Chapter 5

The Immune Response to Viruses

5. The Immune Response to Viruses

The front line protection from virus infection is to avoid contact with viruses. For instance, either wearing a face mask or using an air filter is the front line protection from, for example, the respiratory infection such as influenza virus.

Daily sanitation practices such as washing hands are also important for protection from the virus infection. Human defense systems against infectious pathogens are divided into two kinds (Figure 32). Natural defense physically protects the infection externally, while immunological defense protects the infection internally. The skin and mucous membrane constitute natural defense. Skin, which is dry, acidic, and partly coated with bacteria, is an inappropriate environment for the viral infection. Mucous secretions from respiratory tracts, urinary organ, and eyes also effectively block the virus infection.

Recent appreciation of the importance of the mucous membrane1 in human virus infections such as HIV and herpesvirus infection has drawn attention to mucosal immunity. Once the virus successfully invades the body interior, the immune system is the only defense system for the protection.

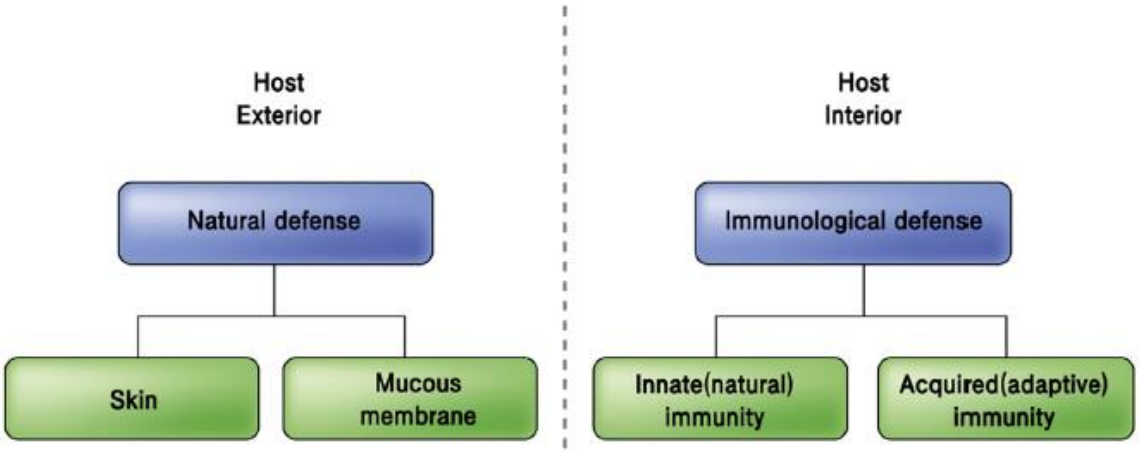

Figure 32: Host defense mechanism against pathogens. Host exterior such as skin and mucous membrane serve as a natural defense or barrier against invading pathogens. Once inside the human body (host interior), the invading pathogens encounter immunological defense, such as innate immunity and adaptive immunity.

5.1. Innate immunity

Innate immune response is a rapid response occurring within only a few hours after the invasion of pathogens. One of the main outcomes is the production of IFN-α, which is the hallmark of antiviral response induced by innate immunity.

5.1.1. Cells Constituting Innate Immunity

As stated above, the innate immune system comprises many types of immune cells, including DCs, macrophages, and NK cells. These cells react to the pathogens via distinct mechanisms.

(1) Dendritic cells: DCs recognize the pathogens, thereby leading to the production of IFN-α/β.

(2) Macrophages: Macrophages engulf and then digest the invading pathogens. They also stimulate lymphocytes and other immune cells in respond to the pathogens.

(3) NK cells: NK cells are a type of cytotoxic T lymphocyte pertaining to the innate immunity. The role of NK cells is analogous to that of cytotoxic T cells in adaptive immune response. NK cells are unique, however, because they have the ability to recognize the cells in the absence of an MHC5 molecule, allowing a faster immune reaction.

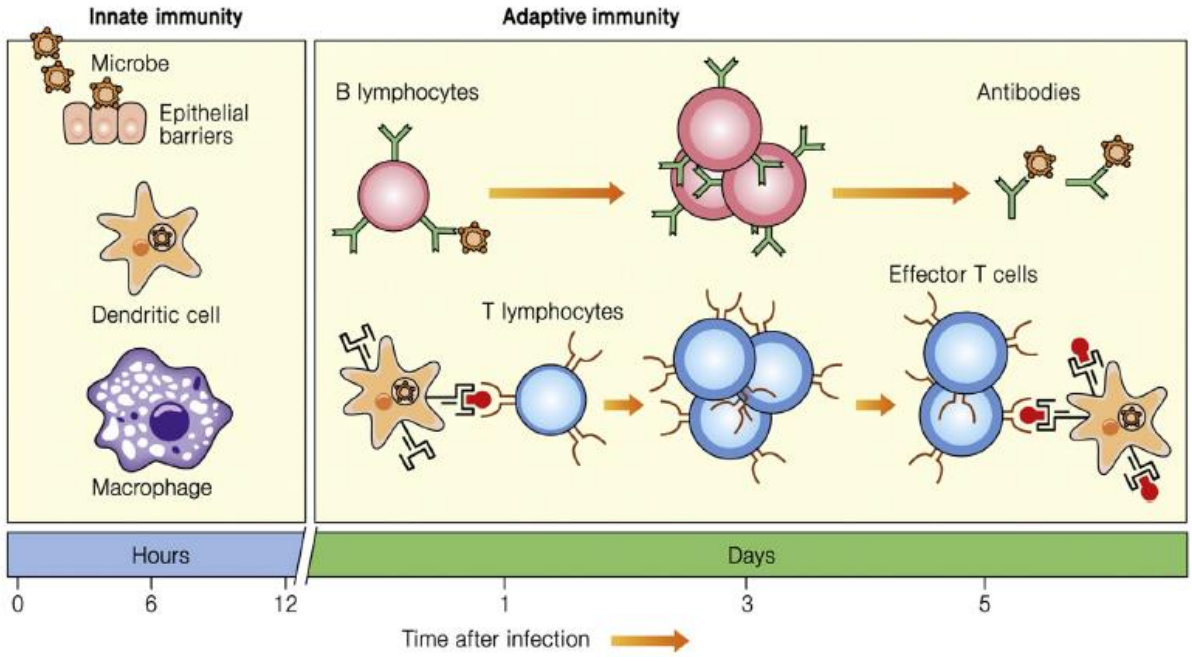

Figure 33: Immune cells involved in the innate immunity and adaptive immunity. Innate immunity is the front-line defense once the microbes bypass the epithelial barriers. Phagocytes such as dendritic cells, and macrophages engulf the invading microbes, whereas NK cells kill the infected cells. Adaptive immunity ensues. B lymphocytes drive humoral immunity, while T lymphocytes drive cellular immunity. Antibodies, the product of humoral immunity, directly bind to the virus particles and block the infection. Cytotoxic T lymphocyte (CTL), the product of cellular immunity, kills the infected cells (Wang-Shick Ryu. 2017)..

5.1.2. Toll-Like Receptors

How do cells constituting the innate immunity recognize the invading microbes? It was speculated early on that a yetto-be-known cellular receptor would recognize the invading pathogens. In support of this prediction, the cellular receptor was indeed identified as toll-like6 receptor (TLR). Fifteen TLRs have now been discovered in mammals (Figure 34). In fact, TLRs are expressed not only in immune cells, such as dendritic cells and

macrophages, but also in nonimmune cells, such as epithelial cells. How does TLR selectively recognize diverse invading pathogens? In fact, TLRs recognize pathogen-associated molecular patterns (PAMP), which are found on the invading pathogens.

In general, double-strand RNA, single-strand RNA, and CpG dinucleotide DNA of the invading viruses are recognized by TLRs that are located at endosomes. Specific TLRs that recognize the many human viruses have been uncovered. For instance, TLR7/8 recognizes the genomic RNA of influenza virus. Often, these endosomal TLRs (ie, TLR3, 7, 8, and 9) are called "nucleotide-sensing TLR." On the other hand, bacterial cell wall components such as LPS and diacyl lipopeptide are typically recognized by TLR4 and TLR2/6, which are located on the plasma membrane.

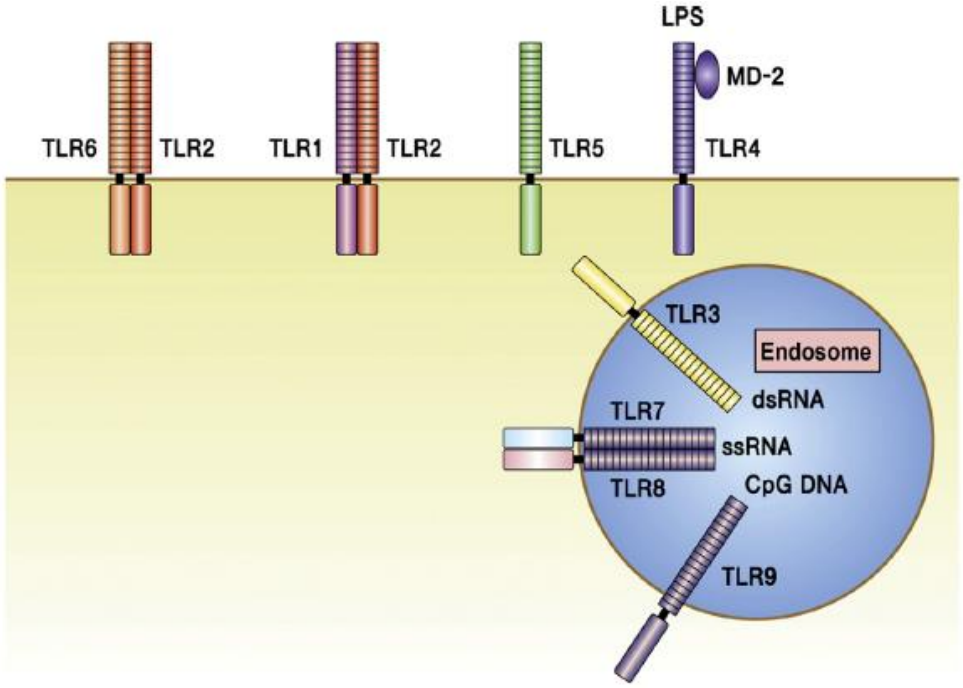

Figure 34: Toll-like receptors and their ligands. Some TLRs (TLR1, 2, 4, 5, and 6) are located on the plasma membrane, while other TLRs (TLR3, TLR7/8, TLR9) are located on endosomes. The PAMPs that are recognized by each TLR are denoted. Note that the endosomal TLRs recognize viral nucleic acids such as ssRNA, dsRNA, and CpG DNA. In fact, many TLRs act as either homo- or heterodimers such as TLR1/2, TLR2/6, and TLR7/8 (Wang-Shick Ryu. 2017.).

5.1.3. RIG-I (retinoic acid inducible gene)

It was first discovered as the retinoic acid-inducible gene, as the name implies. These RNA helicases serve as an "RNA sensor" in the cytoplasm of the virus infected cells. They are also known as "intracellular receptor," as opposed to TLRs that detect extracellularly located PAMPs. Since both TLRs and RIG-I/MDA5 recognize the molecular pattern of the invading pathogens, these receptors are collectively called "pattern recognition receptors" (PRR12)

Subsequently, in addition to TLRs, RIG-I10 and MDA-511 were identified as receptors sensing the viral RNAs (Figure 35).

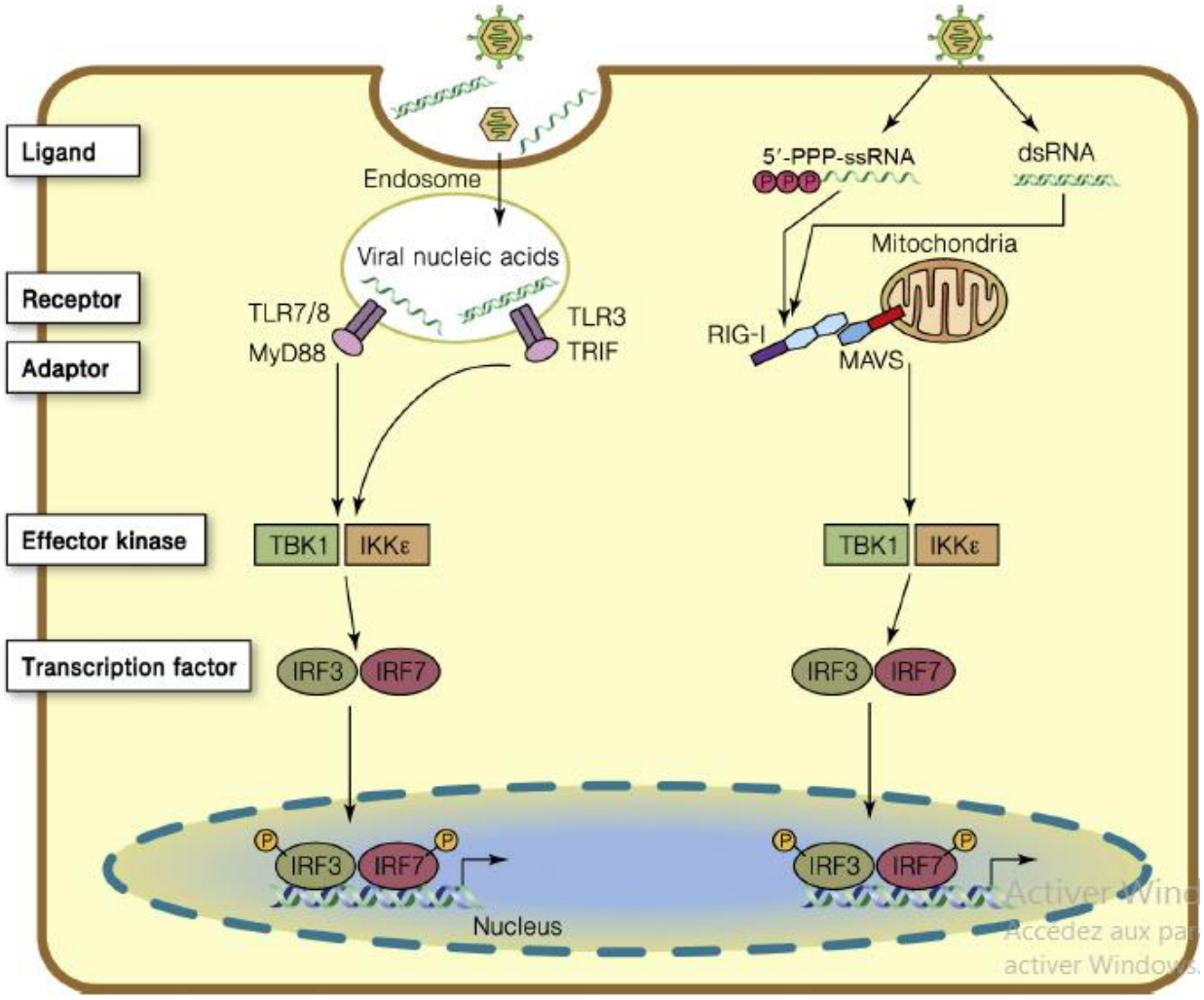

Figure 35: Toll-like receptor signaling versus RIG-I signaling. TLR3 and TLR7/8 are shown as the representative of TLR signaling, while RIG-I is shown as representative of RIG-I/MDA5 signaling. Viral RNAs in endosome are recognized by TLR3 or TLR7/8, whereas the viral RNAs in the cytoplasm are recognized by RIG-I/MDA5. Note that five steps of the signaling, from ligand to transcription factor, are highlighted on the left. On left, common nomenclatures for the molecules involved in each step of TLR/RIG-I signaling are highlighted: in particular, the adaptor refers to a protein linking the receptor and an effector kinase via protein_protein interaction (Wang-Shick Ryu. 2017.).

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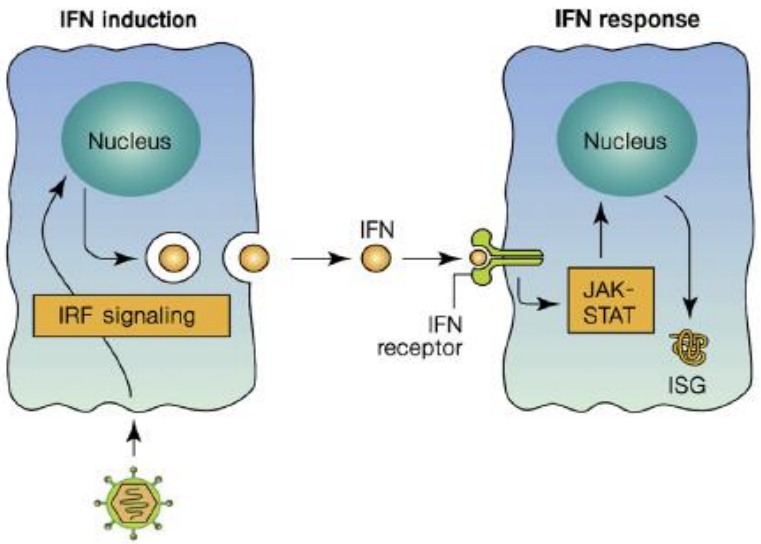
Table 3 : Pattern Recognition Receptors That Recognize Viruses (Louten , 2016)

Pattern Recognition Receptor	Ligand	Virus Recognized
Toll-like Receptors		
TLR2	Envelope proteins	Measles virus
TLR3	Viral dsRNA	Respiratory syncytial virus, reovirus, West Nile virus
TLR4	Envelope proteins	Respiratory syncytial virus, mouse mammary tumor virus
TLR7	Viral ssRNA	Influenza A virus, vesicular stomatitis virus
TLR8	Viral ssRNA	Human immunodeficiency virus, vesicular stomatitis virus, influenza A virus
TLR9	Viral dsDNA	Herpes simplex virus-1, herpes simplex virus-2, mouse cytomegalovirus
RIG-I-like Receptors		
RIG-I	Recognizes virally transcribed ssRNA and short dsRNA	–ssRNA viruses: Influenza A virus, influenza B virus, Sendai virus, respiratory syncytial virus, measles virus, rabies virus, vesicular stomatitis virus
		+ssRNA viruses: Hepatitis C virus, Japanese encephalitis virus
		dsDNA viruses: Epstein–Barr virus, herpes simplex virus-1, adenovirus
MDA5	Long dsRNA	+ssRNA viruses: Picornaviruses (encephalomyocarditis virus, Theiler's virus, murine norovirus-1, murine hepatitis virus)
LGP2	Regulates other RLRs	

5.1.4. Antiviral Function of Interferon

The first line of defense against viral infection is the IFN response, which triggers the induction of a broad array of antiviral proteins. Cells mounting innate immune response produce IFNs. IFN was discovered in 1957 by Alick Isaacs and Jean Lindenmann as an antiviral cytokine that blocks the infection of influenza virus (figure 36). In fact, there are three types:IFN-α, β, and γ. IFN-α is mainly produced in immune cells, such as dendritic cells, while IFN-β is produced in most types of cells. On the other hand, IFN-γ is produced in activated T lymphocytes and NK cells. IFN-α and IFN-β share a receptor, while IFN-γ uses a distinct receptor. The former are called "type I IFN," while the latter is called "type II IFN."

IFN induces the antiviral state of the infected cell via its binding to the IFN receptor (Figure 36). It is worth noting two points. First, IFN, a cytokine that is extracellularly secreted, acts on not only the virus infected cells, but also neighboring cells, a phenomenon called the "paracrine effect." In other words, IFN renders neighboring cells into an antiviral state so that the virus spread is prevented. Second, the antiviral functions of IFN are not



virus-specific. Therefore, IFN produced by a certain virus could block the infection of other unrelated viruses.

Figure 36: IFN induction versus IFN response

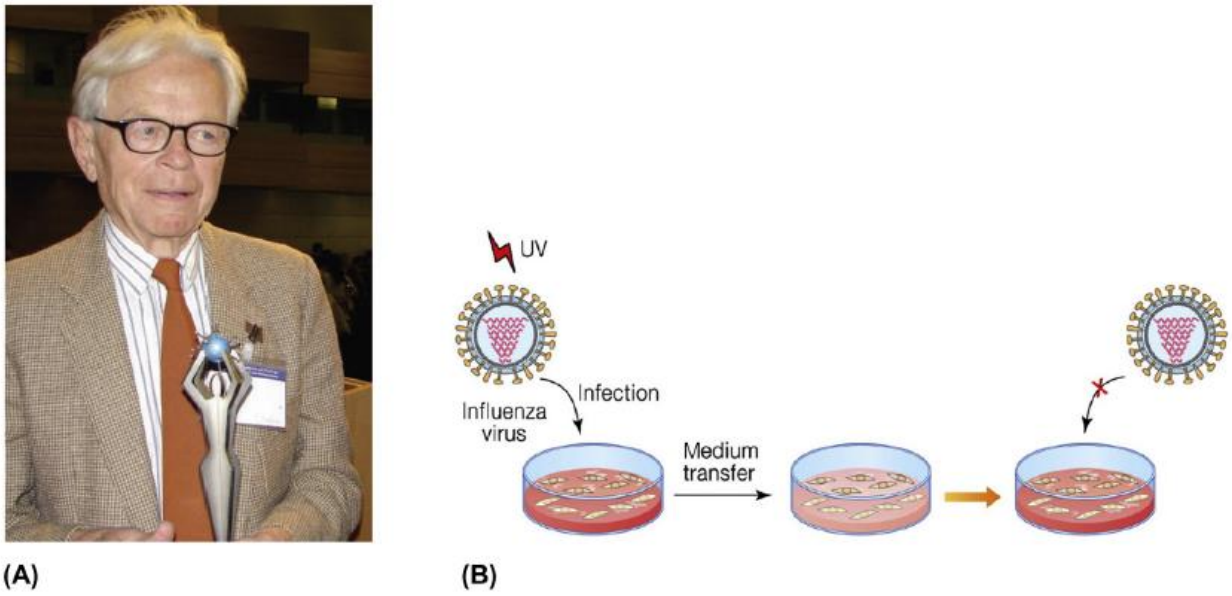


Figure 37: (A) Photo of Jean Lindenmann (1924_2015). (B) A schematic showing an experiment that leads to the discovery of interferons. The culture medium taken from cells that had been infected by the UV-inactivated influenza virus blocked the infection of influenza virus (Wang-Shick Ryu. 2017).

5.2. Adaptive immunity

Adaptive immunity embraces two arms: humoral immunity and cellular immunity (Figure 38). Humoral immunity is executed largely by antibodies (ie, immunoglobulins) that are produced by plasma cells, a differentiated B lymphocyte, while cellular immunity is executed by helper T lymphocyte (Th) and cytotoxic T lymphocyte (CTL), a differentiated CD41 and CD81 T lymphocyte, respectively. Antibodies block viral infection by neutralizing the virions via direct binding, while CTL kills the virus infected cells by introducing toxic substances. In addition, the adaptive immune response results in memory lymphocytes so that the host can respond quickly and effectively to subsequent reinfection.

In other words, the persistency and the memory of immune response are the defining features of the adaptive immunity (figure 39) .

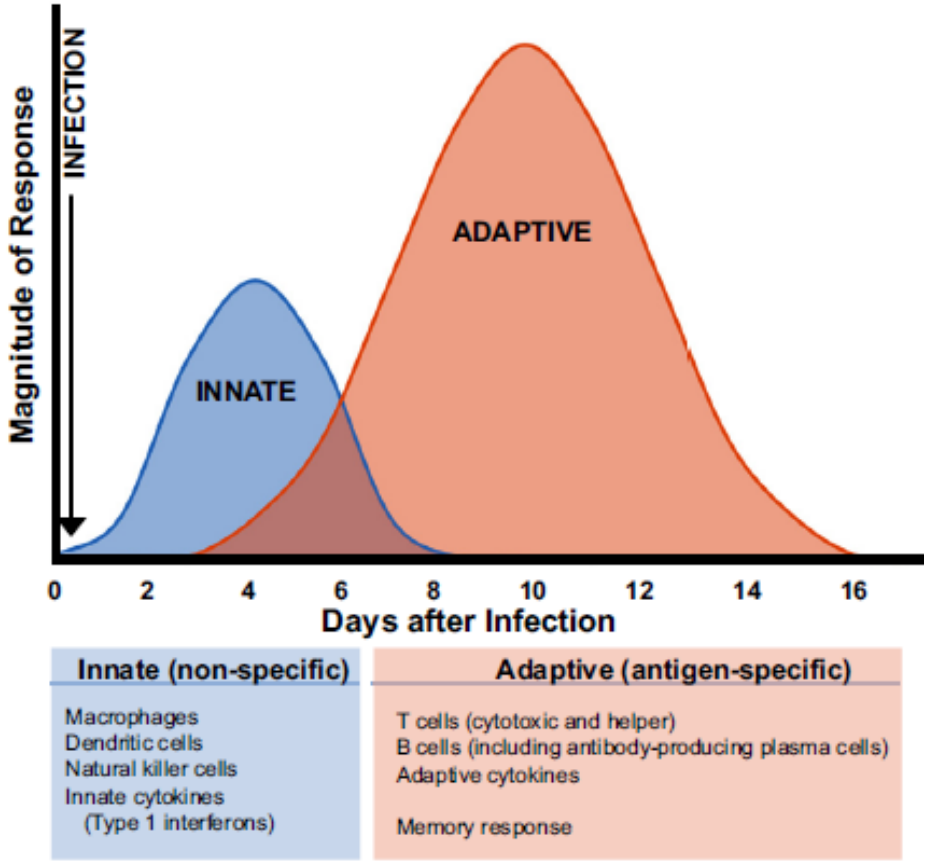
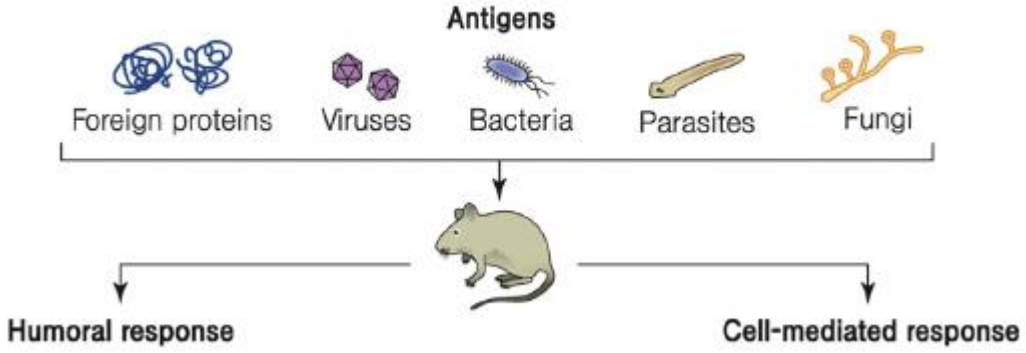


Figure 38: The innate and adaptive arms of the immune system (Louten , 2016)



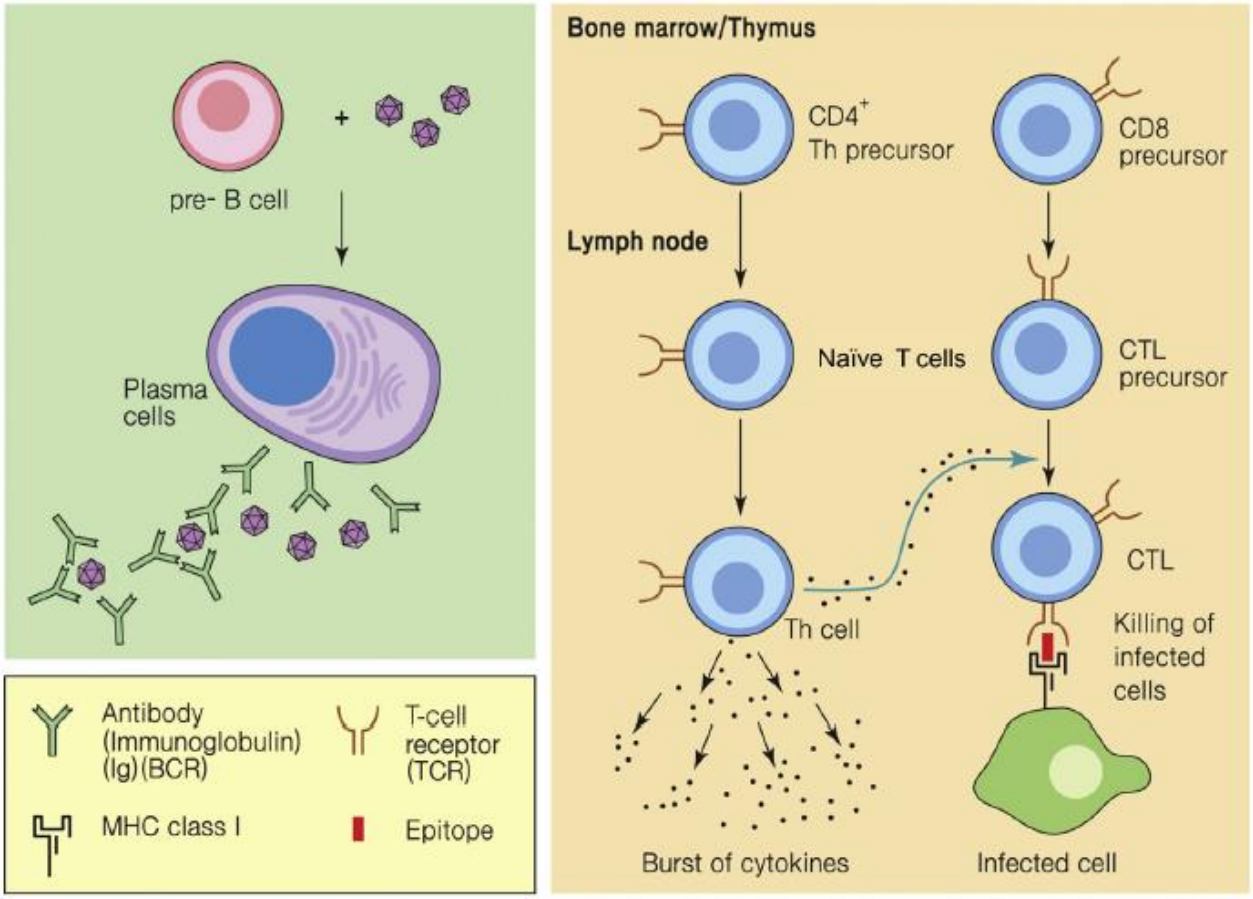


Figure 39: Humoral immunity versus cellular immunity (Wang-Shick Ryu. 2017).

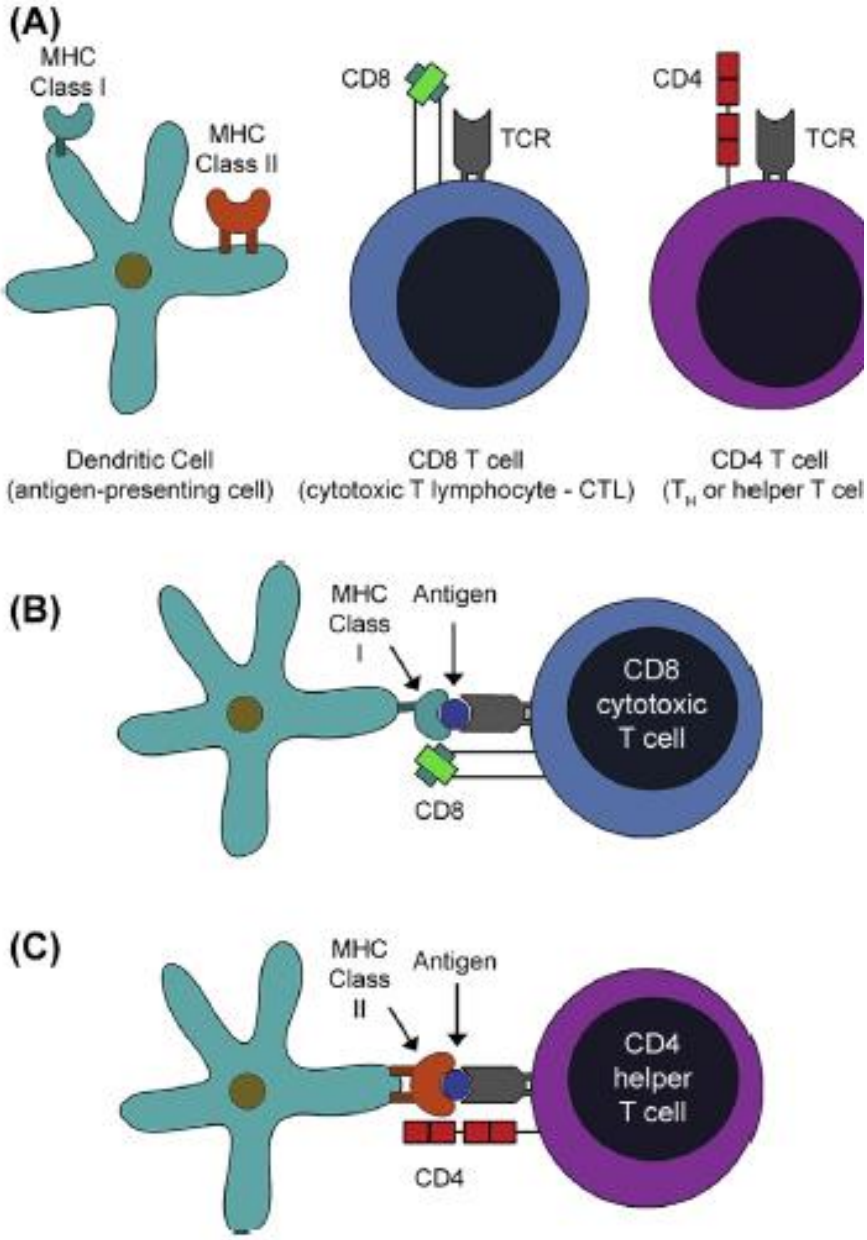


Figure 40: Important cell surface molecules found on immune cells. (A) Dendritic cells are specialized antigen-presenting cells. They possess MHC class I and MHC class II molecules, which they use to present antigen to T cells. (B) CD8 T cells (CTLs) recognize antigen presented by MHC class I, (C) while CD4 T cells (helper T cells) respond to antigen presented by MHC class II molecules. The TCR cannot recognize antigen unless it is presented by one of these two molecules. When a match is made, the T cell becomes activated (Louten , 2016)

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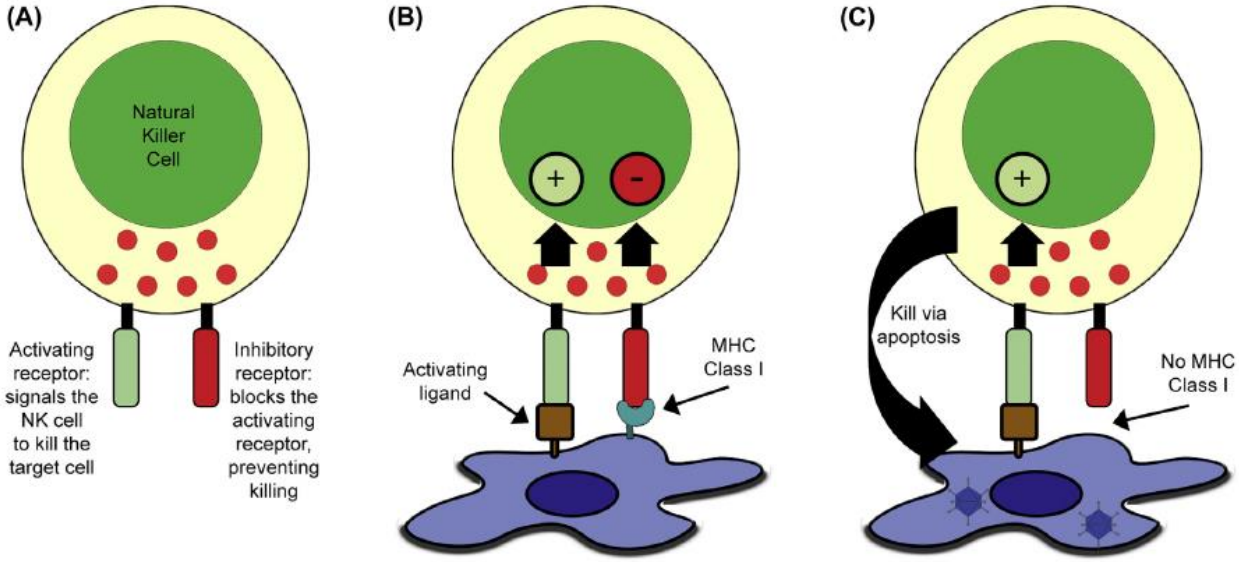

Figure 41: Activation of natural killer cells**.** (A) A natural killer cell has two receptors on its surface, an activating receptor and an inhibitory receptor. (B) The activating receptor binds a stress-induced activating ligand found on the target cell. This sends a positive "killing" signal to the NK cell. However, if MHC class I is present on the cell surface, it binds the inhibitory receptor. This sends a negative signal to the NK cell, preventing it from killing the target cell. (C) If the virus has interfered with the MHC class I molecule, the inhibitory receptor will not be engaged. Without the negative signal, the activating receptor sends the killing signal to the NK cell, which induces apoptosis of the target cell (Louten , 2016)

5.3. Immune Evasion

The immune system is a dynamic collection of cells and proteins that safeguards the host from pathogens, including viruses. Over evolutionary time, however, viruses have also evolved mechanisms to interfere with the host immune response. Nearly every facet of the immune system is thwarted by some virus. RNA viruses, with error-prone RNA polymerases, mutate quickly and can escape immunological memory in this fashion, whereas large DNA viruses, such as herpesviruses and poxviruses, have large genomes that encode immune evasion proteins. (Table.4, figure 42)..

Table 4: Viral Immune Evasion Mechanisms (Cann,.2016)

Immune Evasion Strategy	Mechanism	Virus (protein, RNA)
Infection of Immune cell	$CD4^+$ T-lymphocyte infection	HIV
Immune tolerance induction	T-cell exhaustion	HBV, HCV, HIV
Immunosuppression	Inhibition of antigen presentation	HSV-1 (ICP47), CMV (US3)
	Inhibition of TLR signaling	HCV NS3/4A
	Inhibition of RNG-I signaling	HCV NS3/4A
		Influenza virus NS1
	Blockade of IFN action	Adenovirus (VA RNA)
Immune escape	Antigen drift and shift	Influenza virus HA
Latent infection	Immuno-privileged tissue (neuron)	Herpesvirus

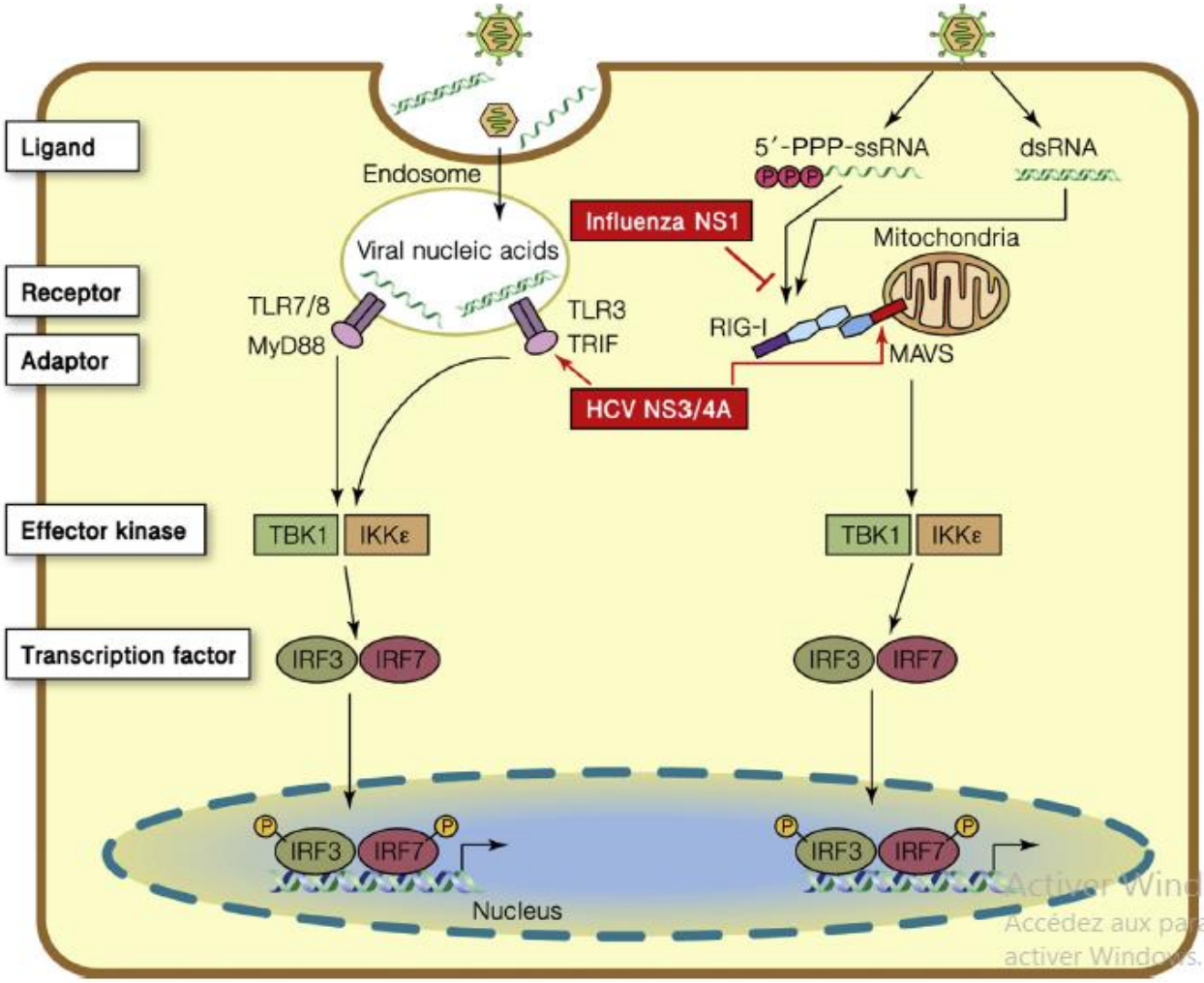

Figure 42: Blockade of innate immunity by viruses. Two representative examples are depicted here. HCV NS3/4A serine protease cleaves TRIF and MAVS, which are the adaptor molecules in TLR- and RIG-I signaling, respectively. Influenza virus NS1 protein blocks the TRIM25-mediated ubiquitination of RIG-I, thereby suppressing RIG-I signaling (Wang-Shick Ryu. 2017.).

5.3.1. Antigenic Variation

Viruses contain antigens, the small portions of viral proteins that are picked up and processed by dendritic cells and presented to T cells. The human body has an estimated 10^{18} different T cells, each with a slightly different TCR, and the T cell that specifically recognizes the viral antigen will be the only T cell able to respond

to it. Similarly, there are around 5×10^{13} B cells with different B cell receptors, and only those that specifically recognize the viral antigen will respond.

The memory T cells and B cells that are generated will have the same antigen specificity as the initial effector T cells and B cells. The error-prone RNA polymerases of RNA viruses often introduce point mutations into viral genes when they place an incorrect nucleotide while copying or transcribing its RNA.the host ribosome reads the RNA in 3-nucleotide codons and creates a protein out of amino acids based upon the sequence of nucleotides. If the viral mRNA has a mutation, this can result in an amino acid different than the original one being incorporated into the protein.

This leads to a slightly different protein being translated, and as a result, a slightly different antigen is created. This is known as **antigenic variation** (Figure 43). The T cell that originally responded to the initial antigen may be unable to recognize the new antigen because of this slight difference, and the memory T cells that were generated will also not be responsive. Consequently, a new T cell with a TCR that recognizes the new antigen will need to be activated. The entire process of antigen presentation and T cell activation will need to be repeated. The same phenomenon occurs with B cells, and so antibodies created against the initial antigen may not recognize the mutated antigen. This constant acquisition of small mutations in viral proteins is called **antigenic drift**.

Viruses with RNA polymerases lacking proofreading ability create small mutations that result in the creation of new strains of the virus that contain antigens unrecognized by previously activated T and B cells. This happens frequently with influenza viruses, which introduce point mutations into their hemagglutinin and neuraminidase proteins, which become unrecognizable by previously generated antibodies. Similarly, there are over 100 different strains of rhinovirus, so the immune response against the cold you catch one year will not provide immunological memory against a different strain of rhinovirus the following year.

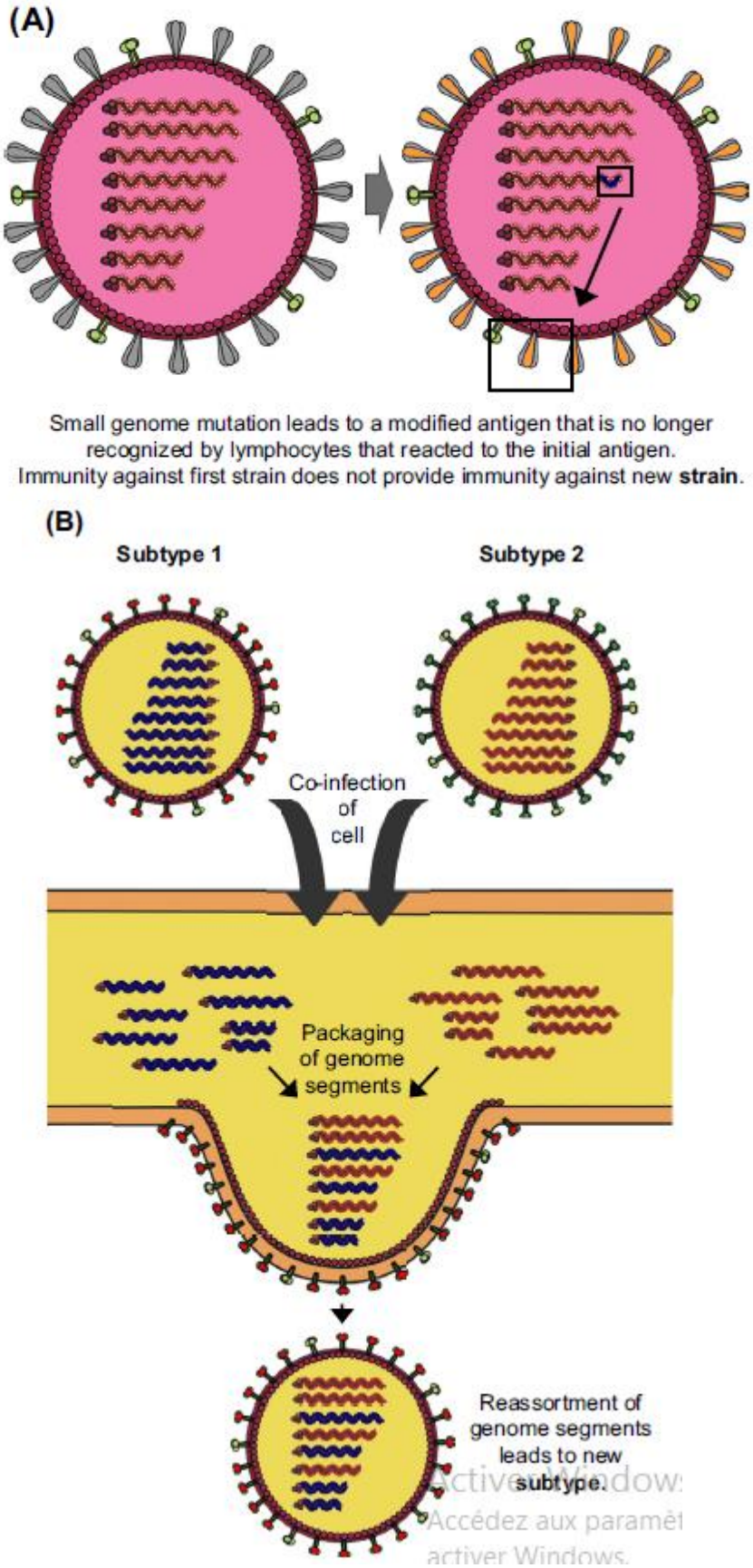

Figure 43: Antigenic drift and antigenic shift.

(A) Error-prone viral RNA polymerases introduce point mutations into viral genes that result in the translation of a slightly different protein antigen, creating a new strain of virus. The result is that the T and B lymphocytes that specifically responded to the initial antigen may be unable to recognize the new antigen. A new primary response by other T and B lymphocytes would have to occur upon infection with the new strain as no memory

response would be available. The constant acquisition of small genome mutations is known as antigenic drift. (B) If two different subtypes of the same segmented virus infect a cell, reassortment of the viral segments can occur during assembly of new virions. This results in the formation of a new subtype. Reassortment is of particular concern when an animal and human reassortant is created.

5.3.2. Latency

When a virus infects and replicates within target cells, viral proteins are produced that act as antigens and are displayed within MHC class I on the surface of the cell. This facilitates the recognition of the infected cell by CTLs. Some viruses, however, enter a state, known as **latency**, where they no longer replicate within the cell but remain dormant until the immune system is weakened. Viral replication does not occur during latency, and so there are no viral proteins produced to act as antigen and alert the immune system of the infected cell.

Viruses can become latent in the initial cell type they infected or in a different cell type near the initially infected cell. For instance, Epstein–Barr virus (EBV) infects and establishes latency within B lymphocytes; on the other hand, varicella zoster virus (VZV), which causes chickenpox, infects epithelial cells of the skin but establishes latency within a sensory nerve at the posterior (dorsal) root ganglion, where the nerve joins the spinal cord. VZV replicates locally within skin epithelial cells but then infects nearby sensory nerves within the skin. The virus travels up the neuron to the dorsal root ganglion, where it remains dormant within the nucleus. When the immune system is weakened, the virus reactivates, travels down the nerve, and begins replicating again within the skin epithelium, causing the disease shingles.

6.3.3. Virus-Encoded Evasion Mechanisms

Viruses encode the genes necessary for their replication, but some viruses also encode genes whose protein products interfere with the host immune response.

Table 5 : Virus-Encoded Genes That Interfere With the Host Immune Response (Louten , 2016)

Viral Gene Function	**Examples**

Interferes with innate cell functions	**1.** Vaccinia virus, Ebola virus, HSV-1, measles virus, and human cytomegalovirus infect dendritic cells and prevent them from functioning properly **2.** Hepatitis C virus, Kaposi's sarcoma-associated herpesvirus, and vaccinia virus interfere with PRR pathways **3.** Human cytomegalovirus expresses a "dummy" MHC class I molecule that binds to NK cell inhibitory receptors, preventing killing
Interferes with MHC class I (preventing CTL activation)	**1.** Human cytomegalovirus US3 and adenovirus E19 protein prevents peptide loading onto MHC class I **2.** HSV-1 ICP47 and human cytomegalovirus U6 proteins prevents peptides from being loaded onto MHC class I
Inhibits inflammatory response	**1.** Vaccinia encodes secreted cytokine receptors to soak up host cytokines **2.** EBV prevents the infected cell from expressing adhesion molecules so cytotoxic cells can't easily bind the infected cells
Inhibits humoral response	**1.** HSV-1 and human cytomegalovirus encode their own antibody receptors that soak up antibodies **2.** Measles virus prevents the activation of B cells