The Major Players in Adaptive Immunity

1. Humoral Immunity

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How do we remain healthy, for the most parts, in the midst of an environment teeming with opportunistic and infectious microbes, potential carcinogens and allergens? The fact is that our immune system, by and large, does a fine job in protecting us. It is therefore important to understand the organization of the immune network, which is broadly categorized into two groups: innate and adaptive. Cells involved in innate immunity are the first to come into contact with invading microbes, similar to the border security force, and respond rapidly but in a non-specific manner. On the other hand, the cells involved in adaptive immunity are slower to respond but act in a very specific manner. Though the primary response is slow, the secondary response is much faster and demonstrates memory. This article will focus on some important features and key players involved in the adaptive immune response. The first part deals with the humoral immune response mediated mainly by immunoglobulins produced by the B cells. The second part deals with T cells, the Major Histocompatibility Complex (MHC)-encoded molecules, and Recombination Activating Genes (RAG) responsible for generating diverse B-cell receptors (BCR) and T-cell receptors (TCR). With the advent of newer and smarter infectious agents, it is important to understand the working of the immune network as more research in this area may facilitate the development of better protective strategies.

The immune system is responsible for protecting us against microbial infections and the spontaneous generation of tumours. If the immune system is compromised, resident microbes gain advan-

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tage, which may be deleterious to the host. An example of this is evident in patients suffering from acquired immune deficiency syndrome (AIDS)[1] who show high susceptibility to opportunistic infections leading to greater mortality. Another example is that of David Vetter who suffered from congenital immunodeficiency (lack or reduced numbers of immune protective cells or mechanisms) and spent his entire life (thirteen years) inside a sterile plastic bubble; hence, the name 'Bubble boy'.

How does the immune system work? Broadly, its function depends on three ingredients: cells, receptors and effector molecules. Similar to a nation's defence organization, the immune system possesses different cells which play distinct roles during the immune response (*Figure* 1). Immune cells possess key cell-surface receptors that recognize microbial constituents such as lipopolysaccharides (LPS) and flagellin, present on the bacterial cell surface. The immune system can distinguish between self and non-self and mount a response by recognizing the unique molecular signatures present on invading microbes, tumours or foreign cells. Activation of specific receptors on immune cells results in signalling events that culminate in the production of various effector molecules such as free radicals, cytokines, and immunoglobulins and lead to the elimination of invading microbes, tumours or foreign cells. As the doubling time of microbes is short, multiple strategies are required to generate a rapid and effective immune response.

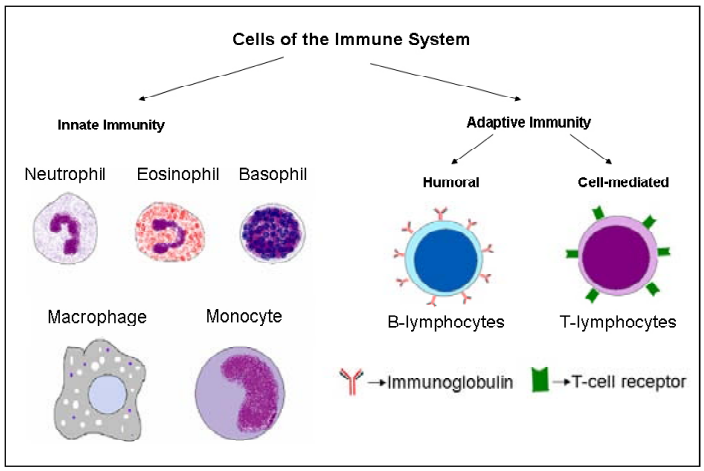

Figure 1. Cells of the immune system. Pluripotent stem cells give rise to the immune system, which may be classified into two groups: innate and adaptive. The left-hand side shows cells involved in innate immunity, which act as the first line of defence: granulocytes (neutrophils, basophils and eosoniphils), monocytes and macrophages. The right-hand side shows cells involved in adaptive immunity: B cells secrete specific Ig whereas T cells are responsible for cell- mediated immune responses. There is plenty of cross talk between cells involved in both forms of the response, e.g., macrophages process antigens and present peptide/MHC molecules to T cells to initiate the adaptive immune response. T cells, upon activation, secrete cytokines, e.g., IFNγ, which stimulate macrophage functions. (See text for details)

[1] *Acquired Immune Deficiencey Syndrome (AIDS)*: A set of syndromes caused by infection with human immunodeficiency virus (HIV). The numbers of $CD4^+$ T cells decrease as the infection progresses, resulting in enhanced susceptibility to opportunistic infections, tumours and other complications.

There are two types of immune responses: innate or inborn and adaptive.

The Innate Immune Response

The components of the innate system are responsible for the first line of defence and include anatomic, physiologic, phagocytic and inflammatory barriers. This response is quick but lacks specificity. During infections, the numbers of blood cells known as neutrophils[2a] increase and physicians often use this information obtained from blood tests for confirmatory diagnosis. Increase in the numbers of eosinophils[2b] is evident in parasitic helminthic infections, e.g., infection with *Ascaris*, a parasitic roundworm which resides in the small intestine. In addition, eosinophils and basophils[2c] also accumulate at inflammatory sites during allergic reactions induced by external agents. Cells belonging to the monocyte/macrophage[3] lineage ingest and kill microbes. They are also important in processing and presenting antigens[4] (substances from invading microbes or tumour cells or allergens that elicit an immune response) to T cells to initiate the adaptive immune response, which is discussed below.

Adaptive Immunity

The adaptive immune response is much slower but is characterised by specificity and memory. In fact, these two characteristics are important hallmarks of this response. Lymphocytes, which constitute 20–40% of white blood cells or leukocytes, are the major cells responsible for adaptive immunity. These are further grouped into B and T lymphocytes (*Figure* 1). B cells are responsible for humoral (present in fluids) immunity whereas T cells mediate cellular immunity. B cells possess B-cell receptors (BCR), also known as membrane immunoglobulins, on the cell surface which, upon binding to antigen, result in differentiation of B cells to plasma cells. These plasma cells secrete copious amounts of soluble immunoglobulins (or Ig, also known as antibodies) which bind to antigens. Therefore, the active ingredient here, i.e., antibodies, can be separated away from cells without any loss in functional activity

[2a] *Neutrophils*: Cells of the innate immune system that usually are the first to migrate to a site of infection. Their primary function is to phagocytose and kill pathogens

[2b] *Eosinophils*: are a class of granulocytes with a bilobed nucleus and cytoplasm which stains with the acidic dye, eosin. They are part of the anti-microbial innate defense network and are also involved in allergic reactions together with basophils and mast cells. An abnormally enhanced number of blood eosinophils, known as eosinophilia, iis indicative of infections, usually parasitic.

[2c] *Basophils:* are non-phogocytic granulocytes which form <1% of the total circulating white blood cell population. Their nuclei are lobed and their cytoplasm stains with a basic dye, methyulene blue. Basophils and mast cells are closely related and are the main mediators of the allergic response. they store histamine and release it during an allergic reaction when their Fc receptors are cross-linked by allergen coated IgE antibodies.

[3] *Macrophages*: Cells of the innate immune system whose major functions are phagocytosis, antigen processing and presentation.

[4] *Antigen Presenting Cells*: A class of immune cells which includes dendritic cells, macrophages and B cells. They digest cellular or microbial-derived proteins to peptides and present them in complex with MHC to T cells.

(defined as the capacity to bind antigen). Blood serum (liquid component of blood without any clotting factors) contains polyclonal antibodies which are secreted by plasma cells with multiple specificities. As serum antibodies are produced due to stimulation by antigens, the presence or absence of specific antibodies has a potential clinical value. Diagnostic kits have been developed to detect antigen-specific antibodies and are used to diagnose microbial infections. Those infected with *Salmonella* possess high amounts of serum antibodies that clump or agglutinate *Salmonella* antigens and this test is known as the Widal test, which is often used in the diagnosis of typhoid. Similarly, the presence of antibodies against HIV proteins is the basis for diagnosing HIV infections.

For cellular immunity mediated by T-lymphocytes, cell–cell contact is required. T cells express the T-cell receptor (TCR) which recognises peptides (short segments of amino acids derived from self proteins, microbial proteins, tumour[5]-specific proteins or self proteins modified by binding to allergens) on presenting molecules known as the major histocompatibility complex (MHC)-encoded proteins located on the cell surface of antigen-presenting cells (APC). In addition, there is a separate group of lymphocytes known as natural killer (NK)[6] cells which are the key effectors of 'antibody-dependent cell-mediated cytotoxicity' (ADCC)[7] and are important for immunity against tumours. NK receptors do not undergo rearrangement.

How is the antigenic specificity of the immune response achieved? B and T cells express on their surface, receptor proteins known as BCR or TCR which determine antigenic specificity. Each B or T cell expresses a unique BCR or TCR (a heterodimer of two proteins, either $\alpha\beta$ or $\gamma\delta$); the total number of molecules that they can recognize is very large as the number of different combinations possible are large. This concept that each lymphocyte expresses a unique cell surface receptor for antigen recognition is important as it allows only selected cells containing the appropriate receptors to proliferate and mount responses during an immune reaction. F McFarlane Burnet was the first to enunciate this concept known

[5] *Tumour*: Uncontrolled growth of cells due to loss in cell cycle regulation. Plasma cells that become tumorigenic are known as myeloma.

[6] *Natural Killer (NK)*: Lymphocytes that do not express B cell or T cell antigen receptors but can kill tumour cells and participate in ADCC.

[7] *Antibody-Dependent Cell-mediated Cytotoxicity (ADCC)*: A process by which antibody bound infected cells are recognized and killed by host NK cells, macrophages, neutrophils or eosinophils which possess receptors for Ig.

as the 'clonal selection hypothesis' for which he was awarded the Nobel Prize in 1960 [1]. This concept has practical applications too and has led to the development of the 'hybridoma technology' which enables the isolation and purification of antibodies with unique specificity known as 'monoclonal antibodies'[8]. We will examine lymphocytes and important associated molecules necessary for their (lymphocytes) optimal function in greater detail below.

[8] *Monoclonal Antibody*: Originating from a single cell and all cells derived from this original clone possess the identical antigenic specificity.

The Humoral Response

B cells derive their name from 'Bursa of fabricius', the organ in birds in which B cells mature. This was first demonstrated by the observation that the removal of the Bursa in newly hatched chickens impairs their ability to generate a humoral response, i.e., produce antibodies against a specific antigen [2]. Later it was discovered that B cells originate and mature in the bone marrow of mice and humans. Each B cell expresses a unique antigen-binding receptor called the BCR on its cell surface. Circulating B cells that have not been exposed to antigens are known as naïve B cells[9], which get activated upon binding of their BCR to a cognate antigen. B cells internalize antigens, digest them and present peptides derived from these antigens together with MHC molecules (see below) to T cells. Activated T cells induce the expression of cell surface proteins (e.g., CD40L) and secrete factors known as cytokines which stimulate the activated B cells to divide rapidly and differentiate into memory B cells and plasma cells (*Figure* 2). This process occurs in specialized areas in lymph nodes or spleen known as germinal centres. Increase in the numbers of germinal centres in lymph nodes and spleen correlates with an active humoral response. Plasma cells are the main effector B cells which secrete large numbers of immunoglobulin molecules (some estimates have it as more than 2000 Ig/second). The first immunoglobulin to be secreted in this response is always IgM**;** subsequently, different cytokines instruct B cells to secrete different classes of Ig, i.e., IgG, IgA and IgE, with the same antigenic specificity (see *Figure* 2). Memory cells have a longer life span and they respond quickly upon a second exposure to the same antigen.

[9] *Naïve Cell*: Mature T or B cells that have not encountered antigen.

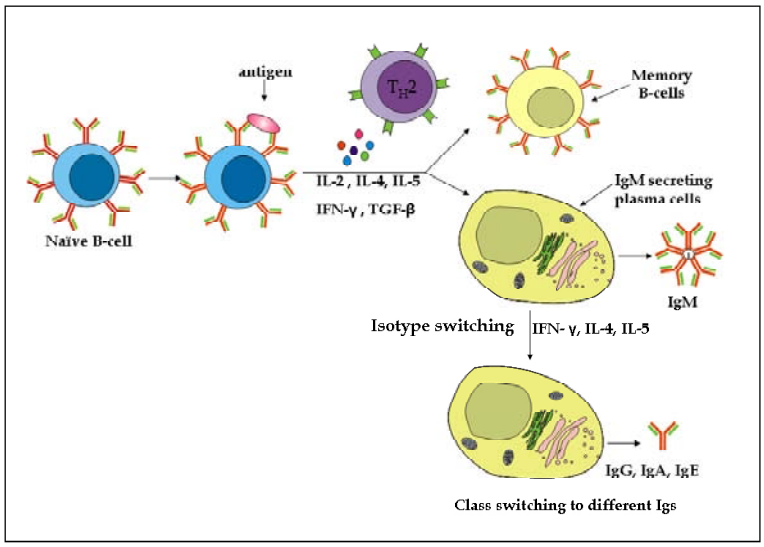

Figure 2. Overview of cellular events during the generation of the humoral response. A naïve B cell recognizes antigen and this binding delivers the first signal. Also, B cells process antigens and present peptides with MHC molecules to $CD4^+$ T cells. Consequently, T cells get activated and express the cell surface molecule CD40L which binds to CD40 on B cells, thus delivering the second signal. These signals, together with cytokines produced by activated T cells, play important roles: IL-2, IL-4 and IL-5 are important for proliferation whereas IFN-γ and TGF-β are important for differentiation of activated B cells to plasma cells and memory B cells. Initially, IgM is secreted by plasma cells; subsequently, isotype switching occurs under the influence of different cytokines, resulting in secretion of different Ig isotypes such as IgA, IgG and IgE.

What are Immunoglobulins?

In general, immunoglobulins or antibodies have a similar overall structure consisting of two polypeptides: heavy chain (higher molecular mass) and light chain (lower molecular mass). A typical antibody molecule is 'Y' shaped and is composed of two heavy chains and two light chains (IgD, IgE and IgG) that are held together by covalent (disulphide bonds) and non-covalent forces, giving rise to two antigen binding sites (*Figure* 3). G M Edelman and R R Porter were awarded the Nobel Prize in 1972 for their pioneering studies that led to the unravelling of the structure of the antibody molecule. Secretory IgA is dimeric (consisting of four heavy chains and four light chains) whereas IgM is pentameric (consisting of ten heavy chains and ten light chains). There are two types of light chains: κ (kappa) and λ (lambda) and five types of heavy chains: α (alpha), δ (delta), ε (epsilon), γ (gamma), and μ (mu). A single Ig molecule possesses one type of heavy chain associated with either light chain but never both. Human immunoglobulins are divided into five classes of antibody known as isotypes (*Box* 1): IgA (α), IgD (δ), IgE (ε), IgG (γ), and IgM (μ), depending on the type of heavy chain which has been denoted within brackets. IgG is present in maximal amounts in serum followed by IgM and IgA whereas the amounts of IgD and IgE are

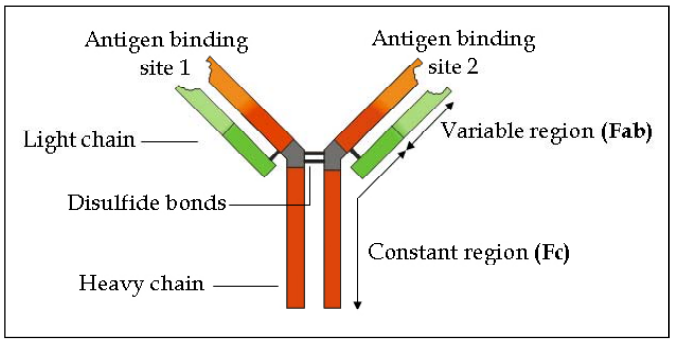

Figure 3. Structure of a typical Ig molecule. In most cases, Ig are heterodimers of two heavy and two light chains. The number of antigen binding sites can vary from two (e.g. IgA, IgD, IgE and IgG,) to four (e.g. secretory IgA) to ten (e.g. IgM). The amino terminals of both heavy and light chains are variable and are responsible for antigen binding (Fab). The rest of the chains remain constant and form the Fc fragment, which is responsible for Ig-mediated effector functions, e.g. opsonization and complement activation (See text for details).

low. IgG and IgA are further classified into subclasses depending on minor amino acid differences. IgG is subdivided into IgG_1, IgG_2, IgG_3, IgG_4 based on minor chain differences and numbered according to decreasing serum concentration. IgA is subdivided into IgA_1 and IgA_2. IgA_1 is a monomer and forms the major serum IgA while IgA_2 is a dimer and the main component of secretory IgA.

Each heavy and light chain can be further divided into constant and variable regions. The first 110 amino acids of different immunoglobulins show sequence variability (hence the name) and form the antigen-binding pocket. This characteristic is important as it allows flexibility in binding to different antigens. Multiple alignment of amino acid sequences from different immunoglobulins demonstrate that three regions within the variable region show greater variability and are termed as 'complementarity determining regions' (CDR), out of which CDR3 shows the greatest variability. On the other hand, there is not much variability in the 'constant region' of antibody molecules. This region contains the site for carbohydrate addition, i.e., glycosylation and can bind to Fc receptors on a number of cell types such as NK cells and direct different biological activities (*Box* 1).

What are the Mechanisms Involved in Generating Antibody Diversity?

For a long time, immunologists were puzzled by the ability of the immune system to generate the vast array of antibodies against a large number of antigens – it would appear that this potential is

Box 1. Different Human Immunoglobulins and their Properties

Class (heavy chain)	Subclass	Molecular form	Major biological functions
IgG(γ)	IgG_1 IgG_3 IgG_4	Monomer	Placental transfer to protect the fetus. Can activate complement system. Opsonization. IgG_1 and IgG_3 are often produced against protein antigens whereas IgG_2 is produced against capsular polysaccharides present in some bacteria. In case of deficiency of any of the 4 subclasses, there is increased risk of asthma or infections.
IgA(α)	IgA_1 IgA_2	Monomer Dimer is Secretory	Found in external secretions, e.g. breast milk, saliva, tears and mucus. Protects new born babies from infections. Important line of mucosal defence against infections. Some individuals have complete absence of IgA, but normal levels of other Ig. This is called as selective IgA deficiency which is a common immunodeficiency disease. The majority of these cases are asymptomatic although in some cases cases there are increased incidences of infections, autoimmunity and allergy.
IgM(μ)	–	Pentamer	First class of Ig to be produced during a primaryimmune response. Contains 10 antigen binding sites. Activates complement system more efficiently than IgG. Deficiency results in susceptibility to infections, especially by encapsu lated bacteria (e.g. *Streptococcus pneumoniae* etc.) and increased autoim mune disorders, cancer etc. Inability of IgM to switch to other classes causes hyper IgM syndrome with elevated levels of IgM and reduced levels of other Ig subtypes.
IgE(ε)	–	Monomer	Responsible for allergic reactions. Binds to Fc receptor and induces mast cell degranulation. Serum IgE levels increase upon parasitic helminth infections or allergy. Low IgE levels are often associated with immunodeficiency associated with low amounts of other Ig isotypes. Hyper IgE syndrome is characterized by recurrent *Staphylococcal* infec tions, skin and pulmonary abscesses and increased numbers of blood eosinophils.
IgD(δ)	–	Monomer	It is present in very low amounts is serum and not much is know of its roles. IgD and IgM are membrane bound Igs on naïve B cells. IgD deficiency individuals do not show any obvious phenotype.

infinite! S Tonegawa's group (Nobel laureate 1987) showed for the first time that the immunoglobulin genes rearrange during B cell development. In the germ-line DNA there are multiple gene segments that encode a portion of an immunoglobulin's heavy or light chain. The genes encoding these chains are arranged as 'multi-gene families' situated on different chromosomes. These regions contain coding sequences that are separated by non-coding regions which are brought together after gene rearrangement [3]. The light chain family contains ~76 variable genes (V) and 5 joining genes (J) and a unique constant (C) gene (Chromosome 2p). There are ~73–74 V_l genes, 7–11 J_l and 7–11 C_l genes that may encode the human light chain (Chromosome 22q). On the other hand, there are 123–129 V_H, 27 diversity (D) and 9 J_H genes and 11 C_H genes that can encode the human Ig heavy chain (Chromosome 14q). Hence, the ability of any of the V_H gene segments to combine with any of the 27 D_H gene segments and any of the 9 J_H segments and 11 C_H genes gives rise to large numbers of possible combinations ($V \times D \times J \times C$). Similarly for κ and γ there will also be large numbers of combinations, and this mechanism allows humans to synthesize millions of different types of Ig.

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This recombination of V, D and J coding genes of B- and T-cell receptors are performed by enzymes collectively called 'VDJ recombinase'. The products of two genes known as Recombination Activating Gene (RAG)-1 and RAG-2 form this recombinase (*Figure* 4). Apart from VDJ recombination, several other mechanisms have been identified in humans that generate additional antibody diversity. These include junctional flexibility, P-nucleotide addition (occurs due to variation in endonucleatic cleavage resulting in addition of nucleotides to form a palindromic sequence), N-nucleotide addition (addition of nucleotides in the free 3'end of DNA by terminal deoxynucleotidyl transferase), class switching and combinatorial association of light and heavy chains. These processes result in expression of immunoglobulin molecules on the B-cell surface.

Further modifications contributing to Ig diversity occur upon B cell

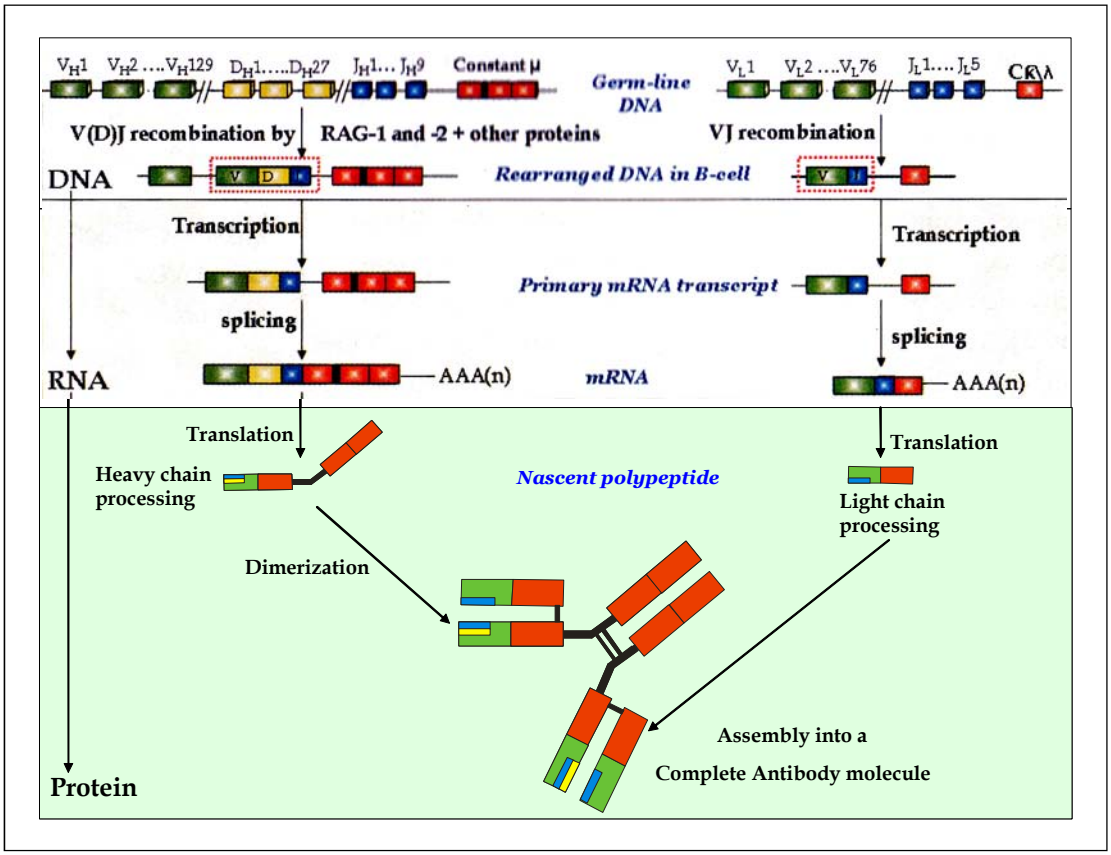

Figure 4. Overview of RAG mediated Ig gene rearrangement. Germline DNA contains three Ig multigene families: κ light chain, λ light chain and the heavy chain, which are located on different chromosomes. During B cell maturation, gene segments of these families are rearranged to yield functional Ig molecules. The light chain families possess V, J and C segments whereas the heavy chain family has V, D, J and C segments. With the help of RAG proteins V, D and J segments are rearranged to give rise to the variable region of the heavy and light chains. Rearrangement of V, D and J segments in different permutations and combinations generates Ig diversity. The rearranged variable segments then join the constant region fragments to produce the final heavy and light chain transcripts. These are translated to form the heavy and light chain proteins which assemble to form a functional Ig molecule.

activation. For example, it is well known that IgM is on the B-cell surface but is secreted by plasma cells. This process involves RNA splicing and the heavy chains of secreted Ig possess a stretch of 20 hydrophilic amino acids at the C-terminus, whereas membrane Ig possess a stretch of 40 amino acids comprised of a hydrophilic segment followed by a hydrophobic transmembrane

domain and a short hydrophilic cytoplasmic portion. The hydrophobic segment is encoded by two exons, M1 and M2, present in the heavy chain encoding DNA of all immunoglobulin isotypes. If the mRNA transcript is spliced such that M1 and M2 are included, then the translated protein is a membrane immunoglobulin. However, if M1 and M2 are excluded during splicing then the immunoglobulin molecules are secreted. Most likely, the process of activation and differentiation into plasma cells directs altered splicing of the mRNA transcript.

IgM is the first isotype to be secreted by plasma cells and, with time, other isotypes with the same antigenic specificity appear in circulation. During this process, the variable region remains the same (hence there is no alteration in antigenic specificity) whereas the constant region of the heavy chain of the immunoglobulin changes (known as 'isotype switching'). The switch to other isotypes is determined by the cytokines secreted by the T_H cells. For example, Interleukin[10] (IL)-4 induces switching to IgG1 and IgE whereas IL-5 induces switching to IgA. DNA sequences known as 'switch sites' are located upstream of the heavy chain constant regions. The order of heavy chain constant regions in the DNA is: IgM, IgD, IgG, IgE followed by IgA. IgM and IgD do not have any upstream switch sites and are the first isotypes to be expressed on the cell surface of naïve B cells. If a switch occurs from IgM to IgE, then the DNA sequence from IgM to IgG is looped and excised and the functional transcript will now contain the heavy chain constant region of IgE.

[10] *Interleukins*: A group of low molecular proteins secreted by a variety of cells that regulate different immune functions: proliferation, death, differentiation, antibody secretion, etc. IL-2 increases proliferation of T cells whereas IL-4 regulates IgE production by B cells.

During B-cell response it is often found that antibodies produced early during the immune response bind to the antigen with low affinity whereas those produced later bind with stronger affinity. This process is known as 'affinity maturation' and the mechanism involves 'somatic hypermutation'. Random mutations occur in the variable regions of immunoglobulins of individual B cells that enhance the binding of the antibody with its cognate antigen. This process results in the selection and secretion of high-affinity antibodies by plasma cells.

In summary, rearrangement of immunoglobulin genes results in the generation of large numbers of B cells with different membrane Ig molecules. Upon binding to an antigen, B cells are triggered, followed by secretion of antibodies and isotype switching and binding affinities are increased further by somatic hypermutation. These multiple strategies, i.e., "first bind, adapt and, subsequently, bind with greater affinity" allows greater flexibility to the immune system to generate specific antibodies against a limitless number of antigens.

Antibodies Play Different Effector Roles

The variable regions of antibodies bind to antigens via non-covalent interactions, e.g., hydrogen bonds, ionic bonds, hydrophobic interactions and van der Waals interactions. It is this binding that neutralizes toxins and viruses rendering them biologically non-functional. Attenuated viruses (e.g., small pox, polio) or toxoids (inactivated toxins, e.g., tetanus exotoxin, diphtheria exotoxin) are used to elicit specific antibodies and memory cells by the host via a process known as 'active immunization'. In essence, vaccination allows the host to produce high amounts of neutralizing antibodies that bind to pathogens and render them non-functional (i.e., they are not capable of replicating) and prepare the host to counter a possible infection. In fact the most effective vaccines are ones that generate good neutralizing antibodies. Sometimes, preformed antibodies (for example from horse or goat) are administered against rapidly acting toxins or snake/insect bites. Such 'passive immunization' is helpful in conditions when there is not much time for the host to produce antibodies and neutralize toxins (e.g., botunilum toxin) or snake venoms. Anti-snake venoms contain immunoglobulins that will neutralize venoms of many common poisonous snakes found in India, e.g., cobra, kraits and vipers. This aspect is important as there is no time to ascertain the identity of the snake that has bitten the patient and quick therapy is a must in such cases.

***Complement Activation*:** The antigen-antibody complexes activate the complement[11] system, which consists of several plasma

proteins that are present in blood in an inactive state. Once activated, they clear pathogens by cell lysis. Some antibodies bind to microbes and enhance their phagocytosis (this process is known as opsonization) by promoting adhesion to neutrophils and macrophages. Some antibody-coated target cells are lysed by NK cells by ADCC, as described earlier. Here the constant region of the antibody is bound to CD16[12] receptors on NK cells that get activated and attack antibody-bound cells leading to their lysis.

Protection of Mucosal Surfaces: Some Ig have specialized roles, e.g., secretory IgA is the major class of Ig present in mucosal secretions (tears, saliva, intestinal secretions, etc.) and plays a key role in protecting such environments in the body. The mechanism by which secretory IgA is present in mucosal secretions is due to an interesting cell biological process. Plasma cells secrete IgA which is recognized by polymeric Ig receptor (pIgR) present in epithelial cells. These receptors present on the basolateral surface bind to IgA and transport these molecules to the luminal surface. Here, there is cleavage of the receptor and IgA is released in the lumen together with a remnant of the pIgR known as the 'secretory component'.

Ig and allergy: An immunoglobulin isotype known as IgE is important for allergic responses. IgE binds to specific receptors present on the membranes of tissue mast cells and blood basophils. Once an allergen cross-links a receptor-bound IgE on cells, it induces degranulation, resulting in release of several effector molecules, including histamines[13], leukotrienes, etc. These compounds lead to dilation of blood vessels, increased mucous secretions (runny nose) and more severe effects leading to drop in blood pressure, collapse of the respiratory, circulatory systems, etc.

Immunoglobulins and Autoimmunity: In some cases, antibodies are formed against the host, leading to autoimmunity (*Box* 2). Rheumatoid arthritis is one such autoimmune disorder, where antibodies are formed against the Fc region of IgG. Patients suffering from rheumatoid arthritis possess high amounts of an IgM known as rheumatoid factor. It binds to the circulating IgG, to

[11] *Complement*: A number of serum proteins which, upon activitation, cause lysis of antibody bound infected cells or pathogens.

[12] *Cluster of Differentiation (CD)*: Cell surface molecules usually present on leukocytes that are identified using monoclonal antibodies, e.g. CD3 is present on T cells whereas CD19 is present on B cells.

[13] *Histamines*: Derivatives of the amino acid Histidine that are released by mast cells upon exposure to allergens. Binding to their receptors leads to smooth muscle contraction, mucus secretion, etc. Anti-histamines are widely used to counter the effects of allergy.

form IgM–IgG complexes, which get deposited in joints causing inflammation. Antibodies against self molecules are also produced in other autoimmune diseases, e.g., Systemic Lupus Erythromatosus and Graves disease (*Box* 2).

Box 2. A List of Some Autoimmune Disorders

Disorder	Description
Insulin Dependent Diabetes Mellitus (IDDM)	Develops due to autoreactive T cells attacking and destroying insulin secreting beta cells in the pancreas. Eventually, the body's insulin production drops and blood glucose levels rise. The most common therapy used is administration of insulin. This disease affects ~0.2% of the world's population.
Graves' disease	People suffering from this disease produce auto-Ig to the thyroid-stimulating hormone (TSH) receptor. Binding of these Ig to the receptor results in overproduction of thyroid hormones, thyroxine and triiodothyronine, in afflicted individuals.
Myasthenia Gravis	Patients suffering from myasthenia gravis produce auto-Ig, which bind to acetylcholine receptors on muscle cells, and block the binding of acetylcholine. This leads to the inhibition of neurotransmitter signaling and destruction of antibody bound muscle cells due to complement activation. Eventually, there is weakening of skeletal muscles (muscle fatigue) and it is a struggle to perform normal chores, e.g. eating.
Systemic Lupus Erythromatosus (SLE)	A systemic disorder where an autoimmune response is generated against a wide range of antigens and several organs are affected. Affected individuals produce auto-Ig to DNA, histones, etc. and antigen-Ig complexes lead to hemolytic anemia, glomerulone phritis etc. Women are affected ten times more by SLE than men and it develops between the ages of 20 – 40. One of the hallmark features of SLE is the appearance of a "butterfly" rash on the face.
Multiple Sclerosis	It is one of the most common causes of neurologic disability. Autoreactive T cells form lesions along the myelin sheath of nerve fibres leading to numbness and paralysis of limbs.
Rheumatoid arthritis	It is a fairly common autoimmune disease and arises due to production of auto-Ig (mainly IgM) reactive to Fc regions of IgG. IgM-IgG complexes deposit in bone joints and trigger inflammation.

Monoclonal Antibodies: As mentioned previously, serum Ig are polyclonal as they originate from different plasma cells. However, as the original B cell is clonal in origin, it is possible to isolate and propagate such clones using the hybridoma technology developed by G Kohler and C Milstein (Nobel Prize, 1984). The advantage is that B-cell hybrids grow continuously in culture and produce Ig that are of single antigenic specificity, known as monoclonal antibodies. It involves the fusion of antibody-producing plasma cells (Ig secretion) with a non-Ig secreting myeloma cell line (ability to grow continuously in culture). These hybrid cells are selected for growth and secretion of Ig; subsequently, they are further screened and selected for the production of the desired Ig. Monoclonal antibodies are used extensively for research and diagnostics, e.g., development of ELISA kits for detection of human chorionic gonadotropin in the urine of pregnant women, detecting immune cell numbers (e.g., $CD4^+$ T cells in blood) during pathological conditions, e.g., AIDS. In addition, such antibodies are used for therapy, e.g., treatment with an Ig (known as Herceptin) to an oncoprotein (c-erbB-2) is effective against some breast cancers. Also, one of the lines of treatment of B-cell lymphomas is using an Ig to CD20 known as Rituximab.

Serum Ig are polyclonal as they originate from different plasma cells.

Consequences of Reduced or Excess Antibody Production

As seen in the above section, antibodies play several roles and to evaluate their physiological roles, it is useful to study the effects of reduced or increased production of antibodies. One such deficiency disease is Bruton's X-linked agammaglobulinemia (XLA), where a male child is unable to produce Ig (*Box* 3). This defective gene XLA encodes Bruton's tyrosine kinase (Btk) which plays a role in B cell maturation. Due to mutations in Btk, patients possess very low levels of immunoglobulins in blood and suffer from recurrent infections during childhood. Another immunodeficiency condition is 'Common Variable Immunodeficiency' (CVID) in which reduced amounts of antibodies are found. The symptoms observed are similar to XLA but the reasons for this are multiple, due to mutations in different genes involved in antibody production.

Antibodies play several roles and to evaluate their physiological roles, it is useful to study the effects of reduced or increased production of antibodies.

Box 3. Some Disorders due to Mutations or Deficiencies in Key Immune Response Genes

Disorder	Description
Autoimmune polyendocrinopathy – candidiasis – ectodermal dystrophy (APECED)	Results due to mutations in the autoimmune regulator, *AIRE*. Studies on AIRE in mice have revealed that it is highly expressed in thymic medullary epithelial tissue where it regulates expression of peripheral tissue antigens. Individuals suffering from APECED display chronic mucocutaneous candidiasis, Type I diabetes and other autoimmune features.
DiGeorge Syndrome	Characterized by an absence of the thymus and afflicted individuals are immunodeficient, suffer from hypoparathyroidism, congenital heart disease and display characteristic facial abnormalities.
Severe Combined Immunodeficiency (SCID)	It is a broad family of disorders characterized by both B and T cell deficiencies that may develop due to a variety of reasons. The most common cause of SCID is mutation in the common gamma chain of the cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. The second most common reason is Adenosine deaminase (ADA) deficiency, which affects purine metabolism and reduces lymphocyte proliferation. Mutations in RAG affecting VDJ recombination also cause SCID. Most SCID patients suffer from recurrent infections and usually die early in life due to absence of the adaptive immune response.
X-linked hyper IgM syndrome	Characterized by abnormally high levels of IgM and absence of IgA, IgE and IgG. The defect arises due to inability of T-helper cells to express CD40L. Therefore, T-dependent B cell activation and class switching to other antibody isotypes does not occur. Affected individuals suffer recurrent infections especially those associated with the respiratory tract.
X-linked Agammaglobulinemia (XLA)	Mature B cells fail to develop from pre-B cells due to defects in a signal transduction component known as Bruton's Tyrosine Kinase (Btk). Characterized by very low levels of IgG and absence of other isotypes. People suffering from XLA lack peripheral B cells and cannot make Ig. Consequently, they suffer from recurrent bacterial infections.
Bare Lymphocyte Syndrome	Occurs due to low or no surface MHC class I or class II expression. Patients have low numbers of peripheral T lymphocytes and suffer from recurrent infections, especially diarrhoea and candidiasis. This disorder is usually caused due to defects in a transporter (TAP), which is essential for surface expression of MHC class I, or transcription factors, e.g. CIITA, required for MHC class II expression.

In general, plasma cells have a short life-span although some long-lived plasma cells have also been found. Plasma cells have a finite life span; however, in cases when a plasma cell becomes tumorigenic, i.e., keeps on dividing even without antigen stimulation, it gives rise to a condition known as 'multiple myeloma'. Here immortal plasma cells secrete Ig in great excess which are termed as myeloma proteins and constitute about 95% of serum antibodies. In some cases, myeloma cells secrete huge amounts of light chains which are named 'Bence-Jones proteins' (after the name of the physician who made this observation). Interestingly, sequencing of Bence-Jones proteins played a pivotal role in elucidation of the amino acid sequence of immunoglobulin.

The second part of the article will discuss cell-mediated immunity.

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