ANTIGENS

What are antigens?

Antigens are substances that cause an immune response in the body by identifying substances in or markers on cells. Your body produces antibodies to fight antigens, or harmful substances, and tries to eliminate them.

Antigen vs Antibody

Antigens are molecules capable of stimulating an immune response. Each antigen has distinct surface features, or epitopes, resulting in specific responses.

Antibodies (immunoglobins) are Y-shaped proteins produced by B cells of the immune system in response to exposure to antigens. Each antibody contains a paratope which recognizes a specific epitope on an antigen, acting like a lock and key binding mechanism. This binding helps to eliminate antigens from the body, either by direct neutralization or by 'tagging' for other arms of the immune system.

Utilizing Antibodies and Antigens in Diagnostics

When infected with a pathogen such as SARS-CoV-2, the body produces antibodies that bind specifically to the antigens to help eliminate the pathogen. This binding can be harnessed to develop antibody and antigen-based diagnostic tests. An antibody test reveals if a person has already been exposed to an infection, by detecting antibodies in their blood or serum. This can be done by a laboratory-based test such as an ELISA (Enzyme-Linked Immunosorbent Assay) or CIA (chemiluminescent immunoassay), or a point-of-care test based on lateral flow technology.

Antibody tests are not usually used to diagnose current infection as it takes the body some time to produce antibodies. During the period before the adaptive immune system kicks in, the fast-acting and non-specific innate immune response combats infection. A negative test result may occur if the test is taken too soon after infection before antibodies have been produced by the body. False positive test results could also occur due to cross-reactivity.

Antibody tests are useful to help track the spread of a disease, identify those who should be prioritized for vaccinations, and highlight potential donors for convalescent plasma therapy. An antigen test reveals if a person is currently infected with a pathogen. Once the infection has gone, the antigen disappears. Unlike nucleic acid-based tests such as PCR, which detect the presence of

genetic material, antigen tests detect proteins, such as those found on the surface of a virus. Accuracy can be a problem, with antigen tests typically having a much lower sensitivity than PCR. However, they usually provide test results rapidly, are relatively cheap, and can be more amenable to point-of-care use, which could make them more suitable for testing in the community and in remote regions.

The Role of Antigens & Antibodies in Vaccinations

Vaccines contain antigens which stimulate the B lymphocytes of the immune system to respond by producing plasma cells which secrete disease specific antibodies (Primary response). Some of the B cells become memory B cells, which will recognise future exposure to the disease. This results in a faster and more intense production of antibodies, which effectively work to eliminate the disease by binding to the antigens (Secondary response).

Since the Nobel-prize winning work of Kolher and Milstein in the 1970's, which enabled the infinite production of monoclonal antibodies in culture using hybridoma technology, the quest to develop therapeutic antibodies has been on. In the last few years, therapeutic antibodies have become the main class of new drugs in development, and by December 2019, 79 therapeutic monoclonal antibodies had been approved by the US FDA. The range of conditions they can be used to treat includes several types of cancers, autoimmune conditions, and infectious diseases such as Zika. A number of studies are currently investigating their potential as a treatment for COVID-19, including via convalescent plasma therapy. Therapeutic antibodies work by binding with high specificity to the target antigen and stimulating an immune response, which may involve inhibition of ligand binding or tagging the cell for binding by cytotoxic T cells.

Types of therapeutic antibodies include:

- Immunoglobulins
- Antibody fragments
- Antibody-drug conjugates
- Bi-specific antibodies
- Radioimmunoconjugates

Antibodies can be produced using a variety of techniques, including hybridoma technologies, transgenic mice, and in vitro display technologies. The evolution of antibody engineering has led to the development of increasingly humanized antibodies, with the benefit of low immunogenecity.

T CELL RECEPTOR FOR ANTIGEN

T cells also have a receptor for antigen on their surfaces. This receptor is not an immunoglobulin molecule but it is composed of two different polypeptide chains which have constant and variable regions analogous to the immunoglobulins. Diversity in the T cell receptor is also generated in the same way as described for antibody diversity (e.g. by VJ and VDJ joining of gene segments and combinatorial association). However, no somatic mutation has been observed in T cells.

Major Histocompatibility Complex (MHC) and T-Cell Receptors - Role In Immune Responses - Structure Of Class I And Class II MHC Molecules - Important Aspects of MHC

The MHC GENE COMPLEX:

The MHC complex contains a number of genes, which control several antigens, most of which influence allograft rejection.

These antigens (and their genes) can be divided into three major classes: class I, class II and class III. The class I and class II antigens are expressed on cells and tissues whereas as class III antigens are associated with proteins in serum and other

body-fluids (e.g.C4, C2, factor B, TNF).

While antigens from class I and class II gene products play a critical role in transplantation, those from class III gene products have no direct role in immune responses that determine graft survival.

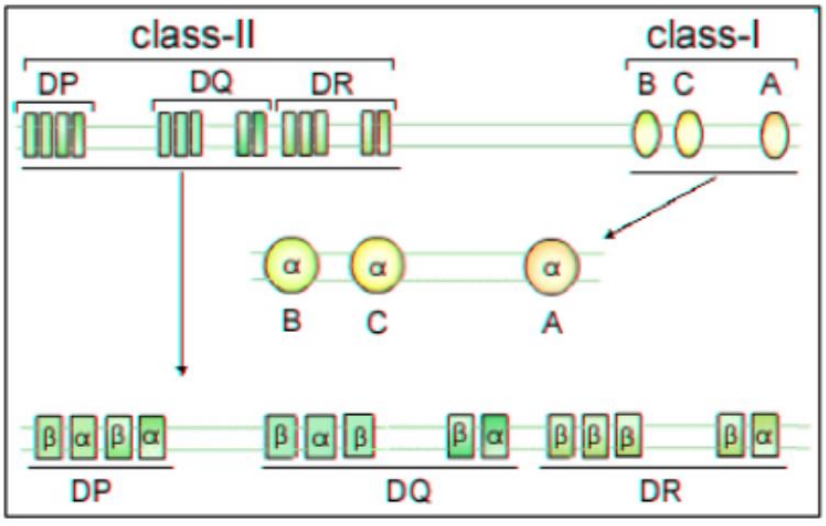


Figure 1. The human MHC gene complex

Human MHC:

The human MHC is located on chromosome 6.

Class I MHC:

The class I gene complex contains three major loci of highest significance, **B**, **C** and **A** and some undefined loci of less significance (Figure 1). Each these loci codes for a polypeptide, α-chain that contains antigenic determinants that are **polymorphic** (has many alleles). Each α-chain associates with a β-2 microglobulin molecule (β-chain), encoded by a gene outside the MHC complex. The α-β-chain complex is expressed on the cell surface as the class-I MHC antigen. Without a functional β-2 microglobulin chain, the class I antigen will not be expressed on the cells surface. Individuals with defective a β-2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.

Class II MHC:

The class II gene complex also contains at least three loci, **DP**, **DQ** and **DR**; each of these loci codes for one α- and a variable number of ß-chain polypeptides which associate together to form the class II antigens. Like the class I antigens, the class II antigens are also polymorphic. The DR locus contains more than one, possibly 4, functional β-chain genes.

MHC Polymorphism: MHC complex is the most polymorphic in the genome. This means that there is an astonishing allelic diversity found within MHC. In humans, the most conspicuously- diverse loci, HLA-A, HLA-B, and HLA-DRB1, have roughly 250, 500, and 300 known alleles respectively. This helps protect the species from extinction that could result from infections and other diseases. However, it is for this very reason, it is extremely difficult to match the donor and the recipient.

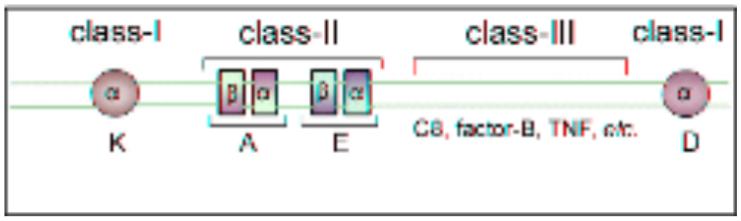
Mouse MHC:
The mouse MHC is located on chromosome 17.

Class I MHC:

It consists of two major loci, **K** and **D**. Unlike the human MHC, the mouse class I gene complex loci are not together but they are separated by class II and class III genes (Figure 2).

Class II MHC:

Figure 2. The mouse MHC complex



The class II gene complex of mouse contains two loci, **A** and **E** each of which code for one α- and one ß- chain polypeptide, which form one class II molecule. The mouse class II gene complex is also known as the **I-region** and the genes in this complex are referred to as **Ir** (immune response) genes since they determine the magnitude of immune responsiveness of different mouse strains to certain antigens. Products of A and E loci are also termed IA and IE antigens, collectively known as Ia antigens.

MHC ANTIGENS: Nomenclature:

HLA specificities are identified by a letter for locus and a number (A1, B5, *etc.*), and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10 DR8). Specificities which are defined by genomic analysis (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, etc.)

Specificities of Mouse MHC (H-2) are identified by a number. Since laboratory mice are inbred, each strain is homozygous and has a unique haplotype. The MHC haplotype in these strains is designated by a 'small' letter (a, b, d, k, q, s, *etc.*). For example, the MHC haplotype of Balb/c, an inbred strain of mouse, is $H2^{d}$.

Inheritance:

MHC genes are inherited as a group (**haplotype**), one from each parent. Thus, a heterozygous human inherits one paternal and one maternal haplotype, each containing three class-I (B, C and A) and three class II (DP, DQ and DR) loci. A heterozygous individual will therefore inherit a maximum of 6 class I specificities (Figure 3: top). Similarly, the individual will also inherit DP and DQ genes and express both parental antigens. Since the class II MHC molecule consists of two chains (α and β), with some antigenic determinants (specificities)

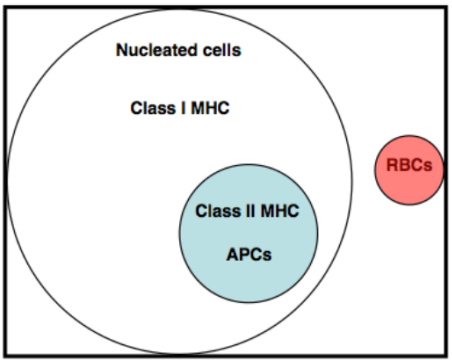


Figure 1. Distribution of class I and class II MHC molecules on human cells.

on each chain, and DR α- and β-chains can associate in *cis* (both from the same parent) or *trans* (one from each parent) combination, an individual can have additional DR specificities (Figure 3: bottom). Also, there are more than one functional DR β-chain genes (not shown in the figure). Hence, many DR specificities can be found in any one individual.

T Cell Receptor and Major Histocompatibility Complex: Role in Immune Responses

I. Historical Overview

Gene products encoded in the Major Histocompatibility Complex (MHC) were first identified as being important in rejection of transplanted tissues. Furthermore, genes in the MHC were found to be highly polymorphic (i.e. in the population there were many different allelic forms of the genes). Studies with inbred strains of mice showed that genes in the MHC were also involved in controlling both humoral and cell-mediated immune responses. For example, some strains of mice could respond to a particular antigen but other strains could not and these strains differed only in one or more of the

genes in the MHC. Subsequent studies showed that there were two kinds of molecules encoded by the MHC – Class I molecules and class II molecules. Class I molecules were found on all nucleated cells (not red blood cells) whereas class II molecules were found only on antigen presenting cells, (APCs) which included dendritic cells, macrophages, B cells and a few other types (Figure 1).

It was not until the discovery of how the T cell receptor (TCR) recognizes antigen that the role of MHC genes in immune responses was understood. The TCR was shown to recognize antigenic peptides in association with MHC molecules. T cells recognize portions of protein antigens that are bound non-covalently to MHC gene products. Cytotoxic T cells (Tc) recognize peptides bound to class I MHC molecules and helper T cells (Th) recognize peptides bound to class II MHC molecules. The three dimensional structures of MHC molecules and the TCR have been determined by X-ray crystallography so that a clear picture of how the TCR, MHC gene products and antigen interact has emerged.

Structure of Class I MHC Molecules (Figure 2)

Class I MHC molecules are composed of two polypeptide chains, along α chain and a short β chain called β2-microglobulin. The α chain has four regions. First, a cytoplasmic region, containing sites for phosphoylation and binding to cytoskeletal elements. Second, a transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane. Third, a highly conserved α3 immunoglubilin-like domain to which CD8 binds. Fourth, a highly polymorphic peptide binding region formed from the α1 and α2 domains.

The β2- microglobulin associates with the α chain and helps maintain the proper conformation of the molecule.

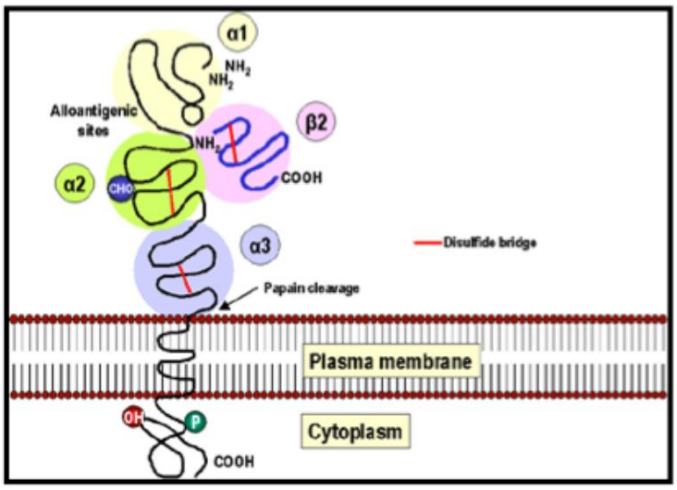


Figure 2. Structure of Class I MHC molecules.

An analysis of which part of the class I MHC molecules is most variable demonstrates that variability is most pronounced in the α1 and α2 domains, which comprise the peptide binding region (Figure 3). The structure of the peptide binding groove, revealed by X-ray crystallography, shows that the groove is composed of two α helices forming a wall on each side and eight β-pleated sheets forming a floor. The peptide is bound in the groove and the residues that line the groove make contact with the peptide (Figure 4). These are the residues that are the most polymorphic. The groove will accommodate peptides of approximately 8-10 amino acids long. Whether a particular peptide will bind to the groove will depend on the amino acids that line the groove. Because class I molecules are polymorphic, different class I molecules will bind different peptides. Each class I molecule will bind only certain peptides and will have a set of criteria that a peptide must have in order to bind to the groove. For example, Figure 5 shows that one class I molecule will bind peptides that have a leucine (L) as the carboxy-terminal amino acid and either tyrosine (Y) or phenylalanine (F) as the 4th amino acid from the carboxy-terminal end. As long as these two conditions are met a peptide will bind, regardless of what the other amino acids are. Similarly a different class I molecule will bind any peptide that has a tyrosine (Y) as the second amino acid from the amino terminal end and either a valine (V), isoleucine (I) or leucine (L) at the carboxy- terminal end (Figure 5). Thus, for every class I molecule, there are certain amino acids that must be a particular location in the peptide before it will bind to the MHC molecule. These sites in the peptide are referred to as the "anchor sites".

Within the MHC there are 6 genes that encode class I molecules HLA-A, HLA –B, HLA-C, HLA-E, HLA-F and HLA-G. Among these HLA-A, HLA –B, and HLA-C are the most important and are most polymorphic. Table 1 shows the degree of polymorphism at each of these loci.

Table 1. Polymorphism of class I MHC genes	
Locus	Number of alleles (allotypes)
HLA - A	218
HLA - B	439
HLA - C	96
HLA - E, HLA - F and HLA - G	Relatively few alleles

Structure of Class II MHC Molecules

Class II MHC molecules are composed of two polypeptide chains an α and a β chain of approximately equal length. Both chains have four regions: first, a cytoplasmic region containing sites for phosphoylation and binding to cytoskeletal elements; second, a transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane, third, a highly conserved α2 domain and a highly conserved β2 domain to which CD4 binds and fourth, a highly polymorphic peptide binding region formed from the α1 and β1 domains.

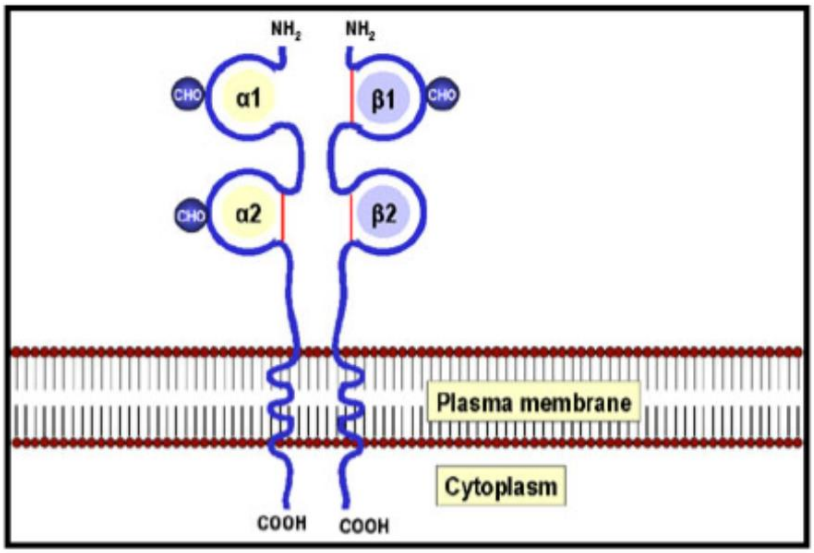


Figure 6. Structure of Class II MHC molecules.

As with Class I MHC molecules, an analysis of which part of the class II MHC molecule is most variable demonstrates that variability is most pronounced in the α1 and β1 domains, which comprise the peptide binding region (Figure 7). The structure of the peptide binding groove, revealed by X-ray crystallography, shows that, like class I MHC molecules, the groove is composed of two α helices forming a wall on each side and eight β-pleated sheets forming a floor (Figure 8). Both the α1 and β1 chain contribute to the peptide binding groove. The peptide is bound in the groove and the residues that line the groove make contact with the peptide. These are the residues that are the most polymorphic. The groove of Class II molecules is open at one

end so that the groove can accommodate longer peptides of approximately 13-25 amino acids long with some of the amino acids located outside of the groove. Whether a particular peptide will bind to the groove will depend on the amino acids that line the groove. Because class II molecules are polymorphic, different class II molecules will bind different peptides. Like class I molecules, each class II molecule will bind only certain peptides and will have a set of criteria that a peptide must have in order to bind to the groove (i.e. "anchor sites").

Within the MHC there are 5 loci that encode class II molecules, each of which contains a gene for an α chain and at least one gene for a β chain. The loci are designated as HLA-DP, HLA –DQ, HLA-DR, HLA-DM, and HLA-DO. Among these, HLA-DP, HLA –DQ, and HLA-DR are the most important and are most polymorphic. Table 2 shows the degree of polymorphism at each of these loci.

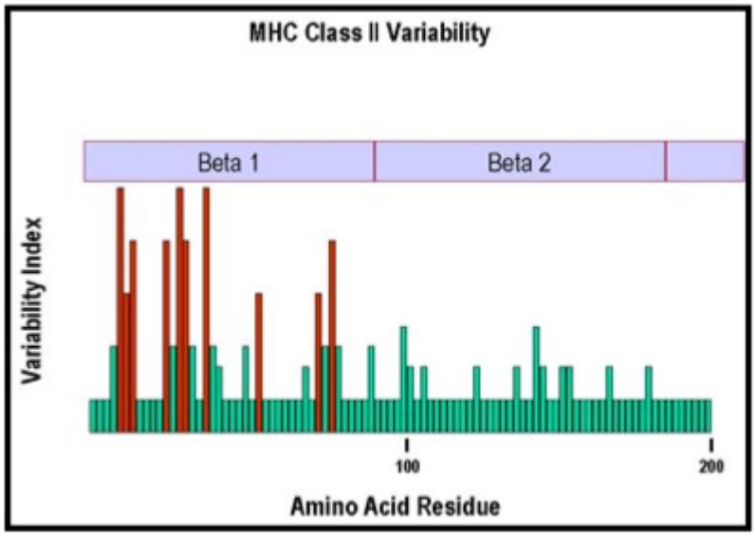


Figure 7. Variability of residues in Class II MHC molecules.

Table 2. Polymorphism of class II MHC Genes	
Locus	Number of alleles (allotypes)
HLA - DPA HLA - DPB	12 88
HLA - DQA HLA - DQB	17 42
HLA - DRA HLA - DRB1 HLA – DRB3 HLA – DRB4 HLA – DRB5	2 269 30 7 12
HLA - DM and HLA - DO	Relatively few alleles

Important Aspects of MHC

A. Although there is a high degree of polymorphism for a species, an individual has maximum of six different class I MHC products and only slightly more class II MHC products (considering only the major loci).

B. Each MHC molecule has only one binding site. The different peptides a given MHC molecule can bind all bind to the same site, but only one at a time.

C. Because each MHC molecule can bind many different peptides, binding is termed degenerate.

D. MHC polymorphism is determined only in the germline. There are no recombinational mechanisms for generating diversity.

E. MHC molecules are membrane-bound; recognition by T cells requires cell-cell contact.

F. Alleles for MHC genes are co-dominant. Each MHC gene product is expressed on the cell surface of an individual nucleated cell.

G. A peptide must associate with a given MHC of that individual, otherwise no immune response can occur. That is one level of control.

H. Mature T cells must have a T cell receptor that recognizes the peptide associated with MHC. This is the second level of control.

I. Cytokines (especially interferon-γ) increase level of expression of MHC.

J. Peptides from the cytosol associate with class I MHC and are recognized by Tc cells. Peptides from within vesicles associate with class II MHC and are recognized by Th cells.

K. Polymorphism in MHC is important for survival of the species.

Structure of the T cell receptor (TCR) (Figure 9)

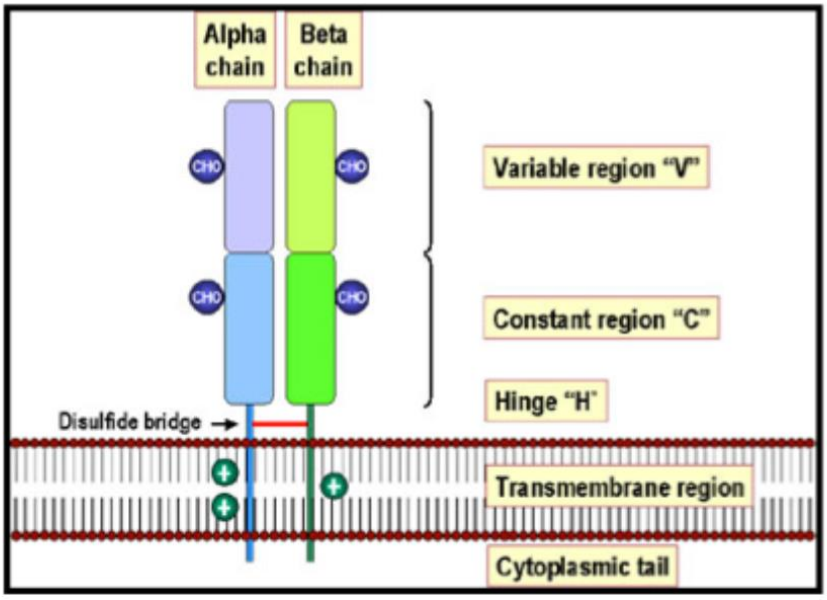


Figure 9. Structure of the T Cell Receptor

The TCR is a heterodimer composed of one α and one β chain of approximately equal length. Each chain has a short cytoplasmic tail but it is to small to be able to transduce an activation signal to the cell. Both chains have a transmembrane region comprised of hydrophobic amino acids by which the molecule is anchored in the cell membrane. Both chains have a constant region and a variable region similar to the immunoglobulin chains. The variable region of both chains contains hypervariable regions that determine the specificity for antigen. Each T cell bears a TCR of only one specificity (i.e. there is allelic exclusion).

The genetic basis for the generation of the vast array of antigen receptors on B cells has been discussed previously (see lecture on Ig genetics). The generation of a vast array of TCRs is accomplished by similar mechanism. The germline genes for the TCR β genes are composed of V, D and J gene segments that rearrange during T cell development to produce many different TCR β chains (Figure 10). The germline genes for the TCR α genes are composed of V and J gene segments which rearrange to produce α chains. The specificity of the TCR is determined by the combination of α and β chains.

REFERENCES AND FURTHER READING

1. "Antibody". National Human Genome Research Institute, US National Institutes of Health. 2020. Retrieved 13 October 2020.
2. "Immune system and disorders". MedlinePlus, US National Institute of Medicine. 28 September 2020. Retrieved 13 October 2020.
3. Male, David K. (2006). Immunology. Elsevier Health Sciences. p. 10. ISBN 978-0323033992.
4. Gavin, AL; Hoebe, K; Duong, B; Ota, T; Martin, C; Beutler, B; Nemazee, D (22 December 2006). "Adjuvant-enhanced antibody responses in the absence of toll-like receptor signaling". Science. **314** (5807): 1936–38.
5. Gallucci, S; Lolkema, M; Matzinger, P (November 1999). "Natural adjuvants: endogenous activators of dendritic cells". Nature Medicine. **5** (11): 1249–55.
6. "Antigenic characterization". US Centers for Disease Control and Prevention. 15 October 2019. Retrieved 13 October 2020.
7. Strebhardt, Klaus; Ullrich, Axel (Jun 2008). "Paul Ehrlich's magic bullet concept: 100 years of progress". Nature Reviews Cancer. **8** (6): 473–80.
8. Lindenmann, Jean (1984). "Origin of the Terms 'Antibody' and 'Antigen'". Scand. J. Immunol. **19** (4): 281–85.
9. Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, Bebris L, Florens L, Dobano C, Witney AA, Appella E, Hoffman SL, Yates JR, Carucci DJ, Sette A (August 2003). "Identification of Plasmodium falciparum antigens by antigenic analysis of genomic and proteomic data". Proceedings of the National Academy of Sciences of the United States of America. **100** (17): 9952–57.
10. Parham, Peter. (2009). The Immune System, 3rd Edition, p. G:2, Garland Science, Taylor and Francis Group, LLC.
11. Janeway CA, Jr (1 November 2013). "Pillars article: approaching the asymptote? Evolution and revolution in immunology. Cold spring harb symp quant biol. 1989. 54: 1–13". Journal of Immunology. **191** (9): 4475–87.
12. Gayed, PM (June 2011). "Toward a modern synthesis of immunity: Charles A. Janeway Jr. and the immunologist's dirty little secret". The Yale Journal of Biology and Medicine. **84** (2): 131–38
13. Parham, Peter. (2009). The Immune System, 3rd Edition, p. G:11, Garland Science, Taylor and Francis Group, LLC.
14. Kuby Immunology (6th ed.). Macmillan. 2006. p. 77

15. Schumacher, Ton N.; Schreiber, Robert D. (April 3, 2015). "Neoantigens in cancer immunotherapy". Science. **348** (6230): 69–74.
16. Wang, Qing.; Douglass, Jacqueline (September 16, 2019). "Direct Detection and Quantification of Neoantigens". Cancer Immunol Res. **7** (11): 1748–54.
17. K. Abbas, Abul; Lichtman, Andrew; Pillai, Shiv (2018). Cellular and molecular immunology (Ninth ed.). Philadelphia: Elsevier. p. 97.