Project Number: 101006468

Project Acronym: PAFSE

Project title: Partnerships for Science Education

EDUCATIONAL SCENARIOS
UNIVERSITY OF CYPRUS

AUGUST 2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101006468.

Contents:

This document contains the five topics of educational scenarios developed by the University of Cyprus, which include:

1. Specifications for an educational scenario on the topic “Looking after myself and others – Healthy Eating”
2. Specifications for an educational scenario on the topic “Looking out for my community, Vaccines development and the science that responds to hesitancy”
3. Specifications for an educational scenario on the topic “Looking after myself and others – Tobacco”
4. Specifications for an educational scenario on the topic “History of pandemics: what do we know about powerful viruses and their impact?”
5. Specifications for an educational scenario on the topic “Workings and malfunctions of human Immunological memory”

For each topic includes:

- A description of an educational scenario
- the proposed activities per educational scenario,
- an assessment of impact questionnaire, specific for each educational scenario
- a list of associated learning objects

1. Specifications for an educational scenario on the topic "History of pandemics: what do we know about powerful viruses and their impact?"

4.1. Introduction to the module

This topic will investigate the historical virus-related pandemics, shedding light on the existing knowledge that we have on powerful viruses, and their impact on the aspect of health, society, economy, and governance. Students of the age range 12 – 15 years old (preferably students of 15-years old) will be given the opportunity to expand their knowledge by learning about different classes of virus pathogens, in terms of their size, morphology, ways of invading the human immune system and causing disease, and how these may develop into new variants to re-emerge years after and cause reoccurrence of disease. They will also be presented with a timeline of historically documented pandemics, all related to virus causative agents.
Using differentiated instruction to teach immunological concepts related to infection and disease, to a diverse group of learners, of various ages, provides a comprehensive learning module designed to expose high school students to immunological concepts related to infectious agents that can cause spreading of disease leading to epidemics and possible pandemics, by using active hands-on and minds-on teaching strategies. This module includes:

Activity 1: a student created collaborative poster
Activity 2: an Immune-response Action Model
Activity 3: a simulation of virus antigenic drifting/shifting to clearly demonstrate and assess acquired knowledge on how a future pandemic may arise.
Activity 4: discussion and proposal of possible strategies for dealing with future pandemics, at local community, national and international level, that is presented to the local school community, as well as the wider educational community, promoting open schooling.

4.2. Expected student prior knowledge

Students should have a basic Cell Biology knowledge of the characteristics and the Variety of living organisms, including:

- ✓ knowledge and ability to describe the common features shown by eukaryotic organisms (i.e., animals, fungi and protoctists, and prokaryotic organisms such as bacteria),
- ✓ an understanding of the term pathogen and know that pathogens may include fungi, bacteria, protoctists or viruses,
- ✓ an understanding that viruses are not living organisms, but very small particles (smaller than bacteria) that have a parasitic nature since these can multiply only inside the living cells that they infect (including cells of animal, plant, and bacterial origin),
- ✓ knowledge of structures and functions in living organisms, including levels of cell organisation, cell structure, biological molecules (including amino-acids, proteins, enzymes, DNA, RNA),
- ✓ an understanding of the different ways of transport of substances in and out of living cells, with emphasis on receptor-mediated active transport.

4.3. Expected outcomes

Learns will have the opportunity to refresh their prior knowledge, but mainly will acquire new knowledge about:

- ✓ the meaning of the term epidemic and pandemic and the difference between the two,
- ✓ the past pandemics of the current and previous centuries,
- ✓ the present and newly emerging viral pathogens,
- ✓ classifying viral pathogens in terms of their size and morphology,
- ✓ understanding how such pathogens emerged in the first place,
- ✓ the different ways that viruses use to evade human defence mechanisms and cause disease,

- ✓ how existing viruses may develop into new variants to re-emerge years after and cause recurrent epidemics and sometimes pandemics,
- ✓ different remediation approaches that are used or can be used to mitigate the occurrence of future virus-related pandemics.

Learners will also acquire the use of transferable skills such as critical thinking, problem solving, analysis, reasoning, interpretation, adaptive learning, creativity, continuous learning, self-direction, responsibility, perseverance, self- regulation (metacognition, forethought, and reflection), integrity, self- monitoring, self- evaluation, self- reinforcement, and apply all these to their everyday life within their community.

4.4. Relation to other topics

This module could complement the teaching of the existing curriculum of general biology of the immune system, and the activities proposed within this learning module are designed to give students opportunities to explore, learn, and peer teach concepts related to more specialized functions of the immune system against invading virus pathogens. The specific assignments are geared toward a general biology course, but the strategies are applicable for higher-level biology classes (A-level, undergraduate students) if the content is scaled up. The order in which these activities should be applied follows a scaffolding approach where students uncover new knowledge at each level and then use it to bridge their understandings to new learning.

4.5. Pedagogical methods utilised in the teaching of this module

The goal of this module is to help students build on prior learning and develop further skills and attitudes. Meanwhile, this also expands the current knowledge of the educators, enabling them to present this module in a way that is relevant to the students' needs.

A range of different pedagogical methods are implemented through all the different activities catering for a broad range of different learners. To begin with, the current learning module is based on the pedagogical approach of inquiry-based learning, where students are encouraged to ask questions and complete research while learning various concepts of basic virology and immunology. In this way, individual learners acquire the skills necessary to develop their own ideas, as well as question themselves and group members in a constructive way. In the initial poster preparation activity, students are asked to collaborate with their peers and conduct their own research on the given topic to produce a poster or a power point presentation, on what is known about past centuries virus-related pandemics. During this first activity, student will have to apply and develop their own critical thinking, learning, and writing skills through peer-to-peer interaction and interpersonal engagement.

In the second activity, students are asked to create an Immune-response Action Model (IRAM). This activity allows students to actively participate in inquiry-based learning where they work on a simple materials model that simulates the dynamic biological process of immune response mechanisms following virus evasion. This method of learning increases student accountability for their own learning and allows multiple opportunities for the educator to check for understanding. Additionally, the modelling of the immune response against an incoming virus will help students take an abstract concept and make it tangible and more concrete.

The third activity proposed involves problem-based learning, during which students will be asked to simulate virus antigenic drifting and shifting, by simple creative classroom methods (Marintcheva B., 2016), and via and discuss the consequence of this ability of a virus pathogen to re-emerge and cause continuity of disease. The activity is a continuation of the IEAM; students acquire knowledge by devising a solution to a real-world problem. As they do, they acquire knowledge, as well as communication and collaboration skills.

The final activity comes to conclude on the sequence of all previous activities and promotes the application of open schooling, where the educator asks from students to proposed and discuss possible strategies for dealing with future virus pandemics. In this way, the educator wraps up the delivery of this module with problem-based learning, where students acquire knowledge by devising a solution to

a real-world current problem and as they do so, they acquire knowledge, as well as essential communication and collaboration skills. During this final activity, the students will be requested to demonstrate their individual understanding by preparing their own essay work, presenting their own and acquired knowledge regarding the specific topic taught. The combined work of the students will be presented in the form of an article, where the thoughts and conclusions of the students will be featured and discussed further by their educator. This article shall be made available within the school community (published in school's newsletter) as well as the wider educational community (published in the local educational news website, paideia-news.com) and therefore promoting open schooling.

4.6. Background science

4.6.1. Brief review on the history of virus-related pandemics

Along the centuries, novel strains of viruses have been the causative agents of global pandemics (Figure 1, Table 1). Examples such as influenza have resulted in pandemics which increase illness, loss of lives, causing economic, social, and political disruption in affected countries. Figure 1 provides an outline of the most notable pandemics recorded throughout the history of humankind and associated with various types of parasitic pathogens. Those being virus-associated have been indicated in red.

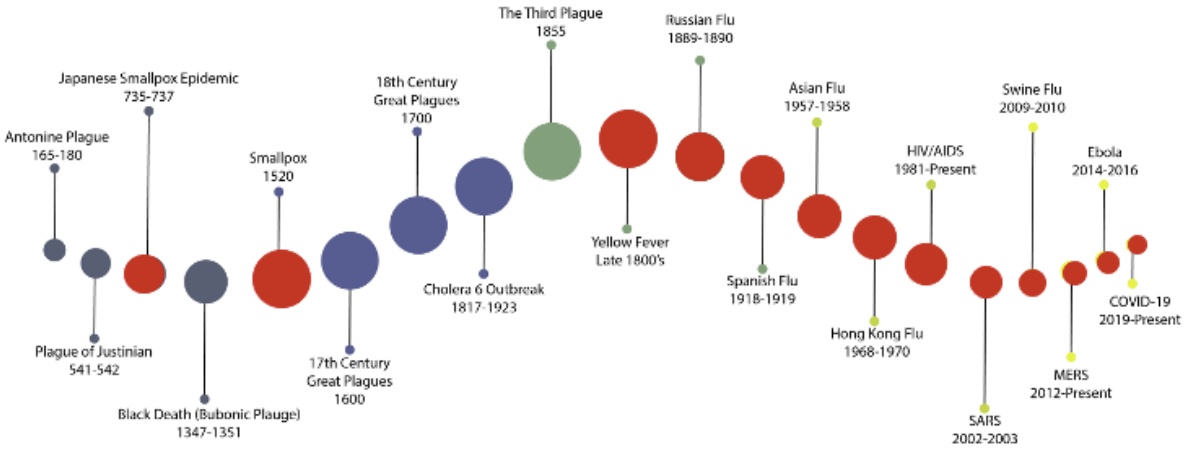


Figure 1: A schematic view of historical pandemics
Indicated in red are pandemics caused by viral pathogens including, smallpox, yellow fever, influenza, human immunodeficiency virus-1, severe acute respiratory syndrome Corona Virus-1 (SARS-CoV-1), Middle East respiratory syndrome virus, Ebola, severe acute respiratory syndrome Corona Virus-2 (SARS-CoV-2), respectively. (A modification of the image produced by Gabrielle Rodriguez, taken from https://trinitonian.com/2020/09/10/a-history-of-sports-and-diseases).

Spanish flu that had originated in Kansas (US) in 1918, also known as the "Great Influenza epidemic" was caused by the avian (bird-related) influenza A-virus, H1N1. The virus had spread rapidly around the globe in four successive infection waves, infecting around 500 million people, and resulting in a highest of 100 million deaths. Similarly, in 1957, the Asian flu was the result of another strain of the influenza A-virus, subtype H2N2, which originated in Guizhou province in the southwest region of China and resulted in an estimated one to four million deaths worldwide. N3H2, a variant arising from influenza H2N2 during the Asian flu pandemic, was the causative agent of the Hong Kong flu pandemic in 1968, which resulted in the deaths of almost four million people, globally. Apparently, N3H2 was a variant of H2N2 that had emerged following an antigenic shift that occurred in the virus during its transmission, a genetic alteration that resulted in a dramatic change in the structure of its protein antigens and its degree of virulence. Interestingly, the swine flu pandemic in 2009 was the outcome of a virus that appeared to be a new strain of H1N1 that had resulted from a previous triple re-assortment of bird, swine, and human flu viruses which further combined with a Eurasian pig flu virus, to give rise to a variant that resulted to an estimated total of 284,000 deaths globally.

It seems that zoonotic transmission of pathogens from animals to humans has always acted as a pivotal mechanism by which emerging infections have afflicted humans throughout history. Pandemics of the past have various characteristics in terms of morbidity (suffering from disease) and mortality (death resulting by disease). The recent pandemic caused by a novel corona virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), in 2019, was first identified during an outbreak in the Chinese city of Wuhan, and was very similar to Spanish, Hong Kong, and Asian as well as swine influenza pandemics in terms of spreading globally through person-to-person transmission. Humans have witnessed another two such deadly pandemics so far in the twenty-first century which have been associated with corona viruses and include SARS-Cov-1 and the Middle East respiratory syndrome (MERS), both being of zoonotic origin. It is therefore evident that since the time of the human hunter-gatherer, human populations have been suffering for millions of years from infectious diseases similar or identical to diseases of other wild primate populations, some of which could have emerged only within the past 11,000 years, following the rise of agriculture (Wolfe et al., 2007). The increasing interactions between humans and animals have facilitated the transmission of zoonotic pathogens, and the expanded cities, extended trade territories, increased travels as well as effects on ecosystems due to increased human population raised the emergence and spread of infectious diseases leading to higher risks for outbreaks, epidemics, and pandemics. Interestingly, some scientists point out that major infectious diseases seem to have arisen overwhelmingly in the past in Europe, the Mediterranean, and some in the Middle East (Old World), often from diseases of Old-World domestic animals (Wolfe et al., 2007).

Table 1: History of pandemics linked to viral infection (Modified from, Piret J. and Boivin G., 2021)

Occurring period	Disease	Causative agent (virus)
1520	Smallpox	Variola major
1889 – 1893	Russian flu	Influenza A –H3N8
1918 – 1919	Spanish flu	Influenza A – H1N1
1957 – 1959	Asian flu	Influenza A – H2N2
1968 – 1970	Hong Kong flu	Influenza A – H3N2
1981 – on going	AIDS	Human Immune-deficiency virus (HIV)
2002 – 2003	Severe acute respiratory syndrome (SARS)	SARS-CoV-1
2009 –2010	Swine flu	Influenza A –H1N1
2013 – 2015	Ebola virus disease	Ebola virus
2014 – 2016	Chikungunya	Chikungunya virus
2015 – ongoing	Middle East respiratory syndrome (MERS)	MERS-CoV
2019 – ongoing	COVID-19	SARS-CoV-2

In their review, Wolfe et al., (2007) defined the five stages in the transformation of an animal pathogen into a specialized pathogen, able to infect humans (Figure 2). Stage 1 represents microbes that are present in animals but that have not been detected in humans under natural conditions. Stage 2 represents animal pathogens that, under natural conditions, have been transmitted directly from animals to humans via primary infection, but no secondary human-to-human infection has been reported. Such an example is rabies, caused by Rabies lyssavirus, member of the group of rhabdoviruses. Stage 3 represents pathogens that can undergo only a few cycles of secondary transmission between humans, so that occasional human outbreaks triggered by a primary infection soon die out. An example of such pathogens includes the Ebola virus. Stage 4 can be subdivided into three subsequent parts, including (a) disease that exists in animals, and that has a natural (sylvatic) cycle of infecting humans by primary transmission from the animal host (an example is yellow fever, caused by a Flavivirus), but (b) also undergoes long sequences of secondary human-to-humans transmission without the involvement of animal hosts (an example includes dengue fever, caused by Dengue fever virus, a Flavivirus), that in time (c) the greatest spread observed is between humans (example being Influenza A). Finally, stage 5

includes pathogens that are exclusive to humans, such as measles, mumps, rubella, smallpox and syphilis that most likely became confined to humans either as an ancestral pathogen already present in the common ancestor of chimpanzees and humans and co-speciated long ago, when the chimpanzee and human lineages diverged around five million years ago; or could have risen from an animal pathogen that have colonized humans more recently and evolved into a specialized human pathogen.
Most virus pathogens don't seem to make it to stage 5, and this can be explained in terms of the following:

a) increasing phylogenetic distance (using DNA sequences, protein amino acid sequences, and/or morphology) between the existing host and new host,

b) *variability* among pathogens as some viruses can infect a wide range of hosts, while others can only infect a narrow range, and this variation is related to a pathogen's characteristics, such as its ability to generate genetic variability, or to overcome host molecular barriers of potential new hosts (such as humoral and cellular defences or lack of cell membrane receptors essential for the entry of the pathogen into host cells),

c) high abundance and frequent encounters between the existing hosts with humans in dwellings (i.e., rodents being a source of zoonoses transmission to humans),

d) differences between human and animal behaviour affecting transmission (for example, animals often bite humans, but humans rarely bite other humans),

e) need of the pathogen to evolve adaptations to the new human host and possibly also to a new vector,

f) obstacles to a pathogen's spread between human tissues (i.e., bovine spongiform encephalopathy is restricted to the central nervous system and lymphoid tissue),

g) Presence of barriers between Stages 3 and 4 (i.e., Ebola virus) include those related to human population size and to transmission efficiency between humans.

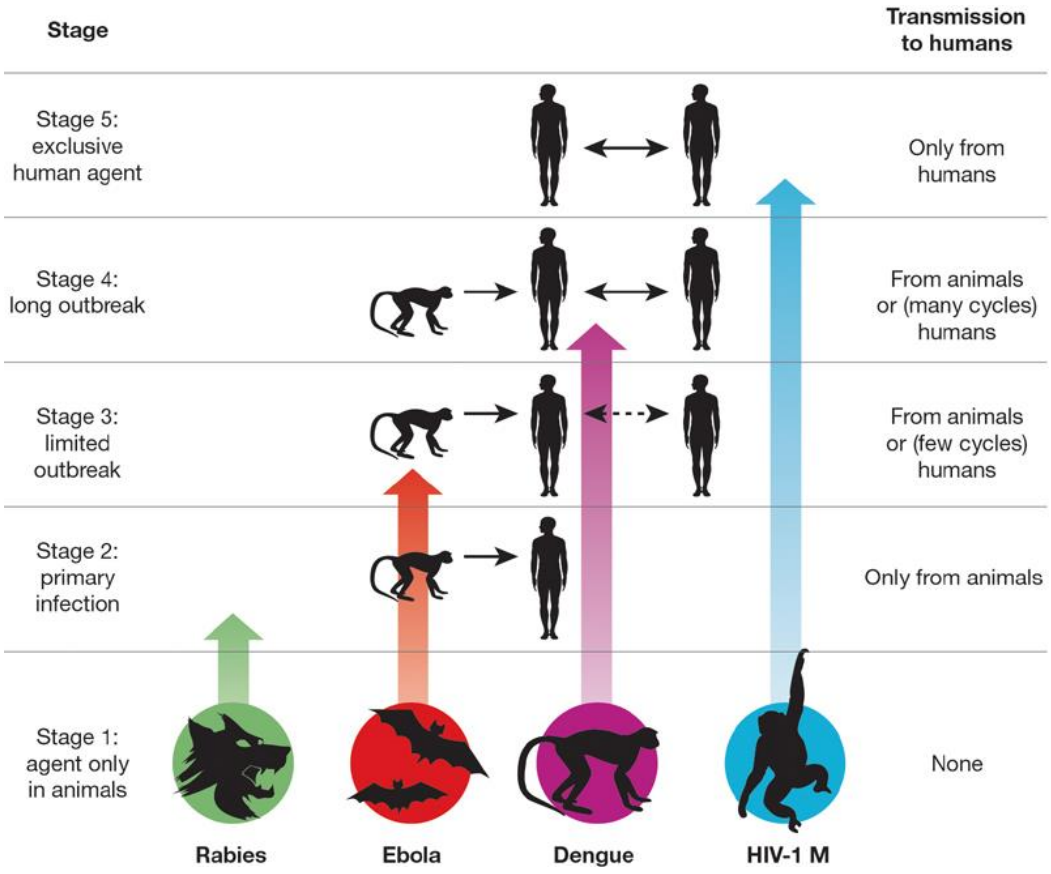


Figure 2: Pathogens of animals evolve to cause diseases confined to humans (Figure taken from Wolfe N. D., Panosian Dunavan C., Diamond J. 2007)

The emergence of novel pathogenic viruses is now made possible by modern developments exposing more human and/or making human-to-human transmission more efficient than before. Such developments include blood transfusion allowing transmission of hepatitis C, the commercial bush-meat trade allowing animal-to-human secondary infection by retroviruses, industrial food production allowing for the secondary infection with bovine spongiform encephalitis (BSE), international travel allowing human-to-human transmission of influenza and coronavirus strains, intravenous drug use allowing human-to-human transmission of human immunodeficiency virus (HIV), vaccine production using viral vectors such as simian virus-40 (SV40), and susceptible pools of elderly, antibiotic-treated, and immunosuppressed patients.

4.6.2. What are viruses?

A virus is characterized as an obligate intracellular parasite, requiring the use of the host cell's biochemical machinery to replicate and sustain its numbers by creating new infectious virus particles, known as **virions.** Viruses are extremely diverse in terms of their structure and genetic complexity, some having RNA genomes, encoding for only a few genes, whereas others have DNA genomes, encoding for up to 200 genes. Regardless of their diversity, viruses share several common characteristics, such as:

- **their small size** – the smallest virus being just 20 nm in diameter and belonging to the family of "pico-rna-virus", which is a large group of the smallest known animal/human viruses. The prefix "pico" refers to its small size and "rna" refers to its core of ribonucleic acid (RNA). The family of picornaviruses includes enteroviruses, which attack the intestinal tract and often invade the central nervous system, rhinoviruses, which infect the nasal epithelium, and the virus agent of foot-and-mouth disease. Amongst the enteroviruses are polioviruses, echoviruses, and Coxsackie viruses. Echoviruses cause fever with rash and meningitis, whereas Coxsackie viruses cause sore throat or

fever with chest or abdominal pains. Looking at larger viruses, influenza, and the human immunodeficiency virus (HIV), these have a more typical size of about 100nm in diameter, compared to the 10–30μm diameter of an average human cell. This is a 100 to 1000 times smaller than the size of cells that they infect (Figure 3).

- **their intracellular parasitic nature** – they enter the host cell via receptor-mediated attachment. Within the host intracellular environment, the virus disassembles and deposits its genetic material that encodes the instructions for the proteins that will spontaneously assemble into the new virions, into the cell nucleus. It will use the cell's energy and machinery to create and assemble new virions piece by piece, completely from scratch that will be then released from the initially infected cell into the extracellular environment to infect adjacent cells.
- **their genetic material being either of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) nature** – some viruses have **genomes** that can be composed of DNA *or* RNA, but not both, this being either double-stranded (ds) or single stranded (ss). The size of viral genome can vary greatly, with a typical size falling in the range of 7000–20,000 base pairs (bp). Smaller-sized virions naturally can hold less nucleic acid than larger virions, but large viruses do not necessarily have large genomes.

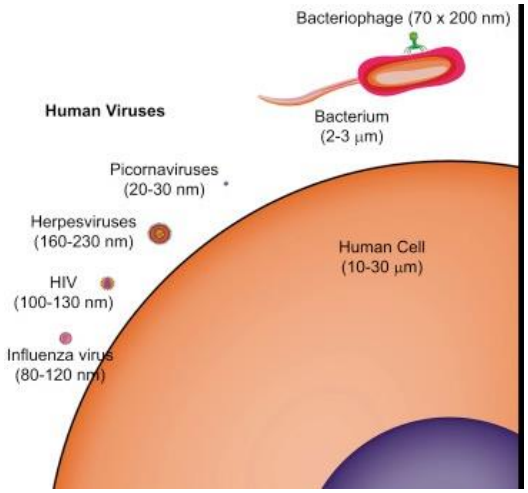


Figure 3: Comparing the size of a virus to its host cell.

Human viruses can vary in size but are generally in the range of 20–200 nm in diameter, in comparison to bacteria that are generally 2–3 μm in length, and average human cells, having a diameter of 10–30 μm. (Figure taken from Louten J., 2016).

4.6.3. Architecture of viruses

The structure of a typical virus consists of a capsid which is essentially a protective protein coat. The term is derived from Latin term "capsa", meaning box. Usually, the capsid is composed of one or more different types of proteins that are repeated to create the entire capsid structure. This continuous repetitive pattern of proteins to create the entire capsid, results from the fact that most viruses have very small genomes with only a few genes encoding for capsid proteins. These capsid proteins can self-assemble into the capsid structure without requiring additional information. This was first reported in 1955, by Fraenkel-Conrat and Robley Williams, who witnessed the *in-vitr*o, automatic and spontaneous reassembly of RNA genome and protein subunits of tobacco mosaic virus, to generate infectious virions. The repeating protein pattern forms a strong, but slightly flexible capsid that is physically very difficult to break open and sufficiently protects the nucleic acid that is packed inside it. Both the virus nucleic acid and capsid compose the nucleocapsid (Figure 4).

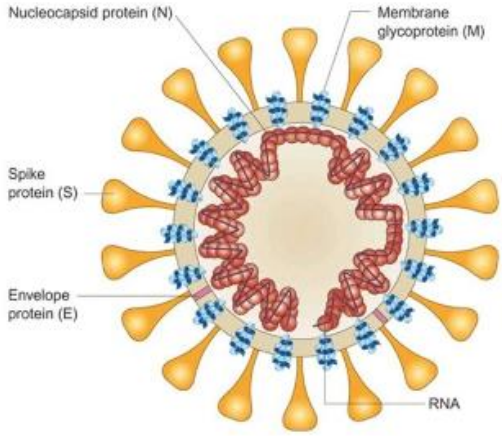


Figure 4: Structure of SARS-CoV-2 virus

Viral capsid proteins protect the fragile genome, composed of nucleic acid, from the harsh environment. In the case of SARS-CoV-2, the capsid and nucleic acid are embedded together to form the nucleocapsid.. (Figure taken from https://www.lubio.ch/).

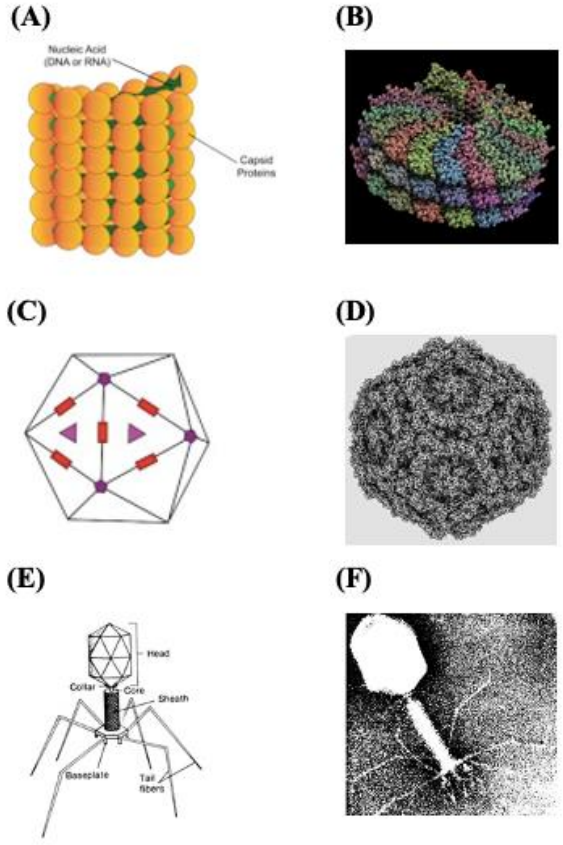


Figure 5: Variability in the structure of the capsid in different viruses.

(A) Viral capsid proteins wind around the nucleic acid, forming a helical nucleocapsid. (B) Helical structure of tobacco mosaic virus. (C) Icosahedron faces (fuchsia triangles), edges (red rectangles), and vertices (violet pentagons) are indicated on the white icosahedron. (D) llustration of human hepatitis B virus, as viewed on the twofold axis of rotation (Louten J., 2016). (E) Model of bacteriophage T4, (F) Electron Micrograph of bacteriophage-T4 (Todar K., www.textbookofbacteriology.net).

The shape of the capsid varies between different viruses. This can be a simple helical shape as seen in the tobacco mosaic virus (Figure 5A/B) or an icosahedral form (Figure 5C/D), as seen in most viruses, to even more complex structures of some isolated examples of viruses such as the bacteriophage-T4 (Figure 5E/F). The capsid provides protection for the viral genome against the environment and functions in receptor recognition, targeting the virus to a susceptible host and cell type. Some viruses have an additional phospholipid-envelope, derived from the infected host's cell membrane, surrounding the protein capsid. Inserted into the phospholipid-envelope there are usually viral encoded proteins know as spike projections, these being typically glycoproteins usually involved in receptor recognition and viral tropism (Figure 4). A classic example is the neuraminidase and haemagglutinin glycoproteins expressed on the surface of the influenza-A virus. Other, more recent examples include the S (spike), E

(envelope) and M (membrane) proteins creating the viral envelope of ARS-CoV-2 virions (Figure 4). The spike protein is responsible for allowing the virus to attach to and fuse with the membrane of a host cell, with the S1 subunit catalysing the attachment, and the S2 subunit allowing fusion with the cell membrane of the target cell.

4.6.4. Classification of viruses

Classification of viruses allows scientists to study the origin of viruses and how they have evolved over time, reporting contrast between viruses, but also revealing newly discovered viruses by allowing comparison to similar, current viruses. There are currently over 2800 different viral species with very different properties that are classified using the Baltimore classification system that categorizes viruses based on the type of nucleic acid genome (RNA or DNA) and replication strategy of the virus. According to the Baltimore classification system, there are seven classification types, referring to single-stranded RNA viruses that possess a positive RNA-strand (+) or a negative RNA-strand (−). A positive RNA-strand can be immediately translated into protein (for example, messenger RNA in cells, mRNA), whereas a negative RNA-strand cannot be translatable into proteins, as it first needs to be transcribed into a positive RNA-strand. Some viruses possess accessory components such as the enzyme reverse transcriptase, allowing the performance of reverse transcriptio**n**, converting their RNA template into DNA, before inserting this into the host cell's genome.

In summary, the seven classes of viruses include, dsDNA viruses, ssDNA viruses, dsRNA viruses, positive-sense ssRNA viruses, negative-sense ssRNA viruses, RNA viruses that reverse transcribe, DNA viruses that reverse transcribe.

4.6.5. Viruses and host co-evolution

There is a constant competition between a host and a virus pathogen, with the later applying evolutionary pressures to their host, also influencing themselves in return. As an example, the high level of compatibility identified between the phylogenetic trees of mammalian herpes-viruses and their hosts, indicates co-evolution over many millions of years. Similarly, retroviruses have been shown to influence host evolution in more direct ways, through the integration of retroviral pro-viruses into the host's germ-line DNA, leading to permanent residence of the virus genome within the host genome, providing a survival benefit to the host, in situations where such integrated defective pro-viruses may interfere with non-defective, invading viruses.

Base substitution mutations are also a major mechanism of virus evolution, as well as recombination and re-assortment of virus and host genes, which can give rapid rise to viruses with novel properties, just like in the case of influenza viruses, where antigenic shift resulting from re-assortment can give rise to pandemic strains. Evolution of a virus can also occur within an individual host during infection, where potentiators, such as the virus escaping the host's immune response and the use of antiviral drugs, may have important implications for the control of viral diseases of humans (Stern et al., 2016).

4.6.6. Mode of infection

A typical virus infection involves the initial attachment of the virion to the cell membrane of its target. This is mediated by specific receptor intermolecular binding by both the pathogen and its target. This specificity identifies the tropism of a virus for a particular host.

For example, the HIV enters cells by means of a complex of two non-covalently associated viral glycoproteins, gp120 and gp41 (Figure 6A). These form trimers within the viral envelope. The gp120 subunits of trimeric gp120/gp41 complexes bind with high affinity to the cell-surface molecule CD4, which is expressed on CD4 T-cells, and to a lesser extent on subsets of dendritic cells, macrophages, and monocytes. Before fusion and entry of the virus, gp120 must also bind a co-receptor on the host cell, this being the chemokine receptors CCR5 and CXCR4. While CCR5 is predominantly expressed on subsets of effector memory CD4 T-cells, dendritic cells, and macrophages, CXCR4 is expressed primarily by naive and central memory CD4 T-cells.

Similarly, the virus SARS-CoV-2 infects mainly lymphatic epithelial cells and type II pneumocytes with the initiation of human body's innate response by producing interferons (IFNs), which in turn, activate the expression of the angiotensin-converting enzyme-2 (ACE-2) that acts as receptor for virus

attachment to host cells. Interaction between the virus Spike-protein (S) and ACE-2 leads to its cleavage by host proteases into two subunits, a receptor-binding fragment (S1) and a fusion fragment (S2), during biogenesis or virus assembly. The single-stranded RNA in the viral genome is translated by host machinery to produce viral polypeptides, which result in the formation of a replication transcription complex (RTC, see step 5 of Figure 6B), which continuously replicates and produces a series of sub-genomic messenger RNAs that encode the accessory and structural proteins. The viral genomic RNA and proteins are assembled to form the virus particles in the Endoplasmic Reticulum and Golgi Intermediate Compartments (ERGIC). The vesicle-containing virus then fuses with plasma membrane of the host, releasing the viral particles out of the cell (Figure 6B), to infect neighbouring cells and tissues. The details of this process depend on the virus and the metabolic state of the host cell, i.e., picornaviruses require around eight hours producing new virions, whereas the human Cytomegalovirus (hCMV) requires 48 hours.

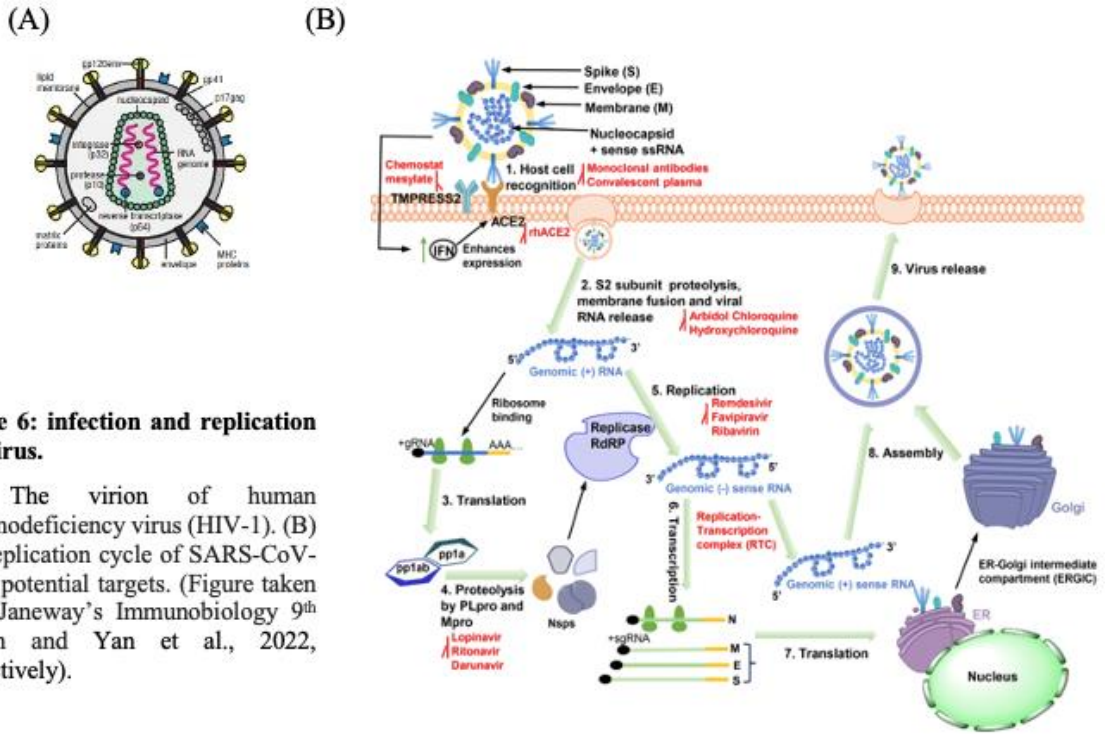


Figure 6: infection and replication of a virus.

(A) The virion of human immunodeficiency virus (HIV-1). (B) The replication cycle of SARS-CoV-2 and potential targets. (Figure taken from Janeway's Immunobiology 9th edition and Yan et al., 2022, respectively).

It should be noted that viruses are extremely divert in their ability to infect and cause disease in a host. Entry usually occurs from mucosal epithelial surfaces, and then into the bloodstream. Replication takes part within epithelial host cells, producing vast numbers of virions released into interstitial tissues and bloodstream, spreading infection, and causing viraemia. Recovery from infection can involve the elimination of the virus by the host's immune system. However, some viruses persist within the host's tissues in a non-infectious (latent) form following acute infection recovery and can be reactivated under low immune surveillance to produce new infectious virions. Such an example is the human herpes virus. Other viruses, such as the human hepatitis B virus, can persist in infectious form within the host, despite of the presence of active immunity.

4.6.7. An overview of the human immune defence mechanisms against invading viruses

The defence against incoming viral pathogens involves a four-level barrier (Figure 7). This includes the anatomical barriers of the skin, oral mucosa, respiratory epithelium, and the intestine, with the complementary action of a chemical barrier composed of a group of antimicrobial proteins, and the cellular barriers of the innate and specialized adaptive immunity. Anatomic and chemical barriers are the initial defence against infection. The skin and mucosal surfaces introduce an avoidance strategy that prevents the exposure of internal tissues to microbes. Additional resistance mechanisms that further strengthen host defences involve mucosal surfaces and a variety of antimicrobial proteins that they produce and act as natural antibiotics to prevent microbes from entering the body (complement system).

Once the anatomical barrier is breached, early non-specific, innate immune defence becomes active. The innate immune response includes the first line of the cellular barrier defence involving the action of phagocytic cells, including macrophages, dendritic cells, neutrophils, Natural killer (NK) cells and their molecular secretions. Macrophages, dendritic cells, and neutrophils, can all act as phagocytes able to internalize external agents, degrade and/or process and present to specialized cells of the adaptive immune system, T and B lymphocytes, which constitutes a specialized cellular barrier.

The innate immune response involves the early phase of a viral infection and is often a race between the host's defence systems and the virus (Figure 8, innate immune system). As soon as the integrity of the anatomical barrier is breached, the cellular barrier becomes activated, regulating phagocytosis and secretion of chemical components that can inhibit viral replication, but also eliminate virally infected cells.

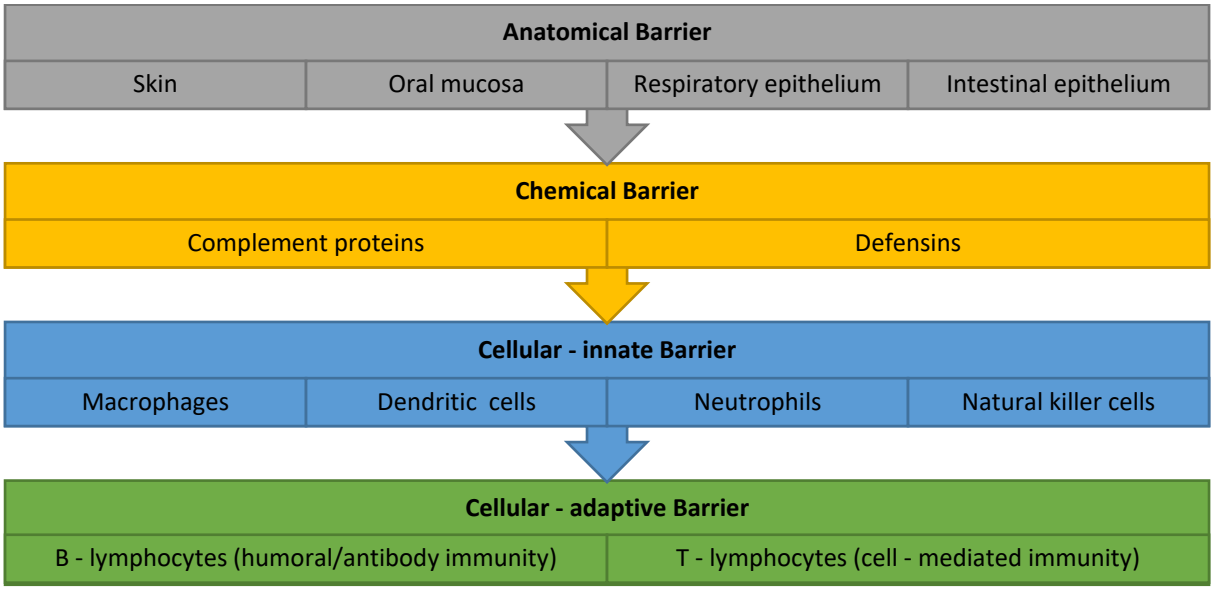


Figure 7: The four-level barrier of defence against incoming pathogens.

The secretion of interleukin-8 (IL-8) by phagocytic cells (macrophages, neutrophils) and epithelial cells during inflammation plays a key role in the enhanced recruitment of neutrophils and other immune cells to the site of infection. Additionally, virus infected cells can and will also secrete interferon (IFN), another important chemical component, as part of activating antiviral mechanisms in neighbouring cells, enabling them to resist viral infection, as well as promote macrophage activation, enhance antigen presentation, orchestrate activation of the innate immune system, coordinate lymphocyte–endothelium interaction, regulate T-helper-1/T-helper-2 balance, and control cellular proliferation and apoptosis of senescent/infected cells (Tau et al., 1999).

Interferon-alpha (IFN-α) and Interferon-beta (IFN-β) are produced by cells in response to infection with virus and both contribute to the direct inhibition of viral replication by the activation of antiviral

mechanisms in neighbouring cells, enabling them to resist infection, by blocking the translation of the virus genome into viral protein, and the activation of a specific endonuclease that degrades viral RNA. In addition, Interferon-gamma (IFN-γ) enhances the efficiency of the adaptive immune system by up-regulating the viral – antigen presentation and activating macrophages and Natural killer cells.

Activated Natural killer (NK) cells are cytotoxic lymphocytes that can directly lyse cells that become infected with a virus or any other intracellular pathogen, and act as essential components of the immune response mediating protective immunity. Their cytolytic/cytotoxic function can be initiated through a variety of processes, including degranulation (the release of enzymes, such as perforins that will cause pore formation in cell membranes of target cells) and death receptor ligation, and is critical for the clearance of diseased and dysfunctional cells. Additionally, NK cells can produce a variety of inflammatory cytokines in response to activation receptor stimulation as well as inflammatory cytokine-induced activation signalling (Abel A. M., et al., 2018), promoting thus the action of adaptive immune responses.

The adaptive immune response involves the activation of pathogen-specific defence mechanisms against incoming pathogens (Figure 8, B and T lymphocyte responses). This specialized cellular barrier includes both T and B lymphocyte associated responses, which are regarded as the "special forces" of the immune system. In athymic "nude" mice (absence of a functional thymus gland that is the organ specializing in T lymphocyte maturation), infection with virus can be lethal, since these animals lack mature T lymphocytes and are thus susceptible to viral attacks. Spreading of lesions and eventual infestation of the central nervous system resulting in death was the result when such animals were infected with herpes simplex virus (HSV). Transfer of mature HSV-specific T lymphocytes, early on in their infection with HSV, ensured the survival of these animals (Kapoor A. K. et al., 1982). Since those studies, it is now clear that T lymphocytes play an important key role in the protection against virus attacks. In fact, $CD4^+$ T lymphocytes mediate viral immunity in many ways. These cells are responsible for the activation of $CD8^+$ cytotoxic T lymphocytes, which are the principal surveillance system operating against viruses in a highly efficient and selective manner, focusing on and destroying virally infected cells. In a similar way, $CD4^+$ T lymphocytes also have cytotoxic activity, specifically targeting and eliminating infected antigen presenting cells (macrophages, dendritic, epithelial cells). $CD4^+$ T lymphocytes are also responsible for the recruitment of macrophages at the site of virus exposure. Additionally, T lymphocytes will also mediate most antibody responses, since these are thymus-depended meaning that B lymphocytes require an interaction with specialized $CD4^+$ T lymphocytes for their immunoglobulin class switching and maturation into antibody producing plasma cells.

Anti-viral antibodies are the result of humoral immunity brought about by B lymphocytes. Such antibodies provide a major barrier to virus spread between cells and surrounding tissues, restricting access of the virus within cells (neutralization), identifying viral antigens on infected cells through a process known as opsonisation, promoting cell destruction via the action of the complement proteins, and resulting in the massive clustering and coagulation of virus particles together ensuring their elimination. Immunoglobulin-A (Ig-A) is the major form of antibody observed at mucosal surfaces, whether these are part of the respiratory or the intestinal system, and a major molecular component of the immunological barrier to incoming pathogens. Ig-G is found in the bloodstream and is important in restricting virus spread throughout the body. Antibodies may be generated against any part of a viral protein, however, only those directed against viral glycoproteins of the envelope structure of the virus or towards those expressed on the cell membrane of infected cells are of importance in controlling the infection.

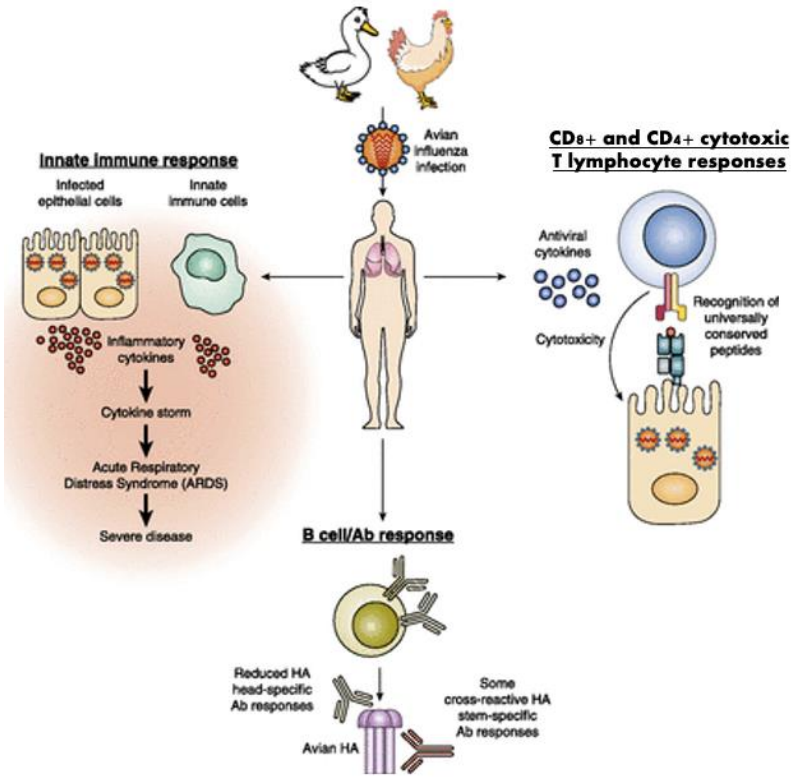


Figure 8: The immune response to avian influenza virus infection.

Infection with novel avian influenza viruses triggers innate and adaptive immune responses. The humoral response (antibodies) results in the production of antibodies. Cytotoxic $CD8^+$ T lymphocytes that recognize conserved peptides can kill virus-infected cells and also produce antiviral cytokines, promoting viral clearance and recovery. (Figure taken from Koutsakos et al., 2019).

4.6.8. Strategies of viruses for evading immune defences

Viruses have evolved various strategies for evading the immune system and escaping recognition by antibodies, the most effective one being antigenic variation. This involves the mutation of specific regions of key proteins that are the usual targets of antibodies. Antigenic variation is a common strategy used by a broad range of viral pathogens to avoid host immune responses. However, the rate of genomic mutation and the diversity of antigenic variants vary markedly among different viral species, as viral antigenicity is tightly linked with the capacity of a virus to be bound by specific antibodies, especially neutralizing antibodies. Such antigenic variation is seen with HIV and influenza viruses (Figure 9) and antibody mediated immunity lasts until a new virus variant emerges.

Some viruses such as the HSV can make glycoproteins that result in binding a different site of antibodies, alternative to the usual antigen-binding site, thus escaping immune recognition, but also interfering with immune complimentary activation and elimination of infected cells. Other viruses, such as Epstein-Barr virus (EBV), will produce short stretches of RNA that compete with the action of the host's protein kinase of blocking viral protein translation. This is the virus's own defence mechanism against the action of IFN-α and IFN-β. There are also viruses, such as the cytomegalovirus (CMV) that encode for proteins that inhibit the transport of specific antigen presenting molecules to the cell surface of virally infected cells and prevent the expression and presentation of viral antigens to $CD8^+$ cytotoxic T lymphocytes. Part of the numerous mechanisms developed by viruses to evade immune responses, includes the ability to encode for proteins that target the function of the host's cytokines and immune signals triggered by cytokines (Alcami A., 2016). An example of such a virus is EBV that has been shown to encode for protein that mimic the molecular structure of host's specific cytokines.

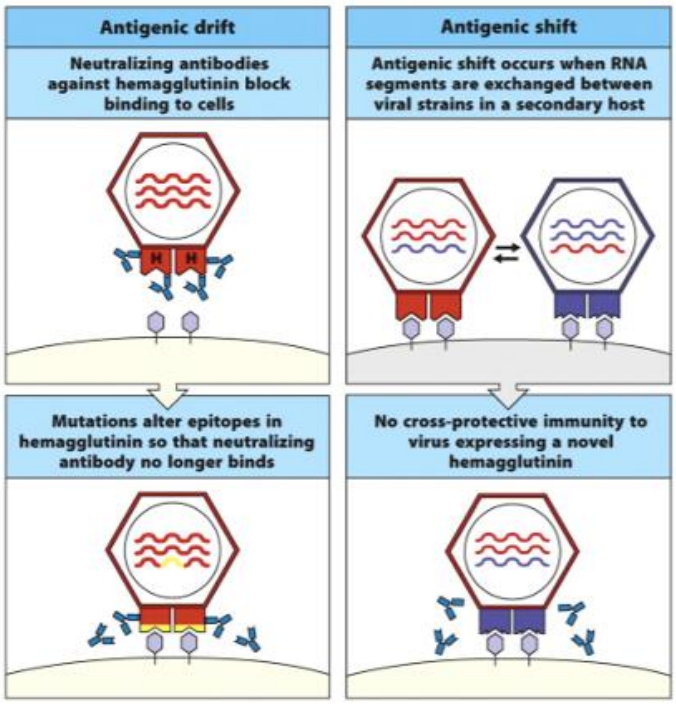


Figure 9: Antigenic drift and shift occurring in viruses, providing the engine for genetic diversity.

Antigenic drift can results in the virus changing its surface antigens slightly, whereas antigenic shift results in radical changes that result in the generation of novel strains.
(Figure taken from Janeway's Immunobiology, 9th Edition)

4.6.9. Defining the term pandemic

Let us begin with a question, "what is a pandemic?" Some would casually, say that a pandemic is defined as a disease outbreak that spreads across countries or entire continents, or even globally, and it affects more people and results in more deaths than an epidemic. Back in the early 19th century, the terms epidemic and pandemic were considered synonyms, which lead into an increasing abandonment of the term. Since the 1889 and 1918 influenza pandemics, as a result of globalization, change of lifestyles, social and economic improvement, the meaning of the word pandemic has been clarified and made widely understood, but it soon drifted into looseness and vagueness as it began to be used popularly to signify large-scale occurrences of other infections (not related to influenza) and to chronic and lifestyle-associated diseases (such as cancer incidences); it thus returned to its previous vague status, describing almost anything that increased and appeared to spread within or among groups of people, such as obesity, cardiovascular disease, traffic accidents, factory closings, even fear.

In their review, David M. Morens, Gregory K. Folkers, and Anthony S. Fauci (2009), refer to a vast number of scientific, modern definitions of the tem pandemic that include "extensively epidemic", "epidemic that has spread over a very wide area and usually affecting a large proportion of the population", and "distributed or occurring widely throughout a region, country, continent or globally", among others. All these definitions convey the initial idea that a pandemic is a very large epidemic, however, such definitions remain vague. Even though there seems to be little disagreement that a pandemic is a large epidemic, the question remains on whether pandemics must be also new, explosive, and/or severe, must they be infectious, what if they rapidly spread globally without causing high attack rates? All such questions need to be taken into consideration when coming to identify potential pandemics. Figure 10 provides an easy way, allowing the distinguishing between the terms endemic, epidemic and pandemic, that one could use to explain and clarify the difference between each term, ensuring the correct understanding and use of each term.

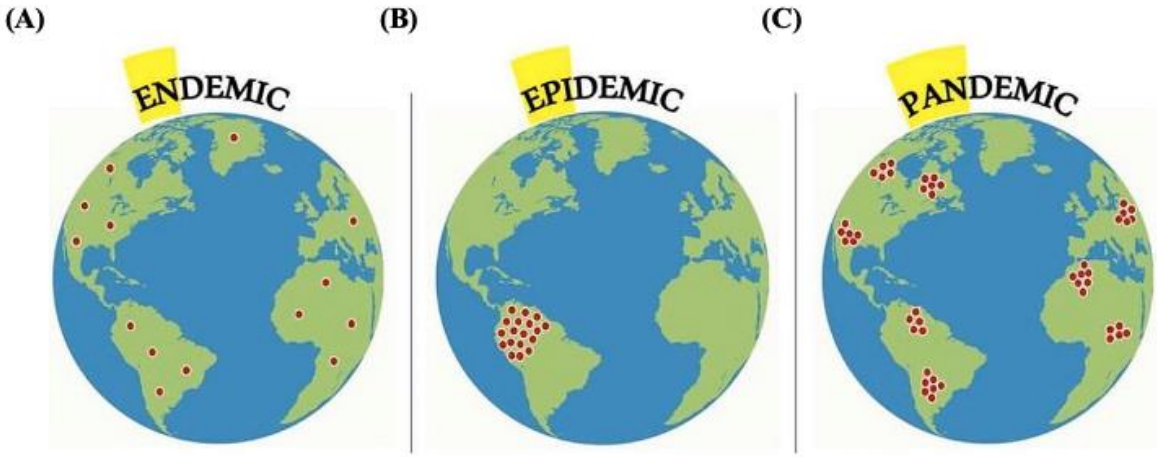


Figure 10: Understanding how to classify a disease based on extend of spreading globally. In situations, occurrence of transmissions is noted but, in (A) an endemic the number of cases remains constant, in (B) epidemic the number of cases increases, in (C) pandemic the number of cases increases and spreading occurs worldwide. (Diagram taken from https://abilenetx.gov/978/Epidemics-and-Pandemics).

Identifying and describing pandemics

Taking into consideration some, amongst many known diseases, including Acquired Immune Deficiency Syndrome (AIDS) caused by infection with the Human Immunodeficiency Virus-1 (HIV-1), cholera, dengue, influenza, plague, severe acute respiratory syndrome (SARS), one needs to identify what these have in common to describe disease. Factors to consider include the following:

geographic extension of the virus, spreading of disease caused by the virus, attack rates and explosiveness, minimal population immunity, novelty of the virus, infectiousness, contagiousness, severity following infection.

a) Considering the **Geographic extension of the virus**, a pandemic can be categorized as being:
   - Trans-regional, where the virus occurs at two (2) or more adjacent regions of the world,
   - Inter-regional, where the virus occurs at two (2) or more nonadjacent regions of the world, and
   - Global, where the virus occurs at most regions around the world.

b) The **spreading of disease caused by a virus** can be via transmission, and can be traced from place to place, as it has been done historically for centuries, including widespread person-to-person transmissions in diseases caused by respiratory viruses, such as influenza, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). One should not exclude animal-to-person transmissions, such as in the initial occurrence of SARS and MERS and the Avian Influenza (H5N1), where the disease causative virus is zoonotic, meaning that the virus has an initial animal origin, and this has "jumped" from animal to humans.

c) **High attack rates but also explosive spreads** (that can occur in very short time) comprise some of the characteristics exhibited by identified pandemics. It is important to understand that not every disease spread can be generally called a pandemic, and there are cases of diseases that had not been classified as pandemics presumably because attack rates have been moderate and symptomatic cases have been relatively low.

d) **Minimal population immunity** is a concept that applies to most pandemics, with individuals of the age of 60 and above having a modest degree of protection to existing/ circulating pathogens. Pre-existing immunity drops when it comes to viral pathogens that are characterised by high

mutation rates but can also use animals and humans as their hosts. In such cases, such as influenza and corona viruses, one's immune system constantly requires "updating".

e) **Novelty of the viruses** arises for those pathogens that can undergo frequent antigenic shifts, and therefore continually alter their genetic makeup, making it difficult for the immune system to keep up with such changes. Examples of pandemics arising from new variants of such viruses include the reoccurring of influenza and corona viruses, the emergence of HIV/AIDS when it was recognized back in 1983, and the historical epidemics of diseases, such as plague.

f) **Infectiousness** of a viral pathogen is an important parameter to consider, as this may result in possible high rates of transmissibility; in simpler words, the ability of a pathogen to be passed easily from person-to-person, through air or contaminated utensils or food, and induce disease.

g) **Contagiousness** is a term usually used to describe direct person-to-person transmissions.

h) **Severity following infection**, is a common characteristic for pandemics, however, not all pandemics are associated with high death rates and high severity.

4.6.10. Viruses that have staged pandemics over the last century

Knowledge of past pandemics has been constantly ignored and not utilized in ways that would benefit humankind, resulting in repeated failure of attempts to prevent emerging infectious diseases, with the most recent event being the SARS-CoV-2 outbreak. According to Dr Hunasanahally Puttaswamygowda Gurushankara, from the Department of Zoology, School of Biological Sciences, of Central University of Kerala, pandemics' history has highlighted the persistent socioeconomic classes, xenophobia, and pervasive fear of the invisible enemy pathogen (Gurushankara H. P., 2021). As already discussed in section 3.1, animals are the reservoir of vast pandemic disease pathogens and act as vectors for transmission to humans (Table 2). Factors such as urbanization, habitat destruction, habitat loss, and trade and consumption of high-risk, wild animals have been responsible for almost all disease outbreaks. Examples of human viral infectious diseases that have existed for centuries include measles, influenza, smallpox (eradicated in 1980), dengue, HIV, and many others, all of which have originated by animal-to-human host-switching. In fact, human beings are the ultimate cause of pandemics.

During the Neolithic revolution, where humans began to abandon their nomadic hunting and gathering nature, and started settling down in stable locations, growing crops and raising domestic animals for food, labour, and clothing to survive, it is under such conditions of intense human-animal proximity and environmental alterations that enzootic and zoonotic diseases arose. Viral agents that are the cause of many pandemic diseases evolved from animal pathogens that switched hosts to become human infectious agents. For example, the emergence of deadly "bird flu" associated with the poultry-adapted influenza A-viruses H5N1 and H7N9, SARS-Cov-1 that came close to causing a global pandemic in 2002 and 2003, and now in 2019 and 2020, SARS-CoV-2 that has caused the newest pandemic, COVID-19, are all diseases that originated from China's numerous live-animal markets. The establishment of such multiple large live-animal markets in a densely inhabited region, or at least the greatly increased human-wild animal contacts that such markets represent, has within two decades caused the emergence of four fatal zoonotic diseases, including one barely prevented near pandemic (SAS-CoV-1), and one we have clearly failed to prevent (SAS-CoV-2).

The most devastating realization is that the best containment measures that were used in the far past, are the same exact approaches that are currently used in the 21st century to fight the new global disease. As stressed by many scientists, from prehistoric to 21st-century pandemic, humans have not understood many fundamental aspects of emerging infectious diseases, including the origin and evolution of the novel emerging pathogens. It seems that some of these viruses have all the essential components and the ability to directly infect and be transmitted between humans, and, therefore, are poised for human emergence (Morens D. M. et al., 2020). As frankly stated by Morens et al., (2020), since the Athenian plague that was the first, historically identified transregional pandemic, there has been a steady stream of new pandemics of even greater mortality. Confronting them and then quickly forgetting the lessons that they left behind have become a recurring theme in human existence, and repetitive nature of

struggles to combat these diseases is illustrated in countless history books, with sometimes striking similarities in avoidance and control strategies across the centuries (Figure 11).

(A) (B)

Figure 11: Protection methods used throughout centuries.

(A) Doctors, to protect themselves from highly contagious diseases doctors wore protective hoods like the plague-cotton-velvet mask, that completely covered the face and the wearer breathed through two small holes in the 'beak', which held herbs or sponges soaked in vinegar. (B) Protective mask worn in 2020, during the pandemic caused by SARS-CoV-2. (Images taken from Morens et al, 2020)

Table 2: Comparison of virus related pandemics (Table reconstructed from Roychoudhury S. et al., 2020)

	SARS-CoV-2	**SARS-CoV-1**	**MERS-CoV**	**Ebola**	**H1N1**	**HIV**
Outbreak year	2019	2003	2012	1976 onwards	2009 (North America)	1981 onwards
Location first reported	Wuhan, China	Southern China	Saudi Arabia	Central Africa	North America	West-Central Africa
Outbreak countries	More than 215 countries	29 countries	More than 27 countries	Africa, the Americas, Southeast Asia, Europe, Eastern Mediterranean, Western Pacific	Africa, Europe, the Americas, South-East Asia	More than 130 countries
Natural reservoir	Not identified	Bat	Bat	Fruit bats, porcupines, and non-human primates	Human, avian, swine	Chimpanzee
Case Fatality Rate	2–3%	10%	34.4–37%	50–63%	0.02–0.4%	80–90%
Community attack rate	30–40%	10–60%	4–13%	5–30%	10–20%	23%
Clinical symptoms	Fever, Cough, Dyspnea, Myalgia, Malaise	Fever, Cough, Dyspnea, Chills, Diarrhea	Fever, Cough, Dyspnea, Myalgia, Headache	Fever, Fatigue, Muscle pain, Headache, Sore throat, Vomiting, Diarrhea, Rash, Low WBCs and platelet count and elevated liver enzymes	Fever, Chills, Cough, Sore throat, Runny nose, Body aches, Headache, Fatigue, Diarrhea, Nausea, Vomiting	Fever, Cough, Muscle aches, Fatigue, Bloating, Headache, Memory loss, Poor appetite, Diarrhea, Nausea

4.7. Educator's implementation guide

The four activities within this learning module have been designed to provide students with the opportunity to explore, research, learn, reflect and peer teach concepts related to the general knowledge of virus pathogens, the general mechanisms viruses use to evade the immune system, and how viruses can be the cause of seasonal epidemics and sometimes pandemics.

The specific assignments are geared toward a general biology course for students within the age range of 12-15 year, but the strategies are applicable for higher A-level, undergraduate and postgraduate level biology classes if the content is scaled up. The order in which they should be applied follows a scaffolding approach where students acquire new knowledge at each level via performing their own research and then use this to bridge their understandings to new learning.

This section includes all four proposed learning activities and descriptions of how these could be implicated as an extension to the general biology topics taught at the different stages of education, including lower secondary (12-15 years).

4.7.1. Collaborative poster (Activity-1)

Getting students to work on their collaboration skills through the creation of group posters is a great way to help young minds to structure their understanding in a visual form; using various ways of presenting the information they collect, including tables and timeline diagrams. During this activity students should be guided on how to choose valid resources for extracting information. Creating a poster encourages student's creativity while assisting student's self-assessment via a set of instructions. This strategy is applicable to any topic as well as beneficial for students' use to express creativity and to familiarize students with this type of performance task throughout the year.

The educator initiates students to what they will be teaching through an introductory discussion, using the information of Section 6 "Background Science", and/or by using the additional resources recommended within this learning scenario (video providing an overview of historical pandemics, caused by various pathogen types and not exclusive to viruses, https://youtu.be/B7ivFcGbFJM). Within this video, the pupils are shown a map of the world and are asked to observe to what extend disease spreads in each pandemic example given.

Activity-1: Creating a historical timeline of past virus-associated pandemics	
Learning objectives	Students will: ➢ gain an overall historical view of general pandemics (via the recommended video resource) ➢ be able to apply and demonstrate their acquired knowledge via the creation of a collaborative poster, where they will be requested to produce a timeline of pandemics caused specifically by virus pathogens, providing information such as dates, identity of causative pathogen.
Time requirements	➢ Preparation of students at home, the day prior to the activity, requires a maximum of 20-25 minutes. 15 minutes for watching the assigned video and 5-10 minutes to prepare notes of the names, causative infectious agents, and dates of pandemics. ➢ The collaborative poster is designed to be implemented over 2 teaching periods (80-100min in total). One teaching period for creating the poster and one for presenting this to the rest of the class.
Description of activity sequence / Educator's Instructions	**The day prior to introducing this activity:** ➢ The educator asks all students to watch at home the video of the overview of historical pandemics and record names of disease and dates (give link or send via email). ➢ For challenging those more able students, the educator could suggest that they could research the individual appearance of each virus they note down. **On the day of the activity**: 1 Display the learning objectives of this given activity on the whiteboard. 2 Begin the lesson by displaying Figure 10 (from subsection 4.6.9) 3 Ask the students to explain with their own words the main difference between an endemic/epidemic/pandemic, based on what they observe. 4 Ask students the question "what do you think are the causative infectious agents of such spreading of diseases?" and get them to think and explain

	how a spreading of disease can accumulate from an endemic case to an epidemic and a pandemic. 5 Organise students in groups of 3-4 and get them to prepare a collaborative poster, summarising a timeline of all historical pandemics that were associated with viruses. Giving them the time to think about how to represent the information that they have gathered, discuss, and reach a consensus on which information and dates they should include in their poster. 6 As groups plan and create their poster, a set of instructions is essential to ensure that there is a discussion amongst them and that they stay on task and use images and record dates down correctly. Each student in the team uses a single and distinct colour marker, meaning each member uses a different colour to represent his/her work on the poster. Each group member also signs the poster in his/her respective colour when the group agrees that the poster is complete. 7 After the posters are complete, groups present the information to the whole class, or groups share the information in a gallery walk format.
Materials required	➢ Colour markers ➢ A3 carton (per poster) or larger ➢ Ruler(s) ➢ Web based resources displayed or printed and given to students to work from
Learning Objects (LO) / **Digital Educational Resources** **(DER)**	➢ Video overview of historical pandemics, caused by various pathogen types and not exclusive to viruses - https://youtu.be/B7ivFcGbFJM **(DER1)** ➢ The educator can use and/or give to students: o Figure 1 from subsection 4.6.1, for historical sequence of pandemics (**DER2: https://trinitonian.com/2020/09/10/a-history-of-sports-and-diseases/**) o Table 1 from subsection 4.6.1, for reference to time periods of various virus-associated pandemics (**DER2**) o Figure 10 (**LO1: http://abilenetx.gov/978/Epidemics-and-Pandemics**) from subsection 4.6.10, as a sketch representation of the differences between the term endemic, epidemic, and pandemic ➢ For background information on the general scope of pandemics, the educator can refer to subsections 4.6.9-4.6.10. ➢ For background Information regarding the architecture of virus, the educator can refer to subsection 4.6.3.
Assessment/evaluation of learning outcomes	➢ Student groups are asked to present their poster to the whole class. ➢ Student posters are displayed on the class board and groups are allowed to view each other's work and along with their educator, they ask questions on the presented work.

4.7.2. Immune-response Action Model (Activity-2)

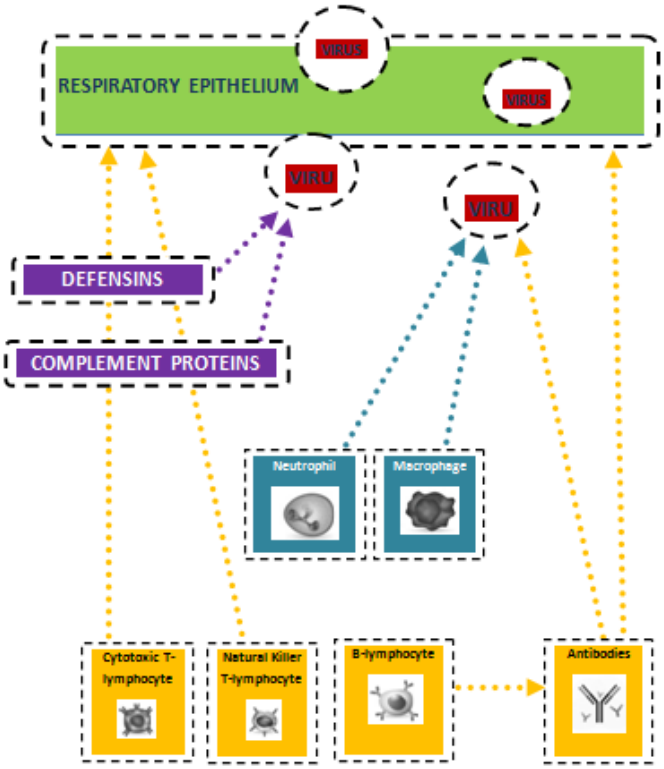
Biology action modelling is a strategy that allows students to participate in active learning where they construct a simple materials model that simulates a dynamic biological process, in this case, the

response of the human immune system towards an incoming virus. This approach to learning increases student accountability for their own learning and allows multiple opportunities for the educator to check for understanding of the contents delivered. In addition, the modelling will help students take an abstract concept and make it tangible and concrete to them.

The immune-response action model demonstrates how a virus can initially enter the human body, infecting initially the epithelial cells covering the surfaces of these entry points, and how the different cells of the human immune system will collaborate to mount an effective immune response that will contain the infection and eliminate the pathogen and any infected cells. Students will be able to model the immune-response mechanisms against a virus, from the point of entry into the body, at the different stages of breaching the anatomical, chemical, and cellular barriers of the immune system.

Activity-2: Constructing an Immune-response Action Model	
Learning objectives	Students: ➢ will learn the different routes of entry that viruses use to inter the human body, and how they can infect human epithelial cells found at those entry points. ➢ will be reminded of and/or reintroduced to the important cell categories of the immune system and their specific functions, and how these interact with each other to bring about an effective immune response. ➢ will be able to link the different categories of immune cells/components to the three main barriers of immune protection (anatomical/ chemical/cellular). ➢ will explore how antibodies and different immune cells and immune components help protect against viral pathogen. ➢ will be able to model the interaction between and incoming virus, epithelial cells, complement proteins, B-cells, antibodies, and T cells to manipulate the fundamental immune response mechanisms triggered when a virus enters the human body.
Time requirements	➢ Activity-2 will require a prior introduction to the cells of the immune system and their specific functions (see educator's instructions for more details). This could be a 20-minute session, where the educator will introduce their students to the fundamental barriers of the immune system and the different categories of immune cells/ specific functions. They will also explain the importance of collaboration between these components to ensure an effective immune response against an invading virus. ➢ Students shall be given printouts of the different categories of immune components and will be asked to cut out to use for constructing their model. This should take 5-10 minutes. ➢ The actual construction of the immune-response action model should take 20-30 minutes to complete, allowing students to familiarise themselves with the different categories of immune components and their functions towards protection, the interactions between the different cell categories and the outcome leading to immunological protection.

Description of activity sequence / Educator's Instructions	**The day prior to introducing this activity:** ➢ Educator needs to familiarise themselves with the theory related to this module by reading through subsection 4.6.6 and 4.6.7 of the current learning scenario. ➢ Printouts of the different categories of immune components need to be prepared (see relevant appendix section). ➢ A list of set out steps should be printed for each student groups to help them with the construction of their model (see relevant appendix section). ➢ Print out of assessment of learning sheet for students to complete at the end of the activity. **On the day of the activity**: 1 Educator introduces their students to the fundamental protective barriers (anatomical/chemical/cellular) and the different categories of immune cells, explaining their specialised functions in promoting active immune responses against an incoming virus pathogen. They should follow the suggested sequence of events and refer to Figure 7, in subsection 4.6.7, for their own guidance: > A virus entering the human body experiences the first line of defence that involves the anatomical barrier (epithelial cells). > The action of the anatomical barrier is further aided by the complementary action of the chemical barrier (mucus, enzyme secretions from the epithelial cells and complement proteins). > Even after the collaborative action of these two barriers if a virus succeeds in infecting the epithelial cells, it will have to face the action of the cellular barrier (innate). At this point immune cells known as phagocytic cells will internalise the virus and break it down by internal cellular mechanisms. > The action of the innate cellular barrier is also aided by the collaborative action of the chemical barrier; chemical complement proteins that bind to the virus better facilitate the actions of phagocytic cells. > However, if the innate immune cells become overwhelmed by an increasing virus load and cannot clear out the infection, then their actions are re-enforced by signalling the help of the specialised branch of the cellular barrier, known as adaptive. > The cellular adaptive barrier involves the specialised action of lymphocytes, including both T-lymphocytes and B-lymphocytes. > T-lymphocytes will regulate the action of immune cells of both the innate and adaptive cell barrier but will also act on their own and eliminate virus infected cells either directly (Natural Killer cells) or via regulation (Cytotoxic T lymphocytes). > B-lymphocytes are mainly responsible for the secretion of virus specific antibodies. These cells require T-lymphocyte regulation to produce and secrete virus-specific antibodies. Secreted virus-specific antibodies are a component of the chemical barrier of the immune system. At this point it should be made clear to students that all barriers of the immune system complement each other via their collaborative functions to produce an effective immune response. 2 Educator asks students to colour their cut-outs:

	> Anatomical barrier components in green > Chemical barrier components in purple > Cellular innate barrier components in blue > Cellular adaptive barrier components in orange 3 Educator arranges their students in groups of 3-4 and asks them to use their coloured cut-outs to manipulate the immune response towards an incoming virus, by sticking these on an A3 (or larger) paper. The action of each component should be indicated by drawn in arrows having the same colour as the acting component.  4 Educator can assess individual student learning by getting students to complete the assessment of learning sheet at the end of this activity.
Materials required	➢ Coloured pens/pencils/markers (green, purple, blue, orange) ➢ A3 carton (or larger) ➢ Scissors ➢ Glue ➢ Prints of immune components per group
Learning Objects (LO) / **Digital Educational Resources** **(DER)**	➢ The educator can use and/or give to students: o Figure 7 from subsection 4.6.7, for reference to the four main barrier of defence towards incoming pathogens o List of sequence of events from "**Description of activity sequence / Educator's Instructions" (New LO2)** ➢ For background information on mode of viral infection and the effective mounting of an effective immune response, the educator can refer to subsections 4.6.7 and 4.6.8. ➢ Use of **New LO2** for cut-out prints and assessment sheet (found at the end of Activity-2).

Assessment/evaluation of learning outcomes	1 Guiding questions for the groups should be used to monitor student progress and to check for understanding as students interact with the model. Examples could include: > Ask students to decide which virus point of entry they would like to explore. > As them to classify their virus (i.e., is this a respiratory, intestinal virus etc) > Ask students to identify the anatomical barrier that their virus is encountering at first entry. > Ask them to identify the most important cell from the cellular adaptive barrier and explain their choice. 2 At the end of the activity the educator can give out a handout listing the series of events during an effective immune response towards an incoming virus and ask students to number the correct order to assess individual student learning.

Material required for Activity-2: Immune-response Action Model (New LO2)

Pathogenic component

VIRUS

Components of the immune system

a) The anatomical barrier components

SKIN

RESPIRATORY EPITHELIUM

INTESTINAL EPITHELIUM

b) The chemical barrier components

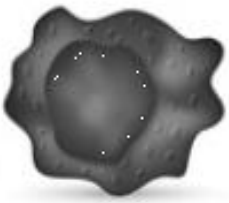
COMPLEMENT PROTEINS

DEFENSINS (enzymes)

c) The cellular innate barrier components

Neutrophil

Macrophage



d) The cellular adaptive barrier components

T-lymphocyte $CD4^+$

Cytotoxic T-lymphocyte $CD8^+$

Natural Killer T-lymphocyte

B-lymphocyte

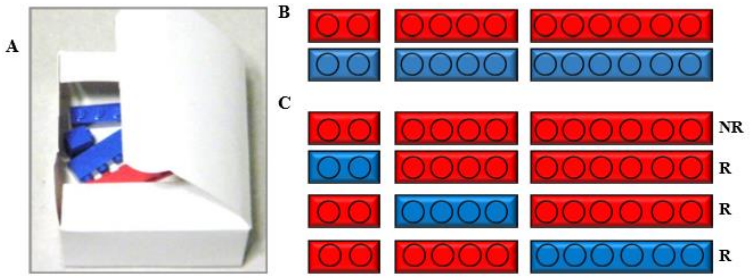
Antibodies

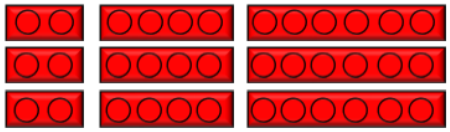
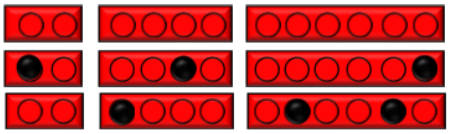
4.7.3. Simulation of virus antigenic drifting/shifting (Activity-3)

The presented activity offers a straightforward and efficient way to visualize the mechanisms of antigenic shift and drift in an evolving virus. Pedagogically, the influenza virus proposed by Marintcheva B., (2016) is an excellent choice for demonstrating these concepts in the classroom (LO3). Antigenic drift and shift are central to understanding viral diversity and evolution and the occurrence of re-infections and have direct application to vaccine design and development. Students often struggle to fully understand how both phenomena of viral drift and shift work mechanistically and thus have limited opportunity to gain an appreciation of the scientific principles behind a vaccine's development and its effectiveness. Marintcheva has developed a simple exercise using conventional LEGO bricks to physically model antigenic shift and drift to aid student understanding. The exercise can be executed in any type and level of classroom for about 10 minutes. The material used for this activity is economical and easy to store in the classroom. During this activity, students work in pairs and take turns manipulating the LEGO bricks and recording their data. The example of the virus used is that of Avian Influenza and for keeping the activity simple, this model of virus has only three genome segments, each visualized by a LEGO brick of a different size. A small non-transparent box is used to model the host cell. The educator needs to first explain to the students the concepts of antigenic shift and drift be referring to subsection 4.6.8 (could also use Figure 9, to explain this is in a graphical way).

In short, **antigenic drift** is defined as a random genetic mutation occurring in an infectious agent such as a virus, resulting in **minor changes in proteins called antigens**, expressed on its surface, which stimulate the production of antibodies by the immune systems of humans and animals. A proportion of human/animal populations will have immunity to these minor changes of viral antigens. On the other hand, **antigenic shift** is defined as a genetic alteration occurring in an infectious agent that causes a **dramatic change in an antigen** on its surface, which stimulates the production of antibodies by the immune systems of humans and other animals. Pre-existing immunity to such a dramatic antigenic change is minor and the newly emerged viruses have the potential to cause epidemics or pandemics, since very few, if any, humans possess immune memory cells that can protect them against the new antigens.

From the antigenic shift modelling activity, students will realize that roughly only 25% of their constructed brick-viruses are non-recombinant (of single colour) and 75% are recombinant (having different combinations of the two colours). If the newly acquired fragment (i.e., the one with a different colour) is significantly different from its predecessor and antibodies with different specificity will be required to neutralize the virus, then the re-assortment event results in antigenic shift. From the antigenic shift activity, student will randomly choose which studs to colour, and the class ending up with a considerable variety of mutated versions of the genomic fragments and creating a powerful visual of how diverse the progeny of a single virus can be. Over time viruses could mutate significantly by accumulating multiple small mutations, to the point that antibodies that used to neutralize them will no longer be effective. The newest version of a virus comes to existence via antigenic drift. Considering the actual size of the influenza genome (~14,000 nucleotides long) and the error rate of the influenza RNA polymerase, students can appreciate the magnitude of the diversity that can be observed in influenza viruses.

Activity-3: Simulation of virus antigenic drifting/shifting	
Learning objectives	Students: ➢ will be able to understand viral diversity and evolution using a simple hands-on activity. ➢ will be able to appreciate how a virus requires host cells and multiple hosts to evolve into a pathogen that can be linked to a pandemic.
Time requirements	➢ The educator will need to introduce the activity to their students explaining the concepts of antigenic drift and shift. This should require 15-20 minutes of lesson time. ➢ The LEGO activity is a 10–15-minute activity.
Description of activity sequence / Educator's Instructions	**On the day of the activity**: 1 Educator delivers a short presentation introducing the concepts of antigenic drift and shift as important evolutionary mechanisms of viruses. 2 Students are asked to work in pairs and take turns manipulating the LEGO bricks and their recording data. The **LEGO bricks of two different colours** are given in a non-transparent bag/box/container, representing the infected host cell.  Antigenic shift is modelled using a non-transparent box (panel A) as an infected host cell and two sets of Lego® bricks with different colour (panel B) representing the genomes of distinct Influenza strains replicating with the same rate. Recombinant genomes (R) and non-recombinant genomes (NR) are shown in panel C. Modelling **antigenic shift** of a virus within a host cell: 3 Each pair of students is given a box with two viral genomes in different colours. The box represents a host cell harbouring two different strains of influenza virus. 4 The students are asked to construct a virus by assembling a full set of genomic fragments (as shown above) without peeking in the box, and to record the genetic makeup of the constructed virus by describing the fragment colours. 5 Once done with recording, the LEGO bricks are returned to the box and the task repeated to construct ten viruses and to report the number of recombinant and non-recombinant viruses. Modelling **antigenic drift** of a virus in a host cell:

	6 Each pair is given three viral genomes of the same colour and a permanent marker. Students are asked to keep one genome as a reference and to "generate" two new copies, considering the error-prone nature of the **RNA polymerase that makes three errors for every 10 nucleotides copied**. 7 Each pair of students is asked to randomly colour three LEGO studs in two of their genomic virus sequences. Every stud of a LEGO brick is to be considered a nucleotide and every mutation is to be "recorded" by colouring the "mutated stud" with a permanent marker (as shown below). A  B  Panel A shows the three viral genomes given to students, panel B shows a non-mutated genome on the top, and two randomly mutated genomes below (for each viral genome there should be **three black-coloured studs** of LEGO). 8 All students are asked to display their mutated genomic sequences and the educator records all possible new combinations of mutations generated by the whole class. More able students within the class can be challenged further by being asked to connect antigenic shift and drift to vaccine development and effectiveness.
Materials required	➢ Black marker pens ➢ LEGO bricks of two different colours ➢ Non-transparent bags, boxes, containers (that will not facilitate students peaking inside them).
Learning Objects (LO) / **Digital Educational Resources** **(DER)**	➢ The educator can use: o Figure 9 from subsection 6.8, for reference to the strategies of viruses for evading immune defences. o References of **Armitage, H**., (2015), (**DER3:** http://news.sciencemag.org/biology/2015/08/universal-flu-vaccine-horizon) and **Hannoun, C.,** (2013) (**DER4**: www.medscape.com/viewarticle/812621), , to introduce recent developments in the search for a universal flu vaccine in contrast to the established procedure for selecting the composition of the yearly vaccine.
Assessment/evaluation of learning outcomes	➢ To assess the students understanding the educator can pose the following questions during this activity: Would one observe antigenic shift if antigenic drift does not take place? Answer: If antigenic drift did not happen, it is very likely that antigenic shift would not be observed either. The re-assorted fragments would be very similar, and the same antibodies would likely be able to neutralize the original and the recombinant viruses.

4.7.4. Discussion and proposal of possible strategies for dealing with future pandemics (Activity-4)

This final activity comes to conclude on the sequence of all previous activities, where the educator poses the question of what could be a proposed strategy for dealing with a future virus pandemic, bearing in mind both positive and negative impact on the human population, at local community, at national and international level. For this activity, the educator needs to refer to subsections 4.6.9 to 4.6.10 and consider the objectives and outcomes of the previous activities performed with their students.

Activity-4 is a concept mapping engaging activity that helps students tackle complex course concepts and promotes problem-based learning, where students acquire knowledge by devising a solution to a real-world problem, in this case how should future pandemics be dealt with. As they do, they can apply their acquired knowledge, as well as work on their communication and collaboration skills.

Activity-4: Discussion and proposal of possible strategies for dealing with future pandemics	
Learning objectives	Students: ➢ will apply their acquired knowledge from activities 1-3 to come up with proposals and strategies for dealing with future virus-related pandemics. ➢ will improve their collaboration and communication skills by working with their peers. ➢ will develop and improve their critical thinking by being applying acquired and existing knowledge.
Time requirements	➢ The educator introducing their students to this new activity and providing them with a set of instructions ensuring that they keep to task requires 10 minutes. ➢ Student discussions within their allocated groups and recording of their ideas and suggestions require 20 minutes. ➢ The remaining lesson time, 15-20 minutes, the educator collects each groups ideas and records a proposed strategy plan on the whiteboards including of the positive and negative outcomes to the subjects of the community to which this is applied (i.e., school community, national, international)
Description of activity sequence / Educator's Instructions	**Prior to the day of this activity:** ➢ The educator needs to review the theory of this learning scenario and the objectives and outcomes of the previous activities that they have performed with their students. ➢ They could prepare a presentation summarising all learning outcomes from the three activities performed. **On the day of the activity**: 1 Educator presents a summary of all acquired knowledge from previous three activities 2 Educator divides the class into teams and presents them with a course-related problem. 3 One team member writes down a solution and a problem and passes the sheets of paper along to the next team member, who builds upon that idea and then passes it along to the rest of the team. 4 In the end, a spokesperson can present their ultimate solution.

	5 Meanwhile the educator records the outcomes on their whiteboard as the teams get to express their solutions to the whole class.
Materials required	No specialized materials required for this activity.
Learning Objects (LO) / **Digital Educational Resources (DER)**	➢ The educator can refer to the theory (section 4.6) of the current learning scenario, to the previous activity descriptions and to the suggested list of references for information to structure their summary presentation.
Assessment/evaluation of learning outcomes	➢ The educator can assess their students' individual learning by asking them to prepare an essay with the title "History of pandemics: what do we know about powerful viruses and their impact?" using the outcomes all four activities that they have participated in. ➢ The selected parts of the work of the students can be edited into a single article by their teacher and published in the school's newsletter, as well as on their school website, but also on the local pedagogical website paideia-news.com, were the work of the students can be access and read by parents, all education level professionals and stakeholders in the educational field.

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4.9. Educational Scenario Impact Assessment Questionnaire

Context: The knowledge of historical virus-related pandemics sheds light on powerful viruses and their impact on aspects of health, society, economy, and governance. The scenario complements the teaching of the existing curriculum of general biology of the immune system in schools, for students of 3rd grade in lower secondary education, between the ages of 14-15 years old, and is designed to provide them with the opportunity to explore, learn, and peer teach concepts related to more specialized functions of the immune system against invading viral pathogens, aiding them to build on prior learning and develop further skills and attitudes.

Meanwhile, educators will be supported with further inside related to the topic, provided with ideas, activities and tools to support the learning of their students, supporting acquire transferable skills such as critical thinking, problem solving, analysis, reasoning, interpretation, adaptive learning, creativity, continuous learning, self-direction, responsibility, perseverance, self- regulation (metacognition, forethought, and reflection), integrity, self- monitoring, self- evaluation, self- reinforcement, and apply all these to their everyday life within their community.

Additional information: the topic is provided in the specifications of an educational scenario of the "History of Pandemics: what do we know about powerful viruses".

The questions that follow provide and assessment for the impact of the given learning scenario on the pre-existing knowledge of the students, the skills that they have acquired throughout the teaching of this topic and the effect of this on their beliefs, attitude, and behaviour.

As part of acquired knowledge students can:	
1. Identify the history of Pandemics is mostly associated with the evolution of viral pathogens.	1.1. What are the main causative agents of historical pandemics? A. Viruses B. Bacteria C. Fungi 1.2. Which pathogen has always been in the spotlight as the cause of most major pandemics? A. Influenza B. Corona virus C. Human Immunodeficiency virus (HIV)
2. Understand the nature/architecture of viruses and how this changes to allow for evolution	2.1. Viruses are: A. Non-living, intracellular parasites, requiring the use of the host cell's biochemical machinery to replicate and create new infectious virus particles. B. Living, intracellular parasites, requiring the use of the host cell's biochemical machinery to replicate and create new infectious virus particles. C. Non-living, intracellular parasites, that do not require the use of the host cell's biochemical machinery to replicate and create new infectious virus particles. 2.2. Which virus is the smallest in diameter? A. Picornavirus B. Human Immunodeficiency Virus (HIV) C. Influenza virus 2.3. SARS-Cov-2 has a: A. helical form B. complex polymorphic form C. icosahedral form 2.4. Which of the following is not a natural way leading to the evolution of a virus? A. the prolonged use of anti-viral drugs B. escape of potentiator viruses from the human immune system C. base substitution mutations

3. Understand the strategies used by viruses to evade the human immune system.	3.1. SARS-CoV-2 typical infection involves the initial attachment of the virion to the cell membrane of its target via the virus's A. spike protein B. gp120 receptor C. $CD4^+$ receptor 3.2. Which virus can persist within the host's tissues in a non-infectious (latent) form following acute infection recovery and can be reactivated under low immune surveillance to produce new infectious virions? A. Herpes simplex virus (HSV) B. SARS-CoV-2 C. Influenza virus
4. Explain how the immune system responds to current and novel invading viruses.	4.1. The human immune system comprises of a: A. 2 – level barrier B. 3 – level barrier C. 4 – level barrier 4.2. Which is not part of the cellular-innate barrier of the human immune system? A. Natural killer cells B. Macrophages C. B – cells 4.3. The ability of a virus to radically changing its surface antigens thus resulting in the generation of novel strains is termed: A. Antigenic drift B. Antigenic shift C. Antigenic mutation
5. Define, identify, and describe pandemics	5.1. What is the difference between pandemics and epidemics? A. In both an occurrence of transmissions is noted but, in an epidemic the number of cases increases, whereas in a pandemic the number of cases increases and spreading occurs worldwide. B. In both an occurrence of transmissions is noted but, in an epidemic the number of cases remains constant, whereas in a pandemic the number of cases increases but remain local. C. In both an occurrence of transmissions is noted but, in a pandemic the number of cases increases, whereas in an epidemic the number of cases increases and spreading occurs worldwide. 5.2. Spreading of disease must not consider which of the flowing? A. Human-to-human transmissions B. Animal-to-human transmissions C. Animal-to-animal transmissions 5.3. Considering the Geographic extension of the virus, a pandemic can be categorized as being Global when: A. the virus occurs at two (2) or more adjacent regions of the world B. where the virus occurs at two (2) or more nonadjacent regions of the world C. where the virus occurs at most regions around the world
As part of skills being gained/developed students can:	
1. Select appropriate sources to extract valid historical information regarding pandemics and the spreading of disease	1.1. Which data sources may we use to extract historical information regarding pandemics? A. Centre of Disease Control and Prevention (CDC) and WHO database B. Data retrieved by google searches C. Data extracted from Historical online resources
2. Select appropriate sources to explain the historical course of viral pandemics in a scientific perspective.	2.1. To find scientific information about the historical course of viral pandemics I should consult the following sources. A. researchers, scientific publications, CDC database B. newspapers, google, YouTube C. friends, journalists, Facebook

3. Select appropriate scientific data and information to describe the historical course of viral pandemics.	3.1. I feel able to identify scientific sources to describe the historical course of viral pandemics. 1) strongly disagree… 5) strongly agree. 3.2. I know the main sources to consult to assess the progress of viral pandemics. 1) strongly disagree… 5) strongly agree
4. Propose plausible actions towards promoting protection from possible viral infections in his/her lifestyle.	4.1 Which individual actions can be taken to help containment of a spreading virus within your school community? A. Notify your school community of your unwellness and take a leave to stay home until you recover, while arranging to participate in lessons online. B. No need to notify your school community and go to school as normal. C. Notify your school community and go to school and try and keep a mask on at most times. 4.2 Which individual actions can be taken to help containment of a spreading virus in the vast community? A. Seek the advice of your personal doctor and contain yourself until you are free of all symptoms, and you are no longer infectious to others. B. If feeling that your symptoms are milt, continue to interact within your community. C. Continue to interact within your community, ensuring that you follow precautions such as the use of a face mask.
5. Influence the adoption of choices by others (e.g., their family, peers, friends etc.).	5.1 I feel able to influence the adoption of actions that help achieving the prevention of a future pandemic, including my family, fellow students, and friends. 1) true... 5) false. 5.2 I will try to influence the adoption of actions that help achieving the prevention of a future pandemic, including my family, fellow students, and friends. 1) true... 5) false
6. Selects appropriate scientific data and information to describe the progress of a pandemic	6.1 I feel able to identify scientific sources to describe the progress of a future pandemic. 1) strongly disagree… 5) strongly agree. 6.2 I know the main sources to consult to assess the progress of a future pandemic. 1) strongly disagree… 5) strongly agree.
7. Selects appropriate sources to characterize a pandemic in a scientific perspective.	7.1 To find scientific information about pandemics I should consult the following sources. A. researchers, scientific publications, WHO and CDC data bases. B. newspapers, google, YouTube C. friends, journalists, Facebook, other social media.
8. Identify the problems and challenges of their community in relation to a pandemic and find the relevant resources to address them.	8.1 I feel able to identify the main problems my community faces in relation to a pandemic situation. 1) false… 5) true. 8.2 I can understand how the challenges my community faces are related to health and well-being outcomes. 1) false... 5) true. 8.3 I feel capable of proposing actions that address how to prevent the spreading of a pathogenic virus in my community. 1) true... 5) false.

As part of Beliefs, Attitudes and Behaviour, there are no correct or incorrect answers; we are only interested in knowing the students' perspective on the topic introduced.	
1. Believes that is important to contribute to global efforts for tackling future pandemics.	1.1. My participation and actions will increase the chances of success of the global efforts for preventing a future pandemic. 1) strongly disagree… 5) strongly agree. 1.2. I am physically and financially capable of adopting actions that contribute to the efforts of tackling the spread of disease that can lead to a future pandemic (i.e., contain myself if I am feeling unwell, follow rules and regulations regarding health measures recommended, etc.). 1) extremely unlikely... 5) extremely likely. 1.3. My family and friends think that I should adopt actions that contribute to the global efforts for tackling future pandemics. 1) Extremely unlikely... 5) Extremely likely.
2. Believes that learning about the history of past pandemics and about powerful viruses associated with the spread of disease can lead to positive outcomes at the community level when it comes to handling the spreading of communicable disease more effectively.	2.1. To learn about the history of past pandemics and the causative viruses that lead to the spread of disease will lead to positive outcomes at my community. 1) strongly disagree… 5) strongly agree. 2.2. My community thinks that learning more about past pandemics will bring positive outcomes 1) Extremely unlikely... 5) Extremely likely.
3. Believes that it is crucial to identify obstacles and problems faced by communities regarding the handling of communicable disease.	3.1. The identification of obstacles and problems that my community faces are crucial for solving them. 1) strongly disagree… 5) strongly agree. 3.2. It is possible to identify obstacles and problems that my community faces regarding the dealing with the spread of infectious disease 1) strongly disagree… 5) strongly agree. 3.3. It is common knowledge that it is necessary to identify obstacles and problems that the community faces for solving issues regarding communicable disease. 1) strongly disagree… 5) strongly agree.
4. Has intention to perform sustainable behaviours in his/her/their lifestyle.	4.1. I will try to update myself with current/new information regarding highly contagious viruses. 1) Extremely unlikely... 5) Extremely likely. 4.2. I plan to incorporate health hygiene (i.e., washing my hands often, containing myself if unwell until my symptoms reside) in my day-to-day life. 1) Strongly disagree... 5) Strongly agree.
5. Attitude toward learning about past pandemics and how powerful viruses spread in a community.	5.1. For me to achieve such knowledge is: Harmful : _____:_____:_____:_____:_____: beneficial Pleasant : : : : : : unpleasant Good : : : : : : bad Worthless :_____ :_____:_____:_____:_____: valuable Enjoyable : : : : : : unenjoyable

5. Specifications for an educational scenario on the topic "Workings and malfunctions of human Immunological memory"

5.1. Introduction to the module

This topic sheds light onto the concepts of long-lasting immunological memory and protective immunity, to simplify and improve the understanding of a challenging subject such as Immunology. Educators and students of ages 12 – 15 years old (preferably 15-years of age), are therefore given the opportunity to expand their knowledge by learning about important concepts in Immunology, such as the innate and adaptive arms of the human immune system and the cellular components involved in the establishment of immune memory and the interactions that bring about protective immunity and preventing re-infections. An insight is also given on how immune memory is independent of the frequency or persistence of re-infection, being a long-lasting feature involving both specialised cells of the adaptive and innate immune systems, but also depending on all the rest of the cells of the human body.

Using differentiated instructions to teach immunological concepts related to immunity against infections to a diverse group of learners, of various ages, provides a comprehensive learning module designed to expose high school students to immunological concepts related to the immune memory mechanisms. Such mechanisms are in place to minimise and preventing the spreading of disease and cause of epidemics and possible pandemics. The present learning scenario uses active hands- and minds-on teaching strategies, where students are also introduced to the consequences following malfunction of immune memory mechanisms.

This module includes and proposes the following:

Activity 1: Flipped-classroom activity introducing primary and secondary innate and adaptive immune memory.

Activity 2: In-class short presentation of collaborative work on a selected topic involving immune memory.

Activity 3: Building concept maps of the events leading to established innate and adaptive immune memory.

5.2. Expected student prior knowledge

Students should have a basic Cell Biology knowledge of the characteristics and the Variety of living organisms, including:

- ✓ knowledge and ability to describe the common features shown by eukaryotic organisms (i.e., animals, fungi and protoctists, and prokaryotic organisms such as bacteria),
- ✓ an understanding of the term pathogen and know that pathogens may include fungi, bacteria, protoctists or viruses,
- ✓ knowledge of structures within living organisms and their associated functions (i.e., the bone marrow enclosed withing the spinal cord comprises the lymphopoietic organ of the human body, lymphoid tissues such as the thymus gland and peripheral lymph nodes are organs where immune cells differentiate and develop into specialised cells) including levels of cell organisation, cell structure, biological molecules (including proteins, enzymes, DNA, RNA),
- ✓ an understanding of the different ways of transport of substances in and out of living cells, with emphasis on receptor-mediated transport processes.
- ✓ an understanding of cell membrane features such as the presence of surface bound receptors that can be associated with cell-to-cell interactions, or receptor mediated antigen binding and presentation, (i.e., T-cell and B-cell receptors, the Major-Histocompatibility-Complex, MHC, found on all cells of the body an associated with the presentation of internal and external antigens) etc.

5.3. Expected outcomes

Learns will have the opportunity to acquire new knowledge and enhance their understanding, about:

- ✓ The two arms of the immune system: the innate immune responses are non-specific and general against groups of pathogens, whereas the adaptive immune responses are highly specific against given structural motifs of a given pathogen,
- ✓ The cellular and molecular components of each arm of the immune system: this includes the cells and molecules involved in innate and adaptive responses,
- ✓ The interactions between the immune cells and molecules: chemical and molecular interactions including, signalling, activation, proliferation, differentiation processes, to trigger immune memory mechanisms and protection against re-infection,
- ✓ The problems arising from malfunctions in immune memory: such issues may lead to lack of protection and in some case the trigger and initiation of autoimmune disease, whereby the immune system turns against its own cells and molecules.

Learners will also acquire the use of transferable skills such as critical thinking, problem solving, analysis, reasoning, interpretation, adaptive learning, creativity, continuous learning, self-direction, responsibility, perseverance, self-regulation (metacognition, forethought, and reflection), integrity, self-monitoring, self-evaluation, self-reinforcement, and apply all these qualities to their everyday life within their community.

5.4. Relation to other topics

This module could complement the teaching of the existing general curriculum in biology that focuses on the immune system. The activities included are designed to give students the opportunity to explore, learn, and peer teach concepts related to more specialised functions of the immune leading to immunological memory to past infections. The specific assignments are geared toward a general biology course, but the strategies are applicable for higher-level biology classes (A-level, undergraduate classes) if the content is scaled up appropriately. The order in which these activities should be applied follows a scaffolding approach where students uncover new knowledge at each activity level and then use it to bridge their understanding to new learning.

5.5. Pedagogical methods utilised in the teaching of this module

The objective linked to this module is to help students build on prior learning and develop further skills and attitudes. Meanwhile, the current knowledge of their educators is also enhanced, enabling them to present the given module in a way that is relevant to their students' needs.

A range of different pedagogical methods are implemented through all different activities, catering for a broad range of different learners. The current learning module is based on the pedagogical approach of inquiry-based learning, where students are encouraged to ask questions and complete research while learning various concepts of basic immunology associated with immunological memory. In this way, individual learners acquire the skills necessary to develop their own understanding, as well as question themselves and group members in a constructive way.

The initial flip-class room activity investigates primary versus secondary innate and adaptive immunity, whereby students are asked to watch a series of related online videos and are given a set of pre-lesson questions that they are to complete prior to the actual lesson. This provides an excellent tool that improves tremendously the in-class time with their educator, making the delivery of a lesson more productive. The additional workload imposed by the pre-class activities is worthwhile, as it helps students in their understanding through reinforcement of pre-class material in class, allowing them to adapt an incremental work ethic as opposed to memorising and cramming of information. Additionally, the student responses are used by the educator to provide whole-class feedback and to better focus the in-class plan on the collective needs of the students, transforming the classroom from educator-dominated to student-centered, allowing for bidirectional constructive feedback, creating thus an information loop where in-class and outside-of-class work is highly connected allowing for exploration, collaboration, and interaction among students, while the educator is consistently given feedback of the level of their understanding.

The in-class short collaborative presentation activity follows the “peer instruction” active learning strategy where students are given a narrow list of topics and asked to sign up for a topic of their choice, prepare and present this to their class or in small groups. This approach is particularly useful as this will involve the breaking down of the presented topic into smaller parts, and each student groups will be presenting information about a different part. This approach helps the students to associate cells and molecules and their interactive processes, with different people, therefore acting as an aide memoire for their benefit. This approach also encourages interaction and trust-building between students, being especially important at a time where a portion of learning may take place online while students will be researching on their selected topics.

The final learning activity of building a concept map of the events leading to established innate and adaptive immune memory comes to sum up the previous activities, providing students with a framework within which to organize their newly acquired knowledge, without becoming overwhelmed from all the information involved. This learning technique is particularly well-suited for teaching challenging topic such as immunology-based topics, as students must master an impressive number of new words and concepts in a short period of time. Assigning concept maps after studying a chapter that is particularly jargon-heavy is quite successful and works well in-class group and individual activities, as well as a take home study tool. Assigning students to work on creating concept maps in groups of three or four, also decreases the grading burden, allowing the educator to give more thoughtful feedback to their students, but it also allows students the benefits of discussing with each other what belongs where, and why, and how processes can be interlinked. Thus, in the process of constructing the map, the students are highly engaged in peer-instruction and metacognition learning.

5.6. Background science

5.6.1. What is immunological memory?

Immunological memory is an important evolutionary feature that improves host survival upon re-infection with pathogens. This means that immune cells of the human body can retain memory of past infectious agents and their associated antigens and responds fast and efficiently to any reencounters.

An antigen is basically a molecular structure that can stimulate an immune response. It is important to note that not all molecular structures are antigens, as many will not activate the immune system against them. Immune memory provides a characteristic acknowledged within both the innate and adaptive cellular barriers of the human immune system. Although the mechanisms and properties through which innate and adaptive immune memory induction occur are distinct, their combined effects improve host defence towards pathogenic intruders. Initially, immunological memory was tightly connected to the actions of the cells of the adaptive arm of the immune system, which includes the B and T cell populations. However, since 2013, innate immune memory, otherwise termed "trained immunity", has been explored during vaccine adjuvant development (Vasiliakos P. J., 2013, Basto P. A., et al., 2014, Töpfer E. et al., 2015) and has been acknowledged as an important supportive component of the protection provided by the cellular adaptive barrier.

This topic reviews the main effector components of the human immune system and provides a clear insight to how the two arms of the immune system collaborate to bring about immunological memory to all past but also newly emerging invaders.

5.6.2. The cell players of Immune memory.

The human immune system is comprised by a distinct population of cells along with a vast number of molecular components that all work together to orchestrate an effective protection against any foreign entity that enters the human body. To ensure understanding of all the complicated interactions and mechanisms that take place during an immune response to bring about immune memory, one needs to become familiar with the very basics of immunology.

Starting from fundamental knowledge, all the cells of the human immune system are generated in the bone marrow from progenitor stem cells (Figure 1), some of which develop and differentiate locally, whereas others that require unique environmental conditions migrate to other tissue of the human body to do so. A helpful example is that of B and T lymphocytes, with the latter migrating to the thymus

gland to develop and differentiate into mature, specialized T cell populations that can respond to non-self-antigens. There are two main lineages from which immune cells are derived, these being the myeloid and lymphoid progenitors from which innate and adaptive immune cells, respectively, arise (Figure 1). The cells of the innate immune arm are derived from myeloid progenitor cells, the most important being:

- mast cells, mainly associated with responses to allergens
- myeloblasts, including:
    - monocytes that give rise to macrophages and dendritic cells, these being the major antigen presenting cells of the immune system, and mediators of the adaptive immune arm,
    - eosinophils and basophils, mainly associated with parasitic infections,
    - neutrophils, important phagocytic cells involved in the elimination of bacteria and fungi.

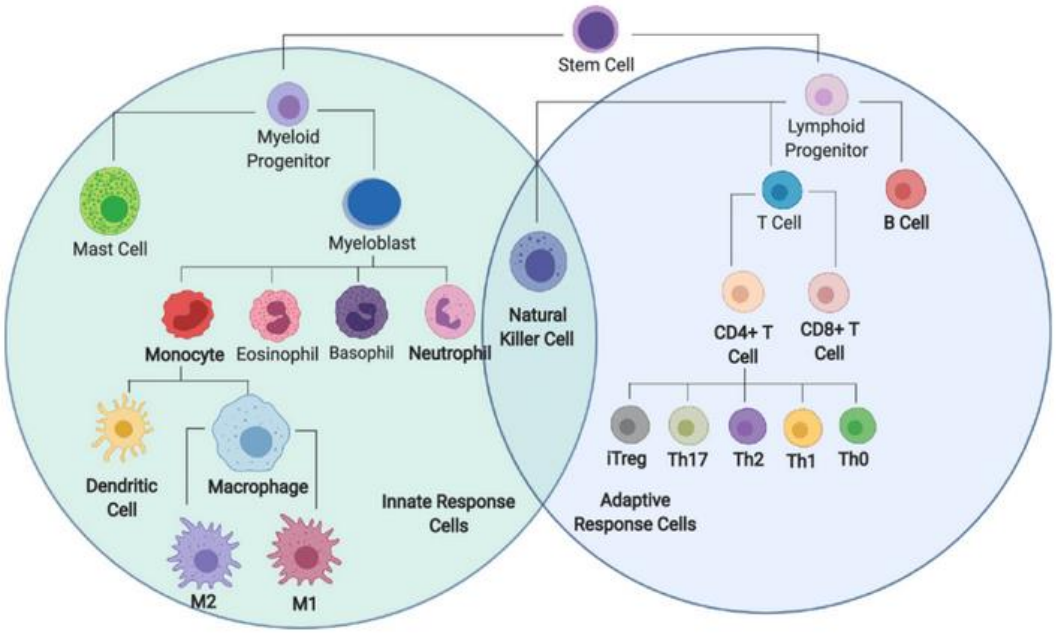


Figure 1: Representation of cells of the immune system.
Immune cells are derived from hematopoietic stem cells in the bone marrow and differentiate into lymphoid and myeloid progenitors that further branch out to differentiate into more specific cell types associated with adaptive and innate immunity. (Figure taken from Torang A., et al., 2019)

The cells of the adaptive immune arm include the T and B lymphocytes, both assigned to pathogen-specific responses and initially linked to long term immunological memory. To our current knowledge, both innate and adaptive immune cells comprise important mediator of immune memory and this topic examines both categories, to explain how memory is implemented and how this can sometimes malfunction to results in re-infection and possible development of disease.

Each immune cell serves a given purpose, this being either auxiliary or specific. However, immune memory is associated with specificity to a given pathogen and is directed against both external and internal molecular pathogen-associated structures, i.e., external surface molecules and/or internal genetic material. Lymphocytic cells of the immune adaptive arm have evolved to directly identify specific pathogen-associated molecular motifs via specialised receptor molecules expressed on their cellular membrane. Activation of B lymphocytes via their B-cell receptor (BCR) leads to the secretion of pathogen-specific antibodies. In a similar way, activation of T lymphocytes via their T-cell receptor (TCR) leads to direct cytotoxic elimination of pathogen and/or the initiation of orchestrated T-cell-mediated responses against it, involving both adaptive and innate arms of the immune system (Figure

2). The action of both B lymphocytes and innate cells is directed against specific external molecular pathogenic motifs, whereas the T lymphocyte-associated immunity is mostly directed towards internal molecular pathogenic motifs (Figure 3), that are exposed following pathogen internatisation, processing and presentation by specialised antigen-presenting cells, such as macrophages, dendritic cells, and organ/tissue specific epithelial and endothelial cells. It is important to note that phagocytic cells are equipped with specialised receptors, known as Toll-like-receptors (TLRs) that are expressed both on their cell membrane but also within their cell cytoplasm and involved in the identification and binding to conserved (unchanged) pathogen-associated motifs. This feature enables phagocytic cells to "remember" conserved molecular patterns expressed by a variety of pathogens, an ability that has been acquired through evolution and continuous exposure to pathogens.

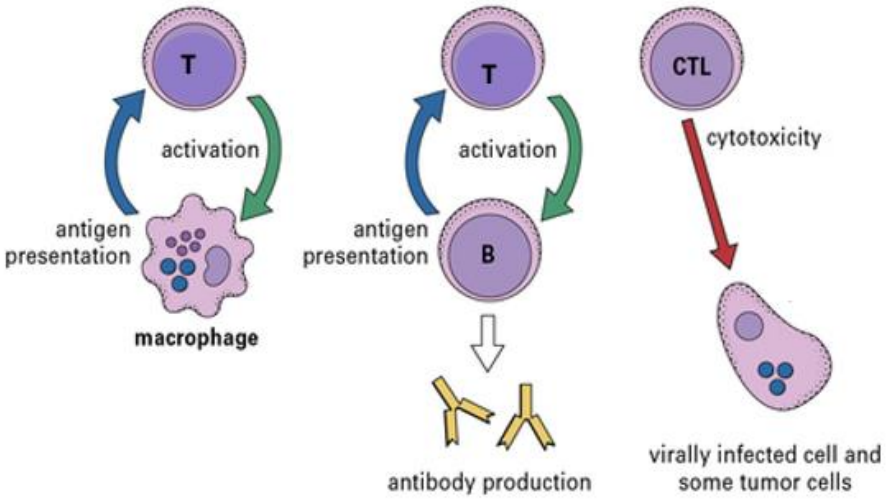


Figure 2: T lymphocyte orchestrated immune response against pathogen.
T lymphocytes can be specifically activated, via their pathogen specific TCR, by a specialised antigen presenting cell (APCs) such as macrophages and dendritic cells, presenting them with the exact specific pathogen antigenic motif, highly compatible to their TCR. An activated T lymphocyte will in turn secrete specific cytokines enhancing the action of antigen presenting cells, as well as the activation of pathogen-specific B lymphocytes and the secretion of pathogen-specific antibodies. Additionally, pathogen-specific cytotoxic T lymphocytes can directly identify and eliminate pathogen via their toxic secretions.

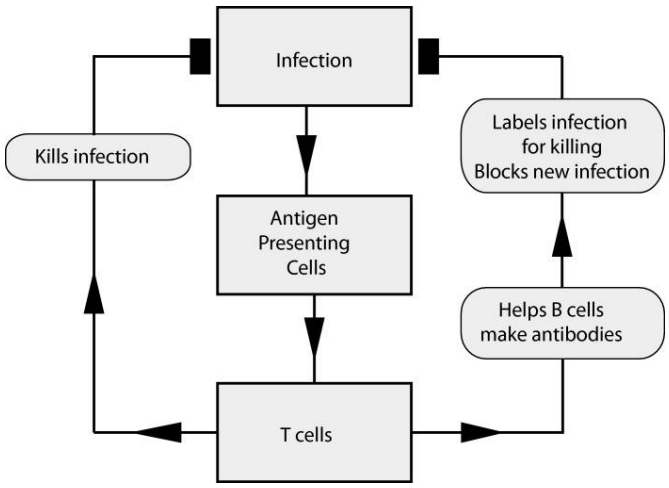


Figure 3: **The adaptive immune response to infection**

Infection, detected by APCs, triggers specific T lymphocytes that co-ordinate killing and antibody production, which stop the infection. (Diagram taken from Nicholson B., 2016)

5.6.3. Activation of immune memory following re-exposure to pathogen.

As already mentioned, immune memory is the ability of the immune system to quickly and specifically recognise an antigen that the body has previously encountered and initiate an analogous immune response against it. Generally, memory responses are rapid secondary, and tertiary subsequent responses to this same antigen, or very similar forms of it. To understand immune memory, one would first need to understand what a primary immune response is. A primary immune response is regarded as the outcome following the first encounter of an organism with a pathogen, and it primarily involves the initial non-specific actions of the chemical and cellular innate barriers of the body's immune system, followed by more efficient and specific immune responses of the adaptive system.

Non-specific innate memory:

The complement system is composed of about 20 proteins that circulate in the blood and tissue fluids and provides an essential feature that complements the action of innate immune responses. Most of its proteins are normally inactive, but in response to recognition of molecular features expressed on microorganisms, these proteins become sequentially activated in an enzyme cascade where the activation of one protein enzymatically cleaves and activates the next protein in the cascade, resulting in the targeted killing of bacteria. Additionally, complement components will flag and give away pathogens, thus enhancing the phagocytic action of macrophages and neutrophils that patrol sites of usual pathogen entry. These phagocytes engulf and digest flagged pathogen, that they can also identify via TLRs expressed on their cell surface. The combined actions of the complement and the innate immune cells is almost immediate and only requires up to twelve hours to ensure control of pathogen invasion.

Studies show that innate memory is achieved via TLR-pattern-recognition, and molecular mechanisms underlying its establishment show strong involvement of transcriptional and epigenetic reprogramming of innate cells, including histone acetylation, methylation, and modulation of miRNAs, which can be shaped by environmentally induced metabolic changes (Saeed S., et al., 2014). The fact that most cells of the human body express TLRs on their surface indicates that innate memory is not a privilege attributed only to immune cells. In fact, epithelial stem cells have been also shown to retain memory of previous inflammatory challenges by displaying an enhanced wound healing capacity upon skin damage (Naik S., et al., 2017) providing scientists with important proof that innate memory is not restricted to immune cells, and that most components of the body can remember past "events of invasion".

Highly Specific adaptive memory:

In situations whereby the innate arm of the immune system is unable to establish control following the entry of a pathogen, an inflammatory response takes place during which phagocytes as well as pathogen infected cells secrete a variety of soluble chemical factors, known as chemokines and cytokines, that are respectively associated with the recruitment and activation of adaptive memory cells (T and B lymphocytes). One comes to question from where these pathogen-specific memory cells originated. At this point it is important to note that all sites of the human body are drained by the lymphatic system, that allows for antigen presenting cells (APCs) to travel to peripheral lymph nodes where they get to present encountered antigens to pathogen-specific T and B lymphocytes. Additionally, antigen can also enter peripheral lymph nodes, where it is captured by local APCs and presented to adaptive cells within the node. Figure 4 provides a diagrammatic summary of the stages following antigen entry and the launching of a specific immune response against it. A typical lymph node is divided into areas of naïve T and B cells. T cells are initially presented with antigen by incoming or local APCs, these being of macrophage or dendritic cell origin. These T lymphocytes possess TCRs that firmly binds to the antigen presented to them in context with the Major Histocompatibility Complex-II (MHC) molecule, expressed on the surface of any APC. Upon interactive binding, a T cell becomes successfully activated and in turn enables the activation of a naive antigen-specific B cell, that is located at adjacent germinal centers within the lymph node's architectural structure. Note that cells need to directly interact with each other, at the barriers of the different regions of the lymph node, and their activation is also facilitated by the secretion of various essential cytokines.

In continuation, an activated B cell will undergo somatic hyper-mutation and re-structuring of their BCR (also known as surface bound antibody receptor) to ensure maximum compatibility (affinity) to the antigen that was presented to them, and will differentiate into a:

- short-lived plasma cells, released into circulation and maintained for a several weeks, producing and secreting antigen-specific antibodies,
- long-lived plasma cells, deposited in the bone marrow throughout life and recruited during memory responses, and
- memory B cells, acting as surveillance cells that circulate throughout the body in a quiescent state until specific antigen is re-encountered and triggers a potent secondary immune response.

These memory B cells respond to antigen much faster (within less than 12h), requiring lower amounts of antigen, and can even be induced in its absence by soluble cytokines secreted by other cells at a site of inflammation, in part because their BCR is already localized on rafts on their cell membrane. Subsequently, just like naïve B cells (these being cells that have not encountered their antigen yet), memory B cells can ingest antigen and express it onto their cell surface, presenting this to helper T cells, receiving thus activation and the ability to undergo expansion and differentiation into antibody producing plasma cells.

Just like memory B cells, T cells also can retain specific memory to incoming pathogens. These memory T cells can thus cut short the 'stealth phase' of pathogen replication that occurs before the initiation of antiviral responses, and mediate extremely potent effector responses, providing thus strong protection upon re-infection, even in the absence of neutralizing antibody.

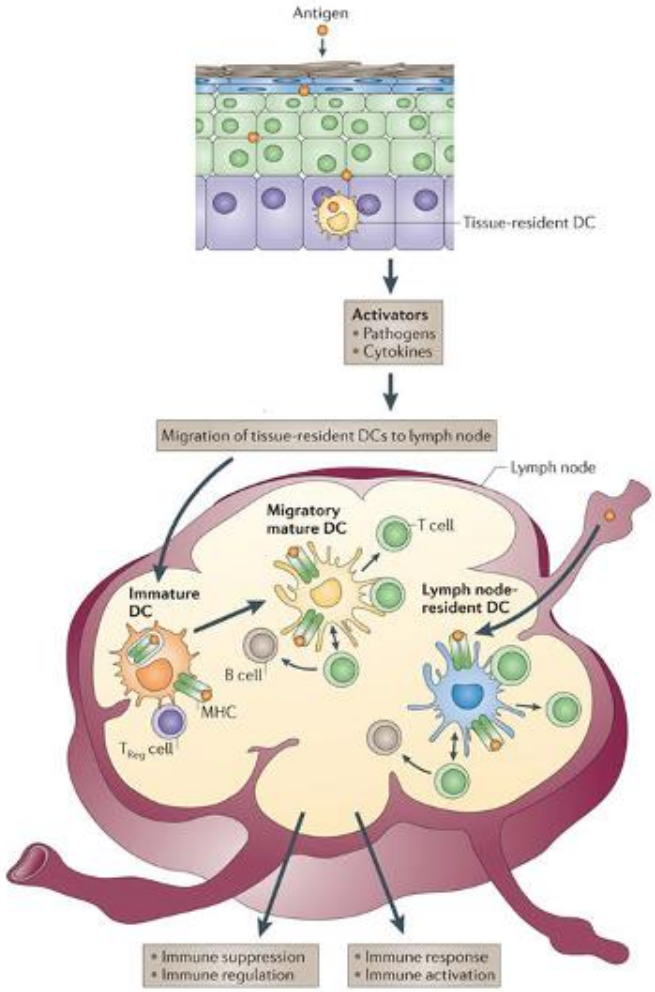


Figure 4: Launching an immune response against an incoming pathogen.

Antigens can enter lymph nodes via the lymphatics, where the antigen is captured by lymph node-resident DCs and macrophages or via tissue-resident DCs and macrophages that in turn migrate to local lymph nodes. DCs and macrophages display antigens in the context of major histocompatibility (MHC) class I and MHC class II molecules or in the context of non-classical CD1 molecules, which allow the selection of rare antigen-specific T lymphocytes (NK T cells). Activated T cells drive DCs towards their terminal maturation, which induces further expansion and differentiation of T lymphocytes into effector T cells.

(Figure from Palucka K., et al., 2012)

5.6.4. Immune Memory is long-lasting.

Protective immunity is defined as the resistance to re-infection, following natural exposure to an infectious agent, or artificial exposure via vaccination. It is therefore a consequence of both innate and adaptive immunity, operating through the epigenetic changes occurring in most cells of the human body and the clonal selection of T and B lymphocytes, respectively. Protective immunity does not depend only on preformed antibody and armed effector T cells. It also depends on the establishment of a population of T and B lymphocytes that mediate long-lived immunological memory, and the given capacity of these cells to respond rapidly to re-exposure to the same antigen or newly altered antigen, that can be also transferred to naive recipients.

Memory T and B lymphocytes can be distinguished by changes in specialised receptor genes, these being their TCR and BCR respectively, due of somatic hyper-mutation, and secondary and subsequent immune responses are characterized by cells expressing surface receptors with increasing affinity for the given antigen. This is termed affinity maturation and summarised in Figure 5. Additionally, memory B lymphocytes will also give rise to antibodies with a similar increasing affinity towards the specific antigen during secondary and subsequent responses (Figure 6). It is important to note that protective immunity does not depend on, nor maintains itself through the numerous re-exposures or persistence of the same pathogen. This is most clear in examples of individuals who were themselves previously exposed to a given infectious agent and are therefore immune. Their memory is not dependent on repeated exposure to infection because of contacts with other infected individuals.

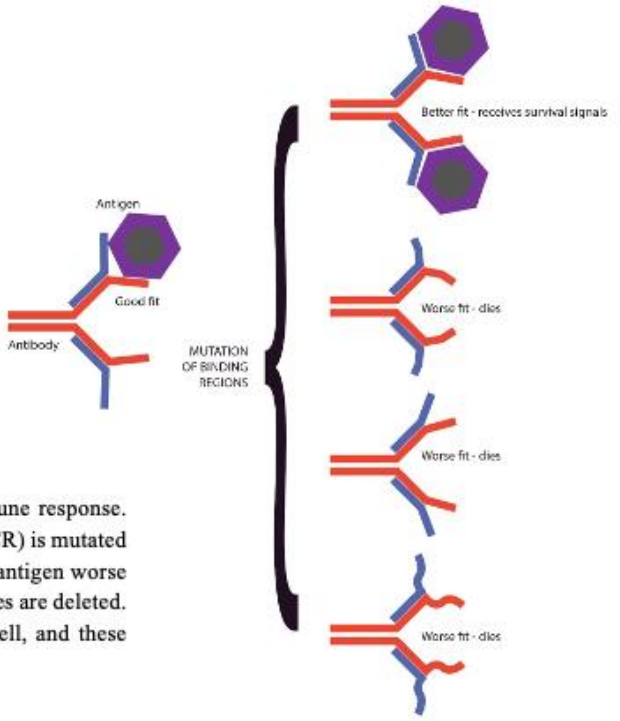


Figure 5: Affinity maturation.

Antibodies that fit the best are selected early in the immune response. The antibody receptor otherwise termed B-cell receptor (BCR) is mutated within daughter B-cells. Many of these mutations bind the antigen worse than the parent antibody, and cells producing these antibodies are deleted. Some antibodies bind the antigen better than the parent cell, and these cells are positively selected.

This was established by observations made on remote island populations, where a virus such as measles would cause an epidemic, infecting all people living on the island at that time, after which the virus seemed to have disappeared for many years. On re-introduction from outside the island, the virus would not affect the original population but causes disease in those people born since the first epidemic that have not been previously exposed to measles. Vaccination provides a means of ensuring that those circulating memory cells are updated to the new "versions" of a pathogen, while been given a head start to undergo somatic hyper-mutation to maintain their alertness to future pathogen encounters.

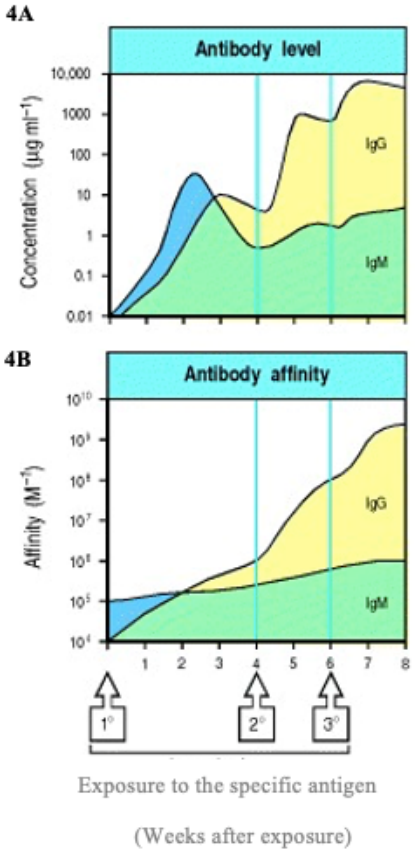


Figure 6: Both antibody affinity and the amount of antibody generated increase with subsequent exposure to the same pathogen-related antigen.

Graph 4A shows the increase in the concentration of antibody with increasing time after primary, followed by secondary and tertiary, exposure (immunisation or natural re-exposure) to the same pathogen-related antigen; Graph 4B shows the increase in the affinity (strength with which an antibody binds it compatible antigen) of the antibodies produced following each exposure. This increase in affinity is seen largely in IgG antibody (as well as in IgA and IgE, which are not shown) coming from mature B cells that have undergone isotype switching (have changed antibody class from IgD which is the initial membrane bound antibody seen on naïve B cells, to either IgG, IgM, IgA or IgE) and somatic hyper-mutation (changes in the gene locus associated with the fragment of antigen binding region of an antibody) to yield higher-affinity antibodies. Although some affinity maturation occurs in the primary antibody response, most arises in later responses to repeated antigen exposure.

(Figure taken from, Janeway C. A. Jr., Travers P., Walport M., et al., 2001)

5.6.5. Malfunctions to Immunological memory.

When an antigen is encountered more than once, the adaptive immune response to each subsequent encounter is faster and more effective. This is a crucial feature of protective immunity, also known as immunological memory, and it is specific towards a particular antigen, as well as long-lived. As previously mentioned in subsection 6.4, immunological memory involves both the adaptive and innate arms of the human immune system, but also involves the trained memory of most cells of the human body.

For an immune system to be effective it must be able to interpret changes in the environment around it and respond appropriately, by solving various issues arising (Nicholson L. B., 2016). For instance, our whole existence depends on the ability of our immune system to be able to discriminate against self and non-self-antigens. The inability to do so efficiently may lead to all sorts of issues like autoimmunity and the development of cancer. Flexibility is another characteristic of an effective immune system, allowing adaptation to strange environmental changes to effectively tackle issues such as infections and cancer that may arise at any point during cell renewal processes and can lead to the development of random unpredictable mutations that will transform a normal cell into a cancer cell. Infectious agents replicate much more rapidly than their hosts and can change their appearance to allow evasion of recognition mechanisms. An effective immune system must be alert and able to always cope with such unpredictability.

One other important feature of the human immune system is its ability to manage infections, and this is achieved through the development of protective barriers. For a pathogen to gain entry it must first breach the innate anatomical and chemical barriers that cover and protect all entries to the human body. When pathogens succeed in penetrating these defences, seeking to cause harm or co-existence with the host organism, they pose many threats, from quiet co-existence to full-blown cell destruction and death. However, the most significant feature of an immune response is the ability to retain memory of previous infections. This both protects from re-infection and limits the spread of infection within a community.

Immune memory can be long-lasting and decays so slowly, with a half-life of approximately 3000 years, which goes well beyond life-long protection.

Rarely, but frequently, individuals are born without an effective immune system. This arises when uncommon mutations prevent immune cells from maturing, and in such cases, individuals have a very limited life expectancy. Less dangerous, but still severe, are mutations that cripple a particular arm of the immune system. There are also situations that an immune system is partly defective, for instance, individuals whose complement proteins do not operate suffer from repeated infections, or those with deficiencies in the action of their natural killer cells are highly susceptible to herpes-virus infections, those who have macrophages that cannot digest bacteria that they encounter develop recurrent abscesses and so on. The importance of efficient innate immune responses is to slow infections down, giving the rest of the immune system time to catch up, readapt and respond appropriately. Such malfunctions of innate immune response will cause "auto-inflammatory" diseases, often manifesting as spontaneous spells of illness and fever.

All above mentioned situations may lead to a defective trained-innate memory. Most common though are defects that arise in B lymphocytes, the cells solely responsible for the production of antibodies. These conditions are often X-linked, meaning that they are encoded on the female X chromosome and therefore are more common in men rather than women. There are also situations that a "stronger-responding" immune memory may lead to complications such as autoimmunity. This is observed in situations involving infections that contain cross-reactive antigens, i.e., pathogen-related antigens that mimic self-antigens, and where tissue damage is caused as immune memory is built against such cross-reactive antigens, therefore posing a threat against the organism's own organs and tissues.

5.7. Educators' implementation guide

The three activities within this learning module have been designed to provide educators and their students with the opportunity to explore, research, learn, reflect and peer teach concepts related to the general knowledge regarding innate and adaptive immune responses, the cellular and chemical components comprising each arm of immune system and how these components come to interact as to bring about immune memory and resistance to re-infection. Students are allowed to explore and understand possible undesirable outcomes of malfunctions in immune memory leading to repeated re-infections and likely outcomes of autoimmunity. The proposed learning activities are geared toward the topic of immunity, which comprises part of a general biology class for students between the age range of 12-15 year, but these proposed strategies are applicable for higher A-level, and undergraduate level biology courses if the content is scaled up. The order in which these activities should be applied follows a scaffolding approach where students acquire new knowledge at each level via performing their own research to present their peers with new concepts, and then use this to bridge their understanding to new learning.
This section includes all three proposed learning activities and descriptions of how these could be implemented as an extension to the general biology topics taught at the different stages of lower secondary education. These include:

- a Flipped-classroom activity introducing primary and secondary innate and adaptive immune memory,
- an in-class short presentation of collaborative work on a selected topic involving immune memory,
- the building of concept maps of the events leading to established innate and adaptive immune memory.

All three activities will ensure that students achieve a basic understanding of the different components of the immune system that are associate with immune memory and how these interact with each other to bring about fast and effective memory responses following re-encounter with the same or mutated pathogens.

5.7.1. Flipped-classroom activity: introducing primary and secondary innate and adaptive immune memory.

Learning and understanding immunology require the engagement of educators and students with new vocabulary and some examples of challenging biological concepts. Stranford A. S. et al., (2020) have found that biology students who regularly spend time before class dealing with new terminology and challenging concepts come to class more prepared to ask interesting, good questions, practice their skills, and apply their self-acquired new knowledge in higher-level thinking endeavours. Students participating in well-designed, pre-class preparation activities, results in being more productive during their class experiences when presented the given topic by their educator. Furthermore, the Flipped-classroom approach aids students in their understanding of challenging concepts, through the reinforcement of pre-class material in class, and helps to entrain an incremental work ethic as opposed to the usual cramming observed amongst young learners.

In this first learning activity, students are provided with a series of online video links to watch prior their actual class introduction, related to the concepts of immune memory. This constitutes their preparation for answering questions shortly before class, where they are requested to submit their answers to these pre-class questions online, 24 hours prior to the class meeting (Stranford et al., 2020). The student responses are used by the educator to provide whole-class feedback and to better focus the in-class plan on the collective needs of their students, transforming their classroom from educator-dominated to student-centred (Simkins S. M. M. et al., 2010). In this way, bidirectional feedback occurs to suitably address the material that is delivered, generating an ideal feedback loop where in-class and outside-of-class work is highly connected, and where the educator is consistently aware of the level of student engagement and understanding. The learning activity assignments can vary, from questions that probe basic vocabulary or the application of concepts, to real-world problems or queries about the assigned online videos. It is ideal to include questions that highlight common confusion and misconception so for the educator to be able to diffuse any issues during the actual delivery of the topic. Examples of such are provided in the section that follows.

Activity 1: Flipped-classroom activity introducing primary and secondary innate and adaptive immune memory.	
Learning objectives	Students will: ➢ Learn about the two arms of an effective immune system, innate and adaptive, ➢ learn about the cellular and molecular components of each arm, ➢ be able to identify the important cellular and molecular components in each, ➢ be able to identify and recall the function of each of these components and how they come to interact to bring about immune memory and prevent reinfection, ➢ be able to explain in simple terms how immune memory arises following primary exposure to a pathogen and how this takes over following future exposure to the same or similar forms of pathogen.
Time requirements	➢ Students are provided with three online video links to watch, a day prior to their introduction to this activity. Each video is approximately 9-minutes long, requiring from the students approximately 30-minutes time to watch. ➢ After watching the related series of online videos, students are requested to answer a series of targeted questions and submit their answers online prior to their next day's lesson. This should take no longer than 15 minutes. ➢ The educator needs to prepare an online questioner for their students, following a selection of the suggested questions found within this activity.

	These could be in the form of multiple choice or structured questions or a combination of both. Students should be provided with these questions 24h prior to their classroom activity.
Description of activity sequence / Educator's Instructions	➢ The educator asks all students to watch at home a series of three online video links or listen to a podcast (**DER5**: https://www.exploratorium.edu/audio/drama-immune-system). All these explain the concept of innate and adaptive immunity, how the arms of innate and adaptive immunity work together to protect against infection and how immune memory is established to prevent re-infection. ➢ The educator prepares a series of questions that will allow them to assess their students' level of outside-classroom learning and understanding on the given topic. ➢ The educator constructs their lesson plan based on the student needs arising from the outcome of their assessment. **Examples of pre-class questions that can be used for this activity**: For 1st online video: Emergency immune response 1. (True/False) Innate immunity involves soluble products and is a part of humoral (antibody-associated) immunity, while adaptive immunity involves the work of B and T cells, or cell-mediated immunity. Please provide a brief rationale for your choice. 2. (True/False) Adaptive immunity is engaged during both a primary and a secondary immune response. Please provide a brief rationale of your choice. 3. (True/False) All antibodies produced during an immune response can stop a virus from entering the bodies epithelial cells. Please provide a brief rationale of your choice. 4. (Optional) Do you have any questions from this part of the viewing preparation for class? Please be as specific as possible. For 2nd online video: Do cells remember? 1. (True/False) Immune memory is only linked to the adaptive immune system, which includes T and B cells. Please provide a brief rationale of your choice. * For this question students will not be aware on the memory mechanisms of the innate immune system. This can be introduced by the educator as part of their lesson planning. 2. (True/False) Adaptive immune memory requires the combined action of both memory T and B cells. Please provide a brief rationale of your choice. 3. (True/False) Immune memory can be only achieved through the concept of vaccination, using either fractions of a virus or an attenuated (weak form) of this virus. Please provide a brief rationale of your choice. 4. (Optional) Do you have any questions from this part of the viewing preparation for class? Please be as specific as possible. For 3rd online video: Why do immune systems forget? 8. (True/False) The immune system of elderly people forgets of past infections. Please provide a brief rationale of your choice. 9. (True/False) Immune memory is short lived and needs continuous boosting. Please provide a brief rationale of your choice. 10. (True/False) Viruses can escape immune memory by mutating their genetic makeup. Please provide a brief rationale of your choice. 11. (Optional) Do you have any questions from this part of the viewing preparation for class? Please be as specific as possible.

Materials required	Electronic device (iPad, personal laptop, other smart device) that will allow internet access.
Learning Objects (LO) / **Digital Educational Resources (DER)**	From the website of British Society of Immunology: ➢ Online Video 1: Emergency response **DER6**: https://www.youtube.com/watch?v=g_RZWDBFJjI ➢ Online Video 2: How do cell remember? **DER7**: https://www.youtube.com/watch?v=IUEWpHAbAGE ➢ Online Video 3: Why do immune systems forget? **DER8**: https://www.youtube.com/watch?v=kN0WROnxaCg ➢ Other online useful links: **LO3**: https://www.playfactile.com/ **LO4**: https://www.gingerlabs.com/ **DER9**: https://www.khanacademy.org/science/high-school-biology/hs-human-body-systems/hs-the-immune-system/a/intro-to-viruses **DER10**: https://www.exploratorium.edu/search/immunity **DER5**: https://www.exploratorium.edu/audio/drama-immune-system
Assessment/evaluation of learning outcomes	➢ Student groups are asked to answer a series of pre-class questions and provide a simple explanation of the rationale for their choice of response. ➢ Student answers are assessed by their educator prior to the introduction of the topic allowing them to assess the level of learning and understanding during this outside-classroom activity. ➢ The educator can plan their teaching of the topic, focusing more on the challenges and needs of their students.

5.7.2. In-class short presentation of collaborative work on a selected topic involving immune memory.

This learning activity, that continues from the previous Flipped-classroom activity, requires that the lesson time is divided into sections, with the educator sharing some in-class presenting time with their students. For instance, the educator might set the stage for the topic to be presented entirely by their students, who shall be in groups and given some of the material to deliver to their peers. Sometimes, the educator will need to interfere to emphasize the main points that have been made by each student group and offer a summary of conclusions. This approach is particularly useful when the subject involves presenting information in the form of a sequence of similar, but slightly different cells and molecules or events occurring during immune memory mechanisms. When student groups are responsible for different parts of the lesson the perceived "sameness" of the material is broken up, helping the students to associate cells, molecules, and biochemical events with different people, and acting as an aide memoire (Stranford A. S. et al., 2020).
The educator could introduce the overall concept of innate and adaptive immunity and then delved into a discussion of how cell members of the two arms of the immune system work together to bring about immune memory. Student groups can then take up the story and present their selected subtopics sharing their acquired knowledge with the rest of the class, referring to concepts of:

- Components of the innate immune system
- Components of the adaptive immune system
- Primary immune response against first encounter with a pathogen
- Secondary immune response following re-infection
- The time span of immune memory to an invading pathogen

Designing and delivering these mini lectures gives the students (usually much needed) additional experience and confidence that they have grasped the newly acquired knowledge, that are able to present to a group of peers. Students should be introduced to different presenting approaches to demonstrate their acquired learning towards the given topic, including preparation of a poster, use of short video, structuring of lists, diagrams, and other creative approaches, other than just PowerPoint presentations that most times are misused by students who get carried away by mechanically copying and pasting information of the internet. This will also help the educator to assess the individual and overall level of understanding of their students.

Activity 2: In-class short presentation of collaborative work on a selected topic involving immune memory.	
Learning objectives	Students will: ➢ Learn how to perform their own research on their chosen topic and prepare for it before they enter the classroom. ➢ Learn to summarise and present important facts regarding immune memory. ➢ Will be able to develop a skill set for effective presentation and communication of their acquired knowledge.
Time requirements	➢ Student groups should be given a week to prepare for their chosen presentation topics, allowing for required communication and collaboration with their peers.
Description of activity sequence / Educator's Instructions	The educator should provide their students with a list of topics to choose from and to prepare short 5–10-minute presentations (poster, lists, diagrams, video, etc.) The topics provided to students should be relevant to the previous activity, focusing on the mediators of innate and adaptive immunity and how are these collaborate and are associated with the establishment of immune memory. Examples of topic presentations could include: 1. Introducing the cells and molecules of the innate immune system 2. The cells and molecules of the adaptive immune system: how these are involved in primary immune responses. 3. Innate immunity and emergency responses 4. Primary versus secondary immune response 5. Innate versus adaptive immune response 6. Immune memory is linked to secondary response 7. The innate immune response and how this adds to the establishment of immune memory.
Materials required	Each student group is to decide on the method of delivering their presentation, therefore this part should be left open to them to choose the material, equipment required, or the approach that they would like to follow for their presentation.
Learning Objects (LO) / Digital Educational Resources (DER)	Online links that could be send to students: ➢ Fighting infection by clonal selection: **DER11:** https://youtu.be/HUSDvSknIgI ➢ Immune encounters: **DER12:** https://www.immunology.org/sites/default/files/Immune%20Encounters.pdf

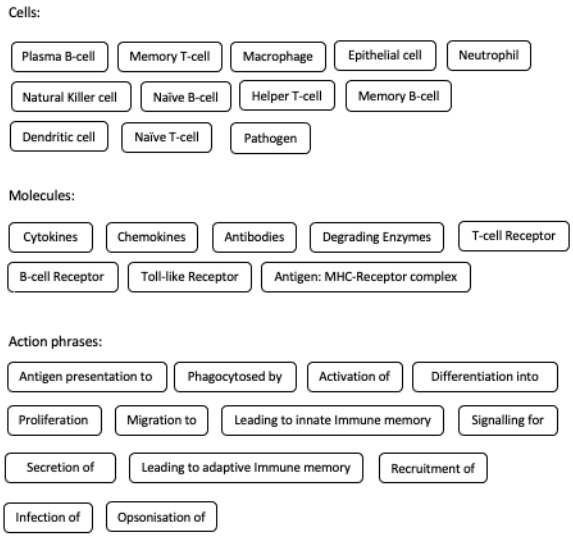
	➢ Educational illustrations: **LO5:** https://www.immunology.org/public-information/immunology-related-activities-and-resources/infectious-educational-illustrations
Assessment/evaluation of learning outcomes	➢ Student presentations are assessed by their educator prior to the lesson allowing them to assess the lever of learning and understanding during this outside-classroom preparation activity. ➢ The educator can prepare a series of questions to assess the level of understanding of their students following each group's presentation. ➢ Following assessment of the presenting material, all groups are asked to collaborate to come up with one final presentation that they are to present during a science forum organised at their local school community, including peers, teachers, parents and selected academic specialists in the fields, leading to an open discussion on the topic.

5.7.3. Building concept maps of the events leading to established innate and adaptive immune memory.

When studying any discipline for the first-time students lack cognitive tools that they could use to "manage" the new facts and ideas they encounter. Lacking a framework within which to organize their new acquired knowledge, students can become overwhelmed and feel like they are swaying in a sea of unrelated facts. The use of concept maps has always been a first-time challenge to most students, but in fact, mastering the correct use of this approach is proven to be extremely helpful in students being able to synthase and model their recently gained knowledge, to merit continued use in science related classes. A concept map typically represents each idea, for instance an organ such as the lymph node, a cell such as a lymphocyte, or a secreted molecule such as an antibody, as a shape, joined to other shapes by lines that indicate the conceptual connection between them. These lines can be labelled with phrases that are used to describe the relationship between the linked shapes. For example:

1. an epithelial cell infected with a viral pathogen
2. will secrete appropriate chemokines
3. to signal for the recruitment of neutrophils
4. and macrophages to the side of infection,
5. which would in turn phagocytose the pathogen
6. and present to antigen-specific memory T helper cells
7. that would activate antigen-specific memory B cells
8. to secrete antigen specific antibody.

This provides a single chain of events following infection with pathogen (Figure 7). Such a technique is particularly well-suited for teaching challenging concepts to students, such as immune memory, that include various cells, molecules, and difficult terminology. Students could be provided with a list of term from which they could select the most appropriate terms to generate their concept maps.

Activity 3: Building concept maps of the events leading to established innate and adaptive immune memory.	
Learning objectives	Students will: ➢ Be able to manage their newly acquired knowledge from previous activities 1 and 2 into a simple structured concept map, summarising the main points. ➢ Be able choose correct terms and action phrases from a list to construct their own concept maps of innate and adaptive immune memory responses against re-invading pathogen, following subsequent re-infection or/and vaccination. ➢ Develop collaborative and communication skills as they will have to work within groups.
Time requirements	➢ This could be a 30-minute classroom activity. Students can be provided with the list of terms to either copy or cut out and use to construct their concept maps.
Description of activity sequence / Educator's Instructions	The educator should provide their students with a list of terms and action phrases to use to construct their concept maps. For example:  1. Students are asked to colour the terms and action phrases of the innate and adaptive immune memory with a different colour. 2. Arrows connecting terms of the same arm of immune system (i.e., innate components) should be in the same colour as that initially selected. Arrows interconnecting innate with adaptive immune memory components should be drawn with a different colour. 3. Completed concept maps can be displayed withing the classroom, allowing peer review and discussion.
Materials required	➢ Printed lists of terms and action phrases ➢ Scissors ➢ Colour pencils/pens ➢ A3 Cartons ➢ Glue / sticky tape
Learning Objects (LO) / **Digital Educational Resources (DER)**	➢ Fighting infection by clonal selection: **DER11**: https://youtu.be/HUSDvSknIgI ➢ Immune encounters: **DER12**: https://www.immunology.org/sites/default/files/Immune%20Encounters.pdf ➢ Educational illustrations: **LO5**: https://www.immunology.org/public-information/immunology-related-activities-and-resources/infectious-educational-illustrations

Assessment/evaluation of learning outcomes	➢ Educator can visually assess the understanding of their students.

5.8. References

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5.9. Educational Scenario Impact Assessment Questionnaire

Context: Basic knowledge of long-lasting immunological memory and protective immunity provides an essential means of simplifying and improving the understanding of challenging topics such as the spreading of communicable disease and prevention of pandemics. The topic of "Workings and malfunctions of human Immunological memory" provides educators and their students with the opportunity to expand their existing knowledge by learning about important concepts in Immunology, including the innate and adaptive arms of the human immune system, the cellular components involved in the establishment of immune memory, and the interactions that bring about protective immunity and preventing re-infections. The topic clarifies how immune memory is independent of the frequency or persistence of re-infection, being a long-lasting feature involving both specialised cells of the adaptive and innate immune systems, that also depends on all the rest of the cells of the human body. High school

students are therefore exposed to immunological concepts related to the immune memory mechanisms in place to minimise and prevent the spreading of disease, preventing thus the occurrence of epidemics and possible pandemics.

Additional information: the topic is provided in the specifications of an educational scenario of the "Workings and malfunctions of human Immunological memory".

The questions that follow provide and assessment for the impact of the given learning scenario on the knowledge acquired and skills acquired by the students throughout the teaching of this topic and the effect of this topic on their beliefs, attitude, and behaviour.

As part of acquired knowledge students can:	
1. Identify immunological memory and its importance in ensuring survival upon reinfection.	1.1. What is immune memory? A. The ability of immune cells to retain memory of past infectious agents and their associated antigens and respond fast. B. The ability of immune cells to retain memory of past infectious agents and their associated antigens and respond slow. C. The initial ability of immune cells to respond to new infectious agents and their associated antigens and respond fast. 1.2. An antigen: A. is a molecular structure that will always stimulate an immune response B. is a molecular structure that will sometimes stimulate an immune response C. is a molecular structure that will never stimulate an immune response 1.3. An effective immune response against a pathogen is achieved: A. by the combined action of both acquired innate and adaptive immune memory. B. primarily by the acquired adaptive immune memory C. primarily by the acquired innate immune memory
2. Recognize the key cell players of immune memory	2.1. Which comprise cells of the adaptive immune system, involved with specialised responses against specific antigens? A. T cells and B cells B. T cells, B cells and macrophages C. Macrophages 2.2. Immune memory is: A. Specific to a given pathogen B. General to all pathogens C. General to a group of pathogens 2.3. Immune memory is brought about by: A. The direct action of pathogen-specific antibodies only B. The direct action of pathogen-specific antibodies and pathogen-specific cells C. The direct action of pathogen-specific cells only 2.4. Phagocytic cells can also remember past pathogens by identifying: A. Conserved molecular structures on the surface of a groups of pathogens. B. Conserved molecular structures on the surface of a specific pathogen only C. Altered molecular structures on the surface of a group of pathogens.
3. Understanding how immune memory is	3.1. Immune memory can be: A. Innate

activated and brought about	B. Adaptive C. Both 3.2. Which of the following is true about innate immune memory? A. innate memory involves immune cells and other body cells B. innate memory is restricted to immune cells only C. innate memory is restricted to body cells only 3.3. Antibodies responsible for bringing about immune memory are secreted by which type of cell: A. B cells B. T cells C. Macrophages 3.4. Immune memory to incoming pathogens begins: A. the lymph nodes that are local to the side of infection B. the bone marrow C. the blood circulation 3.5. Immune memory is: A. Long lasting B. Temporary C. Does not exist
4. Understanding the issues of immune memory malfunctions	4.1. Which is not considered an immune memory malfunction? A. Hypercholestolaemia B. Autoimmune disease C. Natural killer deficiency
As part of skills being gained/developed students can:	
1. Identify the two main arms of the immune system involved in immunological memory and their cellular and molecular components	1.2. Immune memory depends on: A. The innate arm B. The adaptive arm C. Both the innate and adaptive arm 1.3. Innate immune memory involves the action of: A. Macrophages/ dendritic cells/ epithelial cells B. Macrophages/ B cells/ epithelial cells C. B cells/ T cells/ epithelial cells 1.4. Cells involved in adaptive immune memory include: A. B and T cells B. B cells only C. T cells only 1.5. Specific immune memory involves the: A. Secretion of pathogen specific antibodies by B cells and the specific direct killing by T cells B. Secretion of pathogen specific antibodies by B cells and the specific direct killing by phagocytic cells C. Secretion of non-specific antibodies by B cells and the specific direct killing by T cells
2. Identify and recall the function of each of the components of the immune system and how they come to interact to bring about	2.2. The cells of the innate immune system include: A. Macrophages, dendritic cells, Natural Killer cells, basophils, eosinophils, neutrophils B. Macrophages, dendritic cells, Natural Killer cells, basophils, eosinophils, B cells C. Macrophages, dendritic cells, Natural Killer cells, basophils, T cells, B cells

immune memory and prevent reinfection	2.3. The cells of the immune system responsible to produce pathogen specific antibodies are: A. B cells B. T cells C. Macrophages 2.4. The complement system is a component of the innate immune system, composed by 20-proteins that can: A. Stick to the pathogens surface and promote the targeted killing of a pathogen B. Activate the cells of the adaptive immune system C. Are expressed on the surface of immune cells 2.5. Toll-like receptors are involved in immune memory and are found on the surface of: A. All cells of the body B. Only on macrophages and dendritic cells C. Only on B and T cells 2.6. Immune memory to a specific pathogen is: A. Long lasting throughout lifetime B. Short lived and up to 6 months C. Short lived and up to a few weeks
3. Identify that immune memory arises following primary exposure to a pathogen and this results in faster and stronger immune responses to the same pathogen.	3.1. Following primary exposure to a pathogen, re-exposure will result in: A. Faster and stronger immune responses B. Slower and weaker immune responses C. Slower and weaker immune responses
4. Perform own research on their chosen topic regarding immune memory, learn to summarise and present important facts regarding immune memory.	4.1. I feel able to identify scientific sources to use in my research 1) strongly disagree… 5) strongly agree. 4.2. I feel confident to prepare a poster/power point presentation of my chosen topic involving immune memory. 1) strongly disagree… 5) strongly agree 4.3. I can confidently summarise the important facts related to immune memory and present these to my peers 1) strongly disagree… 5) strongly agree
5. Demonstrate understanding and being able to describe the series of events leading to immune memory towards re-encounter with pathogen.	5.1 I can understand the steps following re-encounter with the same pathogen, and how these can lead to faster and stronger responses as a result to immune memory. 1) true... 5) false. 5.2 I can outline and describe briefly these steps leading to immune memory responses following a second exposure to the same pathogen. 1) true... 5) false

6. Development of research, collaborative, and communication skills	6.1 I feel able to identify scientific sources relevant to the consent of immune memory. 1) strongly disagree… 5) strongly agree. 6.2 I know the main sources to consult about immune memory. 1) strongly disagree… 5) strongly agree. 6.3 To find scientific information about immune memory I should consult the following sources. D. researchers, scientific publications, WHO and CDC data bases. E. newspapers, google, YouTube F. friends, journalists, Facebook, other social media. 6.4 I feel able to identify the main problems my community faces when it comes to understanding difficult concepts such as immune memory and how this is the outcome of less severe symptoms following re-exposure to a pathogen. 1) false… 5) true. 6.5 I feel capable of proposing actions that address how to promote immune memory, either using effective vaccination, or testing for pre-existing memory following natural infection. 1) true... 5) false.
As part of Beliefs, Attitudes and Behaviour, there are no correct or incorrect answers; we are only interested in knowing the students' perspective on the topic introduced.	
1. Believes that is important to contribute to global efforts for tackling future pandemics.	1.1. My acquired knowledge and understanding of immune memory will increase the chances of success of the global efforts for preventing a future pandemic. 1) strongly disagree… 5) strongly agree. 1.2. I am physically capable of adopting actions (i.e., school, community presentations) that contribute to the efforts of increasing awareness about immune memory and how this is achieved and can help in decreasing the spread of disease that could lead to future pandemics (i.e., vaccination strategies, testing for specific immune memory against a specific pathogen etc.). 1) extremely unlikely... 5) extremely likely. 1.3. My family and friends think that I should adopt actions that contribute to increase awareness about immune memory and add to the global efforts for tackling future pandemics. 1) Extremely unlikely... 5) Extremely likely.
2. Believes that learning about immune memory and that the human immune system responds fast and effectively to even the most powerful viruses following re-exposure can lead to positive outcomes at the community level when it comes to evaluating severity levels.	2.3. To learn about how immune memory works and how this could help in protecting people from severe symptoms and prevent the uncontrolled spreading of a disease, will lead to positive outcomes at my community. 1) strongly disagree… 5) strongly agree. 2.4. My community thinks that increasing awareness about difficult concepts such as that of immune memory and its contribution to lowering disease severity, will bring positive outcomes. 1) Extremely unlikely... 5) Extremely likely.
3. Has intention to perform sustainable behaviours in his/her lifestyle.	3.1. I will try to update myself with current information regarding highly contagious virus variants. 1) Extremely unlikely... 5) Extremely likely.

	3.2. I plan to update myself about updated versions of vaccines available for communicable diseases. 1) Strongly disagree... 5) Strongly agree. 3.3. I plan to enforce my immune memory to current communicable diseases by being up to date with my vaccinations. 1) Strongly disagree... 5) Strongly agree.
4. Attitude toward learning about how immune memory works and its implications towards controlling the severity of a communicable disease.	4.1. For me to achieve such knowledge is: Harmful : : : : : : beneficial Pleasant : : : : : : unpleasant Good : : : : : : bad Worthless : : : : : : valuable Enjoyable : : : : : : unenjoyable