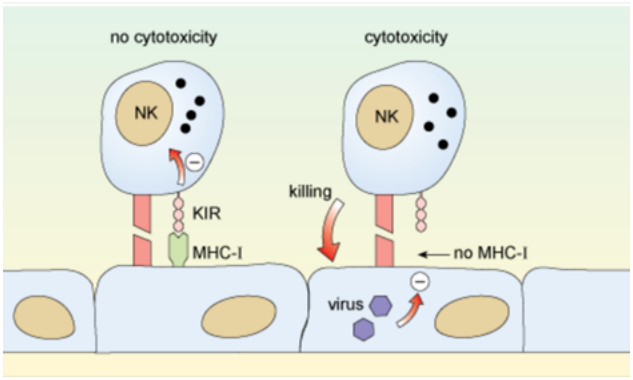


Figure 4 Recognition of infected cells by NK cells

NK cells are normally present and active in the body and are involved in immune defence from the earliest phases of infection. They do not have the high specificity for viruses that the cytotoxic T cells have.

- Why are cytotoxic T cells (CTLs) highly specific in their recognition of virus-infected cells?
- Each CTL has a T cell receptor, which specifically recognises an antigenic peptide presented on a specific MHC class I molecule.

The activity of NK cells improves slightly with repeated encounters with the same antigen, but not in the way that the activity of CTLs does. As such, NK cells are normally considered to be part of the innate immune system.

1.6 B cells and antibodies

B cells recognise antigens through their cell surface receptor for antigen, which is in fact a membrane-bound form of antibody. It is called the B cell receptor (BCR), and like T cells, each individual B cell has just one specificity – meaning that it can recognise a very limited range of antigens. However, taken as a whole, the entire population of B cells can recognise an enormous range of antigens.

- What happens to a B cell if it becomes activated following contact with the antigen it recognises?
- It divides and differentiates into plasma cells, which secrete antibody.

The BCR and the secreted antibody are structurally very similar, and antibodies derived from a single clone of B cells will all have the same antigen-binding specificity. The BCR is a cell surface antigen receptor and is associated with molecules that signal cell activation (Igα, Igβ). The secreted antibody lacks the transmembrane segment that anchors the BCR in the plasma membrane of the B cell. Figure 5 compares the cell surface BCR and secreted form of antibody.

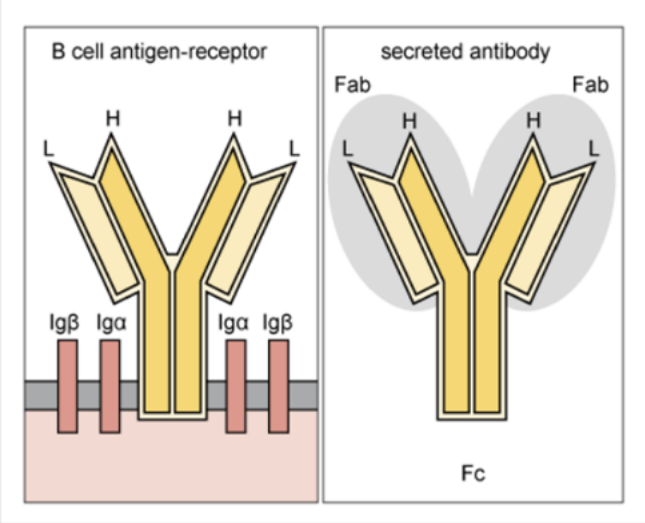


Figure 5 (left) B cell receptor (right) secreted antibody

Secreted antibodies are also called **immunoglobulins** (Ig) and they come in a number of different classes. Three examples are IgG, IgM and IgA, which you will learn about later in the course. But first, let's see how B cells become activated to divide and differentiate into plasma cells.

1.7 T cell help for antibody production

In order for B cells to become active, they normally require help from helper T cells – TH2 cells. Figure 6 shows how B cells interact with TH2 cells by presenting antigen on MHC molecules. Notice, however, that it is slightly different from the antigen presentation shown in Figures 1 and 3; the B cell uses MHC class 2 molecules to present antigenic peptides to the TH2 cell, whereas the cytotoxic T cells are presented with antigenic peptides on MHC class I cells.

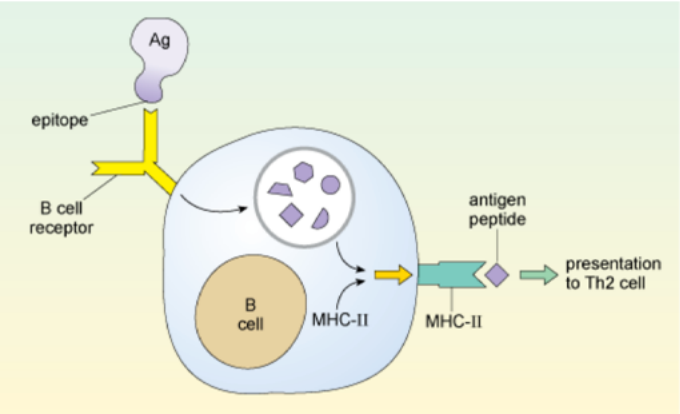


Figure 6 Antigen presentation by B cells

In effect, the antigen is recognised twice: once by the BCR and then by the TCR on the TH2 cell. Both cells must recognise the antigen before the B cell receives an activation signal. The activation signal consists of a combination of direct cell-cell signals and cytokines released by the T cell, which promote B cell division and differentiation. This process is described in Video 3 below.

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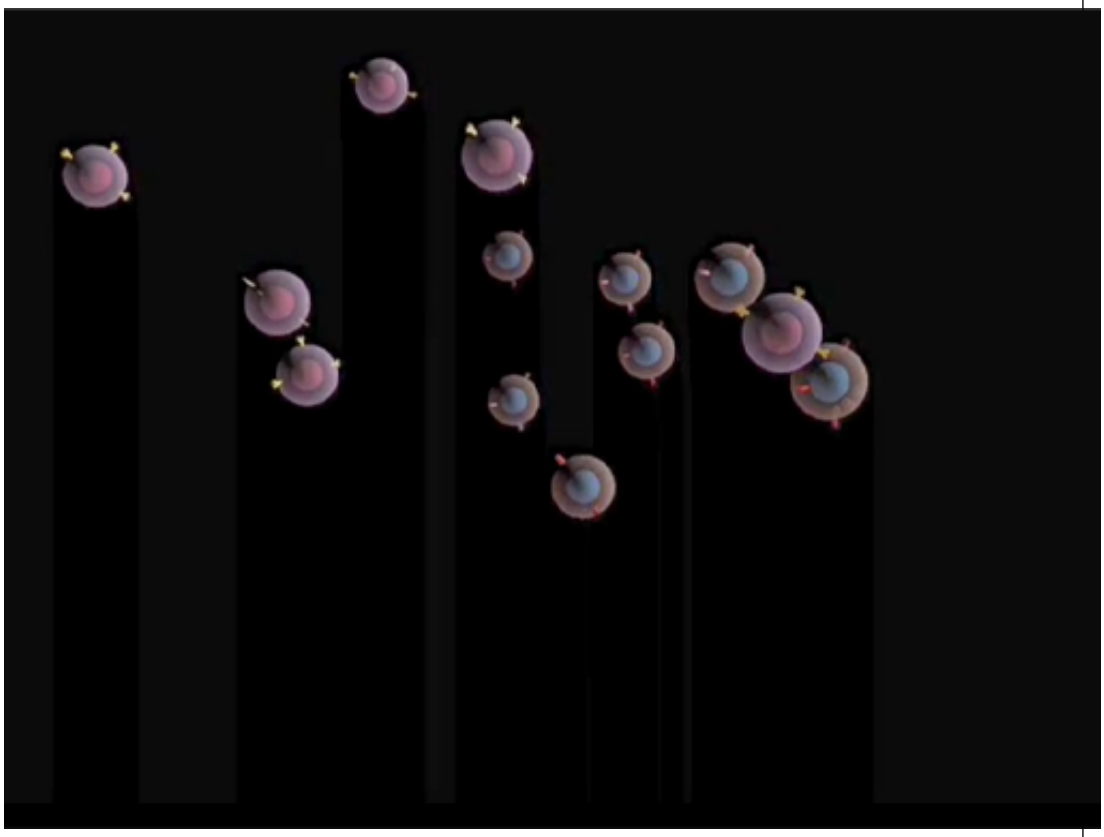
Video 1 Immune defence



2 Immunological memory

An important feature of the adaptive immune response is immunological memory – on subsequent encounters with the same antigen, the immune response is faster and more effective. This effect can be seen in the primary and secondary antibody responses against an antigen, as shown in Figure 7. Note: titre is a measure of how much antibody is present in the serum, and in this case it is shown on a logarithmic scale.

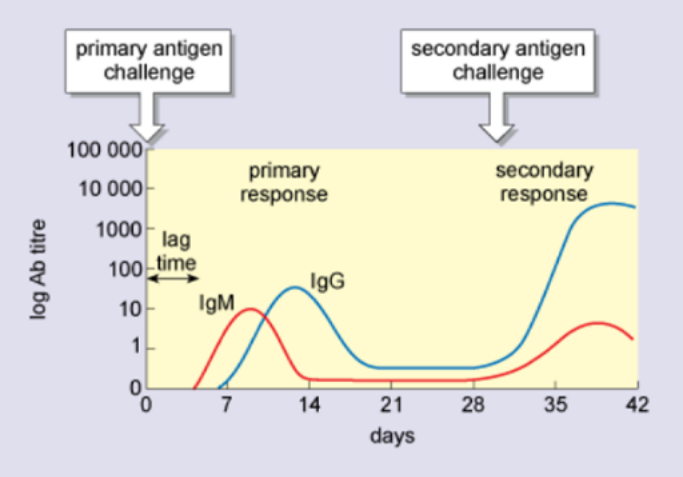


Figure 7 Characteristics of primary and secondary antibody responses

Notice four key differences between the primary and secondary antibody response. On the secondary response:

- The lag time before the appearance of antibodies is shorter.
- The peak titre is much higher.
- IgG antibodies predominate; these antibodies also bind more strongly to the antigen.
- The high levels of IgG antibodies are maintained for longer.

There are three main explanations for the improved secondary immune response:

- The number of lymphocytes that can respond to the antigen increases by clonal division during the primary response.
- Some of the responding lymphocytes have undergone differentiation and maturation steps, which means they can react more swiftly and/or to lower levels of antigen stimulation; these cells act as ‘memory cells’.
- Memory cells are seeded into lymphoid organs and mucosal tissues around the body, so they are on-site to respond as soon as the antigen or pathogen is encountered again.

These findings provide one of the rationales for vaccination. If a person has been vaccinated with a harmless variant of an antigen or pathogen and made a primary immune response against it, they are then able to mount a secondary immune response if they subsequently encounter the real pathogen.

2.1 Phases of the immune response

So far, we have described several different types of immune responses that recognise free virus or virus-infected cells, but how do these responses relate to each other?

Figure 8 shows when these reactions are most important during a response to an acute virus infection.

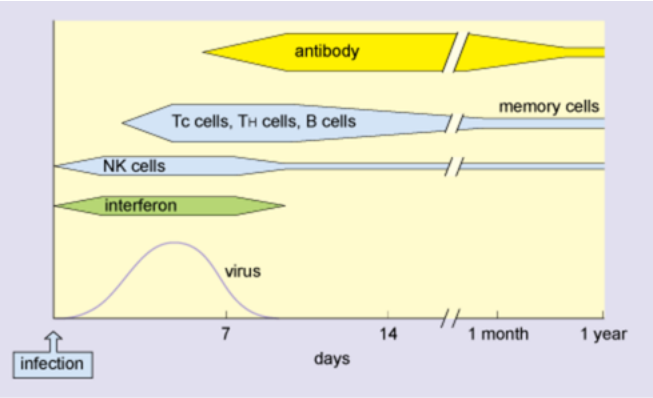


Figure 8 Immune defences against an acute viral infection

Interferon and NK cells are active during the earliest phases of infection. Adaptive immune responses mediated by lymphocytes develop a few days after infection and are involved in clearing infected cells. Antibody is released by plasma cells derived from differentiated B cells and persists for many months, although it gradually declines. Long-term adaptive immunity is primarily provided by the memory cells, which may be either B cells or T cells.

One point you might have missed is that the number of lymphocytes initially available which can recognise a novel infection is relatively small. It takes several days for them to divide repeatedly in order to produce enough lymphocytes of the correct specificity to combat the novel infection. During this early phase of infection, interferons and NK cells are particularly important in slowing virus spread.

2.2 Overview of adaptive immune responses

In this next section, you will watch a video that summarises how viruses infect cells and the variety of immune responses that combat them.

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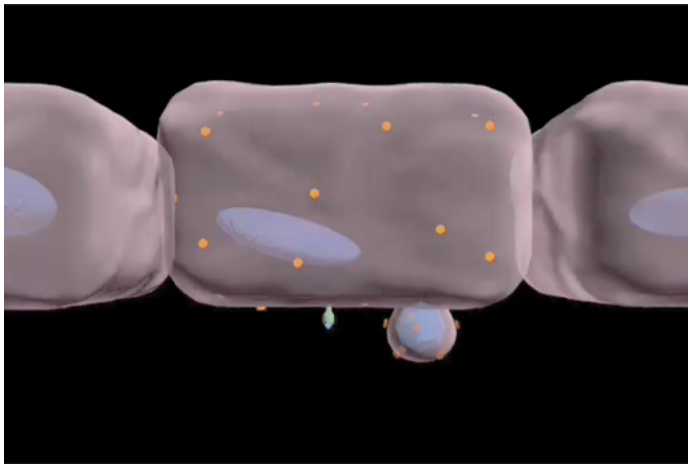
Video 4 Virus infection and immune responses



3 Antibodies

Clearly antibodies are important in protection against virus infection, but how exactly do they work? Before delving into that area, you will take a look at antibody classes because different antibody classes have different functions in immune defence.

- Recall the names of three different antibody classes and identify a distinguishing feature of one of these classes of antibody.
- IgG, IgM and IgA are the three main classes of antibody found in serum – the liquid component of blood. IgM is the first antibody produced in a primary immune response, whereas IgG is the major antibody produced in a secondary immune response (see Figure 7).

An individual B cell initially produces an IgM antibody as its BCR. And if it is activated, it produces secreted IgM. As an immune response develops, and with help from TH2 cells, the B cell may switch to producing an IgG or IgA class of antibody. The antibody retains the same antigen-binding sites at the tips of the Y-shaped molecule, but the stem of the Y (Fc portion) is different for each class. How class-switching is effected at a molecular and cellular level is beyond the scope of this course. The key point is that IgM predominates in a primary response while IgG and IgA antibodies predominate in secondary immune responses.

3.1 Antibody classes

Secreted IgG is produced as a monomer of the basic antibody structure (2 heavy chains, 2 light chains) but IgM is a pentamer and IgA is a dimer, as illustrated in Figure 9 and 10 below.

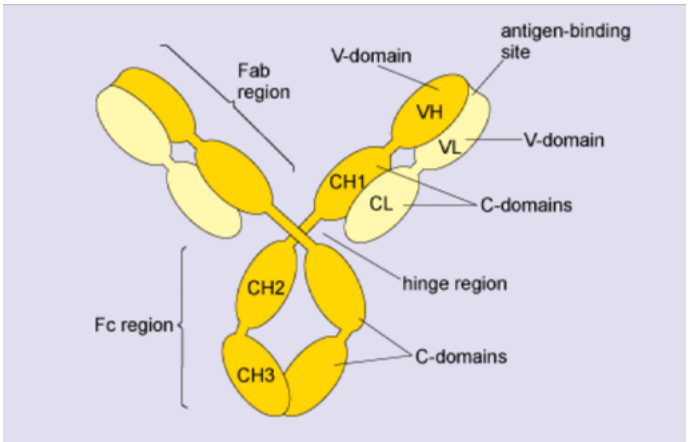


Figure 9 IgG molecule.

In Figure 9, the four polypeptide chains of an IgG molecule are folded into domains. The variable (V) domains of the heavy and light chains (VH, VL) form the antigen-binding sites. The remaining domains are relatively constant (C domains). The hinge confers segmental flexibility.

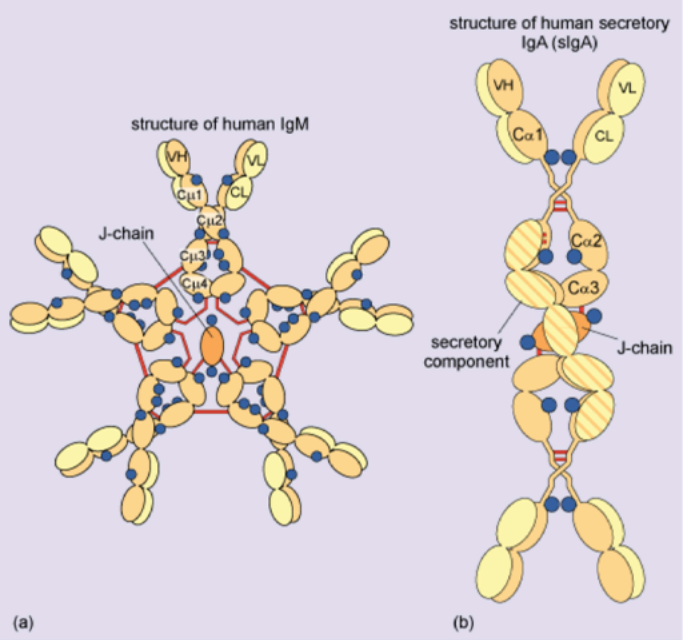


Figure 10 (a) IgM is a pentamer of the basic four-chain immunoglobulin structure whereas (b) IgA is a dimer.

In Figure 10, each molecule is joined by an extra joining (J) chain. When IgA is secreted, it has an additional chain, the secretory component. (Note: carbohydrate units are shown in blue and inter-chain disulphide bonds in red.)

- How many antigen-binding sites does IgM have?
- Ten

The multiple antigen-binding sites of IgM make it very efficient at cross-linking antigens and therefore useful as the first antibody produced in an immune response. However, the affinity of IgM for its target antigens is lower than the affinity of IgG and IgA antibodies produced later in an immune response.

You will now look at how antibodies can protect against virus infection.

3.2 Protection of mucosal surfaces

It was mentioned earlier that IgA antibodies can be produced as secreted molecules. In this case, the antibody is transferred across epithelial cells from the basal (tissue) side to the apical (exterior) side of the epithelium exposed at the mucosal surface. You can see the mechanism outlined in Figure 11.

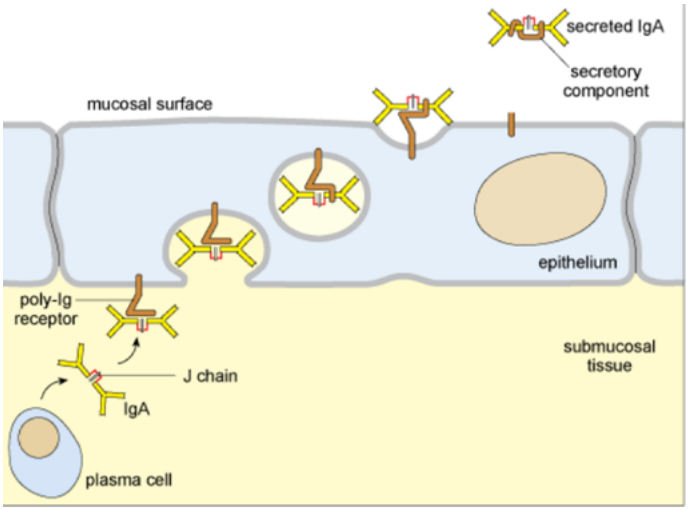


Figure 11 Transport of IgA

Dimeric IgA produced by plasma cells binds to a poly-Ig receptor on the basal surface of an epithelial cell. It is transported across the cell in a vesicle and then released at the mucosal surface as secreted IgA. The secretory component of secreted IgA is derived from the poly-Ig receptor. IgA in mucosal secretions is particularly important in protecting the epithelium against infection with respiratory viruses, such as influenza, rhinoviruses and SARS-CoV2.

3.3 Anti-viral actions of antibodies

Receptor blocking

The simplest way that an antibody can interfere with virus replication is by blocking attachment to the host cell, as shown in Figure 12. Antibodies that do this are called ‘neutralising antibodies’.

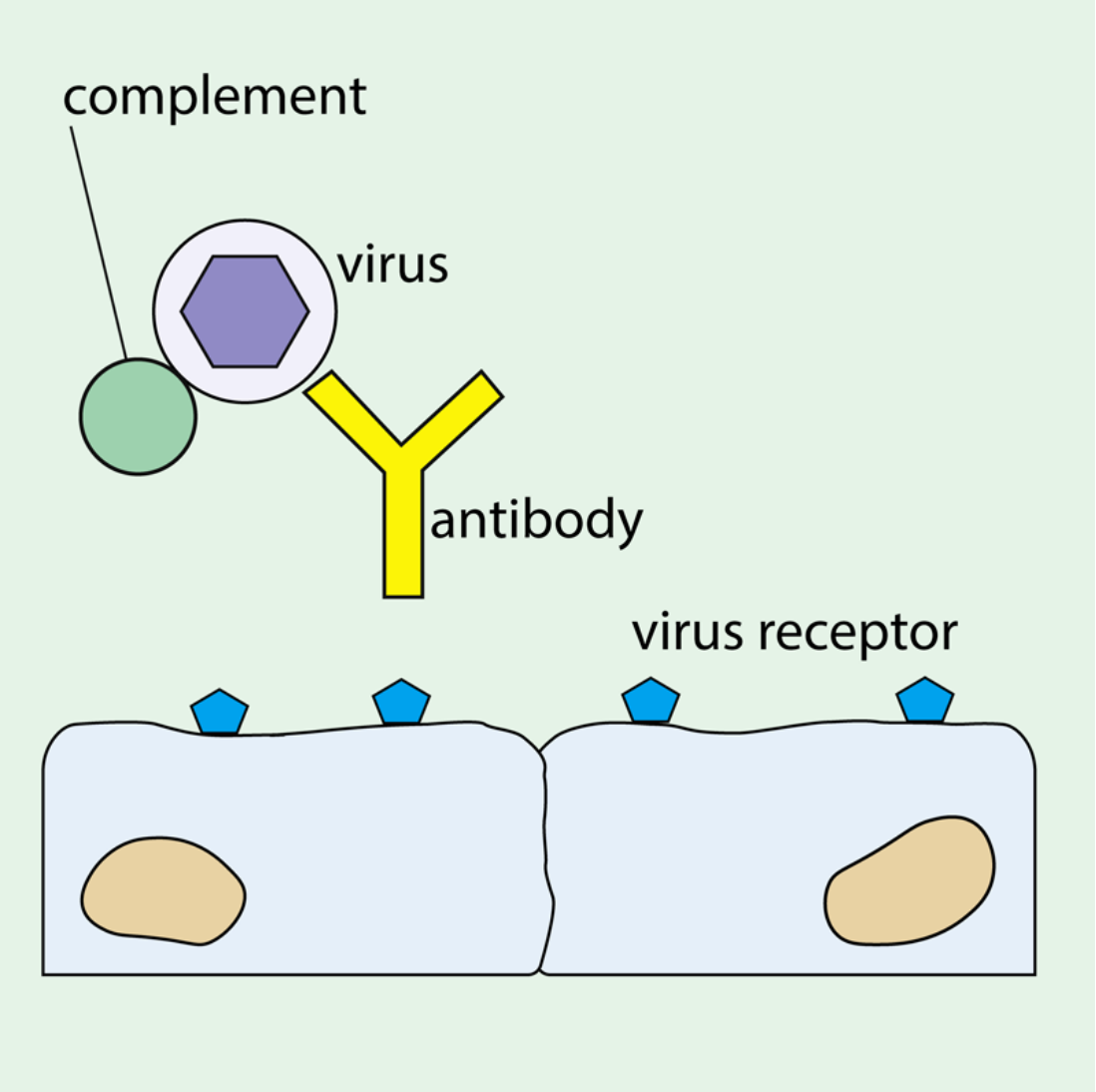


Figure 12 Antibody blocks binding of virus to a host cell and activates complement.

- What type of antibody would you expect to be most effective at blocking entry of SARS-CoV2 into a lung epithelial cell? Why?
- An IgA antibody that binds to the receptor binding domain (RBD) of the spike protein. An IgA antibody will be secreted onto the surfaces of the airways in the lung. An antibody that binds the RBD will be most effective at preventing attachment of the virus to the ACE2 receptor.

Complement activation

IgM and most IgG antibodies can activate the complement system. This is a group of proteins present in blood and tissue fluids that have many functions in controlling inflammation and damaging pathogens. Antibody bound to the virus surface or virus envelope activates the complement system causing deposition of complement molecules on the surface, which you saw earlier in Figure 12 above. This can have a number of anti-viral effects, including:

- It promotes uptake and breakdown of the virus by phagocytic cells, (eg macrophages).
- It directly damages the viral envelope.

If an infected cell has virus molecules inserted in its plasma membrane, then antibodies can bind to these antigens and activate complement. Some complement components can punch holes in the target cell, thus killing it before replicating viruses can be assembled inside.

In addition, complement system molecules can attract leukocytes to sites of infection, and enhance antigen presentation and antibody production.

Promoting phagocytosis and NK cell activity

Phagocytes, such as macrophages are important in destroying pathogens. They internalise them by a different pathway than a virus would normally take. Once inside the phagocyte, pathogens are broken down by a barrage of small toxic molecules and enzymes. Antibodies play an important role in this process by acting as adapters between the virus and the macrophage, as shown in Figure 13.

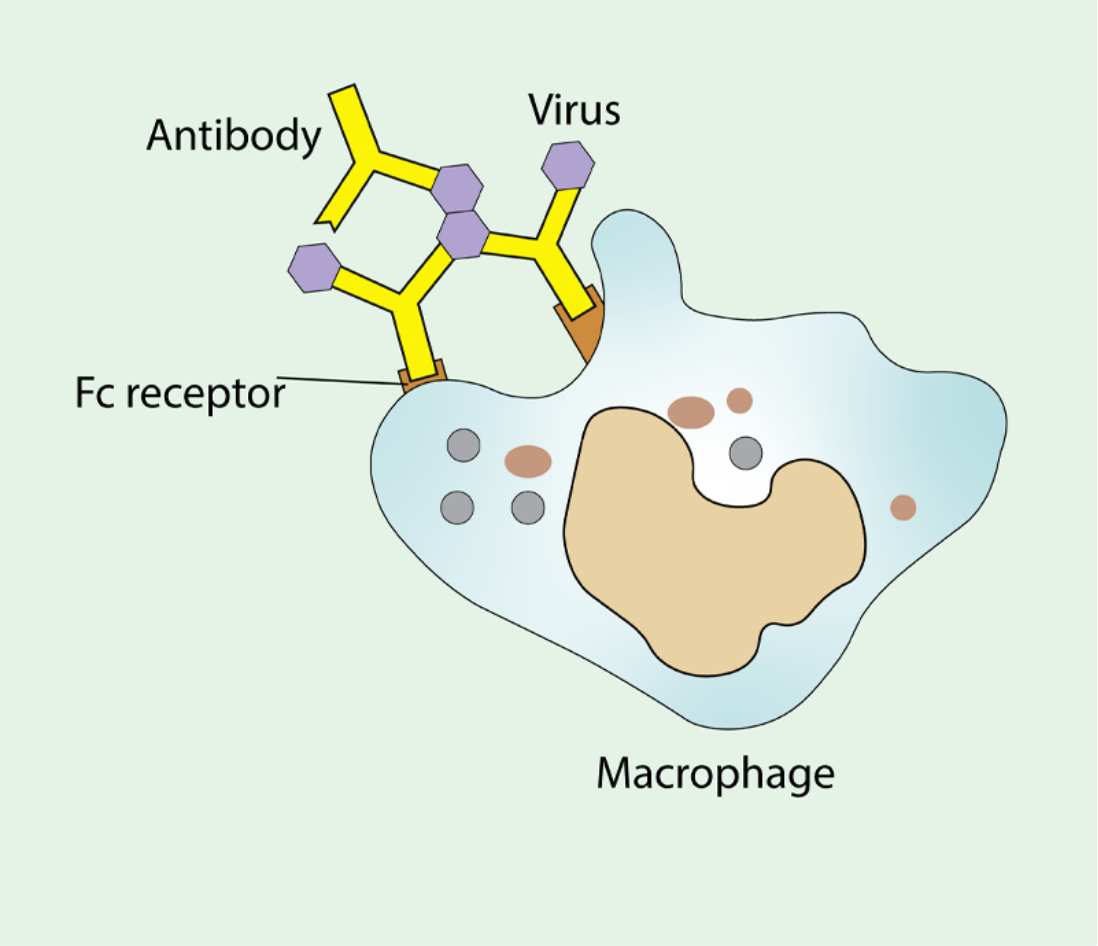


Figure 13 Antibody promotes uptake of virus by a macrophage

Antibody can also promote the activity of NK cells, allowing them to recognise viral antigens that have been inserted into the membrane of an infected cell, which you can see in Figure 14.

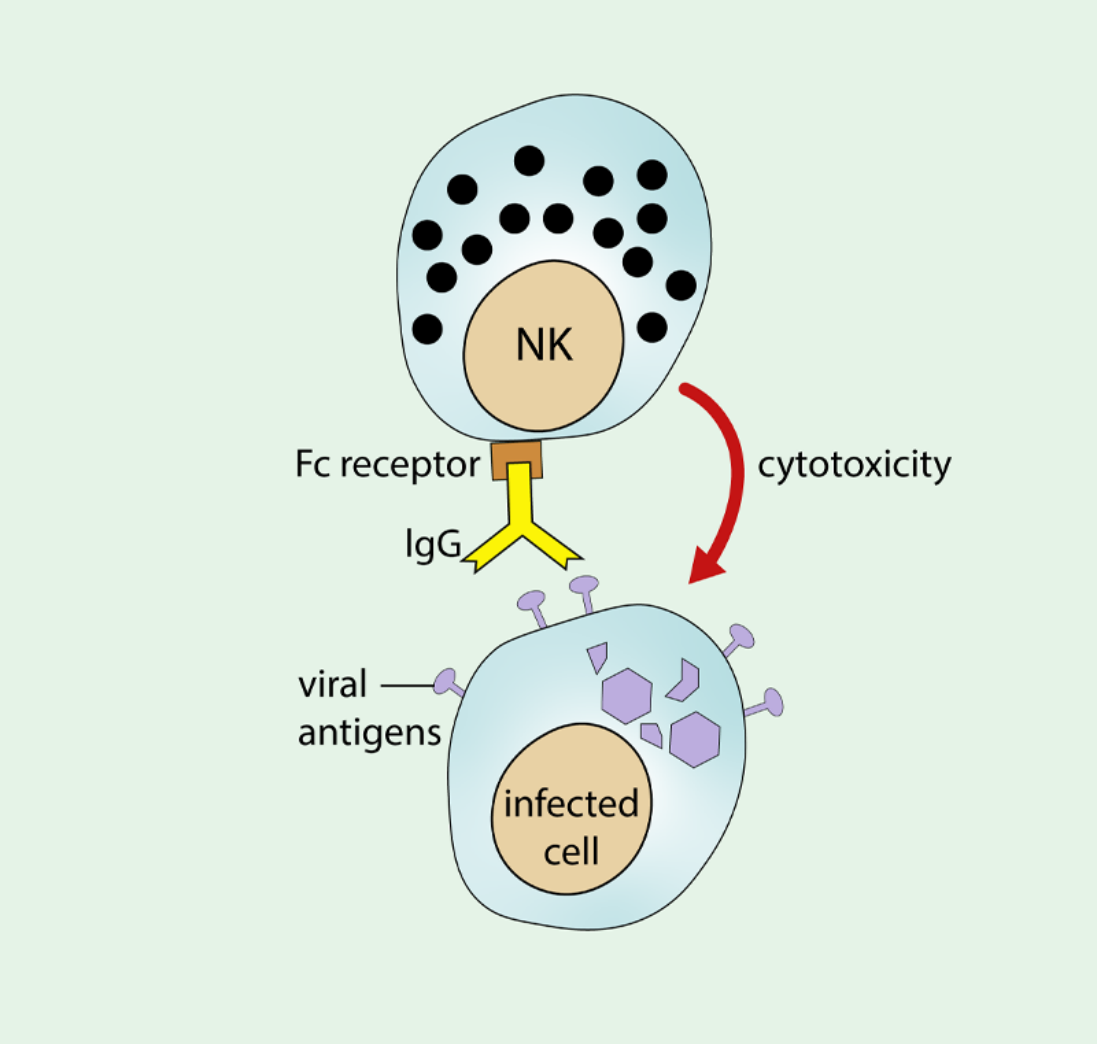


Figure 14 NK cells use IgG as an adapter

Activating intracellular defences

Until recently, it was thought that antibodies could only recognise antigens in extracellular spaces, on the cell surface or in body fluids. It has now become apparent that antibodies can also combat many viruses inside cells. Moreover, this action can be effected by antibodies that are not conventional neutralising antibodies – for example, they may bind to internal proteins of the virus.

Antibodies can enter the cell while bound to a virus, or may be taken up independently by pinocytosis, and they act in a variety of ways. Within an endosome antibodies can inhibit viral uncoating and the fusion mechanism which allows the viral genome to enter the cytosol of the infected cell. Within the cytosol, they can interfere with virus replication or assembly.

In addition, some antiviral functions are mediated by a cytosolic receptor for antibody, TRIM21 (Tripartite Motif 21) which binds the Fc portion of IgG. TRIM21 binds to a virus that has any attached IgG antibody and tags the complex of antibody and viral protein to direct it to the proteasome, where the viral proteins are degraded and can then be presented on MHC class I molecules. Thus, TRIM21 enhances antigen presentation, as shown in Figure 15. This mechanism is most relevant for viruses that enter the cytoplasm intact – i.e., non-enveloped viruses.

It is however possible for TRIM21 to target enveloped viruses that have entered the cytosol from the endosome and lost their envelope and external proteins. If antibodies against the internal viral proteins have independently entered the infected cell, then they can bind to the uncoated virus and engage TRIM21. The virus associated with an antibody in the cytoplasm as an immune complex is recognised by TRIM21 which ubiquitinates the complex. The addition of ubiquitin tags the components of the immune complex for rapid breakdown by the proteasome, so that viral peptides can be presented by MHC class I molecules.

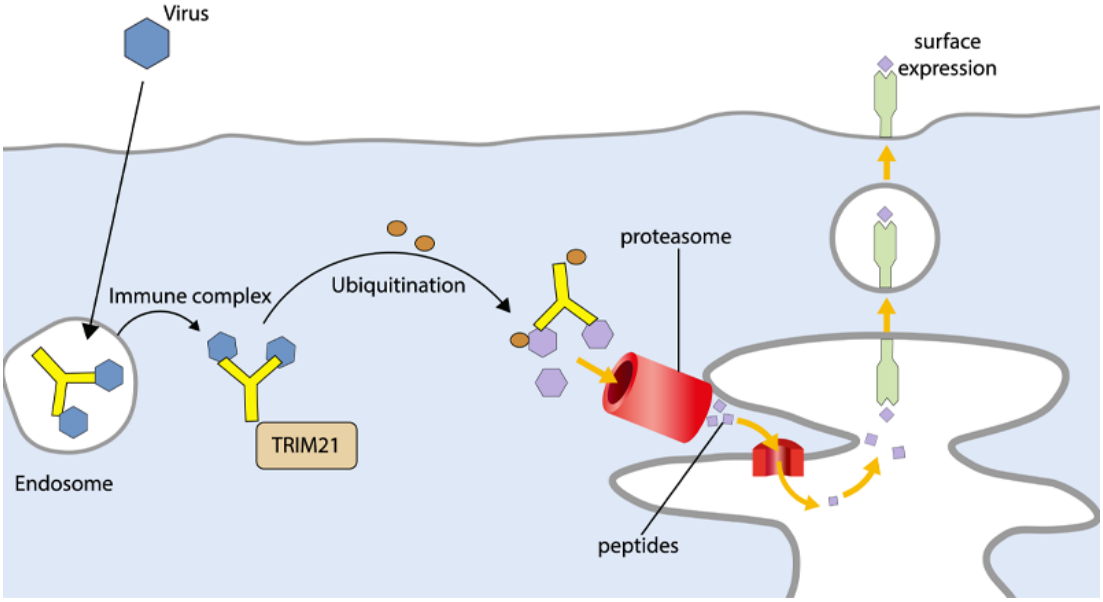


Figure 15 Action of TRIM21

4 Week 2 quiz

Check what you have learned this week by taking the end-of-week quiz.

Week 2 practice quiz

Open the quiz in a new window or tab, then return to this week when you're done.

5 Summary

This week you learnt about the elements of the adaptive immune system that combat a virus infection. Cytotoxic T lymphocytes and NK cells act in a complementary fashion to detect virus-infected cells of the body. If they recognise an infected cell they signal to it to induce apoptosis, - programmed cell death.

B cells can recognise viral antigens and, with help from TH2 cells, they divide and differentiate into plasma cells, which secrete antibody. Individual B cells switch from production of IgM to IgA or IgG. The different antibody classes have different functions. IgM is produced first in an immune response; IgG is the major antibody in the secondary immune response and IgA can be secreted across epithelial cells to protect mucous membranes.

Antibodies protect against virus infection in a number of ways. Neutralising antibodies prevent virus from attaching to target cells. IgG and IgM antibodies can activate complement to damage enveloped virus or virus-infected cells. IgG can promote phagocytosis of virus by macrophages and allows NK cells to recognise infected cells. If they are internalised by an infected cell, IgG antibodies can interfere with virus replication and assembly, as well as promoting presentation of the viral antigens on MHC class 1 molecules, to cytotoxic T cells.

Clearly, antibodies are very important in protection against virus infection. Indeed, the vaccines against COVID-19 were designed to induce high levels of neutralising antibodies against the SARS CoV2 spike protein. But how does one measure antibodies? That is what you will learn about next week, using a technique called ELISA, in a virtual laboratory.

You should now go to Week 3.

Week 3: ELISA – enzyme linked immunosorbent assay

Introduction

In the early days of the COVID-19 pandemic, a fair number of people thought that they had been infected with SARS-CoV2, but they had not had serious symptoms or typical symptoms of infection. But how could they know that it was COVID-19? Perhaps they had just had a regular cold. In the earliest months, qPCR and lateral flow tests for SARS-CoV2 were not widely available. By the time these tests were available, the virus would have long since gone from the body, and these tests would show a negative result. In fact, it was still possible to tell whether a person had come into contact with the virus because they would still have antibodies against it − antibodies last for many months.

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Audio 1 Introduction to Week 3

By the end of this week you should be able to:

- outline the theoretical background and steps used in an ELISA
- carry out an ELISA in a virtual laboratory to detect antibodies to SARS-CoV2 spike protein
- report antibody titres derived from your assay
- interpret your results.

1 Measuring antibodies by ELISA

ELISA is a very versatile assay used for measuring antibodies against pathogens in serum and body fluids. Variants of the assay are used to measure autoantibodies and cytokines. In this course, you will use a standard ELISA to measure antibodies against SARS-CoV2 in serum samples.

ELISAs are usually carried out on 96-well plastic plates which have been 'sensitised' by binding an antigen to the surface of the wells. You can see the basic steps in an **assay** outlined in Figure 1.

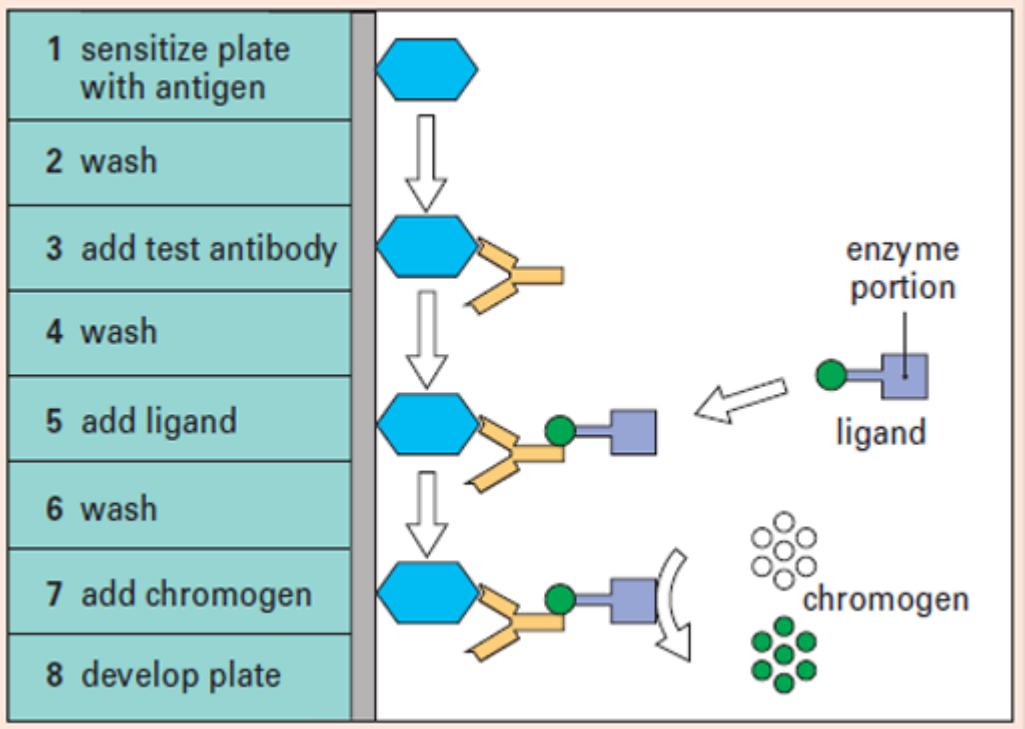

Figure 1 Steps in an ELISA

The antigen bound to the plate determines the specificity of the ELISA test; e.g. SARS-CoV2 spike protein on the plate detects antibodies that are directed to spike protein. The test sera are applied to the plate in serial dilutions as explained in a video guide that you will watch later. A variety of ligands can be used to detect the bound antibodies. The two most often used are a second antibody which binds the first antibody or an antibody-binding protein (protein-G). A variety of enzymes may be coupled to the ligand; the two most often used are horse-radish peroxidase (HPO) or alkaline phosphatase. The chromogen must be matched appropriately to the enzyme.

In these experiments, any antibodies that have bound will be detected with a second antibody coupled to horse-radish peroxidase (HPO), and the chromogen is a substance called tetra-methylbenzidine (TMB), which generates a blue end-product. The enzyme also requires hydrogen peroxide as a substrate. An example of a developed plate is shown in Figure 2. Do not be concerned if you do not understand the results shown in this figure − how to interpret the results will be explained later.

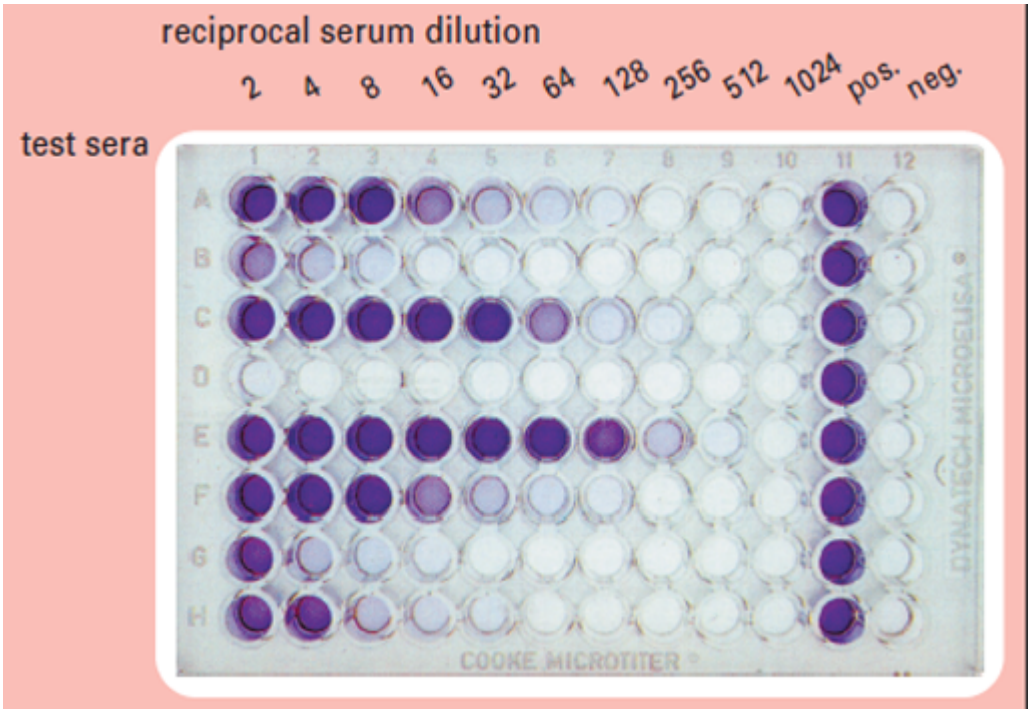

Figure 2 A 96-well ELISA plate developed with chromogen

1.1 The ELISA laboratory

The ELISA laboratory has been designed to replicate the important steps in an ELISA and it allows great flexibility in exactly what you can do. This means that the laboratory can be used to explore technical aspects of the assay, which is beyond the scope of this course. Here, we just want you to accurately measure the levels of antibodies to SARS-CoV2 in sets of serum samples taken in the UK in August 2021. This will allow you to carry out various interesting immunological and epidemiological studies related to the COVID-19 pandemic. To this end, you will use just one standard set of experimental conditions which, if followed accurately, will work well.

As the laboratory recreates the experience of a real laboratory investigation, you should take careful written notes of everything you do, including the samples used, experimental conditions and results obtained. These notes are your ‘laboratory notebook’. Templates for tables will be provided, which you will be able to download, so you may also find it useful to have access to a printer.

This first activity will help you understand how to use the assay.

Activity 1 ELISA: Epidemiology virtual laboratory

Allow 30 minutes

Watch the video guide in Video 1 below, which reprises the theory of ELISA before showing you how to use the ELISA laboratory. Do not worry if you do not get all the details, because you will go through this video again in Activity 2 where you will take notes on the experimental procedures.

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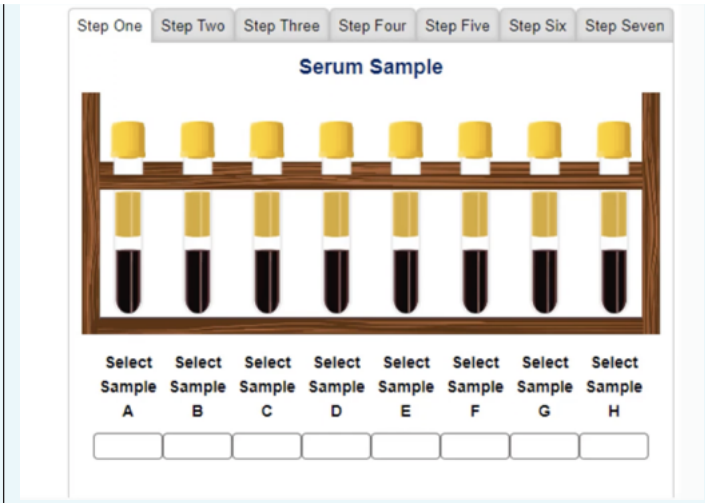
Video 1 ELISA laboratory video guide



1.2 ELISA – experimental conditions

As mentioned previously, you will be using one set of standard conditions in the **ELISA** laboratory to measure IgG antibodies against SARS-CoV2. To do this, you will watch the video guide again, but this time you will **note down the conditions used**. You will use these variable(s)/condition(s) when you use the laboratory later this week.

Now go on to the next activity in which you will identify the assay conditions used in the protocol shown in Video 1 for the ELISA: Epidemiology laboratory.

Activity 2 ELISA: Experimental conditions

Allow 30 minutes

Look at the table below For each action(s) in each step, note down the condition or variable in the blank cells in the table below.

Table 1 ELISA protocol

Step	Action	Variable(s) /condition(s)
1. Serum sample	Select eight samples, one for each row of the plate	
2. Dilution plate	Select transfer volume for serial dilution	*Provide your answer...One answer needed here*
3. Primary antibody incubation	Select ELISA plate Primary incubation time	*Provide your answer...Four answers needed here*

	Number of washes Duration of each wash	
4. Secondary antibody preparation	Select secondary antibody Choose volume of stock reagent	*Provide your answer...Two answers needed here*
5. Second antibody incubation	Secondary antibody incubation time Number of washes Duration of each wash	*Provide your answer...Three answers needed here*
6. Chromogen	Select the chromogen Chromogen incubation time	*Provide your answer...Two answers needed here*
7. ELISA plate reader	Wavelength of filter on plate reader	*Provide your answer...One answer needed here*

When you have filled in the table, click on 'Reveal answer' to check your conditions are correct. These are the conditions you will use in your own assays. Note down these conditions ready for the next set of activities.

Answer

The correct conditions are:

Table 1 ELISA protocol (completed)

Step	Action	Variable(s) /condition(s)
1. Serum sample	Select eight samples, one for each row of the plate	
2. Dilution plate	Select transfer volume for serial dilution	100 µl
3. Primary antibody incubation	Select ELISA plate Primary incubation time Number of washes Duration of each wash	Spike protein 45 minutes 3 5 minutes
4. Secondary antibody preparation	Select secondary antibody Choose volume of stock reagent	Anti-human IgG, HPO conjugate 4 µl

5. Second antibody incubation	Secondary antibody incubation time Number of washes Duration of each wash	45 minutes 3 5 minutes
6. Chromogen	Select the chromogen Chromogen incubation time	TMB 20 minutes
7. ELISA plate reader	Wavelength of filter on plate reader	450 nm

If you had chosen ABTS as the chromogen, then the appropriate filter is 450nm.

- If the samples have very high levels of antibody, what adjustment would you make, so that the results will still be in range of the assay?
- Reduce the volume on the dilution plate transfer – eg 50µl transferred will give a 1:3 dilution series.

- If you use a higher concentration of secondary antibody than the recommended amount (1µg/ml), what effect will that have on the end result?
- The background values will be high.

- If you had used O-phenylene diamine (OPD) as the chromogen, what filter should you use on the plate reader?
- The 645nm filter.

1.3 Measuring antibodies to SARS-CoV2 spike protein

Now it is your turn to try an assay in the ELISA: epidemiology laboratory. To be sure that you are carrying out your assays correctly, you have to directly reproduce the assay that is shown in Video 1.

Activity 3 Antibodies to SARS CoV2 spike protein

Allow 30 minutes

This time open the 'ELISA experiment'.

Then run an assay to detect IgG antibodies against SARS-CoV2 spike protein in the following eight samples, using the experimental conditions you noted down earlier in Activity 2:

- 1-Standard
- 2-Neg.Con
- N9921
- C4443
- C5050
- H1151
- F1949
- Z8207

When you have completed the assay, you should have a plate that looks like Figure 3. (If you have arranged the samples in a different order on the plate, then the appearance will be different, but the results should be the same.) The results from the plate reader will be similar to Figure 4. However, note that the titres will depend on the dilution series chosen, and the identification of positive titres, (highlighted here) depend on the exact experimental conditions and the cut-off value selected.

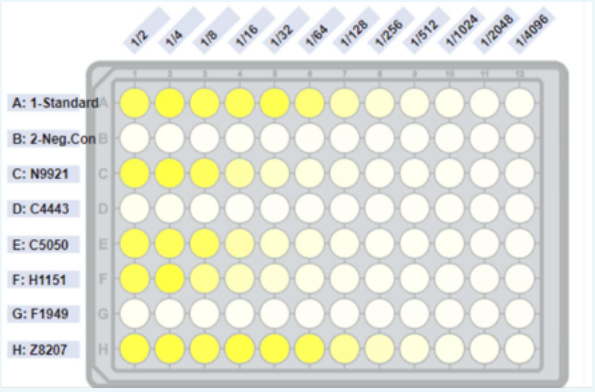


Figure 3 Developed ELISA plate

450nm

Sample	1	2	3	4	5	6	7	8	9	10	11	12
A: 1-Standard	1.305	1.454	1.277	1.422	1.263	1.066	0.607	0.373	0.202	0.152	0.117	0.104
B: 2-Neg.Con	0.106	0.092	0.085	0.084	0.087	0.080	0.081	0.083	0.086	0.087	0.084	0.084
C: N9921	1.331	1.262	1.452	0.713	0.413	0.252	0.157	0.127	0.104	0.094	0.088	0.084
D: C4443	0.141	0.110	0.102	0.095	0.084	0.086	0.083	0.084	0.080	0.083	0.084	0.087
E: C5050	1.415	1.327	1.173	0.638	0.373	0.239	0.158	0.117	0.101	0.096	0.088	0.085
F: H1151	1.328	1.280	0.966	0.519	0.308	0.197	0.136	0.112	0.094	0.085	0.089	0.087
G: F1949	0.112	0.097	0.094	0.090	0.087	0.088	0.087	0.087	0.085	0.080	0.086	0.084
H: Z8207	1.314	1.454	1.334	1.418	1.458	1.427	0.921	0.485	0.272	0.188	0.125	0.103
	2	4	8	16	32	64	128	256	512	1024	2048	4096

Figure 4 ELISA Plate reader results with positive samples highlighted and titres shown beneath. (Note that the titres will depend on the dilution series chosen, and the identification of positive titres, (highlighted here) depend on the exact experimental conditions and the cut-off value selected.)

2 Interpreting and reporting results

The results from this type of ELISA are usually reported as the reciprocal of the highest dilution that shows a positive result. For example, if the highest dilution giving a positive result is 1:64, then the titre would be reported as 64. This has the advantage that a larger number indicates a greater amount of antibody in the serum.

It should be emphasized that the type of ELISA that has been taught here is not highly accurate. If your results are out by one well, then you would report a value that is different by a factor of two from the stated value. The advantage of this assay is not in its accuracy, but in its speed and simplicity. It gives a rapid estimation of whether antibodies to the test antigen are present in significant amounts. As such it is very good for screening large numbers of samples in epidemiological studies.

The results of the ELISA you carried out, show that four individuals (N9921, C5050, H1151, Z8207) had significant levels of antibodies to spike protein. (The low titre in C4443 is more likely due to a high background value in the assay rather than evidence of contact with SARS-CoV2 spike protein.)

Finally, it is important to emphasize that the four positive samples do not necessarily imply that those four individuals have been infected with SARS-CoV2. Vaccination against COVID-19 also induces antibodies to spike protein, so we can say that these individuals have been vaccinated or infected, or possibly both.

3 Week 3 quiz

Check what you have learned this week by taking the end-of-week quiz.

Week 3 practice quiz.

Open the quiz in a new window or tab, then return to this week when you're done.

4 Summary

This week you have learnt the ELISA technique for detecting antibodies, and shown how to use it to quantitate antibodies to SARS-CoV2 spike protein. You then had the opportunity to practice this assay in a virtual laboratory, and a simple method for recording antibody titres and interpreting the results was used. You may also have learnt something about technical aspects of the ELISA, and some basic laboratory science, such as making dilutions.

Next week, you will use the technical knowledge gained here to carry out an epidemiological study and an immunological study using serum samples in the ELISA: epidemiology virtual laboratory.

You should now go to Week 4.

Week 4: Screening for SARS-CoV2 antibodies

Introduction

Knowing the proportion of people in a population that are immune to an infectious disease is really important – but why? One reason is that it can tell you something about how quickly an infection will spread or whether it will die out. Once a sufficient proportion of the population is immune to an infectious agent, then it will die out, or at least that strain of the pathogen will disappear, because there are insufficient susceptible individuals to maintain a chain of disease transmission.

You are going to look into the relationship between transmission rates, herd immunity and vaccination rates later in the course. But before then you are going to investigate the incidence of antibodies to SARS-CoV2 in the sample set provided in the ELISA: Epidemiology laboratory. This will provide the basis for later investigations.

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Audio 1 Introduction to Week 4

By the end of this week, you should be able to:

- outline the course of the COVID-19 epidemic and vaccination programme in the UK
- organise and carry out a laboratory-based investigation for antibodies against SARS-CoV2
- record your data and carry out some simple data analysis.

1 Background to the investigation

In Week 3, you were introduced to the sample set provided by the ELISA: epidemiology laboratory. The set of 60 serum samples were chosen to allow investigation of the level of immunity to SARS-CoV2 in the UK population in August 2021.

This time point was chosen because it is particularly interesting and instructive. At this time a majority of the UK adult population had received at least one dose of the COVID-19 vaccine and a significant minority had been infected with SARS-CoV2. Both of these groups would be expected to have antibodies to the spike protein.

The aim of the investigation is to determine what proportion of the population has antibodies to the spike protein. But first you will learn a little more about the COVID-19 epidemic in the UK to place your investigation in to context.

1.1 The COVID-19 epidemic in the UK

By August 2021, the original strain of SARS-CoV2 first identified in Wuhan, China had been replaced in circulation by a number of 'variants of concern' (VOC), designated by the World Health Organisation (WHO) as variants with one or more of the following characteristics:

- increased transmissibility
- more severe disease
- reduced effectiveness of treatments or vaccines
- failure to be detected by current diagnostic tests.

The new variants were designated by Greek letters (Figure 1), and as of August 2021, the delta variant accounted for nearly 99% of the cases in the UK.

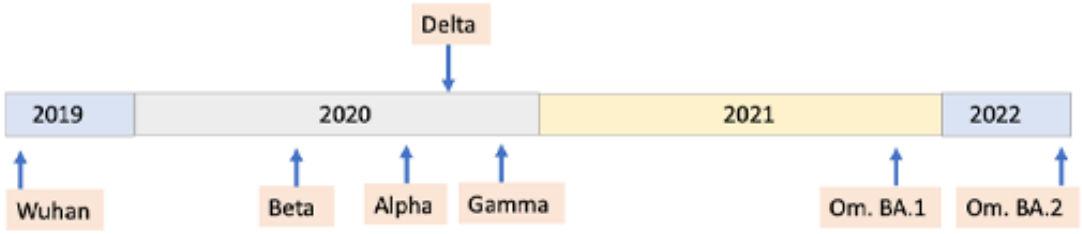

Figure 1 SARS-CoV2 VOCs timeline

At this time point the incidence of infection, which had been low during the spring and early summer was starting to rise in the community, as demonstrated in Figure 2. If you want to learn more about the course of the pandemic in the UK, all data during this period is available from Public Health England, and since December 2021, from the Health Security Agency.

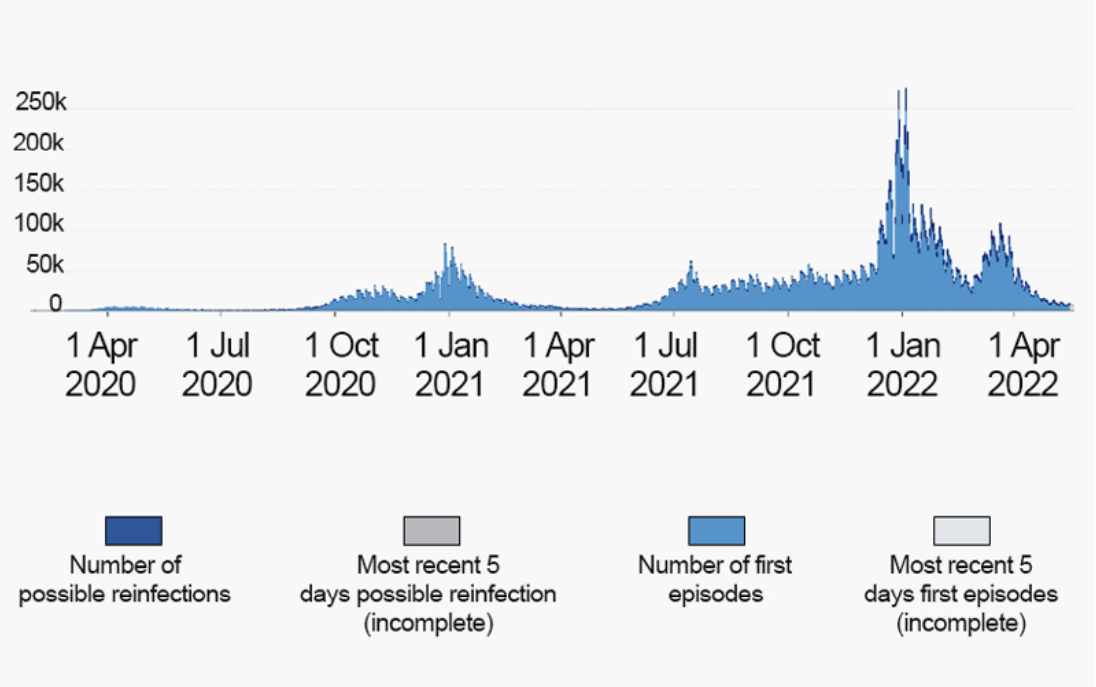

Figure 2 Daily confirmed COVID-19 cases in the UK. Cases by episode of infection and specimen date.

1.2 COVID-19 vaccination in the UK

You will be covering vaccines later in the course, but at this stage it will be helpful for you to see what was happening with the vaccination programme in the UK at the time of the investigation.

A vaccination programme started in the UK in January 2021 with two doses of vaccine, typically spaced at an interval of 2−3 months. The programme started with medically vulnerable individuals and older people and then worked progressively down through the age groups to younger people.

By the Summer of 2021, all of the adult population over the age of 18 had been offered vaccination. Similar programmes were taking place in many European countries during the early months of 2021, although the UK then delayed immunisation of younger age groups (<18 years) until later in the year. This profile of vaccination can be seen in the heat map (Figure 3).

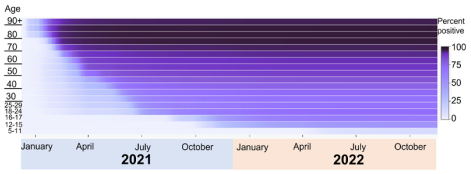

Figure 3 COVID-19 vaccination heat map.

- Describe the incidence of vaccination in the UK population in August 2021, in relation to different age groups.
- Nearly all of the older population (age >70) had been vaccinated. The proportion falls successively with different age groups to ~50% in adults aged 18−-29. School age children and young adults (<18) had low levels of vaccination (<20%).

1.3 Screening programmes

During the COVID-19 pandemic, two main tests were used to detect the presence of the virus:

- PCR test, which detects segments of the virus genome
- lateral flow test, which detects the presence of virus antigens.

Both of these tests can detect the virus during a window of time when a person has recently been infected and is potentially infectious – typically this window lasts for about two weeks. In the UK the PCR tests became available early in 2020 and by May 2020 approximately 100,000 tests were being carried out daily. Lateral flow tests became available in late 2021.

Antibody testing can also be carried out in different ways, including:

- ELISA
- lateral flow tests, configured to detect anti-viral antibodies.

Antibodies can be detected from 1−2 weeks after infection, depending on the sensitivity of the test. This means that there is only a very short period of time when tests for virus and tests for antibodies will both give a positive result.

An important difference between the tests is that the lateral flow tests could be carried out at home, and they give a positive/negative result; the PCR test (virus) and ELISA (antibodies) are carried out by trained staff, usually in a laboratory, and they provide quantitative results.

It is important to note that testing for virus or anti-viral antibodies is carried out on different groups of people. The most reliable type of screening for assessing the prevalence of disease (virus) or immunity (antibodies) is carried out by random testing in the community.

For **notifiable diseases** such as measles, data from general practice and hospitals may give a reliable measure of disease incidence, because virtually all affected individuals have symptoms and will be identified. COVID-19 was a notifiable disease. However there was initially a huge amount of uncertainty as to what proportion of individuals were asymptomatic, and therefore might not have come forward for testing.

The advantage of random screening in the community is that it will identify people who are infected but who have no symptoms and do not know they are infected (virus +), or who had been infected in the past (antibody +).

The samples available in the ELISA: epidemiology laboratory represent a random screen from across the UK taken in August 2021. For the remainder of this week, you will be analysing these samples for the incidence of antibodies to the SARS-CoV2 spike protein, which are referred to as **S-antibodies**.

2 Prevalence of antibodies to SARS-CoV2

In this section you will use the virtual ELISA: epidemiology laboratory to measure the prevalence and titre of antibodies to SARS-CoV2 spike protein. The laboratory includes serum samples from 60 individuals as well as the standard and negative control samples.

You will also use the data you obtain to carry out some simple immunological investigations. For this purpose you are given some additional information on the age and sex of the individuals in Table 1, which can be downloaded here.

2.1 Laboratory investigation

During this course, you will be carrying out a number of investigations on the samples in the ELISA: epidemiology laboratory, so you need to record your results as you proceed. You may wish to download and print a version of Table 2 to enter results or you can note your data in the blank boxes provided below. If you prefer you can also recreate the table in your notebook.

In Table 2 below, there is space for 30 entries and the six results from the first ELISA (Week 3) have already been entered. You will analyse **at least 24** more samples.

Table 2 Data entry table for the investigation of antibodies against SARS-CoV2

Subject	Age	Sex	IgG S-antibody	IgG N-anti
N9921	32	F	256	Provide y
C4443	30	F	2	Provide y
C5050	71	M	128	Provide y
H1151	26	M	128	Provide y
F1949	32	M	<2	Provide y
Z8207	58	M	2048	Provide y
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Activity 1 Selection of serum samples

Allow 20 minutes

Begin by selecting 24 individuals from the list given in Table 1. You should choose subjects with a range of different ages – for example, at least ten subjects older than 50 years and at least ten subjects aged less than or equal to 50 years.

You should also aim to select approximately equal numbers of female and male subjects.

Now enter the identifier of the subjects chosen into column 1 of the data entry table and their age and sex into columns 2 and 3 respectively.

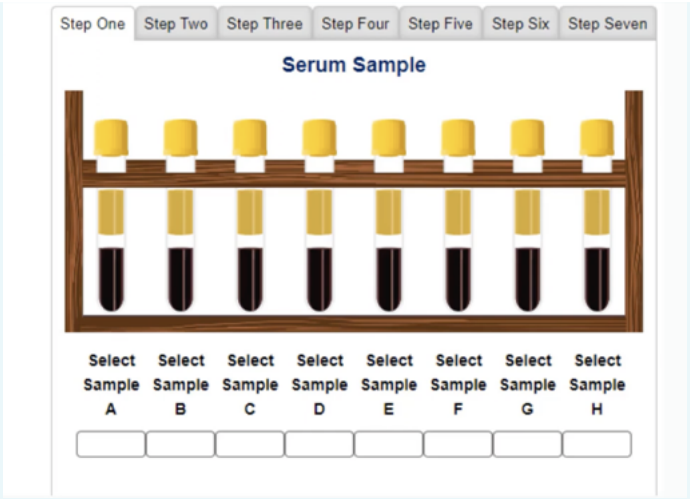
Activity 2 Measurement of IgG S-antibodies by ELISA

Allow 60 minutes

Now that you have selected your samples, go to the ELISA: epidemiology laboratory and carry out assays to determine the titre of **IgG** antibodies against **spike protein** in each of your chosen samples. To do this you should use the assay protocol which you used and noted down in Week 3, Activity 2.

If you are unsure how to carry out the investigation, you may wish to rewatch the relevant parts of the video guide provided again below.

Video content is not available in this format.
Video 1 ELISA laboratory video guide



Each ELISA plate should have a standard control (positive control), a negative control, and space for six serum samples. Therefore, in order to have 24 samples, you will need to run four plates. If you have enough time, run some additional samples to give you more data to work with.

Now enter the titre of each of the selected serum samples in column 4 of your data entry table.

In the final section this week, you will start to analyse and interpret your data.

3 Data interpretation

Screening surveys and epidemiological studies usually require thousands of data points. For example, the first study on home-screening of antibodies to SARS-CoV2 done in the UK using lateral flow tests included 10,000 subjects. Such large studies give robust data but are clearly beyond the scope of this course. Nevertheless, the aim is to give you a flavour of how such studies can be analysed using the data you have obtained in the ELISA laboratory.

The aim of the next activity is to estimate the percentage of individuals who have IgG S-antibodies, from the samples in your data-set.

Activity 3 Prevalence of antibodies to SARS-CoV2

Allow 10 minutes

Take a cut-off point as a titre of 4 (dilution 1:4) and count the number of samples with a titre >4. Then estimate the percentage of individuals who are positive:

$$\text{Percentage positive} = \frac{\text{number with titre} > 4}{\text{total number of samples}} \times 100$$

You may recall that at the time the samples were taken (August 2021) 60-70% of the adult population in the UK had been vaccinated against COVID-19 (Figure 3) and a much smaller percentage had had a natural infection. Individuals with antibodies have a level of immunity against virus infection and considerable protection against severe disease.

From your results, what percentage of individuals had some immunity to COVID-19 during this time?.

Answer

Due to the way in which the vaccination programme was rolled out (older people first), you might also expect to see that a higher proportion of older people would have antibodies than younger people. However, you probably do not have enough samples to confirm whether this is true or not.

Activity 4 Titres of antibodies to SARS-CoV2

Allow 20 minutes

Part 1

Using your data for Table 2, you can now estimate how the titre of antibodies varies across different groups within the population. Statistical analysis is beyond the scope of this course and would require larger data-sets. Nevertheless, you can carry out some simple comparisons. To do this first identify those individuals who are positive for IgG S-antibodies (titre >4). Then carry out two comparisons. In each case compare the median titre in one group with the median titre in the other.

The median is the individual who has the mid-point titre. For example, if the titres of a group of 11 individuals were: 8, 16, 64, 128, 128, **256**, 512, 1024, 1024, 2048, 4096, then the median titre is 256, because this is the person in the mid-point of this group.

Part 2

Now compare the median in female and male individuals. Are they different? Generally titres in males and females are quite similar. It would be surprising if the results in your samples were greatly different, but it might be so if your samples do not represent the overall population well. The larger the number of samples you have taken, the more likely they will accurately reflect the S-antibody titres in the whole population.

Part 3

Next compare the median in older people (age >50) with those in younger people (age ≤50). Are they different? We expect that the titre in the older age group would be lower than the titre in the younger age group, but the interpretation of this is not quite so straightforward.

Can you think of two possible explanations of why the S-antibody titres could be lower in older people?

Answer

It may be that older people produce less antibodies. Alternatively, older people were mostly vaccinated early in 2021, and by this time their antibody titres will be declining. In comparison younger people were mostly vaccinated in Spring and early Summer, and antibody titres are usually highest 2−4 weeks after the second injection. Of course, it is possible that both explanations are true, but one cannot disentangle the two explanations from the data available so far.

This investigation has shown you how data on antibodies can be used for monitoring immunity in the population, but results have to be interpreted with caution. Next week you will start to look at how this type of data informs vaccination programmes.

4 Week 4 quiz

Now it’s time to complete the Week 4 badge quiz. It is similar to previous quizzes, but this time instead of answering five questions there will be fifteen.

Week 4 compulsory badge quiz

Remember, this quiz counts towards your badge. If you’re not successful the first time, you can attempt the quiz again in 24 hours.

Open the quiz in a new tab or window then come back here when you’ve finished.

5 Summary

This week you have been given an overview of the COVID-19 epidemic in the UK and the course of the vaccination programme during 2021. The programme prioritised vulnerable and older people, but by the end of the summer all adults had been offered vaccination.

You then carried out an investigation to measure the prevalence and titres of IgG antibodies against SARS-CoV2 spike protein (S-antibodies) in a group of individuals selected from August 2021. Using your own data, you were able to estimate the percentage of the adult population who had some immunity to the virus at this time. You also carried out comparisons of the antibody titres in females vs males and older vs younger individuals.

This type of data can be used to track epidemics and inform vaccination programmes. Next week, you will learn exactly how infection rates, herd immunity and vaccine effectiveness are related.

You are now halfway through the course. The Open University would really appreciate your feedback and suggestions for future improvement in our optional end-of-course survey, which you will also have an opportunity to complete at the end of Week 8. Participation will be completely confidential and we will not pass on your details to others.

Now go to Week 5.

Week 5: Tracking infection

Introduction

How can one use antibodies to track infection? This week we start to look into the field of serology, the use of antibodies to identify infectious agents or diagnose disease. You will also start to learn some epidemiology, the study of disease in populations and how they spread.

Audio content is not available in this format.

Audio 1 Introduction to Week 5

By the end of this week, you should be able to:

- understand the difference between disease incidence and prevalence
- calculate disease incidence from data provided
- outline how serology can inform epidemiological studies
- identify SARS-CoV2 infected individuals in the virtual laboratory by detection of N-antibodies and estimate the cumulative level of infection in the population.

1 Aspects of epidemiology

Epidemiology is the study of diseases in populations and how they are transmitted. It is a broad and complex field of study, since each disease is unique in its incidence and the way it is transmitted. Because it deals with populations, epidemiology often deals with large data sets which require sophisticated statistical analysis. Furthermore, the findings of epidemiological studies must be considered in relation to environmental, geographic, demographic, cultural and genetic factors, all of which affect the incidence of disease and its transmission.

In this course, the focus is just on the COVID-19 pandemic, and therefore can be very selective about which aspects of epidemiology are covered. The statistical analyses used in epidemiology are beyond the scope of the course. However, it is necessary to introduce you to some basic terminology.

1.1 Incidence and prevalence

The **incidence** of an infectious disease is the rate at which new infections occur within a defined period of time. For a non-infectious disease the rate would be given as the number of newly diagnosed cases in a period of time. For many epidemiological studies the incidence is given as the number of new cases per year, per 100,000 population. For example, the incidence of lung cancer in the UK between 2016−2018 is given as 70 cases per 100,000 people per year. The incidence of this condition is highly dependent on the age of population group studied. For this reason, incidence rates are often ‘age standardised’ so that comparisons can be made between countries with different demographic profiles.

- Apart from age, what other factors might influence the incidence of lung cancer?
- You may have thought of gender, ethnic group, geographic location and social factors such as smoking. Many of these factors can be interrelated.

As you can see, it is important to define exactly what population is being studied and when. If it is not stated, it often means the annual incidence in the entire population of a country or region.

An alternative measure of the occurrence of disease is **prevalence**. This gives the total proportion of the population that are affected at any one point in time. It can be expressed as the number of affected individuals per 100,000 of the population. For more common diseases it may just be given as the percentage of the population affected at any one time point. Chronic conditions (e.g. diabetes) may last for many years, in which case the prevalence at any one time will be greater than the annual incidence.

For acute infectious diseases such as COVID-19, which usually last for 7−10 days, the weekly incidence of the disease is a more useful measure, since it can show how the epidemic is changing from one week to the next. However, if you look again at the incidence of SARS-CoV2 cases in the UK (Figure 1) you will see that the figures actually show the daily incidence. It is therefore important to look at the period of time for which the incidence rate is quoted. For example, if the daily incidence of infection over a period of time is 10,000 cases, then the weekly incidence will be 10,000 x7 = 70,000.

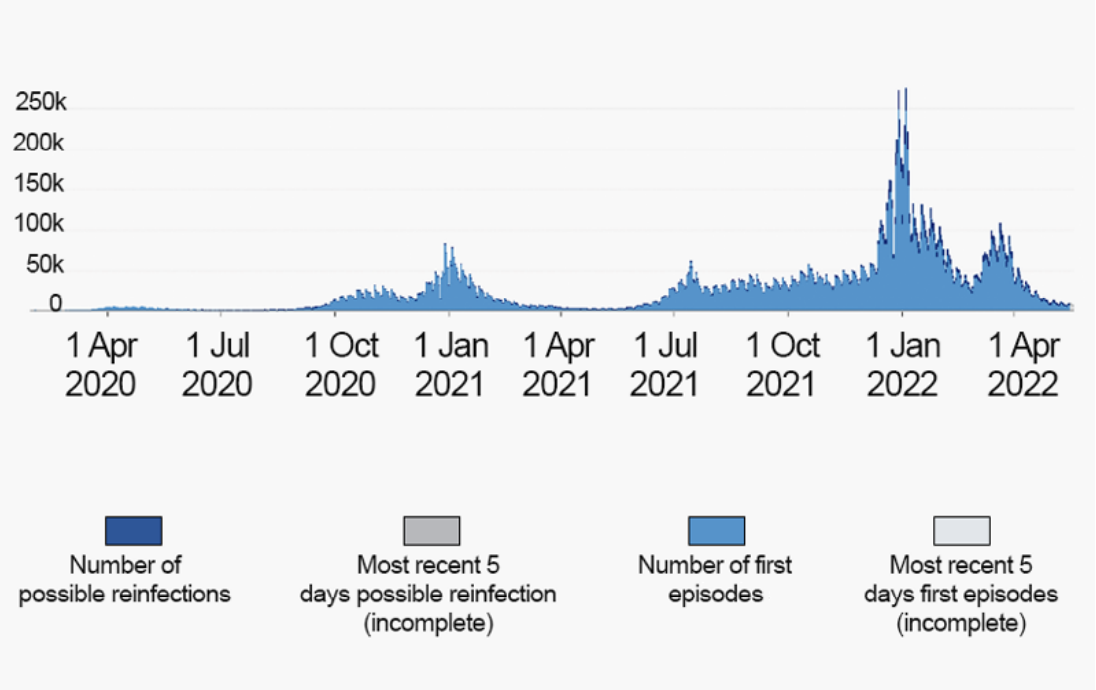


Figure 1 Daily incidence of SARS-CoV2 infections in the entire population of the UK (repeated from Week 4 Figure 2)

Activity 1 Weekly incidence of disease

Allow 10 minutes

An interesting point can be seen in the data on this graph in relation to the weekly incidence of disease – can you see what it is? How could it be explained? Note your thoughts in the box below

Provide your answer...

Answer

The bar chart shows the incidence of new infections detected each day, but it also shows a distinct weekly pattern (the regular notches on the overall profile). There are two possible explanations for this pattern: either infection rates vary on a weekly basis, depending on where people were located and what they were doing; or a more likely explanation is that the laboratories that were testing the specimens, handled or reported more cases during weekdays than at weekends.

Sometimes, during the epidemic in the UK, the level of infection was reported as prevalence, for example, ‘At this time, 1 person in every 100 people in the UK has a COVID-19 infection.’ It is important to see the distinction between incidence and prevalence, since it can lead to confusion in understanding the absolute numbers affected. For example, suppose the incidence of new infections is 10,000 per day and the

average duration of the infection is 10 days, then the prevalence during this period will be 10,000 x10 = 100,000.

For an acute infectious disease, incidence and prevalence are both useful measures of how an epidemic is progressing. But, for many other conditions, incidence and prevalence give different types of information – beware and be aware of the distinction.

1.2 Calculating disease incidence

In this section we will ask you to do some simple calculations of weekly disease incidence, which is made using the formula:

$$\text{incidence} = \frac{\text{number of cases}}{\text{total (number of population x number of weeks)}}$$

Here is a worked example:

In an office with 140 employees, during February 2019 (4 weeks), 17 contracted influenza. What is the weekly incidence of influenza in this office?

Incidence = 17/ (140 x 4) = 0.0304 cases per employee per week.

Activity 2 Calculating disease incidence

Allow 10 minutes

In a secondary school, during the Winter term lasting from January – March 2021 (13 weeks) a number of students were recorded as absent due to COVID-19 infection. The data was broken down according to individual year groups (Table 1). Calculate the weekly incidence of infection in each year group. Don't forget the units.

Table 1 Individual year groups

Year group	Number of students	Number of cases
9	196	16
10	229	42
11	168	34
12	154	24

Which year group shows the highest incidence of COVID-19 infection?

Answer

Did you see that year 11 has the highest incidence (0.0156 cases per student per week), even though the total number of cases was higher in year 10.

2 Detecting infection

As we noted earlier, one of the best ways of measuring the rate of infection in a community is by regularly testing a panel of individuals who are representative of that community. In the earliest stages of the COVID-19 pandemic, it was clear that some individuals could be infected but have no symptoms, but it was not certain whether this was a large or small proportion of the population. This information was, however, very important, since asymptomatic people were more likely to go about their day-to-day activities as normal in the community and potentially infect those they interacted with. This information also informed public health measures such as lock-downs, isolation periods and the wearing of masks.

The incidence of a disease such as COVID−19 is measured by detection of the pathogen, for example by PCR, lateral flow tests or laboratory culture of the infectious agent. These tests show positive for a limited period following infection – typically ~2 weeks for COVID-19. However, if infected people have no symptoms, they are unlikely to go forward for testing. Consequently, measuring the number of infections in people presenting to their doctor or a testing centre is likely to underestimate the true incidence of the disease in the community.

An alternative to detection of the pathogen is to measure antibodies against the pathogen.

2.1 Serology

Serology is the study of antibodies in blood serum and body fluids. It also relates to how antibodies can be used to distinguish between different pathogens and different strains of a pathogen. **Serum**, in this context, is the fluid component of blood containing soluble molecules such as antibodies and other proteins. Serum is formed after blood has clotted, removing the cellular elements of blood (red cells, white cells) and other components involved in formation of the clot (platelets and proteins of the blood clotting system).

Serum should be distinguished from blood plasma which lacks cells, but which retains the components required for clotting. The pie chart in Figure 2 shows the relative amounts of the proteins in plasma.

Figure 2 The protein components of plasma

As you can see, the three major classes of antibody (IgG, IgA, IgM) constitute about 25% of the total plasma protein.

2.2 Seroconversion

Seroconversion refers to the point in time when an infected person has detectable antibodies against the infectious agent. For SARS-CoV2, seroconversion occurs at 7−14 days after the infection and antibody titres typically increase to a maximum at about one month after infection and then gradually decline, provided that the person does not become reinfected.

Different classes of antibody last for different lengths of time in plasma *in vivo*, as shown in Table 2. The measure of persistence is the half-life of the antibody – the amount of time in which half of the original amount is lost. Notice also, that in humans there are four different subclasses of IgG (IgG1 – IgG4) and two of IgA (IgA1, IgA2), which have slightly different characteristics and functions.

Table 2 Properties of human immunoglobulins. The concentration in serum of adults >18 years, is given as the normal range.

Antibody	Half-life (days)	Binding to macrophages	Complement activation	Mucosal transport	Serum conc. g/l
IgM	10	-	+++	+	0.5 -1.9
IgG1	21	+++	++	-	6.0 -16.0
IgG2	20	-	+	-	
IgG3	7	+++	+++	-	
IgG4	21	++	-	-	
IgA1	6	-	-	++	0.8 -4.0
IgA2	6	-	-	++	

While antibodies may only last for a few months, antibody production from B cells and plasma cells lasts for many months after a secondary antigen challenge. and the memory of how to produce these antibodies, residing in memory cells, can last for many years. Consequently, when a person has undergone seroconversion, they usually have detectable antibodies against the pathogen for years. This is a generalisation, and it varies with the pathogen and the individual.

One important question during the COVID-19 pandemic was how long the antibodies and immunity would last. The short answer is that antibodies and antibody production continued for many months following natural infection, and a level of immunity to severe disease lasted much longer than that. The main problem with SARS-CoV2 was not a decline in immunity to the original infection but that the virus mutated to evade the antibodies produced against earlier strains. You will return to this subject in Week 8 and delve further then.

2.3 Seroprevalence

Seroprevalence is a measure of the prevalence of antibodies against an infectious agent. As you discovered in Weeks 3 and 4, this can provide useful information about levels of immunity in the population. In large studies it can also provide regional information. One of the first large-scale studies on COVID-19 antibodies took place in Spain in April – May 2020 and was reported in The Lancet. Figure 3 shows the seroprevalence of IgG antibodies against the SARS-CoV2 nucleocapsid protein, in different regions of the country. It is clear that the levels of infection in the central regions, including Madrid, were much higher than in the peripheral areas.

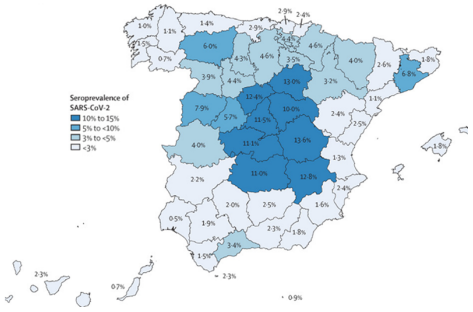


Figure 3 Seroprevalence of antibodies to SARS-CoV2 in Spain, May 2020

Seroprevalence can also be used to track changes in immunity across time. Figure 4 shows the percentage of samples having antibodies to SARS-CoV2 spike protein, from blood donors in different regions of the UK. The donations were screened by an ELISA (Euroimmun) which is similar to the one you carried out in Week 4. Notice that the time of this study covers the period from April to May 2020, when the UK vaccination programme was fully active. The progressive increase in antibodies against spike protein in blood donors, therefore, primarily reflects the increasing proportion who had been vaccinated.

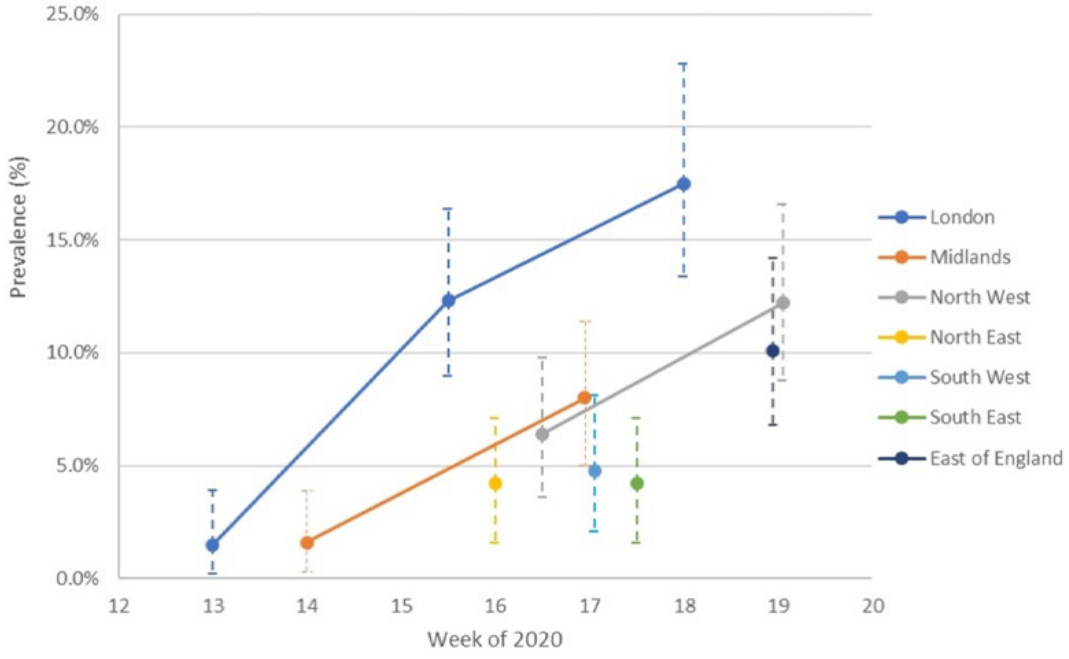


Figure 4 Seroprevalence of IgG antibodies to spike protein in UK blood donors.

Serology can also give information about the proportion of people who are infected but asymptomatic. In the study in Spain, noted above, and in other studies across Europe including Iceland it was found that 30−40% of people who had antibodies against SARS-CoV2 had reported no symptoms and mostly had not been tested for infection by PCR.

A major difference between SARS-CoV2 and the original SARS is the high proportion of asymptomatic cases, which meant that it was much more difficult to identify and isolate infected people, to prevent transmission.

3 Laboratory investigation

Last week, you measured the levels of IgG antibodies against SARS-CoV2 spike protein in a group of 30 individuals in order to assess the level of immunity in the population. This week, you are going to take that investigation one step further. Recall that the antibodies against spike protein could be due to vaccination or natural infection. But how could one specifically identify who had been infected?

If a person has been infected they produce antibodies against all structural components of the virus, including the nucleocapsid. Antibodies against the nucleocapsid are called **N-antibodies**, to distinguish them from the S-antibodies that recognise spike protein.

Activity 3 Measuring N-antibodies by ELISA

Allow 50 minutes

Go back to the data in Table 2 that you produced in Week 4. It should contain your own data on S-antibodies in the samples that you measured. You may have downloaded the table instead, which you can download again if you need to: Table 2.

Now identify those individuals who have a titre of S-antibodies >8. You are going to retest these samples to see if they also have N-antibodies.

Now go to the virtual ELISA: epidemiology laboratory, and measure the titre of IgG antibodies against nucleocapsid.

You should carry out the assay in exactly the same way as previously **except** at step 3 of the assay, you should choose the nucleocapsid ELISA plate (Figure 5)

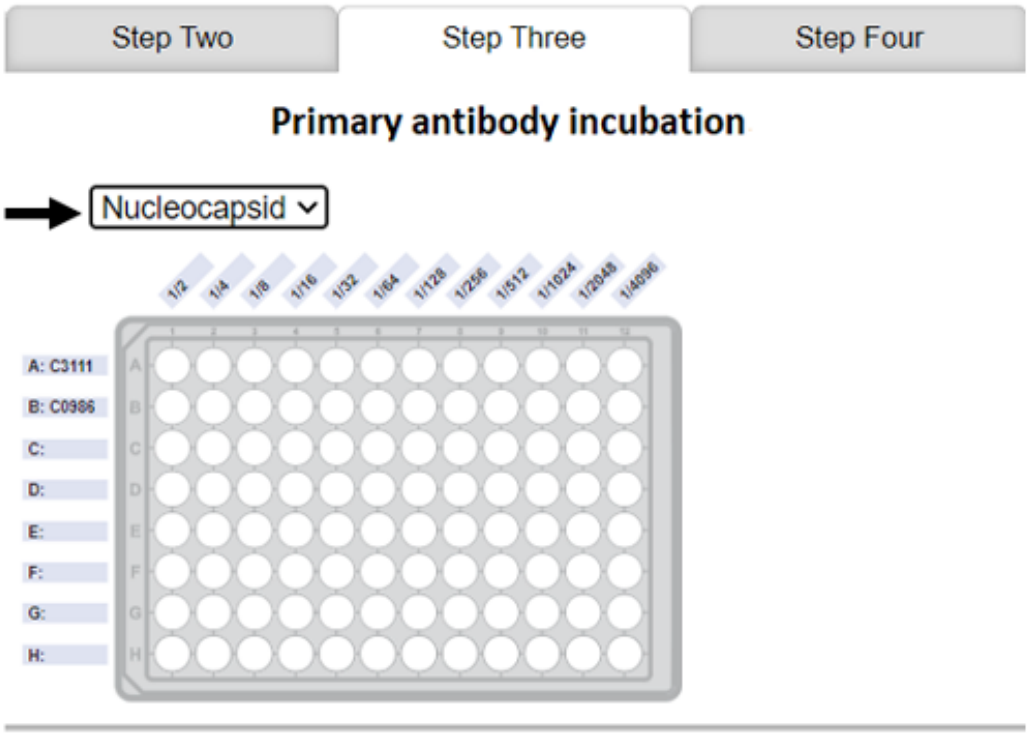


Figure 5 Selection of the nucleocapsid ELISA plate (arrowed) at step 3 of the virtual laboratory

The standard has IgG N-antibodies (titre = 800) which should be used as a positive control, and the negative control serum has no significant N-antibodies (titre <4). You may need to do four ELISA plates in order to measure all of the samples that have S-antibodies. Put your results into the last column of Table 2.

3.1 Data interpretation

You can now make an estimate of the prevalence of N-antibodies in the population, and by inference the number of people who have been infected with the virus. Take a titre of >4 as being a positive result.

$$\text{incidence} = \frac{\text{number with N – antibodies}}{\text{Number tested}} \times 100$$

At this time, about 10% of the UK population had been infected with SARS-CoV2. Because your sample is relatively small, it may not accurately reflect the whole population, but you should have found at least one individual with N-antibodies.

- When you are ready check your results. Do your results correspond? What percentage of the sample had N-antibodies?
- Of the 60 samples available, the following seven samples have IgG antibodies against nucleocapsid:

  C3111, C5930, F7812, H1151, H4439, M6723, N9921

  From these figures we could estimate:

  Prevalence = 7/60 x100 ≈ 12%.

- If a person is seropositive for S-antibodies, but negative for N-antibodies, what can you infer?
- They have been vaccinated with SARS-CoV2 spike protein.

4 Week 5 quiz

Check what you have learned this week by taking the end-of-week quiz.

Week 5 practice quiz.

Open the quiz in a new window or tab, then return to this week when you're done.

5 Summary

This week, we introduced you to some aspects of epidemiology, including the concepts of incidence and prevalence. Incidence is given as the rate of new disease cases over a defined period of time, whereas prevalence gives the total proportion of people affected at any one time.

The value of antibody testing for SARS-CoV2 was outlined. For COVID-19, infected people usually undergo seroconversion 7-14 days after infection, and they then remain seropositive for many months, possibly years. Infection with the virus induces both S-antibodies and N-antibodies. The presence of N-antibodies distinguishes previously infected individuals from those that have been vaccinated against the spike protein.

Seroprevalence, the proportion of people who are seropositive for antibodies, can be used to compare the cumulative level of infection in different regions or over a period of time. This information contributes to understanding disease spread and how it can be controlled. Particularly important is knowing what proportion of infected people are asymptomatic – in the case of COVID-19, up to 40% of cases were asymptomatic. Tests for infection (eg PCR) often underestimate the prevalence of the disease, when there are large numbers of asymptomatic cases, as they are less likely to come forward for testing. For this reason, random sampling in the community is the most reliable way of getting good estimates of disease prevalence.

Finally you used the virtual laboratory to identify sera with N-antibodies as evidence of previous COVID-19 infection. You will take this investigation one step further next week.

Now go to Week 6.

Week 6: Epidemiology

Introduction

Do you remember the regular briefings given by the Chief Medical and Scientific Officers, at the height of the COVID-19 pandemic? There was a lot of concern about whether the number of infections were increasing or decreasing because that determined the public health measures needed to control the epidemic. Later, when vaccines became available, control of the epidemic shifted away from this as the vaccination programme did the heavy-lifting.

Public health measures such as closure of public amenities, reduced travel on public transport and mask-wearing reduced effective contacts between individuals and delayed the progress of the COVID-19 pandemic in different countries.

This week, you will be looking at aspects of epidemiology and modelling that underpinned the response to the pandemic.

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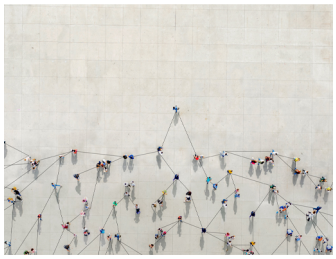
Video 1 Introduction to Week 6



By the end of this week, you should be able to:

- define the meaning of R0, RE and RT
- understand the implications of different values of these variables
- outline the assumptions underlying the determinations of these variables.
- calculate the critical immunisation threshold for different diseases.

1 Epidemiology and modelling

From an epidemiological perspective, each disease is different but there are two main patterns – epidemic diseases and endemic diseases. The SARS-CoV2 pandemic was a classic example of an epidemic disease. It was effectively a new virus in humans and none of the human population had any immunity to it, consequently it produced epidemics in each country affected.

A disease becomes endemic when it transmits continuously in a population, because there are always sufficient susceptible individuals to maintain the infectious agent. Some diseases, such as measles in an unvaccinated large population, are endemic because there are always enough susceptible children to maintain cycles of infection.

In time, an epidemic would develop into a steady-state endemic disease – provided that the virus did not change and immunity lasted for a long-time. However for SARS-CoV2, the virus did mutate and continues to do so. Consequently we have been subjected to repeated waves of infection with new strains of the virus. It is important to distinguish successive waves of an epidemic disease (eg SARS-CoV2), from the regular repeated cycles of infection often seen with an endemic disease such as measles or chicken pox (Figure 1).

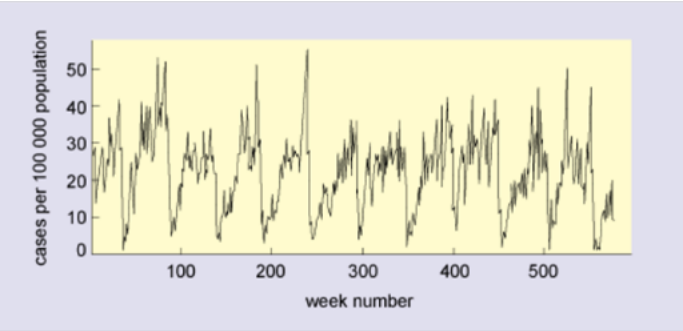


Figure 1 Incidence of chickenpox in France 1991–2001

- Do you think that influenza-A shows an endemic or epidemic pattern of disease?
- Influenza-A is another virus that mutates regularly and produces successive waves of epidemic infection, usually peaking in transmission during the winter months in the Northern and Southern hemispheres.

1.1 The basic reproduction number R_0

An important concept in epidemiology and modelling is the **basic reproduction number (R_0)**. When a person is infected with a disease, there is a period of time when they can transmit the infection to their contacts. The basic reproduction number of the infection is the average number of secondary infections that result from one infected person. This definition is represented in Figure 2.

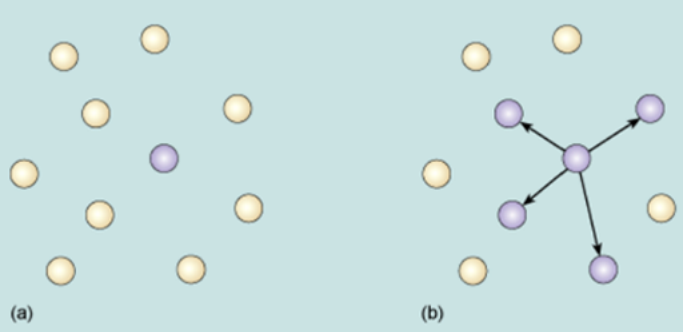


Figure 2 Diagrammatic representation of the basic reproduction number, R0. (a) A single infected person (the purple dot) is introduced into a population of susceptibles (the yellow dots). (b) The initial infective transmits the infection to an average of 'R0' others; here, R0 = 4.

The basic reproduction number is neither a risk nor a rate: it is just a number. It can take any positive value (or zero) and it specifically assumes that all contacts are potentially susceptible to infection. For most diseases this is clearly not true. While it was true at the start of the COVID-19 pandemic, resistance to reinfection gradually built up in the population as increasing numbers of people were infected, recovered and developed some immunity. Later on, the number of susceptible individuals was drastically reduced by the COVID-19 vaccination programmes.

Despite its theoretical nature R_0 is a very useful measure, because it allows comparison of the infectivity of different pathogens or different strains of one pathogen. It is also a key parameter in determining what proportion of the population must be vaccinated in order to stop a disease spreading – this value is called the critical immunisation threshold (qc) and we will return to it later this week.

1.2 Understanding R_0

The basic reproduction number (R_0) is important because it encapsulates the relationship between an infection and its physical and social environment.

The number of secondary infections depends on the ability of the infectious organism to survive outside the host and to migrate from one host to the next, which in turn is contingent on biological and environmental factors. It depends on the infection–host interaction through, for instance, the duration of the infectious period. It is also affected by the frequency and type of contacts that take place within the population, which vary according to environmental, social and cultural factors.

R_0 can also tell us about how quickly an infection is likely to grow in an unvaccinated, fully-susceptible population. Table 1 shows R_0 values for a number of virus diseases, Note that the values given are ranges, which will vary depending on the population and how the communities interact.

Table 1 Range of R0 values for selected virus diseases.

Virus	R_0
Influenza	1 - 2

Hepatitis C	2 - 3
Ebola	1.5 – 2.5
Zika	1.5 – 4.1
HIV	2 - 5
SARS-CoV	2 - 3
SARS-CoV2	2.5 - 6
Mumps	7 - 10
Chickenpox	10 - 12
Measles	12 - 18

As you can see SARS-CoV2 falls around the middle of the range for virus diseases, but as it turned out different variants of SARS-CoV2 have different R_0 values.

1.3 The effective reproduction number R_E

Since most populations will have some level of immunity to an infectious disease, the rate of spread will be less than indicated by the R_0 value. The measure of how a disease spreads in real situations is given by another dimensionless variable R_E – the effective reproduction number (Figure 3), which is often just called the R-value.

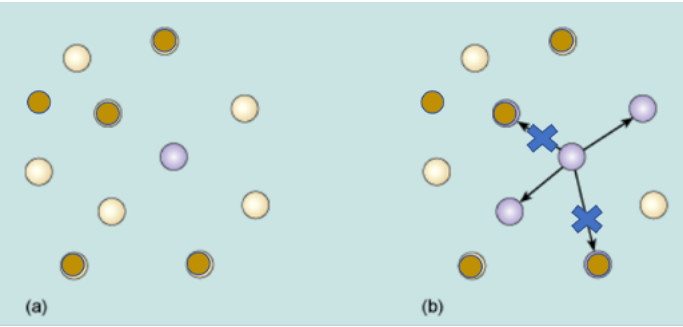


Figure 3 Diagrammatic representation of the effective reproduction number RE . (a) A single infected person (the purple dot) is introduced into a population where half are susceptible (yellow dots) and half are resistant (green dots). For this infection R0 = 4 (b) The initial infective transmits the infection to an average of RE others; RE = 2 because the two contacts who might have been infected (blue crosses) are resistant.

Notice that R_E will never be greater than R_0 and will usually be less than R_0 because of the level of resistance or immunity in the population.

Another point is that R_E can tell us something about whether an infection will develop into an epidemic or just die out. If R_E is >1 then the number of infections will gradually increase over time. Exactly how quickly an epidemic will develop depends partly on the value of R_E, and partly on how long it takes before each infected person becomes infectious themselves, ie the time-course of infection.

Conversely if R_E <1 then the infection is self-limiting, because the number of infected people gradually decreases over time.

1.4 Understanding R_E

Because R_E depends on the particular circumstances of an infection, the value of R_E will be different dependent on the current local conditions.

- With reference to the COVID-19 pandemic: can you think of 5 different conditions that could affect the value of R_E. There is a lot to think about here, so take your time before you reveal the answer.
- The proportion of people in the population who were resistant to infection with SARS-CoV2 varied over time. It was dependent on whether a person had been infected with SARS-CoV2 or had been vaccinated against it. Immunity gradually declines over time, if a person does not become reinfected with the same virus. Also, as the pandemic developed, new variants arose which could partly evade the immunity produced against previous strains. Effectively the new strains resulted in a more rapid decline in resistance than normally occurs and increased susceptibility to reinfection. All of these factors relate to the intrinsic susceptibility of the population.Also R_E depends on how people interact with each other. Even before public health measures were introduced, many people started to reduce their social interactions so the level of effective contacts fell. Public Health measures, such as lock-downs, restricted access to public amenities and mask-wearing also all reduced the number of effective contacts between individuals.

We should also consider the underlying assumption for R_0 and R_E – that the population is homogeneously mixing. This is obviously a simplification. Some people live in large families, others in Institutions, and some people live alone. The intrinsic level of social contact varies greatly for each of these groups. Another important factor is what type of work people were doing. During the pandemic, some people could and did work from home. Others, because of the nature of their occupation, travelled to work and may have been meeting other members of the public as part of their work.

The number of effective contacts and the potential for transmission of an infectious agent depends on all of the factors noted here. Despite all of these variables affecting individuals, R_E is still a very useful concept, when applied to the population, as a whole, since it informs public health policies.

1.5 How R_E changes over time

We have already noted that R_E changes over time due to variable susceptibility, public health measures and vaccination. The value at any one time is important, since if $R_E > 1$ an epidemic is growing, whereas when $R_E < 1$ it is shrinking. Figure 4 shows the estimated value of R_E at different time points between July 2020 and October 2022. The values are given as ranges, and values vary above or below the critical value, $R_E = 1$.

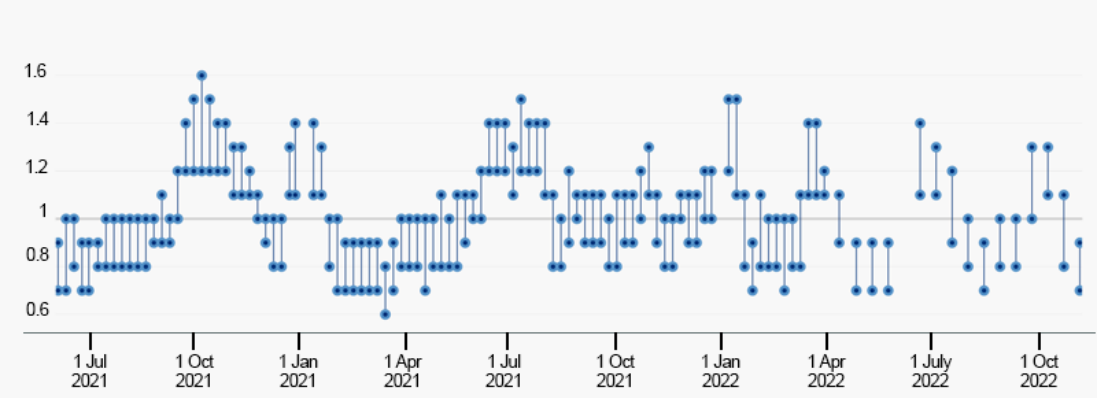


Figure 4 Estimated values of RE in the UK.

It is important to understand that the values shown in Figure 4 are not the numbers of people affected by COVID-19, but they reflect whether numbers affected are increasing or decreasing. It is possible to have very few people affected but a high value of R_E, or many people affected and a low value of R_E. This type of data was fundamental for informing public health policies in the early phases of the pandemic, before vaccines were available. If R_E values were >1, then the numbers that would be infected over the following weeks or months could be projected. In order to prevent the huge numbers overwhelming medical services, public health measures were introduced to reduce contact rates and bring R_E <1.

As one example, look at the R_E values in the month before January 2021 (Figure 4). The sudden rise in R_E at this time corresponds with the government decision to end lockdown restrictions on December 2nd 2020. Once the effect of this policy became evident, the lockdown had to be reinstated on 21st December in London and Southeast England and 26th December in other parts of the UK (Institute for Government analysis, 2021). With public health measures back in force, the R_E value fell below 1.

Once vaccines became available the proportion of susceptible individuals was reduced and vaccination could be relied on to keep infection rates under control. Consequently, during 2021, there was a progressive shift from a reliance on public health measures (to reduce contacts) to the use of vaccines (to reduce susceptibles).

!Warning! Calibri not supported[Note that when R_E values are stated in relation to particular time-points or over a period of time they are often referred to as **R_T**values also called the **net reproduction number**. In many publications values of R_T or R_E are just given as R-values.]

1.6 Estimation of R_0, R_E and R_T

As R_0 is by definition a measure of disease spread in a totally susceptible population, and since such populations rarely exist, estimating R_0 for a disease often presents a challenge. Measurement of R_E is conceptually simpler, because it can be done empirically by measuring new infections.

For uncommon infections such as Ebola, it is possible to directly identify how many people became infected from a single index case. While the numbers are usually small, it gives a direct measure of spread in a defined population where all individuals are susceptible. Also, in the case of Ebola, all infected contacts of the index case develop symptoms and can be identified. Consequently asymptomatic cases do not cause underestimation of R_0.

The R_0 value for COVID-19 could also be estimated from the incidence of new infections. In the earliest stages of the COVID-19 pandemic, virtually everyone was susceptible, so

R_0 was similar to R_E. However, at this time accurate data depended on the availability of reliable tests and screening programmes. In the early stages of the COVID-19 pandemic, testing for infection by PCR was patchy and selective. Moreover, as COVID-19 epidemics developed in different countries, people voluntarily reduced their level of social contacts and public health measures were introduced to limit spread, ie effective contacts were reduced. This means that the most reliable direct estimates of R_0 for SARS-CoV2 come from the period before these behavioural changes and public health policies came into effect.

For endemic infections, which have reached a steady state in a population, it is possible to estimate R_0 indirectly, but this is beyond the scope of this course.

2 Herd immunity and immunisation

This section introduces two important related concepts. Herd immunity describes the level of immunity that develops in a population, due to natural infection. Once a sufficiently large proportion of the population have become resistant, the disease can no longer spread because there are insufficient susceptible people and R_T <1. The level at which this occurs is the '**Herd immunity threshold**' (HIT) and it is different for each disease.

Critical immunisation threshold (qc) is the level of immunity that must be achieved in a population by vaccination, to stop a disease spreading. As you can see, it is conceptually very similar to the herd immunity threshold.

2.1 Herd immunity threshold

In section 1, we discussed disease susceptibility and the proportion of the population who are susceptible. This value is designated by a variable '**S'**. In the illustrated example (Figure 5a) in a population of 12 individuals 9 are resistant and 3 are susceptible.

S = 3/12 = 0.25 and the proportion resistant **= 1 –S = 0.75**

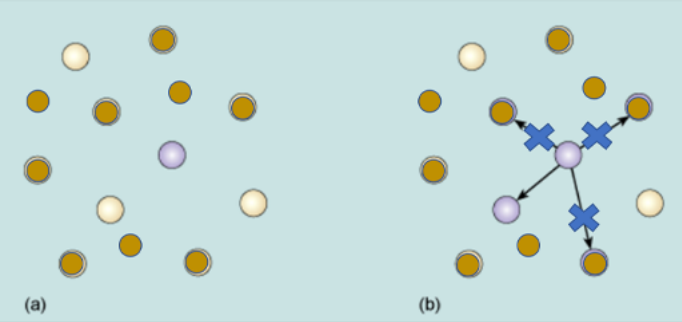


Figure 5 Diagrammatic representation of herd immunity threshold for a disease where R0 = 4. An infective (purple) is introduced into a population (a) where three individuals are susceptible (yellow) and nine are resistant (green). Potentially the infective could infect 4 individuals (arrows) but 3/4 of these contacts are resistant, so there is only one new case of infection (purple dot), ie RE =1.

Recall that if R_T <1 then an infection will die out. The herd immunity threshold and the critical immunisation threshold occur when R_T =1. The value can be calculated from the basic reproduction number R_0.

HIT = $1 - 1/R_0$

Let us consider how this works for a disease with R_0 = 4

HIT = 1 -1/4 = 0.75

If this is now expressed as the percentage of the population that must be resistant to stop a disease from spreading:

HIT = 0.75 x100 = 75%.

For a diagrammatic representation of this example, see Figure 5(b). In this case R_0=4 and there is only one secondary infection (R_E =1), because 3 of the 4 potential effective contacts are resistant.

There is some simplification in the calculation of HIT, since it assumes that a person is either completely susceptible or totally resistant. In practice resistance develops over time following infection or vaccination, and the level of resistance can wane. Nevertheless, considering individuals to be susceptible or resistant is useful for modelling epidemics and planning vaccination programmes.

2.2 Critical immunisation threshold (qc)

The critical immunisation threshold (qc) tells us what proportion of the population need to be vaccinated, in order to control an infection, and it is calculated in exactly the same way as the herd immunity threshold.

$qc = 1 - 1/R_0 \times 100$

Activity 1 Calculation of qc

Allow 15 minutes

In this activity we ask you to calculate qc for 7 different virus diseases expressed as a percentage of the whole population. Use the equation to determine the values of qc and enter them into Table 2.

Round your calculation to the nearest whole number.

Table 2 Calculation of qc values – A range of R0 values is given for SARS-CoV2, but just a single typical value for the other viral infections.

Virus	R_0	qc
Influenza	2	*Provide your answer...*
Hepatitis C	3	*Provide your answer...*
Zika	4	*Provide your answer...*
SARS-CoV2	2.5 - 6	*Provide your answer...*
Mumps	8	*Provide your answer...*
Chickenpox	10	*Provide your answer...*
Measles	16	*Provide your answer...*

When you have completed your calculations, click to reveal the answers.

What do you notice about the relationship between R_0 and qc?

Answer

The higher the value of R_0, the higher is the value of qc.

Table 2 Calculation of qc values – A range of R0 values is given for SARS-CoV2, but just a single typical value for the other viral infections.

Virus	R_0	qc
Influenza	2	50%
Hepatitis C	3	67%
Zika	4	75%
SARS-CoV2	2.5 - 6	60 - 83%
Mumps	8	87%
Chickenpox	10	90%
Measles	16	94%

For diseases such as mumps, chickenpox and measles, the level of vaccination coverage needed to prevent outbreaks is very high. When vaccine coverage of these childhood diseases falls below the required level in the population, outbreaks of the diseases occur. Also, when vaccination levels fall below the qc level, the age at which unvaccinated children contract the disease is older. The reason for this is that on average it will take longer before they encounter an infective with the disease, because the disease is less common in partially-vaccinated populations. Moreover, for some infections, disease is more serious if contracted later in life.

Notice that if a population is protected because the level of vaccination exceeds the qc for a particular infection, then even people who are not immune have some protection, because the infectious agent no longer circulates in the community. However if everyone relies on other people being vaccinated, then the level of population immunity falls below qc and the infection returns. This has occurred in the UK recently for diseases such as measles, which require high levels of vaccine coverage to create herd immunity.

Next week we will look at how vaccines are formulated and how effective they are, but it is worth remembering from you calculations here that the qc values for SARS-CoV2 range from 60 – 83%.

3 Laboratory investigation

This week you will take your laboratory investigation one step further. The aim is to identify anyone who has been recently infected with COVID-19. Recall that last week you identified 7 serum samples that had antibodies against SARS-CoV2 spike protein **and** nucleocapsid, implying that they had been infected with the virus.

Of the 60 samples available those that have both S-antibodies and N-antibodies are:

C3111, C5930, F7812, H1151, H4439, M6723, N9921

Also recall that the first antibodies to be produced following infection are IgM, and it is only later that antibody production switches to IgG and IgA. Therefore, if you can identify sample(s) which have a relatively high IgM titre compared with IgG and IgA, it indicates that the person has been recently infected.

Activity 2 Measurement of S-antibodies of different classes

Allow 30 minutes

In this activity, we ask you to measure the titres of IgM, IgG and IgA S-antibodies in the 7 samples from infected individuals. You should also include the standard in the 8th row of the plate. The assay will require three ELISA plates, one for IgM, one for IgG and one for IgA.

ELISA; epidemiology on-line laboratory

Carry out the assay according to the protocol you developed in week 3, with the following conditions:

At step 1 of the assay you should select the samples; **C3111, C5930, F7812, H1151, H4439, M6723, N9921** and the **standard**

At step 3 of the assay you should choose plates sensitised with **spike protein**.

At step 4, you will need to use **20µl anti-human IgM (HPO)**, 0.5mg/ml (final conc. 1µg/ml)

Or **4µl anti-human IgG (HPO),** 1.5mg/ml (final conc. 0.6µg/ml)

Or **40µl anti-human IgA (HPO),** 1.0mg/ml (final conc. 4µg/ml)

[The S-antibody titres of the standard are IgM-120, IgG-1200 and IgA-140.]

Record you results in a table like the one below (Table 3).

Table 3 S-antibodies of different classes in previously infected individuals

Samples	S-antibodies		
	IgM	IgG	IgA
C3111	*Provide your answer...*	*Provide your answer...*	*Provide your answer...*
C5930	*Provide your answer...*	*Provide your answer...*	*Provide your answer...*

F7812	Provide your answer...	Provide your answer...	Provide your answer...
H1151	Provide your answer...	Provide your answer...	Provide your answer...
H4439	Provide your answer...	Provide your answer...	Provide your answer...
M6723	Provide your answer...	Provide your answer...	Provide your answer...
N9921	Provide your answer...	Provide your answer...	Provide your answer...
Standard	Provide your answer...	Provide your answer...	Provide your answer...

You should see that **one** of these samples has a relatively high IgM titre in comparison to IgG and IgA. This suggests that the person has had a relatively recent infection with SARS-CoV2. When you have examined your data and come to a conclusion, click to check your answer.

Answer

Sample C5930 has IgM-160, IgG-120, IgA-80. The relatively high IgM titre suggests a recent infection. For the other samples the IgG titres are at least 8x higher than the IgM titre.

4 Week 6 quiz

Well done for reaching the end of Week 6. Check what you've learned by taking the end-of-week quiz.

Week 6 practice quiz

Open the quiz in a new window or tab then come back here when you've finished.

5 Summary

This week, we introduced some key concepts in epidemiology including:

- R_0, the number of people infected by a single infective in a totally susceptible population.
- R_E, the number of people infected by a single infective, in real circumstances.
- R_T, the number of people infected by a single infective at some defined time-point.
- The variables imply that when R >1 an epidemic is spreading, but if R<1 it will eventually die out.

The measurement of these variables assumes homogeneous mixing in the population and that all individuals are either completely susceptible or completely resistant to infection – conditions which do not generally occur in the real world. Nevertheless the variables are useful for determining how an infection will spread in the population as a whole.

The herd immunity threshold (HIT) and critical immunisation threshold (qc) define what proportion of the population must be resistant to infection, for it to stop it spreading. These variables can be calculated if R_0 is known. The value varies for each disease. The larger the value of R_0, the greater the proportion of people must be vaccinated to contain a disease.

In your laboratory investigation, you identified one recently infected individual, using the ELISA to measure different classes of S-antibody.

Now go to Week 7.

Week 7: Vaccines

1 Active and passive immunisation

The principle of vaccination is very simple – train the immune system to recognise and react against the infectious agent. This procedure is in fact 'active immunisation', so called because the person who receives the vaccine actively makes their own antibodies and T-cell responses against antigens in the vaccine. For most infections 'active immunisation' is the only form of immunisation available. However there is another form of treatment called 'passive immunisation'.

1.1 Therapeutic antibodies

In 'passive immunisation' a person is given antibodies that have been made in another person, an animal or in a laboratory. The technology for making antibodies in the laboratory was well established before the COVID-19 pandemic started. Consequently, at the start of the pandemic, when there was uncertainty about whether an effective vaccine could be produced in time, pharmaceutical companies put considerable effort into the production of human therapeutic antibodies against the SARS-CoV2 spike protein (Table 1).

Table 1 Examples of therapeutic antibodies.

Antibody	Class	Company	FDA or EU Authorised
Banlanivimab	IgG1	AbCellera Biologics / Eli LIlly	Nov. 2020 – April 2021
Bebtelovimab	IgG1	AbCellera Biologics / Eli LIlly	Feb. 2022 – Nov. 2022
Casirivimab/Imdevimab	IgG1	Regeneron Pharmaceuticals	Nov. 2021 -
Cilgavimab/Tixagevimab	IgG1	AstraZeneca	Dec. 2021 -
Banlanivimab/ Etesivimab	IgG1	Junshi Biosciences / Eli LIlly	Feb. 2021 -

FDA = Federal drug authority in the USA

The antibodies produced were 'monoclonal', meaning that they came from a single clone of B cells, and unlike naturally-produced polyclonal antibodies, monoclonal antibodies bind to just one position on an antigen. In producing therapeutic antibodies, the aim was to have an antibody that bound the receptor-binding domain (RBD) on the spike protein which could prevent the virus binding to its target, the ACE2 receptor.

These antibodies were initially given emergency authorisation, for use in preventing COVID-19 infection in vulnerable individuals and/or for treatment of hospitalised patients with serious disease. As the pandemic progressed it became clear that new variants of

SARS-CoV2 sometimes evaded the protection produced by these antibodies. To reduce the risk of a virus variant evading immunity, formulations with two monoclonal antibodies (eg Casirivimab/ Imdevimab) were developed. Also, authorisation for use of these treatments was sometimes revoked or amended, if they became less effective.

It must be emphasised that these therapeutic antibody treatments are no substitute for active immunisation. A single treatment with a therapeutic antibody can cost £1000 - £1500, which contrasts with the typical cost of a vaccine, £1.50 - £15. Also, for immunological reasons, infusion of therapeutic antibodies may inhibit endogenous antibody production. Hence the reasons that therapeutic antibodies are licensed only for vulnerable or seriously-ill patients.

For the remainder of this week, we will look at active immunisation with vaccines containing virus or viral components.

1.2 Vaccine types

There are several different ways to produce a vaccine against a virus (Figure 1). Traditional vaccines, used the virus itself but chemically inactivated in such a way that it could not produce an infection. Another route was to develop a variant of the virus that could replicate, but which did not produce any symptoms or pathology in the recipient. The two main types of polio vaccine were derived by these two strategies – inactivation or attenuation.

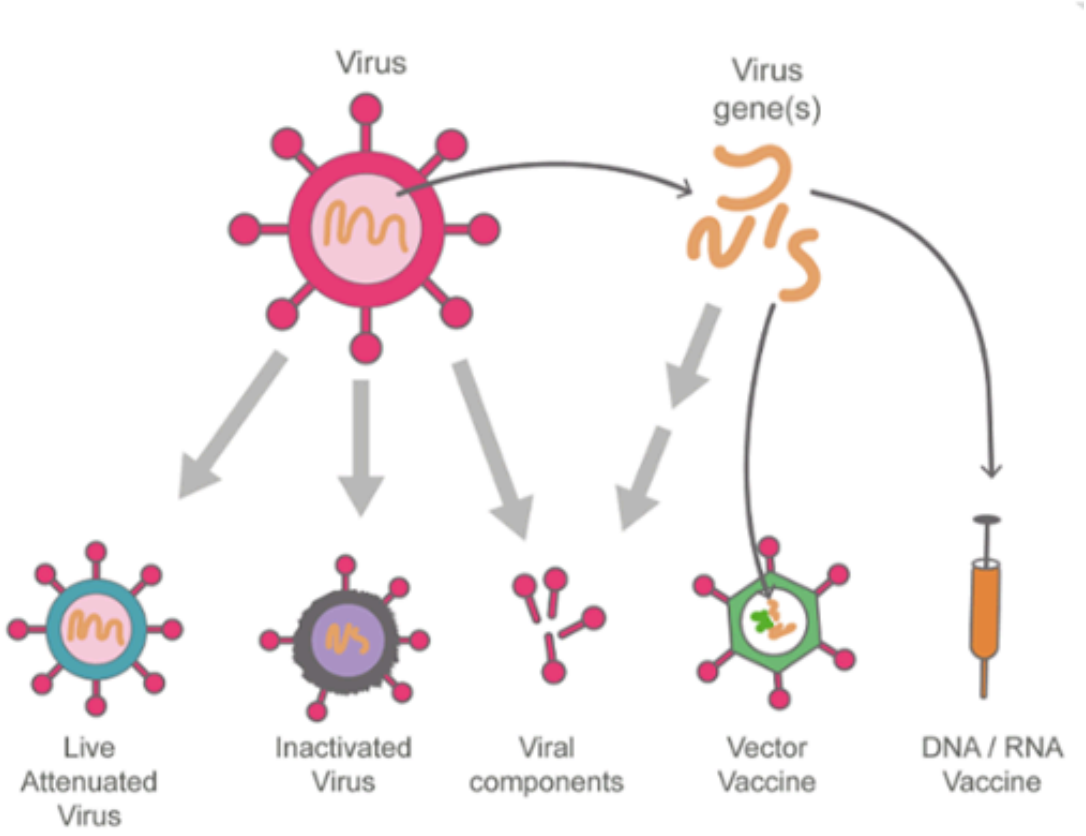


Figure 1 Five different strategies for producing an anti-viral vaccine.

More recently, vaccines have been developed against individual components of a virus, for example against purified spike-protein of SARS-CoV2. One limitation here is knowing which component(s) of the virus are important for inducing immunity. Also, recall that the antigens which stimulate B cells and T cells are often different. Moreover an immune response to a single virus component is often less strong than the response to an inactivated or attenuated whole virus. For this reason, such antigens may be modified to make them more immunogenic, or to favour one type of immune response.

The latest vaccines are produced by genetic engineering. The idea here is to use the genetic material of the virus, to induce production of viral components which then stimulate the immune response. It turns out that this approach, using mRNA for the SARS-CoV2 spike protein, was very successful in the race to develop effective COVID-19 vaccines.

1.3 COVID-19 vaccines

By December of 2020, more than 200 vaccine candidates for COVID-19 were under development, with more than 50 being taken forward into trials on humans. A variety of approaches were made (Table 2).

Table 2 Examples of COVID-19 vaccines taken through to clinical trials.

Vaccine type	Manufacturer (name)
mRNA for spike protein	Pfizer/ BioNTech Moderna CureVac
Vector with gene for spike protein	Oxford/Astra Zeneca (Sputnik V) Johnson & Johnson /Janssen CanSinoBio (Convidecia)
Spike protein	Novavax/ GSK
Inactivated virus	Valneva Sinovac (CoronaVac) Sinopharm (BB1BP CorV)

Interestingly, it was the newest methods – vector vaccines, and mRNA vaccines, which came through first. One strategy for COVID-19 is to take the gene that encodes the spike protein and insert it into a harmless virus vector. The vector has very limited capacity to replicate, but it still produces the COVID-19 spike-protein which induces specific antibody production. This approach has been used by the Oxford/Astra-Zeneca and Russian Sputnik V vaccines.

mRNA vaccines rely on the recipient's cells taking up the gene and expressing it, so that virus antigens (but not virus) are produced by the cells of the body. This approach is relatively new and it was used by the Pfizer/Biontech and Moderna vaccines against COVID-19. This method has been so successful that it has revolutionised vaccine production, and is now being applied to other infectious diseases and cancer therapy.

1.4 Vaccine testing

Vaccines undergo rigorous trials before they are released for general use. The one exception to this rule is where an infection is very dangerous or uncontrolled and it is necessary to put a vaccine into the field as quickly as possible. This was seen with an Ebola virus vector vaccine, in helping control outbreaks of Ebola in the Democratic Republic of Congo and Zaire. Where mortality from a virus infection is high, there is more tolerance of adverse reactions against the vaccine, and the normal extended testing programs can be abbreviated.

A normal testing program is carried out in four phases.

- Phase-1 examines basic safety of the vaccine in healthy volunteers.
- Phase-2 expands the initial trial to a larger and more diverse group of individuals (older, younger, different ethnic groups, etc.).
- Phase-3 determines whether the vaccine is effective in a large cohort (thousands of people) in real-world conditions.
- Phase-4 trials look for any long-term effects of the treatment and may extend over many years.

[Your laboratory investigation this week is to carry out antibody testing on volunteers in a phase-1 vaccine trial.]

Due to the urgency to develop vaccines against COVID-19, volunteers were recruited as quickly as possible and where possible phase 1-3 trials were overlapped to reduce the time before results became available. Also, the vaccination schedules and doses were chosen in these trials as a best estimate of what would produce a good antibody response. Dosing and schedules were later refined pragmatically and as more data on effective schedules became available. For example, the initial schedule for the Pfizer/ Biontech mRNA vaccine was 2 doses given 21 days apart. This schedule was approved in December 2020. However, when it came to roll out the vaccination programme, with limited supplies of vaccine it was thought that greater protection could be given to the population by immunising more people, but spacing out the first and second doses by 6-8 weeks. As it happened, the longer gap between doses actually produced a slightly better antibody response, and the aim of protecting more people sooner was epidemiologically sound.

1.5 Field tests

To determine if a vaccine is truly effective it has to protect people from the naturally occurring infection in a phase-3 trial. The incidence of infection in vaccinated and non-vaccinated people is compared, to see what level of protection is given by the vaccine.

The studies are designed to be double-blind, meaning that neither the clinician administering the vaccine nor the recipient, know whether they have received the real vaccine or a placebo. Data is then collected over several months or years to determine the incidence of infection in the two groups. Once sufficient data has accumulated (number of infections) the coding on the treatments (vaccine or placebo) is opened to see whether there is a difference between the groups and how large the difference is.

If the prevalence of infection is low in the community, then it takes longer to see whether the vaccine is effective, because it requires sufficient infections, to obtain robust data. Ironically, when public health measures reduced the incidence of infection, it could then delay data accumulation on a phase-3 vaccine trial.

All of the approved COVID-19 vaccines have been tested in real-world trials and found to be very effective. But notice that these trials are not exactly comparable, because different trials took place at different times and in different countries where the dominant strains of the SARS-CoV2 virus have been different. For this reason, a simple comparison of 'vaccine effectiveness' in these trials is not straightforward.

As an example, you can see the design and outcomes of the phase-3 trial of the recombinant Oxford/AstraZeneca COVID-19 vaccine (!Warning! Source Sans Pro not supportedAZD1222) at the US National Library of Medicine.

Phase III Double-blind, Placebo-controlled Study of AZD1222 for the Prevention of COVID-19 in Adults

1.6 Vaccine effectiveness

Vaccine effectiveness (VE) is defined as the percentage reduction of infection in a group of vaccinated people, compared with a similar unvaccinated group. The two key parameters are the attack rate in the vaccinated group (ARV), compared with the unvaccinated group (ARU).

VE = (ARU – ARV)/ ARU x100%

Activity 1 A phase III vaccine trial

Allow 10 minutes

The following activity is based on the data from a phase-3 trial of the Oxford/ AstraZeneca AZD1222 vaccine for COVID-19. The trial was randomised, double-blind and all subjects were seronegative for SARS-CoV2 antibodies at the start of the trial.

In this trial 17762 subjects were vaccinated and 8550 received placebo.

73 of the vaccinated group and 130 of the unvaccinated group became infected in the study period.

We now ask you to calculate the vaccine effectiveness using the following steps:

You should first calculate ARV and ARU.

ARV = Number infected / Number vaccinated. ARU = Number infected / Number unvaccinated

Answer

ARV = 73/ 17762 = 0.00411 ARU = 130/ 8550 = 0.0152

Then calculate VE, using the formula:

VE = (ARU – ARV)/ ARU x100%

Click to reveal the answer, once you have made your calculation.

Answer

VE = (ARU – ARV)/ ARU x100% = (0.0152 – 0.0041)/ 0.0152 X100% = 73%

Based on this data the vaccine appears to be effective in reducing the incidence of infection by 73%.

Notice that vaccine effectiveness is normally defined by the attack rate – the reduction in the percentage of infected people. However it is possible to measure ‘effectiveness’ by other means, such as the number of people showing disease symptoms or the number requiring hospitalisation. In the COVID-19 pandemic, the major clinical concerns were the number of people going to hospital, the numbers in intensive care units and the number of deaths.

It was very notable that the COVID-19 vaccines not only reduced incidence of infection, but also reduced hospitalisation and deaths. In effect the spectrum of disease severity, ranging from asymptomatic to hospitalisation was all shifted to the left (Figure 2).

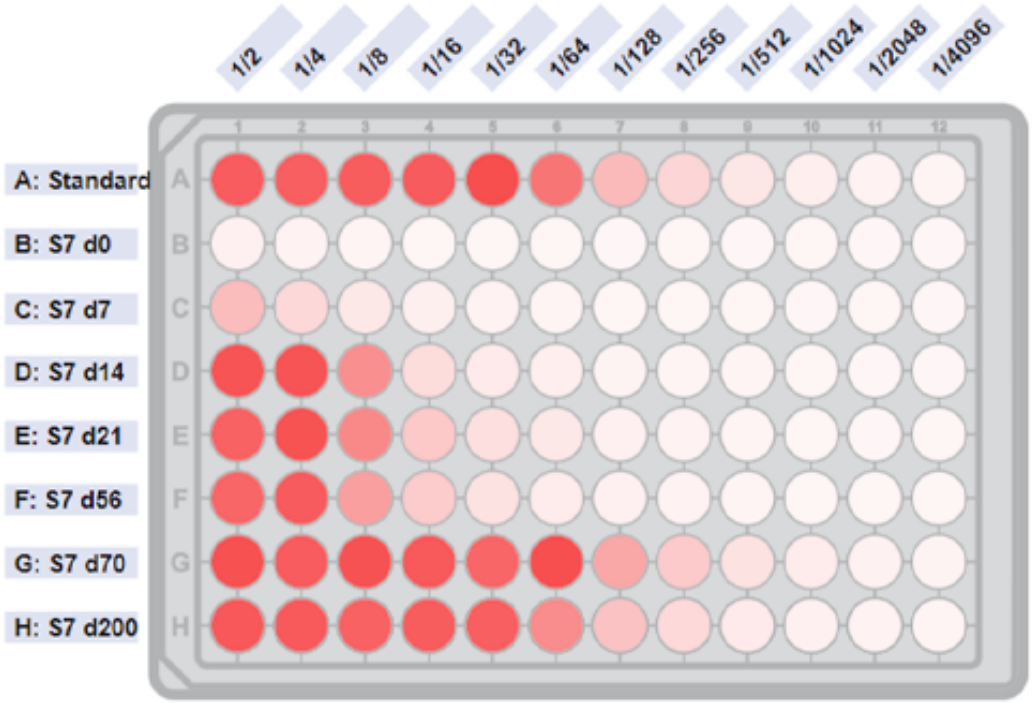


Figure 2 Diagrammatic representation of the role of vaccination. Vaccination shifts the number of people in each of the affected groups to the left reducing the level of serious illness and hospitalisation.

2 Vaccination

This week the laboratory investigation is to measure the development of antibodies in two subjects who have been enrolled in a phase-1 study to assess the effectiveness of a COVID-19 vaccine. You will be using the ELISA again, but with a different set of samples to the previous weeks.

The first 10 minutes of the video describes the samples and data available. It is followed by a section which reminds you how to use the ELISA laboratory. Also note that the video refers to samples from 10 subjects, but in the version of the laboratory available to you, we have only included samples from the two subjects that you are investigating. In this demonstration a different chromogen, OPD was used. You can do this if you like (remember to use the 645nm filter on the plate reader), or you can use TMB as you have done in previous assays.

As you watch the video, be sure to note the vaccination schedule and when the blood samples were taken (Figure 3).

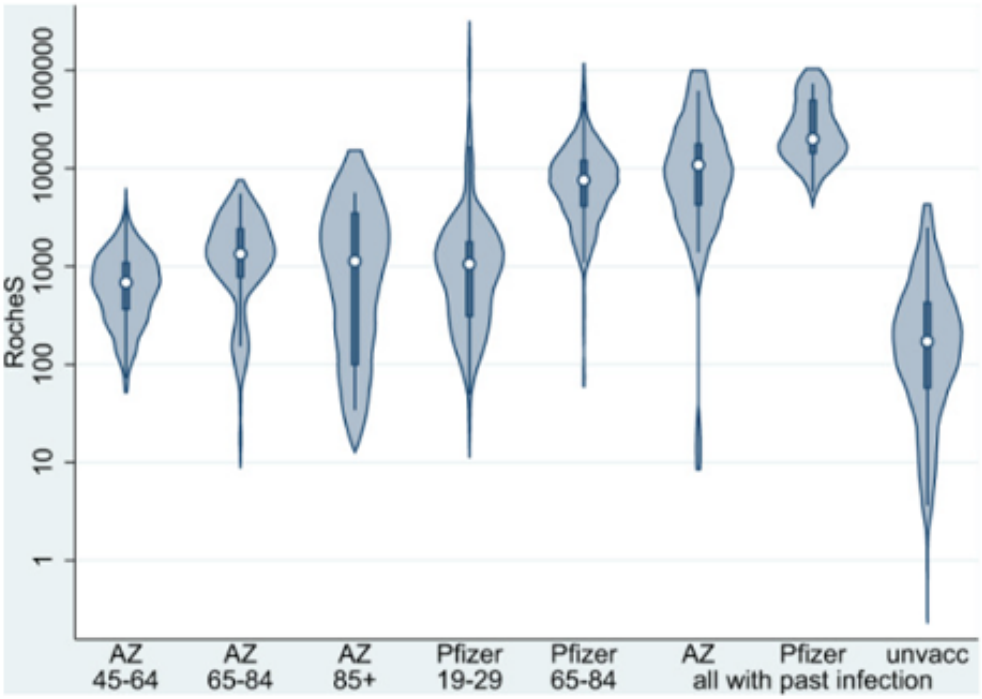


Figure 3 Schedule of a phase-1 vaccine trial

Video content is not available in this format.
Video 1 Detecting antibodies against SARS-CoV-2

This protocol has a primary and secondary dose of vaccine separated by 8 weeks. Samples were taken in the first 21 days to detect appearance of antibodies. A sample was taken just before the second dose of vaccine and again 14 days after the second dose when maximum antibody titres were anticipated. The sample taken 6 months later was intended to detect how long antibodies remained in the serum.

2.1 Laboratory investigation

The aim of this investigation is to detect how antibodies are induced following a primary and secondary injection of SARS-CoV2 spike protein. For a phase-1 vaccine trial, assays like this would be carried out on samples from 100s or 1000s of healthy volunteers.

Activity 2 Quantitation of antibodies induced by vaccination

Allow 30 minutes

For this ELISA, you are only supplied with plates sensitised with spike protein; since the vaccination is against spike protein, there is no point in trying to detect antibodies against other viral components. IgG antibodies are most important for conferring immunity, so we ask you to quantitate IgG S-antibodies, using the ELISA protocol that you developed in week 4.

ELISA website

You will need to use at least two ELISA plates, one for each of the subjects. Set out each ELISA plate with samples from one of subjects, arranged in the order they were taken – day 0, 7, 14, 21, 56, 70, 200. You should also include the standard as a positive control. The day 0 sample will act as negative control in this assay, since the subjects were pre-selected to have no S-antibodies at the start of the trial. The results from each of your subjects should look something like Figure 4. Note that the subject shown in Figure 4 is different from the ones that you are measuring, and that the chromogen OPD has been used, which gives a red end-product.

I don’t see this in OSL so haven’t looked at it, I assume it’s very similar to the other one but happy to look at it if someone sends me the link

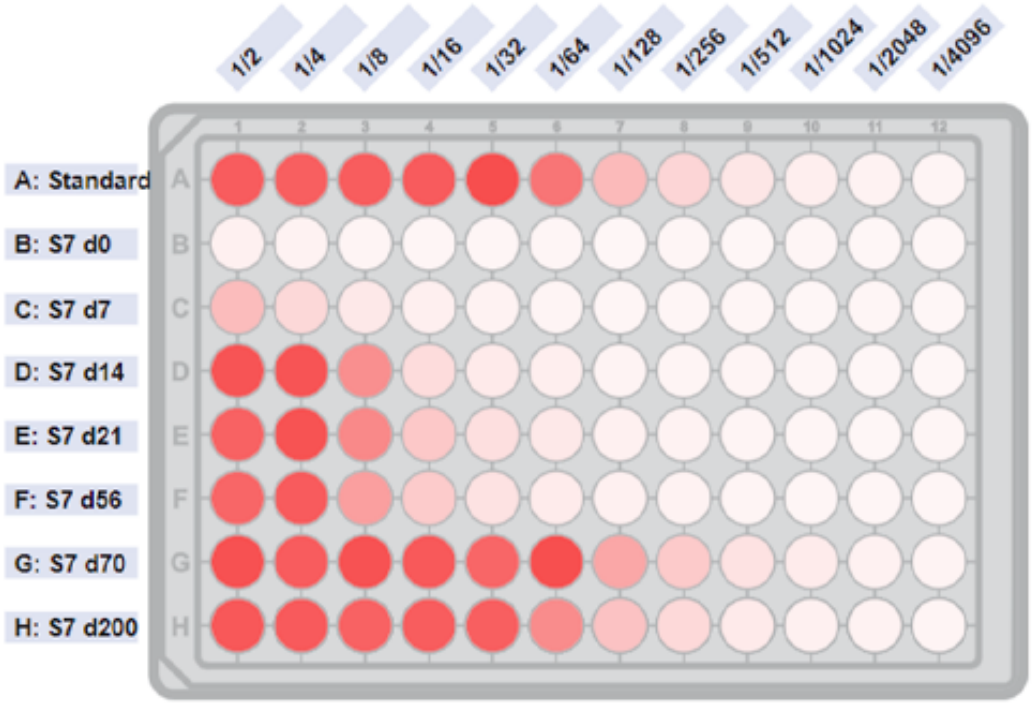


Figure 4 Example of IgG S-antibody detection in a subject (S7) from a vaccination trial.

Notice how IgG antibody titres rise in the 21 days after the first injection at day 0 and then rise sharply following the second injection at day 56. The levels only decline slightly over the following 6 months (d200). The two samples that you have investigated have a very similar time-course profile to this example although the peak titre at day 70 does vary between individuals.

- What was the titre of IgG antibodies to spike protein at day 70 in the two subjects you measured?
- The titres determined at day 70 were: S1 = 800 and S2 = 1600. The titres you measured should lie within one well of these values. For example, you might have estimated S1 titre as 512 or 1024, if you did a doubling dilution series.

If time allows, you could also look at what happens to IgM or IgA titres in the two subjects. We expect IgM to appear slightly before IgG following the primary injection and decline quite quickly. The titre of IgA is likely to follow quite closely the appearance of IgG, but at a lower titre, because the vaccination was given by intramuscular injection, which tends to induce IgG, rather than IgA.

2.2 Comparison of vaccines

As noted previously, it is difficult to directly compare vaccines which have undergone phase-3 trials in different countries or at different times. However it is possible to make some comparisons between vaccines in defined studies. Figure 5 shows antibody production produced by the Oxford/AstraZeneca (AZ) and Pfizer/Biontech vaccines in different age groups and in people with previous SARS-CoV2 infection. Antibodies were measured by an assay that is similar to the ELISA you have used (called RocheS) and the

results on the y-axis are expressed as titres. The results are shown as 'violin plots', where the width of the 'violin' at any particular titre reflects the number of individuals having that level of antibody.

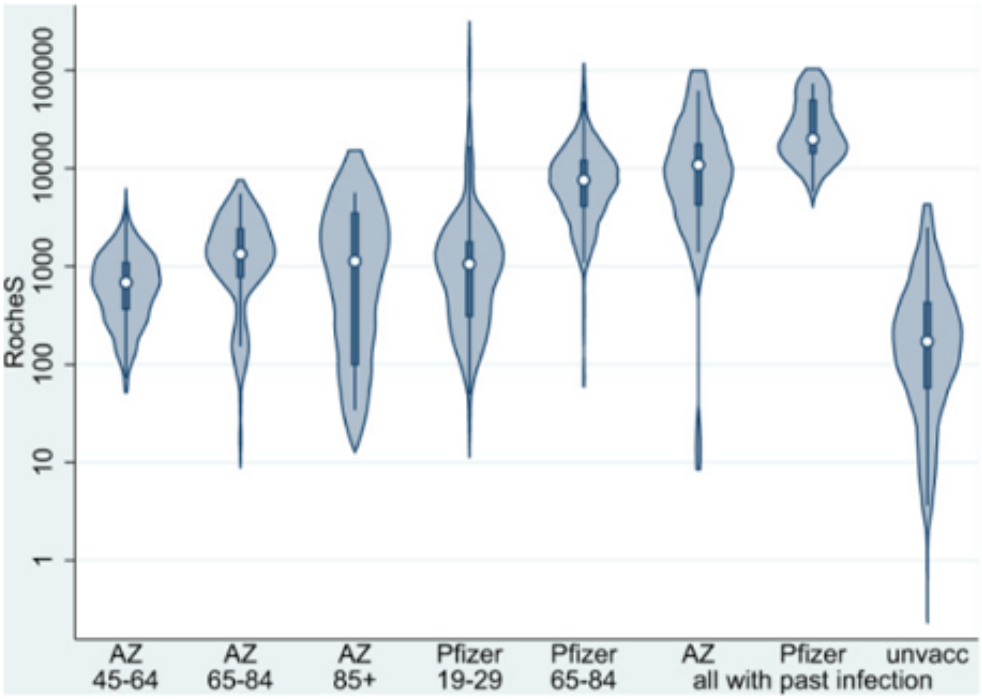


Figure 5 Antibody responses after two doses of ChAdOx1 (AZ) or BNT162b2 (Pfizer) in different subject groups. (Note that the plot of antibody titres is logarithmic.)

- In the age 65-84 age group, which vaccine produced the stronger antibody response? What is the median titre, indicated by the white dot at the centre of the violins.
- In this age group, the AZ vaccine has a median titre of just over 1000. The Pfizer vaccine has a median titre just below 10,000. So the Pfizer vaccine produces a stronger antibody response in this study.

- What effect does vaccination have on antibody titres in people who have been previously infected?
- Both vaccines produce approximately a 100x increase in antibody titres in previously infected individuals, in comparison with previously infected, unvaccinated subjects. This result demonstrates the value of vaccination, even in individuals who have had a natural COVID-19 infection.

3 Vaccination programme

The vaccination programme against COVID-19 in the UK started in January 2021, with priority given to older people and those with weak or suppressed immune systems. Over the following 6 months the programme worked down through the age groups and then paused in late Summer, as there was some debate about whether vaccination was necessary for school-age children. The programme was taken up again later in 2021 for the younger age groups, partly because a wave of COVID-19 infections had start to spread in the community from July 2021. Also by Autumn, the benefits of vaccination in children were becoming clearer – although COVID-19 is not usually a serious disease in children, preventing infection and disruption to their education was at least as important as protection against disease.

In the UK the Pfizer/Biontech mRNA vaccine and the Oxford/AstraZeneca vector vaccines were both used in the initial vaccination programme. Later the mRNA vaccine from Moderna was also used. All of the initial vaccination schedules required two doses of vaccine spaced at an interval of 3-10 weeks. For booster doses, (3rd injection) which started in October 2021, only the mRNA vaccines were used. Hybrid vaccination – when a person is immunised with one type of vaccine and then boosted with another, was found to be just as effective as using the same vaccine throughout. The uptake of vaccination in the UK is shown in Figure 6.

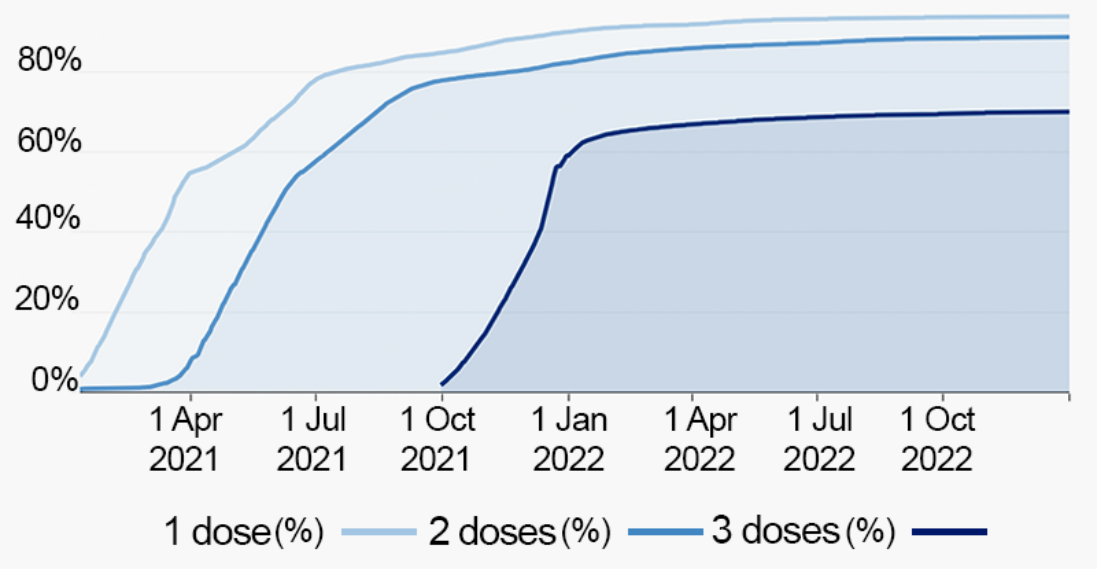


Figure 6 Percentage of the UK population who received 1st, 2nd and booster doses of COVID-19 vaccines.

It should be emphasized that the choice of vaccines to use, the prioritisation and scheduling of the programme was made pragmatically. In the early stages the supplies of vaccines were limited and the ability to deliver them in the community was dependent on the availability of suitably qualified staff who could carry out the immunisation procedure. In these conditions, decisions were taken to vaccinate those most vulnerable to severe disease, protect the health service from being overwhelmed and maximise benefit to the community, by reducing spread of infection.

3.1 Boosters and the duration of immunity

As you may have noted from your laboratory investigation, antibody titres decline gradually after 6 months. This is partly due to the progressive loss of antibodies from the serum and partly because the antibody producing plasma cells stop production and die after 1 or 2 months. To maintain protection, it is necessary to give booster doses of vaccine. How often boosters are needed varies depending on the infectious agent.

A major problem with SARS-CoV2 and influenza-A is that the viruses mutate regularly. Mutation particularly affects the external proteins involved in attachment to target cells, ie the spike protein of SARS-CoV2 and the haemagglutinin of influenza-A. Mutation of these proteins means that protective antibodies against them may no longer bind; the degree to which this occurs varies between individuals.

At this point, it is worth repeating that protection against reinfection is primarily due to antibodies, but protection against disease involves T cells as well as antibodies. The antibodies mostly recognise epitopes exposed on proteins on the surface of the virus, but T cells recognise peptides (presented on MHC molecules) which may come from any part of the viral structural proteins, or even from non-structural proteins expressed in an infected cell. Consequently a variant that has evaded recognition by antibodies, may still be recognised by memory T cells.

Next week will look at viral variants and how they evade immune responses.

4 Week 7 quiz

Well done for reaching the end of Week 7. Check what you've learned by taking the end-of-week quiz.

Week 7 practice quiz

Open the quiz in a new window or tab then come back here when you've finished.

5 Summary

This week we covered vaccination against SARS-CoV2 and the use of therapeutic antibodies for passive immunisation. Therapeutic antibodies are only used for vulnerable patients and for people with very serious illness.

Antiviral vaccines can be made from inactivated virus or viral components. The most recent vaccines use genes encoding viral components incorporated into a vector, or as messenger RNA (mRNA). The vector vaccines and mRNA vaccines are a more recent technology, but they were the first ones to be approved for general use.

More than 50 different COVID-19 vaccines went through trials in humans. Phase-1 trials assess vaccine safety in healthy individuals; phase-2 trials are on a larger scale and include different populations and demographics; Phase-3 trials assess vaccine effectiveness in real-world situations. Effectiveness is assessed as the percentage reduction in infection in the vaccinated group, compared with an unvaccinated group, although other measures of effectiveness may also be assessed, such as reduced number of disease cases or reduced severity of disease.

The vaccination programme in the UK was rolled out in 2021, prioritising older people and medically vulnerable individuals. As immunity was gradually lost, booster doses of vaccine were needed to maintain protection. Viral variants can evade protection produced by antibodies against previous variants, but there is still considerable protection against serious illness.

Now go to Week 8.

Week 8: Variants and immunity

1 Variants of SARS-CoV2

At the start of the pandemic, there was much debate as to whether the original SARS-CoV2 virus, first identified in Wuhan, would mutate to produce new strains. Some viruses, (eg measles) are remarkably stable; the measles vaccine is 97% effective and has not required any substantial modification for many years. In contrast, other viruses such as influenza-A mutate often and it is necessary to produce new vaccines for the new strains each year.

Viruses with an RNA genome tend to mutate more rapidly than those with a DNA genome, and those with a segmented genome such as influenza-A, can also change radically by a process called recombination. Since coronaviruses have an RNA genome, we might therefore have reasonably expected some new variants of SARS-CoV2 to arise.

It turned out that SARS-CoV2 did mutate extensively, but only some of the variants produced were sufficiently advantaged compared with earlier strains, that they were able to spread in the community. Notice that here we are considering changes that could be advantageous for the virus. Viruses evolve in much the same way as other 'life-forms' and a strain with a genetic advantage will eventually replace earlier strains.

- Suggest three factors which could give a mutated virus an advantage over an earlier strain.
- The new strain might infect cells more effectively. It might go through its replication cycle more quickly. The assembly of the virus in infected cells or its release could be more efficient. The new strain might be able to evade the antibodies produced by an older strain.

Notice that production of disease was **not** included in this list. A virus that produces an asymptomatic infection might actually have some advantage, because infected people continue to circulate unknowingly in the community, thereby promoting virus spread. However the production of some symptoms such as coughing or sneezing may be advantageous for a respiratory virus, because they promote production of aerosol droplets that transmit the virus.

It is not simple. As you read through the following sections on new variants and virus evolution, think in terms of what is advantageous for the virus, rather than its human hosts.

1.1 Genomic sequencing

Variants of SARS-CoV2 are detected by genomic sequencing – analysis of the full gene sequence of a virus isolate. Recall that the genome of SARS-CoV2 is 28,000 bases long and it encodes 4 structural proteins and a number of non-structural proteins (Figure 1).

Figure 1 Diagram of the genome of SARS-CoV2 and encoded proteins. (Repeated from Week 1 Figure 8)

Genomic sequencing takes considerably longer than detecting SARS-CoV2 infection by a lateral flow test or PCR. Once a positive sample has been identified, the virus genome is first extracted from the sample, isolated and then sequenced in segments. The sequences of overlapping segments are then assembled by computer, to produce the complete genome sequence of a variant. This process typically takes at least 2 days, but it is essential for tracking the appearance of new variants. At the time of writing (Jan. 2023) the Sanger Institute, which tracks variants in the UK, had sequenced 2.3 million SARS-CoV2 viral genomes.

1.2 Variation in the SARS-CoV2 genome

Mutations in the SARS-CoV2 genome can occur in any of the structural or non-structural genes. For example, the alpha variant (1/Dec/2020) showed 23 mutations from the original strain. 17 of the mutations produced changes in the amino acid sequence of the encoded proteins and 6 did not. Table 8.1 shows the position and type of the 17 ‘non-synonymous’ mutations.

Table 8.1 Non-synonymous mutations in the SARS-CoV2 alpha variant.

Gene(s)	Amino acid
Nsp1 – Nsp16	T1001I
	A1708D
	I2230T
Spike	SGF 3675-7 del.
	HV 69-70 del.
	Y144 del.
	N501Y
	A570D
	P681H

	T716I
	S982A
	D1118H
ORF 8	Q27stop
	R52I
	Y73C
Nucleocapsid	D3L
	S235F

Mutations in the gene sequence are described according to their position and the effect they have on the amino acid sequence. For example the mutation C3267T that occurred at position 3267 in the gene-sequence was a change from a cytosine base (C) to a thymidine (T). This mutation caused a consequent change in the amino acid sequence of the Nsp1-16 protein, T1001I, meaning that at position 1001 of the protein, a threonine residue (T) has been replaced with isoleucine (I). Notice also that there are 3 deletions in the gene encoding the spike protein that have produced deletions (del.) in the protein of 1-3 amino acids.

The key point to take from this table is that the gene encoding the spike protein, which is 10% of the virus genome has more than 50% of the total mutations. In other words, mutation in new variants-of-concern tends to be clustered in the spike protein. Moreover the genome of the variants continued to mutate after it was first identified and sequenced. For example the mutation E484K affecting the spike protein occurred after the initial sequencing of the alpha variant.

Some of the mutations that occurred in one variant appeared in other variants. For example N501Y present in the alpha variant also arose independently in the beta and gamma variants, which implies that this mutation confers some selective advantage in different strains.

1.3 Mutation in the spike protein

As noted previously, mutations tend to cluster in the gene encoding the spike protein, but even there they are not evenly distributed. For example compared with the original Wuhan strain, Omicron BA.1 has a lot of mutations (15) in the receptor-binding domain (RBD), of which five have been shown to enhance binding to the ACE2 receptor. This strain also has a lot of mutation in the N-terminal domain (NTD) , 4 mutations, 3 deletions and one insertion (Figure 2).

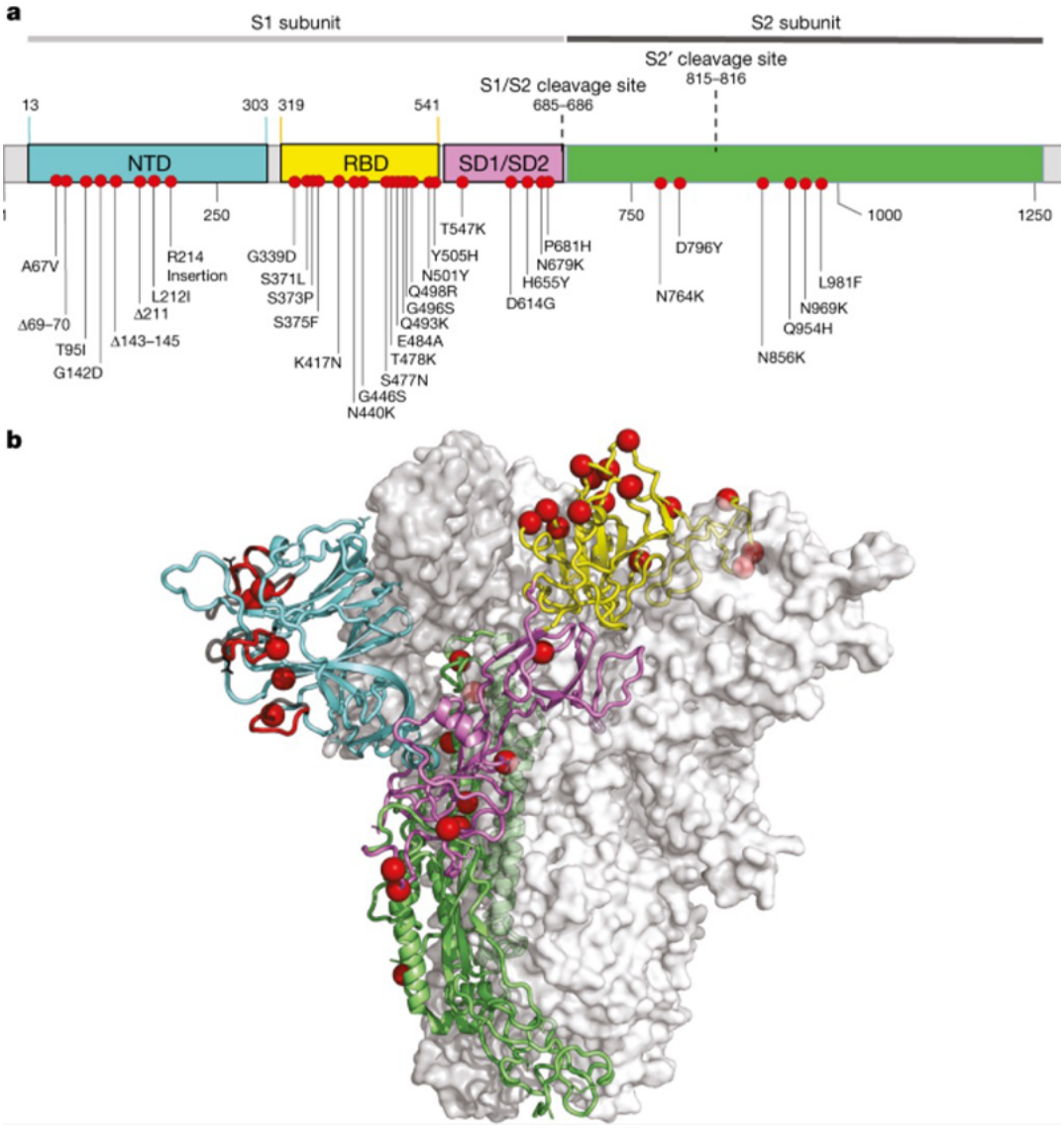


Figure 2 (a) Mutations in the gene encoding the spike protein of the omicron BA.1 variant, compared with the original Wuhan-Hu strain. (b) The position of these mutations (red dots) on a model of the spike protein shows the cluster of mutations in the receptor-binding domain (RBD) which is shown in yellow.

Many of the neutralising antibodies bind to the RBD and NTD regions of the spike protein, so these mutations have allowed the omicron BA.1 variant to substantially evade the antibody response produced by previous variants. Since different people produce antibodies to different regions of the spike protein, the extent to which new variants evade antibodies produced by earlier strains varies between individuals.

2 Variants of concern

Thousands of different genetic variants of SARS-CoV2 have been sequenced. A number of these are identified by the World Health Organisation (WHO) as variants of interest (VOI), perhaps because they have spread locally or have mutations that are characteristic of pandemic strains. If a strain spreads more widely it may be designated as a variant of concern (VOC).

- The four characteristics of a VOC were introduced in week 4 of this course. What are they?

-
  - Increased transmissibility
  - More severe disease
  - Reduced effectiveness of treatments or vaccines
  - Failure to be detected by current diagnostic tests

A VOC strain does not necessarily have all of these characteristics. For example the omicron strain has greatly increased transmissibility over previous strains, but produces less severe disease than the delta strain. Also none of the VOCs to date have been able to completely evade the PCR detection tests or the lateral flow tests.

One common feature of later VOCs was that they could partly evade antibodies produced by infection with earlier strains or the initial vaccine formulations which were based on the sequence of the Wuhan strain spike protein. In addition, mutations that allowed a strain to replicate more quickly or spread more effectively, provided a selective advantage and were seen in later VOCs. The effect of increased transmissibility is seen in table 8.2, which gives estimated R_0 values for different VOCs. Notice that there is a range of estimated values due to the difficulties of estimating R_0 in different populations, discussed earlier.

Table 8.1 Estimated values of R_0 for different SARS-CoV2 variants.

Variant	R_0
Wild-type (Wuhan)	1.4 – 2.5
Alpha	2.8 – 6.4
Beta	2.1 – 3.8
Gamma	2.5
Delta	5.2 – 6.7
Epsilon	1.2 – 1.4
Omicron BA.1	8.2

Remember that R_0 is the rate of spread in a totally susceptible population, so these values represent the basic transmissibility of the different strains. It is noticeable how the alpha delta and omicron strains which caused major waves of infection in the UK all have

increasingly high R_0 values. In comparison the gamma and epsilon variants, which did not spread in the UK, have R_0 values similar to the original Wuhan strain.

2.1 Succession of variants

Each of the VOCs has gradually replaced previous variants as the dominant strain in the pandemic although this has occurred at different times in different countries. This effect can be seen in the spread of the alpha variant, which was first identified in the UK in Kent in November 2020 from a sample taken in September. The variant spread rapidly in the UK and by January 2021 it accounted for more than half of the identified genomic sequences. By October 2021 it had disappeared from the UK, replaced mostly by the delta variant. By March 2022 the alpha variant was declared to be extinct worldwide (Figure 3).

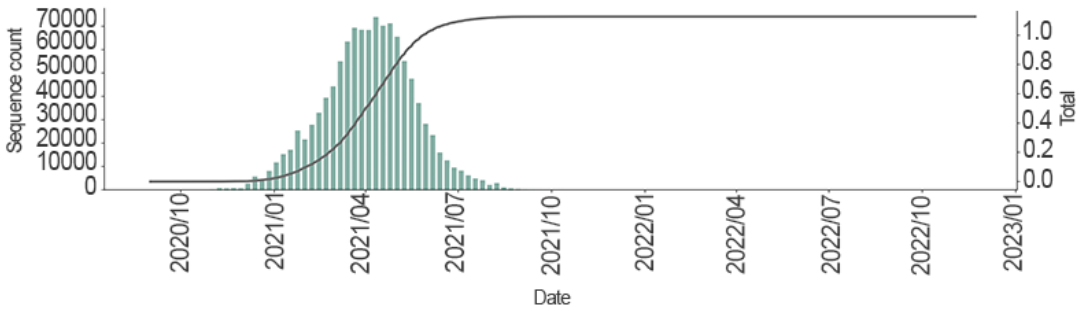


Figure 3 Cumulative sequence count of the alpha variant (B1.1.7) over time. The bars show the weekly number of identified alpha variant sequences and the line shows cumulative number.

2.2 Origin and spread of variants

New VOCs are usually first identified in the country where they originated. However this depends on the availability of good genomic sequencing facilities and a programme of surveillance. For example, the gamma variant was first identified in Tokyo on Jan. 6th 2021, in 4 travellers arriving from Brazil. Presence of the variant was confirmed in Brazil on Jan. 12th 2021 and retrospective analysis of samples implied that it had been circulating widely in Manaus (Brazil) since December 2020.

It is effectively impossible to identify exactly where and who was the first person to develop a new VOC. Variants are most likely to arise where large numbers of people are infected, but this is merely based on probability. Also it has been conjectured that variants are more likely to occur in people who have extended infections, possibly because they have a weak immune response. The delay in clearance of the virus in an immunosuppressed individual could allow the virus more time and opportunity for advantageous mutations to emerge.

It is interesting to note that it was 1 year (2020) before the first VOC (alpha) emerged, and in this year the number of infections was relatively low, so there was less opportunity for variants to develop, and there was less selective pressure on the virus from immunity in

the host population. Several VOCs emerged in 2021 and 2022 as more people became infected.

The rate of spread of pandemic strains of disease has historically been dependent on the fastest form of transport available. For many previous centuries this has been ships and the original term quarantine refers to the isolation of ships carrying (or potentially carrying) infected individuals, for 40 days. More recently, air transport has been the means for the rapid spread of new strains of viruses, including influenza-A, SARS, MERS and SARS-CoV2.

Data on the alpha variant shows how it initially spread in Western Europe and North America with later spread to Asia, South America and Africa (Figure 4). There are however some exceptions such as the early identification of the variant in India. This may reflect the travel links that these countries have with the UK, and it may also relate to the effectiveness of their genomic surveillance programmes.

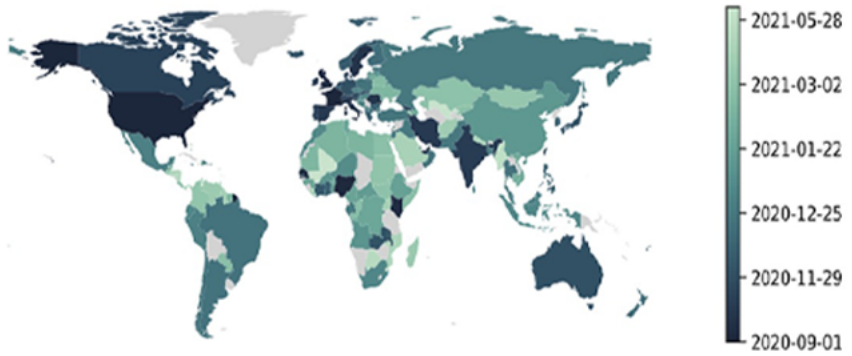


Figure 4 Map showing the date of the first identified alpha variant genomic sequence in different countries. Darker countries have earlier sample dates. This variant spread between September 2020 and May 2021.

2.3 Lineages of SARS-CoV2

Using genomic data, it is possible to reconstruct the lineages for SARS-CoV2, which are analogous to evolutionary family trees (Figure 5). This diagram shows each genome as a single point. The distance of each genome from the origin, reflects the number of mutations that have occurred since the first genome was published. The distance from other points, reflects the genetic distance between them. The lineage was constructed by estimating the fewest number of mutations needed to reach the given genome.

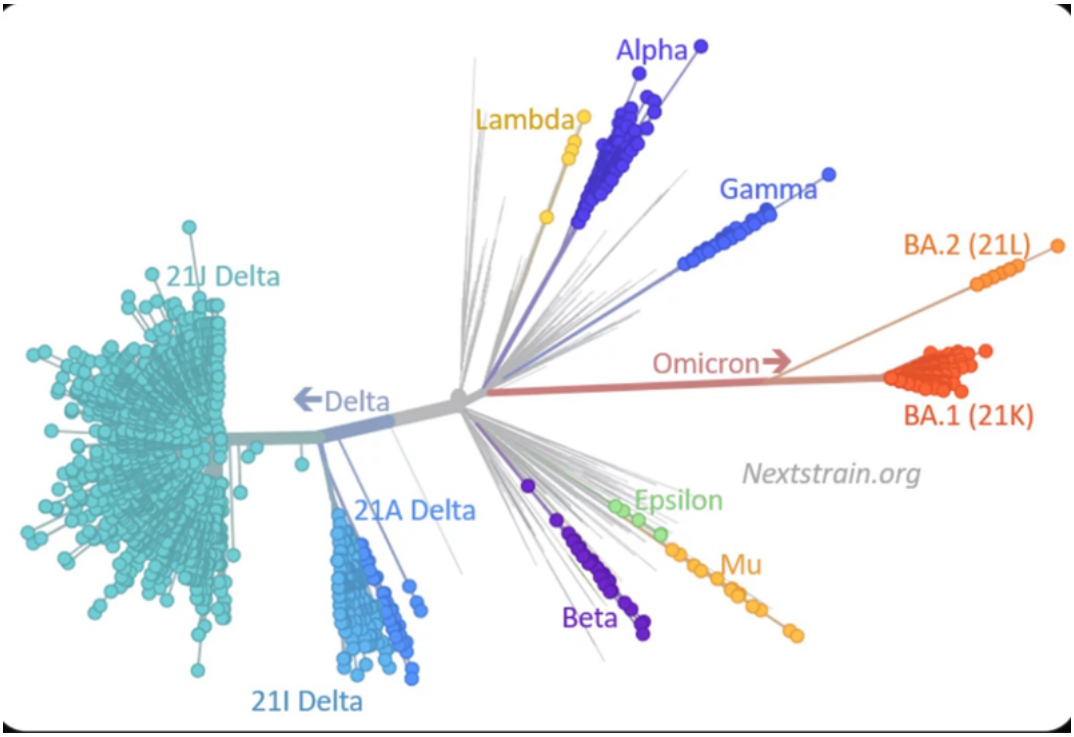


Figure 5 Lineages of SARS-CoV2. The original genome lies at the centre of the branches, identified by a grey dot. Separate lineages have been identified within single VOCs, such as 21A, 21I and 21J in the Delta variant.

The diagram illustrates some interesting points about the evolution of the virus.

- The progressive accumulation of mutations seen in variants such as Gamma.
- The independent development and great diversity (branching) of the Delta variants.
- The VOCS which spread most rapidly (alpha, delta, omicron) also show the greatest diversity.
- A large number of tracked non-VOC lineages arising from the original Wuhan strain.

It is likely that the virus will continue to evolve and produce new strains over the next few years.

2.4 Virus evolution

What drives the evolution of a virus? Different strains of a virus are subject to two main types of selective pressure. In a susceptible host population, a strain that can spread more quickly and/or reproduce more quickly and efficiently has a selective advantage over other strains. The second pressure on a virus is created by immunity in the host population, which may come from infection or vaccination.

With SARS-CoV2, we have seen both of these selective pressures acting on the virus. The increase in R_0 values in later variants indicates that the virus progressively adapted to the human population after it had initially jumped species from bats. The independent appearance of new variants that could evade the immune response against earlier variants, is evidence of the selective pressure produced by immunity in individuals and the population as a whole.

It is worth noting here that a virus cannot mutate indiscriminately and still retain its ability to bind to target cells and replicate. Some viral structural components and enzymes must

be retained. Immune responses (antibodies or T cells) recognising these less-variable viral components are likely to confer some immunity that acts across different strains.

In the last part of the course, we will look in more detail as to how variants have partly evaded immunity produced by previous infections and how vaccines can be modified to provide continued protection against infection and disease.

3 Evasion of immunity

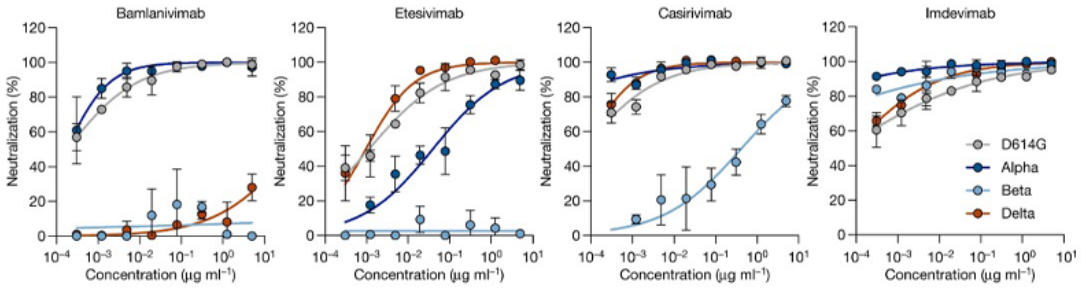
As new variants of SARS-CoV2 emerged a major concern was that any new variant would completely evade the immune response produced by infection or by vaccination. The loss of immunity would lead to faster spreading infection, more serious disease, the necessity to reformulate vaccines and require new vaccination programmes.

As suggested previously, the changes that have occurred in the spike protein have allowed new variants to avoid recognition by antibodies. This is seen most dramatically with the monoclonal antibodies that are used therapeutically.

3.1 Evasion of therapeutic antibodies

Recall that monoclonal antibodies recognise just one position (epitope) on an antigen. If a mutation occurs in the epitope recognised by a monoclonal antibody then there is a high likelihood that the antibody will no longer recognise the epitope or bind to it much less well. Also recall that the therapeutic monoclonal antibodies were mostly selected to bind to the RBD of the spike protein, and this is the region that mutates most often.

Figure 6 shows neutralisation curves for 4 therapeutic monoclonal antibodies for different SARS-CoV2 variants. The antibodies were raised against the original strain (D614G). In these graphs the concentration of serum is plotted against its ability to neutralise the virus *in vitro*. If a low concentration of serum causes good neutralisation (curve to the left of the x-axis) then it demonstrates that the antibody is effective against that variant. Notice how the original wild-type strain (D614G) and alpha strains are neutralised by Bamlanivimab, but the beta and delta strains are not. Etesivimab is effective against the D614G and delta strains, partly effective against alpha and ineffective against beta. Casirivimab is effective against D614G, alpha and delta, but has weak activity against beta. Imdevimab is effective against all four strains.



Neutralization curves of monoclonal antibodies. Dose–response analysis of neutralization of the D614G strain and the Alpha, Beta and Delta variants by four therapeutic monoclonal antibodies (bamlanivimab, etesivimab, casirivimab and imdevimab). Data are mean ± s.d. of four independent experiments.

§

Figure 6 Neutralisation curves of four different monoclonal antibodies, versus four different variants of SARS-CoV2.

As some of the therapeutic monoclonal antibodies were seen to be ineffective against later virus variants, they were either withdrawn from use, or combined with other monoclonal antibodies, so that the new variant was less likely to be able to evade both of the therapeutic antibodies.

3.2 Evasion of natural antibody-mediated immunity

The antibodies produced by a natural SARS-CoV2 infection or by a spike protein vaccine, are polyclonal; they are produced be many different clones of B cells and usually they will

recognise a variety of different epitopes on the spike protein. It is therefore unlikely that a new variant of the spike protein could be so different from an earlier variant that it could completely evade the antibodies produced against an earlier variant or vaccine formulation. However each person produces a different spectrum of antibodies against epitopes on the spike protein, and virus variants will be better able to evade immunity in some people than others (Figure 7)

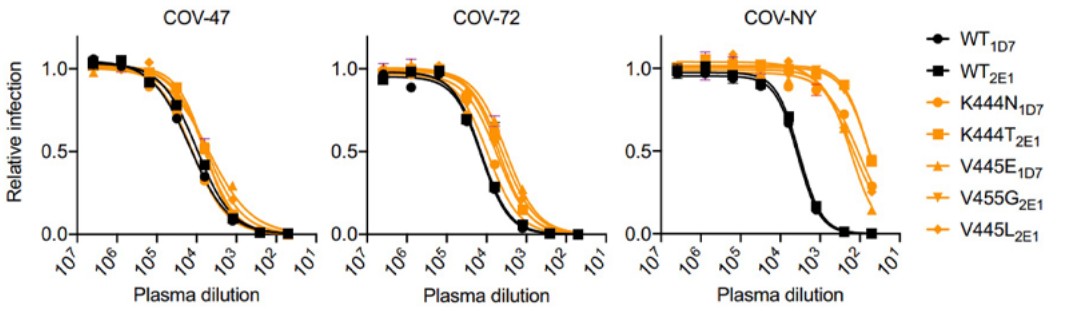


Figure 7 The ability of plasma from 3 different convalescent individuals (COV-47, COV-72, COV-NY) to neutralise laboratory-mutated variants of SARS-CoV2

The three subjects had been naturally infected with an original (wild type (WT)) strain of the virus. In these plots a high dilution of plasma relates to a low concentration of plasma antibodies. The assays were carried out using two wild-type viruses (WT_{1D7} and WT_{2E1}) which had been mutated in two positions (444 and 445) in the spike protein. The plasmas from COV-47 and COV-72 were almost as effective in neutralising mutated virus (orange lines) as the original virus (black lines). However the antibodies in COV-NY plasma were much less effective at neutralising the mutated variants- it requires 100x more antibody to produce a similar level of neutralisation. This result shows that a substantial amount of antibody in COV-NY plasma is binding to an epitope on the spike protein that involves amino acids 444 and 445.

This variation between people in antibody production may explain why some people have been more susceptible to reinfection than others. Variation between individuals also applies to susceptibility to infection following vaccination.

3.3 Persistence of immunity

Recall from week 2, that there are two major components of immune defence against viruses – T cells recognise and destroy virus-infected cells, whereas antibodies prevent virus spread within the body. Antibodies are also important for preventing reinfection, while T cells limit the damage produced by an infection and consequently reduce disease severity.

It has been noticeable during the pandemic, that many people have become reinfected with new strains of the virus, but generally the new infections have produced less-serious disease symptoms. This suggests that T cell immunity persists well, even if the new strains can evade antibody-mediated immunity.

The half-life of antibodies in plasma is a few weeks, depending on the class of the antibody. So there is a natural decline in immunity produced by antibodies. However the long-term ability to make antibodies and the ability of T cells to recognise virus-infected cells lies mostly in the memory populations of T and B cells. So some level of immunity lasts much longer than the antibodies.

At this time (2023) we cannot know how long immunity to SARS-CoV2 would last if there were no reinfections. However it appears that new variants will continue to develop as the disease becomes endemic and less serious. Consequently previously infected or vaccinated individuals will be subjected to regular restimulation of their B cells and T cells and will always have some immunity. This can be enhanced by boosters with vaccines against new variants.

In the final section of the course, we look at the prospects for adapting current vaccines to deal with new variants.

3.4 Adapting vaccines

The majority of the COVID-19 vaccines are designed to induce an immune response against the spike protein. The mRNA vaccines have been particularly successful in this regard. Moreover, it is possible to modify an mRNA vaccine quite quickly; the gene sequence encoding the spike protein of any new variant can be rapidly identified and the new sequence is then used in the vaccine. This approach has been taken by both Pfizer/Biontech and Moderna. In January and February 2022 both companies produced a 'bivalent vaccine' which included both mRNA of the original spike protein and mRNA for the omicron BA.1 spike protein. These formulations were approved in the UK in August 2022 and used in the Autumn booster programme that year. Additional bivalent vaccines have since been developed (Figure 8).

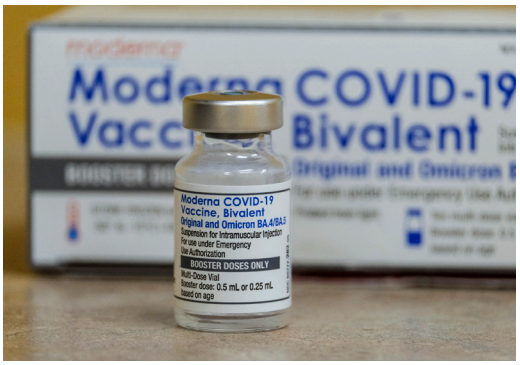


Figure 8 Example of a bivalent Moderna COVID-19 mRNA vaccine against the original strain and Omicron BA.4 and BA.5 strains.

New formulations are screened for safety and monitored in use to see whether they produce more adverse reactions than the original vaccine.

The vector vaccines against spike protein can also be modified relatively quickly, by inserting a gene for the new sequence into the vector. Vector vaccines have some advantages in that they are cheaper to produce and require a simpler cold-chain than mRNA vaccines. However, there is the possibility that an immune response will be produced against components of the vector itself, which means that successive boosts with a vector vaccine become less effective.

Production of a variant spike protein, to use in a component vaccine is certainly possible, but likely to be more complicated and time-consuming than modifying an mRNA or vector vaccine.

Finally, one must not neglect the potential use of inactivated virus vaccines. Although these were the last to come through the development process during the COVID-19 pandemic, they do have one potential advantage. Since they include all the protein

components of the virus (including the core structural proteins that mutate less) they may be more effective at producing cross-strain immune responses.

An important final consideration is not just how quickly a variant-specific vaccine can be made in the laboratory, but also how long it takes to scale-up production. Clearly no-one wants to commit to the production of large quantities of vaccine unless it is genuinely necessary. For future years we can anticipate that new COVID-19 booster vaccines will be produced and given selectively to more vulnerable groups, much as influenza-A vaccines have in the past.

4 Week 8 quiz

Well done for reaching the end of Week 8.

Now it’s time to complete the Week 8 badged quiz. It’s similar to previous quizzes, but this time instead of answering five questions there will be fifteen, covering material from the last four weeks of the course.

Week 8 compulsory badge quiz

Remember, this quiz counts towards your badge. If you’re not successful the first time, you can attempt the quiz again in 24 hours.

Open the quiz in a new window or tab then come back here when you’ve finished.

5 Summary

Since it first appeared, the SARS-CoV2 virus has evolved and new pandemic strains have emerged. Many 1000s of variants have been identified by genome sequencing, but only a few of them are designated as variants of interest (VOI) , and fewer still variants of concern (VOC), that spread widely or produce particularly serious disease. New pandemic strains can displace earlier strains if they can replicate more efficiently, evade immune responses, or spread in the community more effectively.

Genetic variation can occur throughout the genome, but it particularly affects the spike protein, because changes in the spike protein allow the virus to attach more effectively to target cells and/or evade antibodies.

Using genomic sequences, it is possible to reconstruct lineages for the different VOCs. The lineages show how the alpha, delta and omicron variants, which have high R_0 values, have also diversified considerably. As new VOCs have emerged, they spread across the world from their point of origin, eventually replacing previous VOCs, which have gone extinct in the general population.

New VOCs have partially evaded antibody-mediated immunity produced by previous strains or by the earlier vaccines. This means that reinfection with new strains does occur. However the residual antibody and T cell immunity still provides some protection, so that any disease from infection, is much less serious in a person who has been previously infected or vaccinated. Booster doses of vaccine can help maintain this residual immunity, and vaccines are being reformulated to include antigens from later variants.

Audio content is not available in this format.

Audio 2 Valedictory audio

Now you've come to the end of the course, we would appreciate a few minutes of your time to complete this short end-of-course survey (you may have already completed this survey at the end of Week 4).

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Week 2

Figures

Figure 1: adapted from: David Male, R. Stokes Peebles, Victoria Male (eds) (2020), (fig 1.15) in 'Immunology 9th edition', Elsevier

Figure 2: adapted from: David Male, R. Stokes Peebles, Victoria Male (eds) (2020), (fig 6.9) in 'Immunology 9th edition', Elsevier

Figure 5: adapted from David Male, R. Stokes Peebles, Victoria Male (eds) (2020) (fig10.1) in 'Immunology 9th edition', Elsevier

Figure 7:adapted from Roitt, I. (1998) 'Primary and secondary antibody responses', Immunology, 5th edn. Mosby International Ltd

Figure 10: adapted from Roitt, I. et al. (1998) Immunology, 5th edn. Mosby International Ltd

Audio Visual

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Video 4: Virus infection and immune responses 'Immunology Interactive 3.0' by Professor David Male, Professor Jonathan Brostoff and Professor Ivan Roitt. Copyright © David Male. Courtesy David Male

Week 3

Figures

Figure 1: adapted from: David Male et.al (eds) (2020) in 'Immunology 9th edition' published by Elsevier

Figure 2: adapted from: David Male et.al (eds) (2020) in 'Immunology 9th edition' published by Elsevier

Audio Visual

Video 1: ELISA laboratory video guide: courtesy: David Male © David Male

Week 4

Figures

Figure 1: SARS-CoV2 VOCs timeline: courtesy: David Male

Figure 2: Daily confirmed COVID-19 cases in the UK: adapted from: https://coronavirus.data.gov.uk/details/healthcare?areaType=nation&areaName=England https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Figure 3: COVID-19 vaccination heat map.: adapted from: https://coronavirus.data.gov.uk/details/vaccinations?areaType=nation&areaName=England https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Audio Visual

Video 1: ELISA laboratory video guide: courtesy David Male © David Male

Week 5

Figures

Figure 1: Daily incidence of SARS-CoV2 infections in the entire population of the UK adapted from: https://coronavirus.data.gov.uk/details/healthcare?areaType=nation&areaName=England https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Figure 2: The protein components of plasma. Courtesy: David Male

Figure 3: Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study Prof Marina Pollán, MD,(Figure 2) in: Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study - The Lancet

Figure 4: Seroprevalence of IgG antibodies to spike protein in UK blood donors: https://www.gov.uk/government/publications/national-covid-19-surveillance-reports/sero-surveillance-of-covid-19 https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Week 6

Figures

Figure 4: Estimated values of RE in the UK: https://coronavirus.data.gov.uk/details/healthcare?areaType=nation&areaName=England https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Audio Visual

Video 1: Introduction to week 6: © The Open University. Images: Getty images

Week 7

Figures

Figure 3: Schedule of a phase-1 vaccine trial. Courtesy David Male

Figure 5: from Wei, J., Pouwels, K.B., Stoesser, N. et al. Antibody responses and correlates of protection in the general population after two doses of the ChAdOx1 or BNT162b2 vaccines. Nat Med (2022).Open Access Published: 14 February 2022

Figure 6: from https://coronavirus.data.gov.uk/details/vaccinations?areaType=nation&areaName=England
https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

Audio Visual

Video 1: Detecting antibodies against SARS-CoV-2. Courtesy David Male © David Male

Week 8

Figures

Figure 2: (a)and (b) in Viana, R., Moyo, S., Amoako, D.G. *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature* **603**, 679–686 (2022). https://doi.org/10.1038/s41586-022-04411-y open access

Figure 3: from Figure 1 | Cumulative sequence count over time B.1.1.7 in https://cov-lineages.org/global_report_B.1.1.7https://cov-lineages.org/

Figure 4 from Figure 2 | Date of earliest_B.1.1.7 detected in https://cov-lineages.org/global_report_B.1.1.7 https://cov-lineages.org/

Figure 5:
https://www.npr.org/sections/goatsandsoda/2022/02/09/1047616658/take-a-look-at-sars-cov-2s-family-tree-its-full-of-surpriseshttps://www.npr.org/
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Figure 6: from Fig. 1: Neutralization of the SARS-CoV-2 variants D614G, Alpha, Beta and Delta by therapeutic monoclonal antibodies. Planas, D., Veyer, D., Baidaliuk, A. et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 596, 276–280 (2021). https://doi.org/10.1038/s41586-021-03777-9

Figure 7: in Weisblum Y, Schmidt F, Zhang F, DaSilva J et al. Escape form neutralizing antibodies by SARS-CoV-2 spike protein variants. eLife 9:e61312. https://elifesciences.org/ articles/ 61312 This article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.

Figure 8: Photo by RINGO CHIU/AFP via Getty Images

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Glossary

adaptive immune system

The adaptive immune defence refers to the tailoring of an immune response to the particular foreign invader. It involves differentiating self from non self and involves B cells and T cells (lymphocytes). A key feature of the adaptive immune system is memory. Repeat infections by the same virus are met immediately with a strong and specific response.

antigen

Originally defined as any molecule which the body recognised as ‘non-self’, and against which an antibody was produced. This definition was extended to include any

molecule that the body could recognise as foreign. This includes the fragments of molecules that are recognised by T lymphocytes. In the broadest sense, it has always been known that the immune system can recognise self molecules, even if it does not usually react against them. Consequently, the widest definition of an antigen is a molecule that can be recognised by the immune system, of which there are conventional non-self antigens and self molecules or autoantigens.

anti-viral proteins

A group of proteins that are induced by interferon, which when activated, inhibit protein synthesis and viral replication.

chronic

One that continues to produce disease symptoms and tissue damage over many months or years; some chronic infections (e.g. malaria) are characterised by periods of remission and relapse but the pathogen is never completely eliminated from the body.

cytokines

short-lived, short-range signalling molecules primarily synthesised and secreted by leukocytes that affect the activity of other cells participating in an immune response.

glycoproteins

A protein with one or more covalently attached carbohydrate groups (usually short sugar chains). Addition of such groups to proteins, termed glycosylation, is a form of post-translational modification.

innate immune system

The elements of the immune system that are continuously active and that do not depend on immune recognition of antigens by lymphocytes. Innate immune responses do not improve with repeated encounters with the same antigen or pathogen.

interferons

Cytokines that interfere with viral replication by the induction of anti-viral proteins. There are 3 main types of interferon IFNα, IFNβ and IFNγ. IFNγ, produced by active T lymphocytes and NK cells has many additional effects in controlling immune responses and inflammation.

latent

One in which the pathogens persist in or on the host’s body, but without producing symptoms; during the latent period, the host may or may not be infectious (i.e. capable of transmitting the pathogens to others).

nucleocapsid

The core of a virus containing its genetic material (DNA or RNA), within a protein coat (capsid).

receptor-binding domain (RBD)

For Sars CoV2, this is the region of the spike protein that binds to the ACE2 receptor on a cell, as the first step in virus infection of the cell.

sterile immunity

The complete elimination by the host’s immune response of the pathogens responsible for an infectious disease (e.g. the influenza virus is eliminated from the body as the illness resolves).

toll-like receptors

A group of receptors, located on the plasma membrane or on intracellular vesicles, that recognise components of pathogens (PAMPs) and transduce signals for inflammation.

type-1 interferon (IFN)

A cytokine produced by many cell types that signals to other cells to inhibit replication of viruses.

viral envelope

A phospholipid membrane that surrounds the nucleocapsid of some groups of virus. It is derived from the plasma membrane of the virus-infected cell.

viral tropism

The tendency of a particular virus to target specific cells which it can infect and then replicate within.

antigen processing

The process by which antigen is presented to lymphocytes in a form they can recognise. Most CD4+ T cells must be presented with antigen on MHC class II molecules, while CD8+ CTL cells only recognise antigen on MHC class I molecules. Antigen must be processed into peptide fragments before it can associate with MHC molecules

apoptosis

Type of cell death where particular cell populations die in a reproducible manner in every individual. Because of its predictable nature, this form of death was believed to occur as the result of a death ‘programme’, and so was named programmed cell death. Well-known examples are the loss of the cells between the digits (e.g. during the development of fingers). In adult tissues, cell death usually balances cell division, ensuring that tissues and organs retain the same size and structure as old cells are replaced. Apoptosis is a normal response in cells with DNA damage, serving to protect the body from cancer.

B cells

One of two main types of lymphocyte (cf. T cells) which, when activated, synthesises and releases huge quantities of soluble antibodies.

IgA

class of antibody that is prevalent in mucous secretions, and protects against infections in the gut, respiratory tree and genitourinary tract.

IgG

The main antibody in blood and tissue fluid. It has a large number of functions, including neutralising many toxic molecules, preventing viruses from attaching to cells, allowing phagocytes to recognise and internalise pathogens, and protecting the fetus and newborn babies. (It is the only antibody class that can cross the placenta.)

IgM

A class of antibody that is the first to be produced in an immune response.

immunoglobulins

An alternative name for soluble antibodies present in serum and tissue fluids.

immunological memory

the ability of the adaptive immune system to make an improved immune response on repeated encounters with the same antigen or pathogen.

lymphatic system

the connected system of lymphoid organs and lymphatic ducts present throughout the body.

lymphocytes

A major population of leukocytes including T cells, B cells and NK cells.

lymphoid organs

encapsulated organs such as thymus, lymph nodes and spleen and tonsils which contain collections of lymphocytes and cells involved in development of immune reactions.

lymphoid tissues

include both the encapsulated organs of the immune system (eg lymph nodes) and unencapsulated collections of lymphocytes found in mucosal tissues.

MHC molecules

A group of proteins involved in antigen presentation to T cells.

NK cells

A group of lymphocytes that have the intrinsic ability to recognise and destroy some virally infected cells and some tumour cells.

proteasome

an intracellular organelle that breaks down proteins into polypeptide fragments.

T cells

Lymphocytes that differentiate primarily in the thymus and are central to the control and development of immune responses. The principle subgroups are cytotoxic T cells (Tc) and T helper cells (Th).

Assay

A method for quantitating biological material – in this case antibodies.

ELISA

Enzyme linked immunosorbent assay, is a set of techniques used for detection and quantitation of antibodies or antigens.

incidence of infection

The number of new cases of an infection in a given number of people in a defined period of time.

notifiable diseases

Diseases that must by law be reported to health authorities.

S-antibodies

Antibodies against the SARS-CoV2 spike protein.

variants of concern' (VOC)

Variants of the SARS CoV2 virus identified by the WHO as having any combination of these characteristics: increased rate of transmission; producing more serious disease; ability to evade treatments or immune responses produced by vaccines; not being identified by current test procedures.

incidence

The number of new cases of a disease arising in a given period, usually a year, expressed as a proportion of the population at risk (the incidence rate).

N-antibodies

antibodies against the SARS-CoV2 nucleocapsid.

plasma

The non-cellular fluid component of blood containing soluble molecules, including proteins.

prevalence

The proportion of the population with a particular infection or disease at a particular point in time, or during a given period.

seroconversion

The appearance of specific antibodies in the blood serum as a result of infection or vaccination.

serology

The study of antigens and antibodies in patients' sera.

seroprevalence

The proportion of a population which have antibodies against a particular infection at a defined time point.

serum

(Plural, sera.) The part of the blood left behind after cells, platelets and fibrinogen have all been removed, usually by clotting.

Basic reproduction number (R_0)

The average number of individuals directly infected by a single typical infective if the population were totally susceptible.