Chapter 3

The Natural Immune System

The body has many defense mechanisms, which among others are the skin of the body, the membrane that covers the hollow organs and vessels, and the adaptive immune system. The adaptive immune system reacts to a specific foreign body material or pathogenic material (referred to as *antigen*). During these reactions the adaptive immune system adapts to better detect the encountered antigen and a 'memory' is built up of regular encountered antigen. The obtained memory speeds up and improves the reaction of the adaptive immune system to future exposure to the same antigen. Due to this reason defense reactions are divided into three types: non-specific defense reactions, inherited defense reactions and specific defense reactions [127]. The adaptive immune system forms part of the specific defense reactions.

Different theories exist in the study of immunology regarding the functioning and organisational behavior between lymphocytes in response to encountered antigen. These theories include the classical view, clonal selection theory, network theory, and danger theory. Since the clonal selection, danger theory and network theory are based on concepts and elements within the classical view (as discussed in section 3.1), the classical view will first be discussed in detail to form a bases onto which the other three theories will be explained in sections 3.5, 3.6 and 3.7 respectively.

3.1 Classical View

The classical view of the immune system is that the immune system distinguishes between what is normal (*self*) and foreign (*non-self* or antigen) in the body. The recognition of antigens leads to the creation of specialised activated cells, which inactivate or destroy these antigens. The

natural immune system mostly consists of lymphocytes and lymphoid organs. These organs are the tonsils and adenoids, thymus, lymph nodes, spleen, Peyer's patches, appendix, lymphatic vessels, and bone marrow. Lymphoid organs are responsible for the growth, development and deployment of the lymphocytes in the immune system. The lymphocytes are used to detect any antigens in the body. The immune system works on the principle of a pattern recognition system, recognising *non-self* patterns from the *self* patterns [149].

The initial classical view was defined by Burnet [22] as B-Cells and Killer-T-Cells with antigen-specific receptors. Antigens triggered an immune response by interacting with these receptors. This interaction is known as stimulation (or signal 1). It was Bretscher and Cohn [20] who enhanced the initial classical view by introducing the concept of a helper T-Cell (see section 3.3.3). This is known as the *help* signal (or signal 2). In later years, Lafferty and Cunningham added a co-stimulatory signal to the helper T-Cell model of Bretscher and Cohn. Lafferty and Cunningham [120] proposed that the helper T-Cell is co-stimulated with a signal from an antigen-presenting cell (APC). The motivation for the co-stimulated model was that T-Cells in a body had a stronger response to cells from the same species in comparison to cells from different species. Thus, the APC is species specific. Burnet also introduced the theory of *clonal selection* [22].

The rest of this chapter explains the development of the different cell types in the immune system, antigens and antibodies, immune reactions and immunity types and the detection process of foreign body material as defined by the different theories.

3.2 Antibodies and Antigens

Within the natural immune system, antigens are material that can trigger immune response. An immune response is the body's reaction to antigens so that the antigens are eliminated to prevent damage to the body. Antigens can be either bacteria, fungi, parasites and/or viruses [157]. An antigen must be recognised as foreign (*non-self*). Every cell has a huge variety of antigens in its surface membrane. The foreign antigen is mostly present in the cell of micro-organisms and in the cell membrane of 'donor cells'. Donor cells are transplanted blood cells obtained through transplanted organs or blood. The small segments on the surface of an antigen are called *epitopes* and the small segments on antibodies are called *paratopes* (as shown in figure 3.1). Epitopes trigger a specific immune response and antibodies' paratopes bind to these epitopes with a certain binding strength, measured as affinity [127]. Note that the binding between an

epitope and paratope has a complementary match in shape.

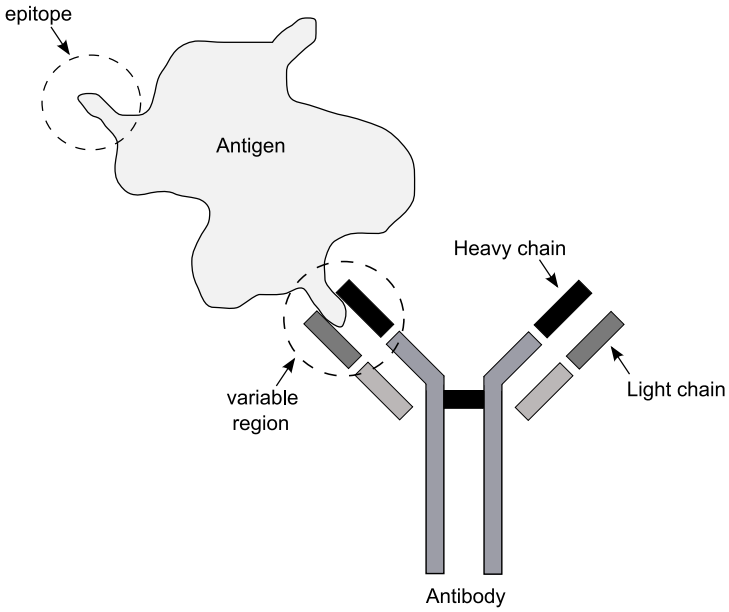

Figure 3.1 Antigen-Antibody-Complex

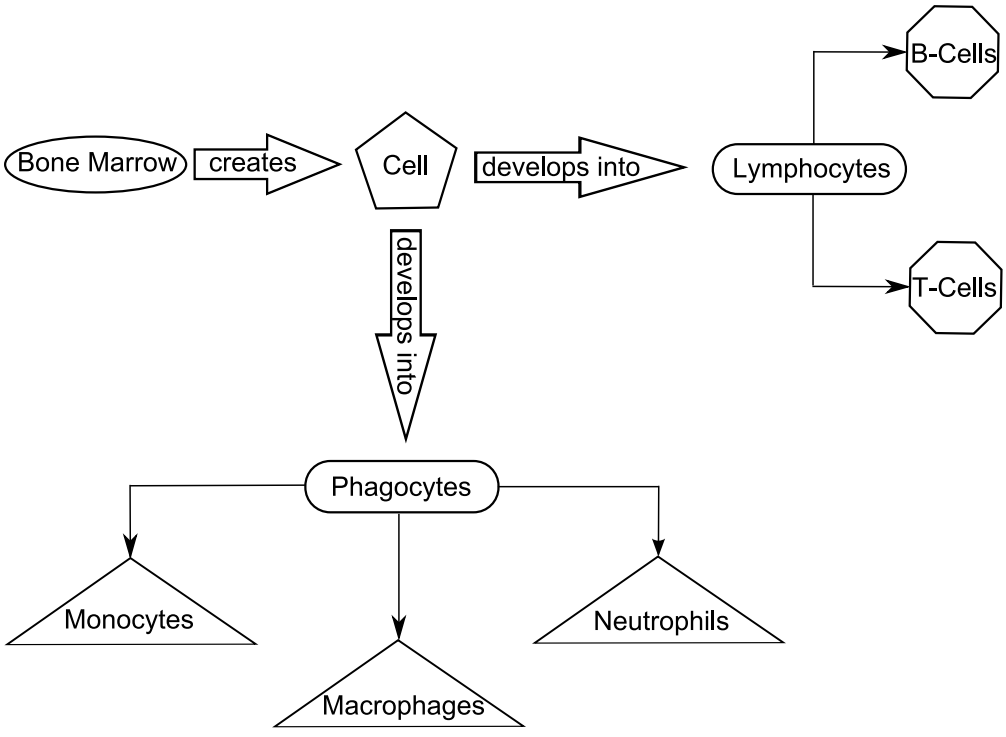

Figure 3.2 White Cell Types

Antibodies are chemical proteins. In contradiction to antigens, antibodies form part of *self* and are produced when lymphocytes encounter antigen (*non-self*). An antibody has a Y-shape (as shown in figure 3.1). Both arms of the Y consist of two identical heavy and two identical light chains. The chains are differentiated as *heavy* and *light* since the heavy chain contains double the number of amino-acids than the light chain. The tips of the arms are called the variable regions and vary from one antibody to another [157]. The variable regions (paratopes) enable the antibody to match antigen and bind to the epitopes of an antigen. After a binding between an antibody and an antigen's epitope, an antigen-antibody-complex is formed, which results into the de-activation of the antigen [127]. There are five classes of antibodies: IgM, IgG, IgA, IgE, IgD [127].

3.3 The White Cells

All cells in the body are created in the bone marrow (as illustrated in figure 3.2). Some of these cells develop into large cell- and particle-devouring white cells known as phagocytes [157]. Phagocytes include monocytes, macrophages and neutrophils. Macrophages are versatile cells that secrete powerful chemicals and play an important role in T-Cell activation. Other cells develop into small white cells known as lymphocytes.

3.3.1 The Lymphocytes

There are two types of lymphocytes: the T-Cell and B-Cell, both created in the bone marrow. On the surface of the T-Cells and B-Cells are receptor molecules that bind to other cells. The T-Cell binds only with molecules that are on the surface of other cells. The T-Cell first becomes mature in the thymus, whereas the B-Cell is already mature after creation in the bone marrow. A T-Cell becomes mature if and only if it does not have receptors that bind with molecules that represent *self* cells. It is therefore very important that the T-Cell can differentiate between *self* and *non-self* cells.

Thus lymphocytes have different states: immature, mature, memory and annihilated (figure 3.3 illustrates the life cycle of lymphocytes). These states are discussed in the subsections to follow below. Both T-Cells and B-Cells secrete lymphokines and macrophages secrete monokines. Monokines and lymphokines are known as cytokines and their function is to encourage cell growth, promote cell activation or destroy target cells [157]. These molecules on the surface of a

cell are named the major histocompatibility complex molecules (MHC-molecules). Their main function is to bring to light the internal structure of a cell. MHC-molecules are grouped into two classes: Type I and Type II. MHC-molecules of Type I is on the surface of any cell and MHC-molecules of Type II mainly on the surface of B-Cells [149]. There are two types of T-Cells: The Helper-T-Cell and Natural-Killer-T-Cell. Each of these types of lymphocytes is described in detail below.

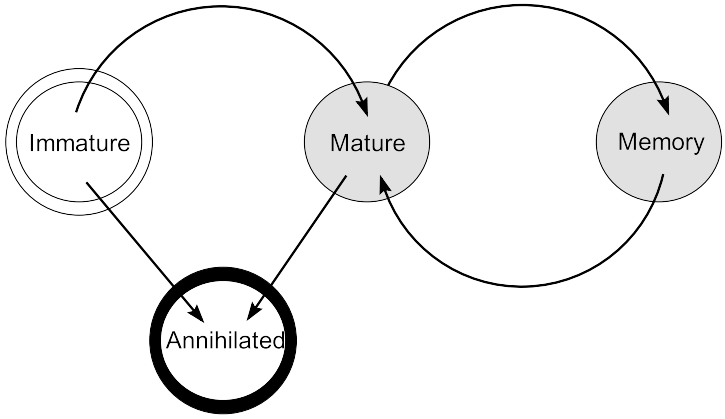

Figure 3.3 Life Cycle of a Lymphocyte

3.3.2 The B-Cell Lymphocyte

B-Cells are created in the bone marrow with monomeric IgM-receptors on their surfaces. A monomeric receptor is a chemical compound that can undergo a chemical reaction with other molecules to form larger molecules. In contrast to T-Cells, B-Cells leave the bone marrow as mature lymphocytes. B-Cells mostly exist in the spleen and tonsils. It is in the spleen and tonsils that the B-Cells develop into plasma cells after the B-Cells are exposed to antigens. After developing into plasma cells, the plasma cells produce antibodies that are effective against antigens [127].

The B-Cell has antigen-specific receptors and recognises in its natural state the antigens. When contact is made between a B-Cell and antigen, clonal proliferation on the B-Cell takes place and is strengthened by Helper-T-Cells (as explained in the next subsection). During clonal proliferation two types of cells are formed: plasma cells and memory cells. The function of memory cells is to proliferate to plasma cells for a faster reaction to frequently encountered antigens and produce antibodies for the antigens. A plasma cell is a B-Cell that produces antibodies.

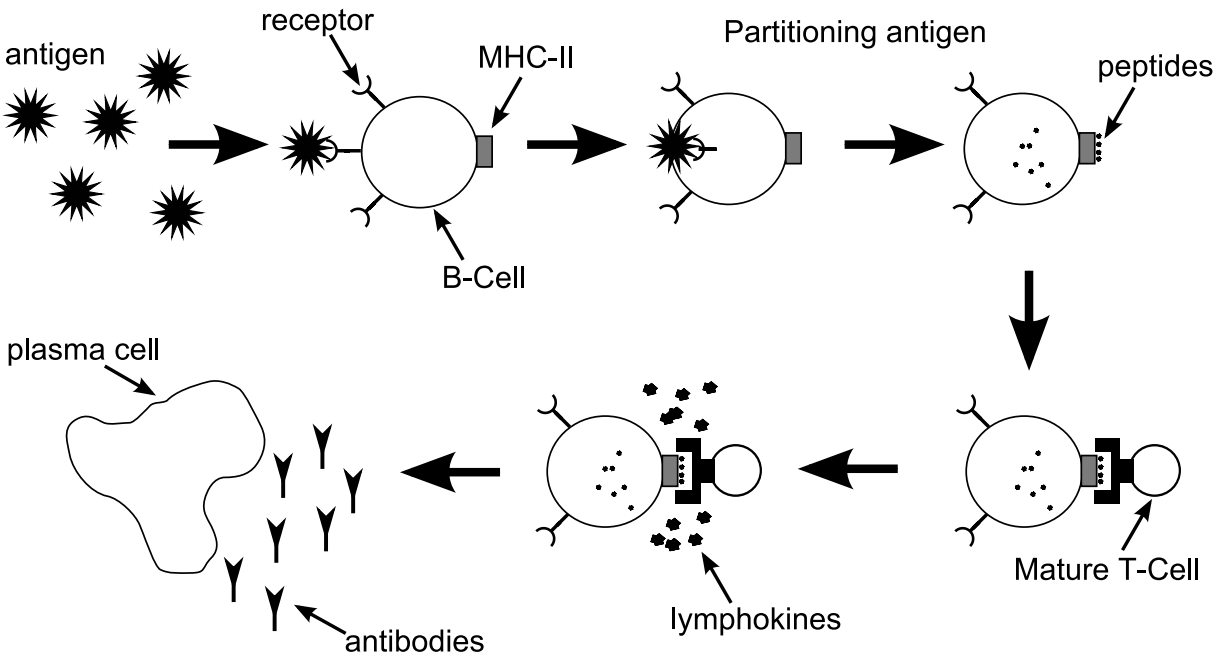

Figure 3.4 B-Cell Develops into Plasma Cell, Producing Antibodies

3.3.3 The Helper-T-Cell (HTC)

When a B-Cell's receptor matches an antigen, the antigen is partitioned into peptides (as shown in figure 3.4). The peptides are then brought to the surface of the B-Cell by an MHC-molecule of Type II. Macrophages also break down antigen and the broken down antigen is brought to the surface of the macrophage by an MHC-molecule of Type II. The HTC binds to the MHC-molecule on the surface of the B-Cell or macrophage and proliferates or suppresses the B-Cell response to the partitioned cell, by secreting lymphokines. This response is known as the primary response. When the HTC bounds to the MHC with a high affinity, the B-Cell is proliferated. The B-Cell then produces antibodies with the same structure or pattern as represented by the peptides. The production of antibodies is done after a *cloning process* of the B-Cell.

When the HTC does not bind with a high affinity, the B-Cell response is suppressed. Affinity is a force that causes the HTC to elect an MHC on the surface of the B-Cell with which the HTC has a stronger binding to unite, rather than with another MHC with a weaker binding. A higher affinity implies a stronger binding between the HTC and MHC. The antibodies then bind to the antigens' epitopes that have the same complementary structure or pattern. Epitopes are the portions on an antigen that are recognised by antibodies. When a B-Cell is proliferated enough, i.e. the B-Cell frequently detects antigens, it goes into a memory status, and when suppressed

frequently, it becomes annihilated and replaced by a newly created B-Cell. The immune system uses the B-Cells with memory status in a secondary response to frequently seen antigens of the same structure. The secondary response is much faster than the primary response, since no HTC signal or binding to the memory B-Cell is necessary for producing antibodies [149].

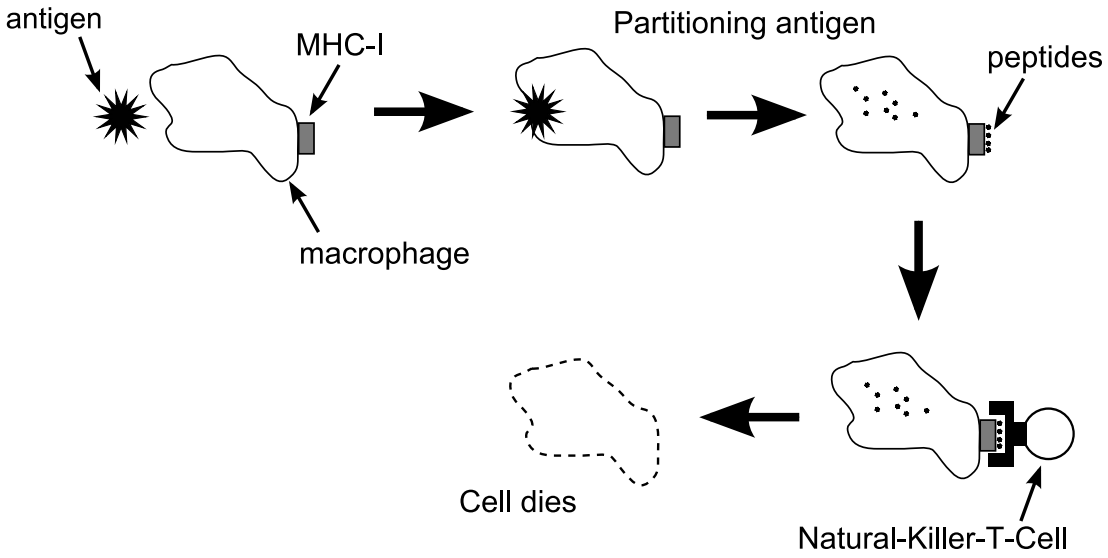

Figure 3.5 Macrophage and NKTC

3.3.4 The Natural-Killer-T-Cell (NKTC)

The NKTC binds to MHC-molecules of Type I (as illustrated in figure 3.5). These MHC-molecules are found on all cells. Their function is to bring to light any viral proteins from a virally infected cell. The NKTC then binds to the MHC-molecule of Type I and destroys not only the virally infected cell but also the NKTC itself [149].

3.4 Immunity Types

Immunity can be obtained either naturally or artificially (as illustrated in figure 3.6). In both cases immunity can be active or passive. Antigens are only encountered in active immunity and activate the immune system. The activated immune system reacts to these antigens by producing memory cells. This implies that memory cells are only produced in active immunity. Although the production of these memory cells in active immunity is much more time consuming compared to passive immunity, active immunity is permanent and passive immunity only temporary.

Passive immunity does however have an immediate reaction to encountered antigen. This section discusses the different types of immunity.

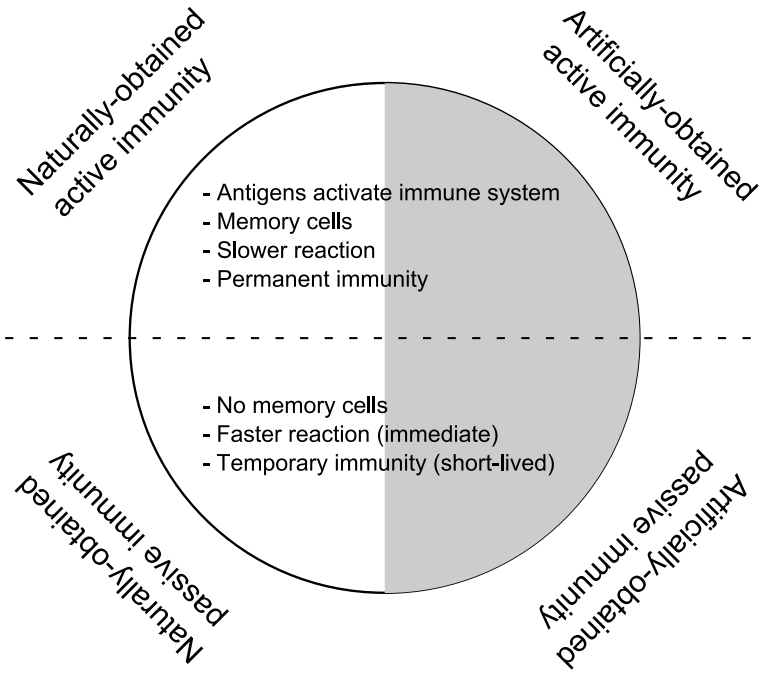

Figure 3.6 Immunity Types

Naturally-obtained active immunity: The immune system of an antigen-infected body reacts to the antigen by producing antibodies. The production of memory cells is an end-result of frequently encountered antigen. Due to memory cells, active naturally-obtained immunity is more or less permanent. This type of immunity can also develop when the body receives foreign red blood cells and actively produces antibodies to deactivate the antigen [127].

Naturally-obtained passive immunity: Naturally-obtained passive immunity is short-lived since antibodies are continuously broken down without creation of new antibodies. New antibodies are not created because the antigens did not activate the *self* immune system. The immunity type develops from IgG-antibodies that are transplanted from the mother to the baby. The secreted IgA-antibodies in mothers-milk are another example of this immunity type and protect the baby from any antigens with which the mother came into contact [127].

Artificially-obtained active immunity: Artificially-obtained active immunity develops when dead organisms or weakened organisms are therapeutically applied. The concept is that special

treated organisms keep their antigens without provoking illness-reactions [127].

Artificially-obtained passive immunity: Artificially-obtained passive immunity is obtained when a specific antibody that was produced by another human or animal, is injected into the body for an emergency treatment. Since the immune system was not activated to generate antibodies and produce memory cells, immunity is short-lived and temporary [127].

3.5 The Process of Affinity Maturation

Learning in the immune system is based on increasing the population size of those lymphocytes that frequently recognise antigens. Learning by the immune system is done by a process known as affinity maturation. As illustrated in figure 3.7, affinity maturation can be broken down into two smaller processes, namely a cloning process (left side of figure 3.7) and a somatic hyper mutation process (right side of figure 3.7). The cloning process is more generally known as *clonal selection*, which is the proliferation of the lymphocytes that recognise the antigens.

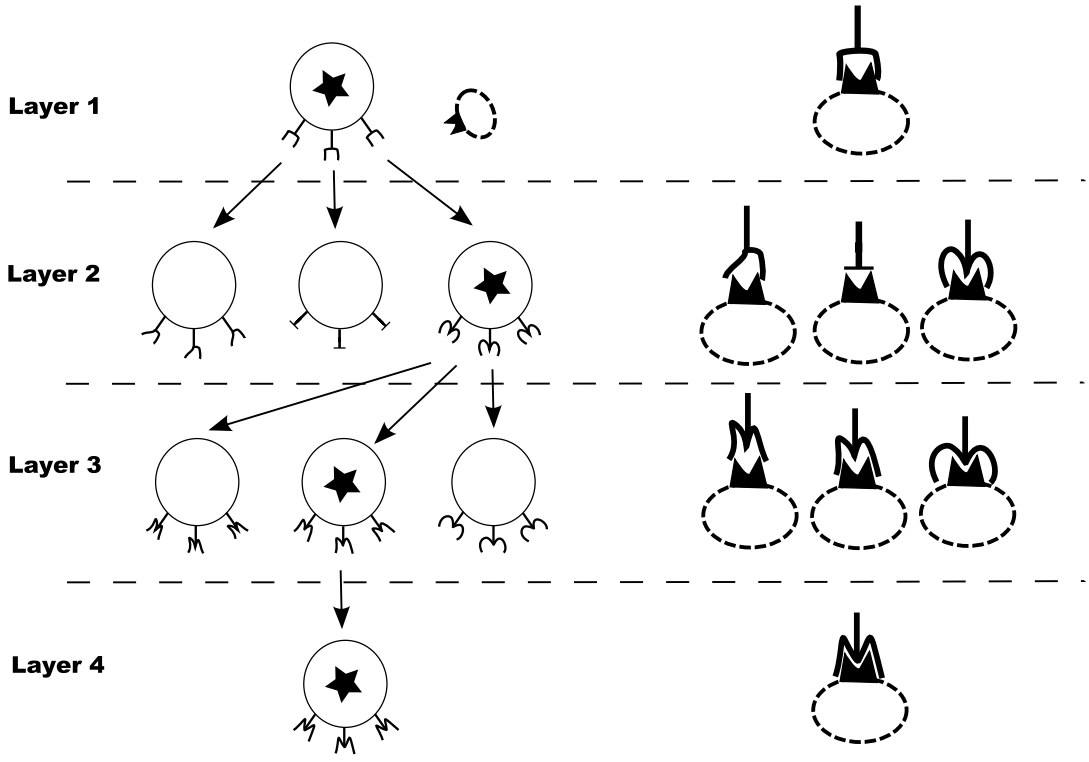

Figure 3.7 Affinity Maturation of Lymphocytes

The interaction of the lymphocyte with an antigen leads to an activation of the lymphocyte where

upon the cell is proliferated and grown into a clone (activated lymphocytes are indicated with a star in figure 3.7). When an antigen stimulates a lymphocyte, the lymphocyte not only secretes antibodies to bind to the antigen but also generates mutated clones of itself in an attempt to have a higher binding affinity with the detected antigen (layer 1 to layer 2, layer 2 to layer 3, etc. in figure 3.7). The latter process is known as somatic hyper mutation. Somatic hyper mutation only occurs in the germinal centers of the different lymphoid organs, which therefore are spatially organised into different zones [123]. Thus, through repetitive exposure to the antigen, the immune system learns and adapts to the shape of the frequently encountered antigen (as illustrated in the right side of figure 3.7) and moves from a random receptor creation (layer 1 in figure 3.7) to a repertoire that represents the antigens more precisely (layer 4 in figure 3.7). Lymphocytes in a clone produce antibodies if it is a B-Cell and secrete growth factors (lymphokines) in the case of an HTC.

Since antigens determine or select the lymphocytes that need to be cloned, the process is called *clonal selection* [127]. The fittest clones are those which produce antibodies that bind to antigen best (with highest affinity). Since the total number of lymphocytes in the immune system is regulated, the increase in size of some clones decreases the size of other clones. This leads to the immune system forgetting previously learned antigens. When a familiar antigen is detected, the immune system responds with larger cloning sizes. This response is referred to as the secondary immune response [149]. Learning is also based on decreasing the population size of those lymphocytes that seldom or never detect any antigens. These lymphocytes are removed from the immune system. For the affinity maturation process to be successful, the receptor molecule repository needs to be as complete and diverse as possible to recognise any foreign shape [149].

3.6 The Network Theory

The network theory was first introduced by Jerne [97, 98] and further developed and formulated by Perelson [148]. The variable region of an antibody can be antigenic and invoke an immune response. Thus, the variable region of an antibody, responsible for binding to an antigen, has an antigenic profile. This antigenic profile is known as the *idiotype* of the antibody. The idiotype of an antibody can invoke an immune response for the creation of anti-idiotypic antibodies by a stimulated B-Cell [98]. As illustrated in figure 3.8, the idiotopic profile of an antibody consists of multiple sites in the variable region of an antibody. These sites are known as *idiotopes*.

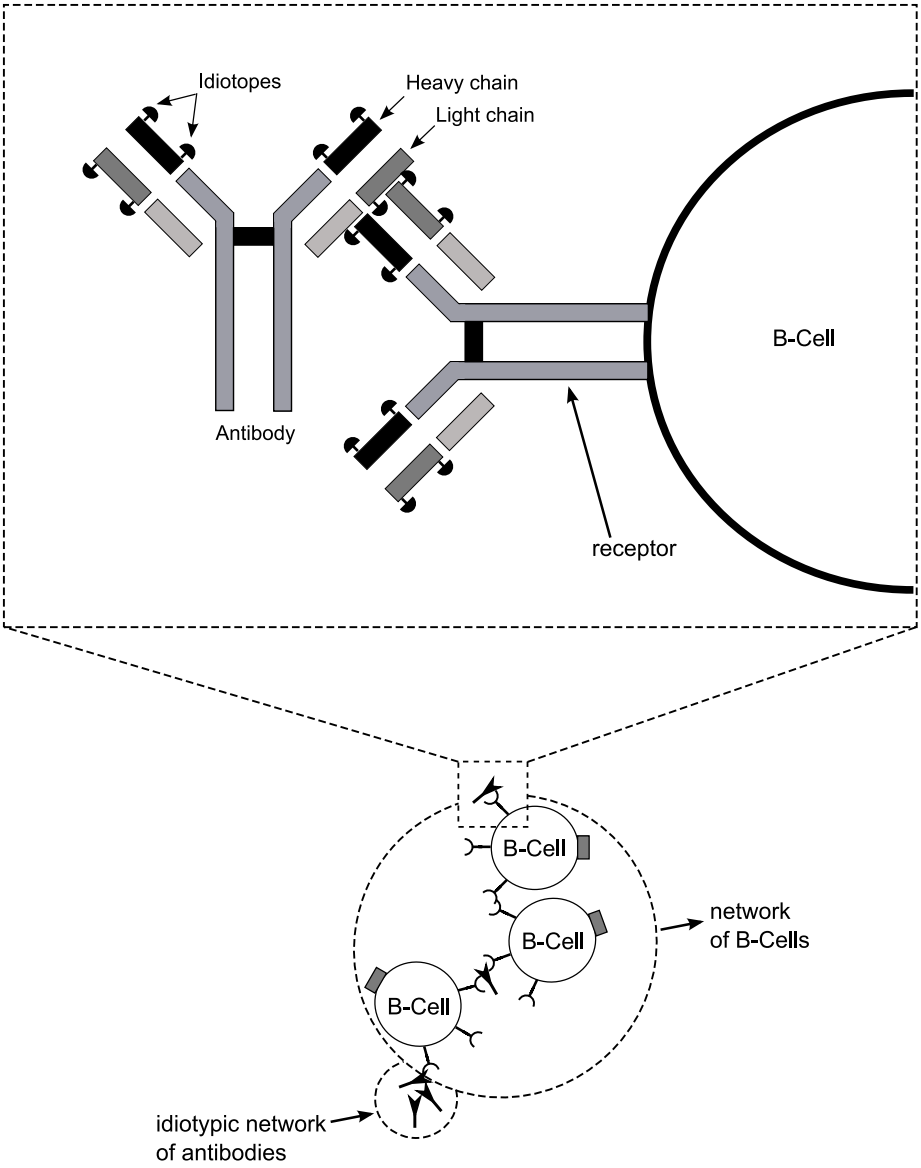

Figure 3.8 Idiotypic Network of Antibodies and B-Cells

In summary, the network theory of antibodies and B-Cells states that B-Cells are interconnected to form an idiotypic network of cells. When a B-Cell in the network responds to a foreign cell, the activated B-Cell stimulates all the other B-Cells to which it is connected in the network. Thus, a lymphocyte is not only stimulated by an antigen, but can also be stimulated or suppressed by neighbouring lymphocytes. That is, when a lymphocyte reacts to the stimulation of an antigen, the secretion of antibodies and generation of mutated clones (as discussed in section 3.5) stimulate the lymphocyte's immediate neighbours, if the neighbouring B-Cells bind to the idiotopes

of the produced antibodies or receptor of the stimulated B-Cell. This implies that a neighbour lymphocyte can then in turn also react to the stimulation of the antigen-stimulated lymphocyte by generating mutated clones, stimulating the immediate group of neighbours [149] (as illustrated in figure 3.8). Therefore, lymphocytes signal (or communicate) each other across spatial distances by means of anti-idiotypic networks.

The interactions or connections between the cells in an idiotypic network determine the resultant architecture or topology of the network. The possible interactions in an idiotypic network can be presented by different network topologies which include but are not limited to the linear topology, the simple cyclic topology, the affinity matrix topology and the Caylee tree topology. These network topologies are discussed in more detail with illustrations in section 4.7.

3.7 The Danger Theory

The danger theory was introduced by Matzinger [124, 125] and is based on the co-stimulated model of Lafferty and Cunningham [120]. The main idea of the *danger theory* is that the immune system distinguishes between what is dangerous and non-dangerous in the body. The *danger theory* differs from the classical view in that the immune system does not respond to all foreign cells, but only to those foreign cells that are harmful or dangerous to the body. A foreign cell is seen to be dangerous to the body if it causes body cells to stress or die. Matzinger gives two motivational reasons for defining the new theory, which is that the immune system needs to adapt to a changing *self* and that the immune system does not always react on *foreign* or *non-self*.

Although cell death is common within the body, the immune system only reacts to those cell deaths that are not normal programmed cell death (apoptosis), i.e. non-apoptotic or necrotic deaths. When a cell is infected by a virus, the cell itself will send out a stress signal (known as signal 0) of necrotic death to activate the antigen presenting cells (APCs) (as illustrated in figure 3.9). Thus, co-stimulation of an APC to a helper T-Cell is only possible if the APC was activated with a *danger* or stress signal. Therefore, the neighbouring cells of an APC determine the APC's state. Hereon the immune reaction process is as discussed within the classical view (see section 3.1), where mature helper T-Cells are now presented with a peptide representation of the antigen and co-stimulated by an activated APC.

The different types of signals from a dying or stressed cell are unknown. According to Matzinger

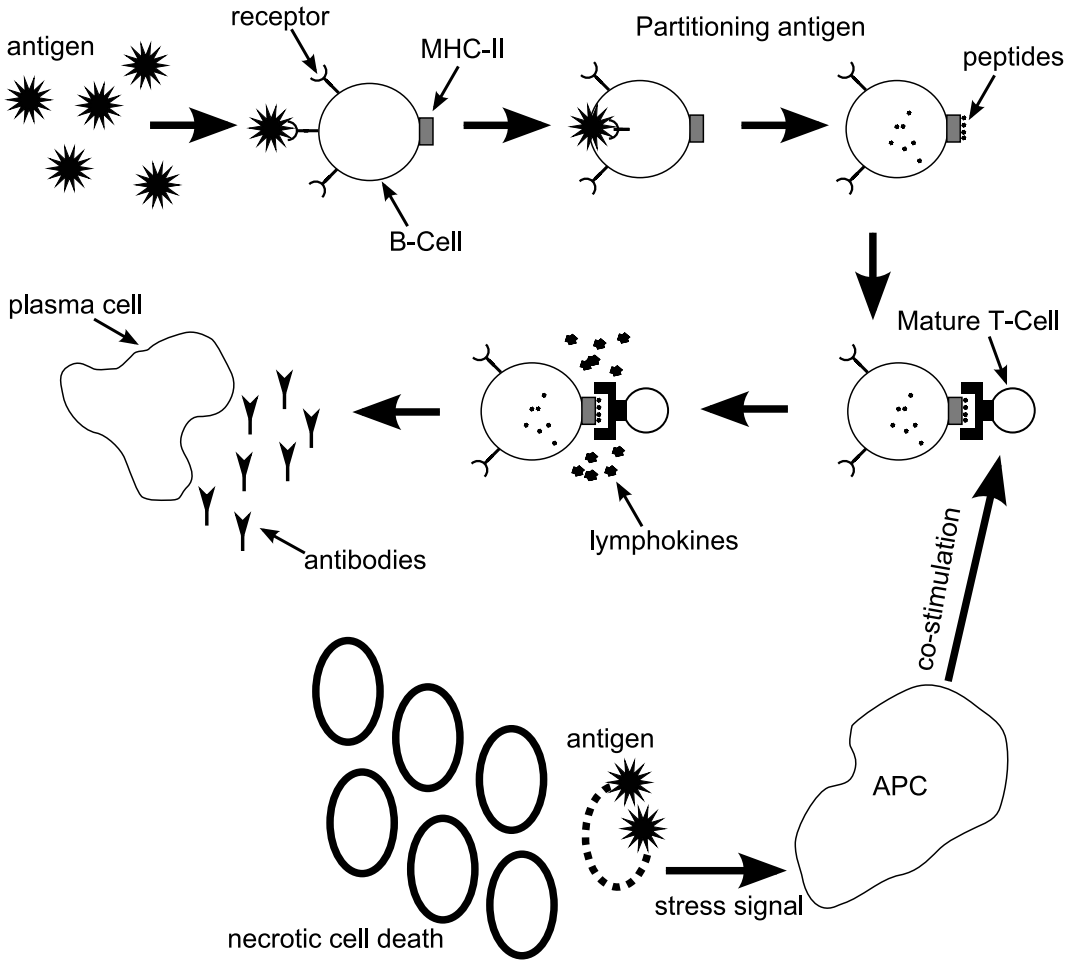

Figure 3.9 Co-Stimulation of T-Cell by an APC

these signals could either be defined as the sensing of a certain protein within a cell that leaked after the cell's death or an unexpected connection lost between connected cells after one of the cells died. Thus, if none of the above signals are fired by a cell, no immune response will be triggered by an antigen to activate the antigen presenting cells (APCs).

Thus, from a *danger* immune system perspective, a T-Cell only needs to be able to differentiate APCs from any other cells. If an APC activated a T-Cell through co-stimulation, then only will the immune system respond with a clonal proliferation of the B-Cell (as discussed in section 3.3.3). The B-Cell will then secrete antibodies to bind with the *dangerous* antigen instead of binding to all foreign harmless antigen.

3.8 The Dendritic Cell System

Dendritic cells were first identified by Banchereau and Steinman [12]. An immature dendritic cell (DC) can be generated when the appropriate cytokines are applied to blood monocytes, but is also developed in the bone marrow. A mature DC is an antigen presenting cell (APC) which initiates an immune response. A difference between immature and mature DCs is that immature DCs have a lower probability to initiate an immune response, and is more specialised in processing encountered antigens, i.e. immature DCs cannot activate T-Cells [12].

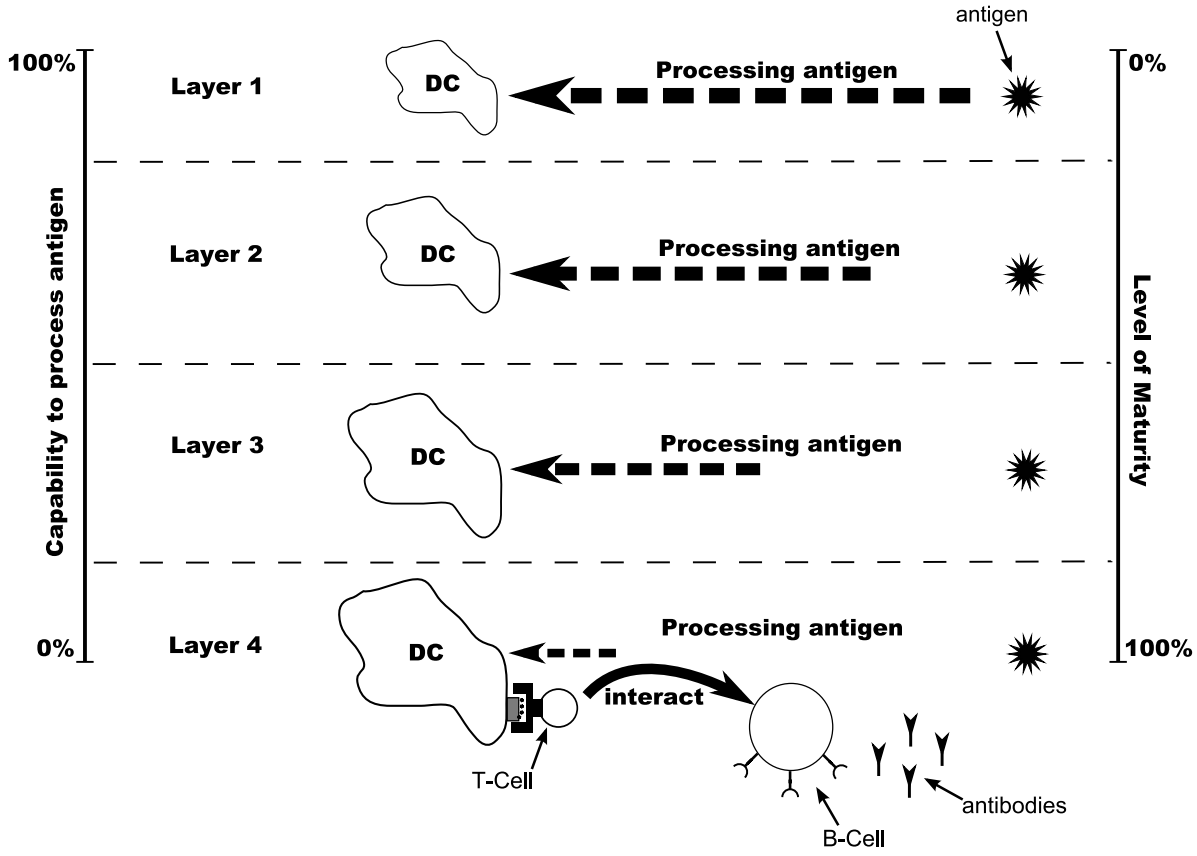

Figure 3.10 Maturation of Dendritic Cells

As illustrated in figure 3.10, initially (layer 1 in figure 3.10), immature DCs process antigen and in time become less capable in processing antigen (as indicated by a shorter dashed arrow in figure 3.10), becoming APCs which stimulate and activate T-Cells. Thus, an immature DC becomes mature by processing encountered antigen (as indicated by an enlarged DC in figure 3.10 at each layer of processing an antigen), which results in the formation of MHC-peptide complexes on the dendritic cell's surface (similar to the antigen presenting B-Cell, as discussed in section 3.3.3).

The MHC-peptide complexes are then presented to an antigen-specific T-Cell (mature T-Cell), co-stimulating and activating the T-Cell and thus initiating an immune response (similar to the APCs as discussed in section 3.7) [163]. The activated T-Cell interacts with B-Cells, which in turn produce antibodies (as discussed in section 3.3.3).

Both the B-Cell and DC are APCs, which are either directly or indirectly responsible for the secretion of antibodies [12]. DCs are responsible for Helper-T-Cell activation, which in turn promotes the proliferation of B-Cells to produce antibodies (as discussed in section 3.5). Thus, DCs *carry* antigenic *information* to lymph nodes where T-Cells reside which can react to the antigen [159].

When an MHC-peptide complex on the surface of a virally infected cell is presented to a Natural Killer T-Cell (as discussed in section 3.3.4), the Natural Killer T-Cell (NKTC) first needs to be activated by a DC before the NKTC can kill the virally infected cell. This is known as *cross-presentation* and enables the DC to initiate an immune response without getting infected by the pathogen [159, 163].

The immune system consists of many different types of dendritic cells (DC) with specialised roles. Follicular DCs (FDCs) directly maintain the growth of stimulated B-Cells [12]. FDCs are found in the lymph nodes. FDCs do not process antigen but capture antigen-antibody complexes which reside on the FDC's surface. FDCs are present in areas of antigen stimulated B-Cells. A stimulated B-Cell proliferates (the process of affinity maturation as explained in section 3.5), and when the B-Cell matches an antibody-antigen complex on the surface of an FDC with a high affinity, processes the antigen and presents the MHC-peptide complex to the T-Cell (as discussed in section 3.3.3). This ensures the survival of stimulated B-Cells with high affinities, while less stimulated B-Cells with lower affinities apoptose (normal programmed cell death) [12].

Since DCs are of crucial importance in the initiation of an immune response, research has been done on vaccines for *tuberculosis* that targets the expansion of DC populations [126]. The increased population of DCs resulted in better T-Cell activation, i.e. amplifying the level of immune activation that led to stable memory formations.

3.9 Conclusion

This chapter introduced the different theories of immunology. These are the classical view, the process of affinity maturation, the network theory, and the danger theory. With reference to the classical view, the co-operation between T-Cell and B-Cell lymphocytes to react to an encountered antigen was discussed. The binding between an antigen and antibody was also briefly discussed. The chapter also gave an overview of the different immunity types. These immunity types can either activate or assist the immune system to react to an encountered antigen. The different immunity types are: naturally-obtained active immunity, naturally-obtained passive immunity, artificially-obtained active immunity and artificially-obtained passive immunity. This chapter was concluded by a brief introduction to the dendritic cell system, with reference to immunology.

The proposed model in this thesis was mainly inspired by the network theory of immunology. The network of lymphocytes learns the structure of an antigen through the process of affinity maturation, where the activated lymphocyte proliferates by generating mutated clones which co-stimulate the immediate neighbours of the activated lymphocyte. The neighbouring lymphocytes in turn could also react and proliferate by generating mutated clones, stimulating immediate neighbours. The network topology of co-stimulated lymphocytes to adapt to the antigen structure inspired the development of the proposed model in this thesis with application to data clustering problems in stationary and non-stationary environments. The proposed model adapts a population of artificial lymphocytes through cloning and mutation operations. Furthermore, co-stimulation between neighbouring artificial lymphocytes is simulated with a pre-defined network topology. Chapter 5 introduces and discusses the proposed model in more detail.

The next chapter discusses some of the most familiar artificial immune system (AIS) models which are inspired by the different theories in the science of immunology.