Software modeling of the Complement System and its role in Immune Response

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Abstract

The complement system refers to a series of proteins circulating in the blood that do the work of complementing antibodies in destroying bacteria. These proteins circulate in an inactive form, but in response to the recognition of the molecular components of the pathogenic micro-organism, they become sequentially active, working in a manner wherein the activation and binding of one protein leads to the activation of the next protein in cascade resulting in the lysis of bacterial cells. There are three pathways of complement activation namely classical, lectin and alternative pathways. The initiation of complement cascade in classical pathway is caused by formation of immune complexes on the surface of the target cell. The alternative and lectin pathways are integral participants of non-specific innate immunity and hence do not require any antibody involvement for activation.

Using software, we have developed a model called SIMISYS version 0.4, which implements the activation of the complement system by the three pathways and demonstrates the lysis of bacterial cells through the common membrane attack pathway. It gives the statistics of the number of complement system components involved and the number of bacterial cells destroyed. The software model illustrates other beneficial immune defense functions carried out by the complement system components, namely chemotactic attraction of phagocytes to the infection site, promotion of opsonization, triggering inflammation and removal of immune complexes from the circulation. Complement deficiencies and disease scenarios involving different kinds of bacteria have been simulated using this model. The performance of each pathway of activation is evaluated individually by running the simulation inputting only the required components and making the necessary changes. The software model simulates thousands of biological entities consisting of human cells, bacterial cells and complement system components. A graphical depiction occurs simultaneously on a small panel of the screen as the simulation proceeds. A console output gets displayed indicating the activated pathway, the complement system components involved in it, the bacteria concentration on the terminal. The software written in C++ language is a modification and enhancement of SIMISYS version 0.3 [6], which models and simulates the basic behavior of the human immune system.

1 The Immune system

Any immune response constitutes recognition of the pathogen or other foreign material and the mounting of a reaction against it to eliminate it. An antigen is recognized as foreign when epitopes of that antigen bind to B cells and T cells by means of epitope-specific receptor molecules whose shapes are complementary to that of the epitope. Immune response can be categorized either as innate immune response or adaptive immune response. The innate immune system is a less specific component of the immune system and forms the first line of defense against infection from foreign micro-organism. Pathogen associated molecular patterns can also be recognized by a series of soluble pattern recognition receptors in the blood that function as opsonins and initiate the complement pathways. Some of the cells involved in an innate immune response include neutrophils, macrophages, mast cells, and basophils. Complement proteins and cytokines are some of the molecules involved.
The adaptive immune system is the more specific component of the immune system and is capable of specifically targeting and eliminating a foreign pathogen. It is capable of remembering the pathogen and can build a fight against the intruder preventing it from causing disease later. Adaptive immune response improves by repeated exposure to a given infection and involves antigen presenting cells such as dendritic cells and macrophages, cytotoxic T cells, helper T cells and B cells and production of molecules like antibodies and cytokines [5]. However, the drawback of the specificity of adaptive immune response is that only a few B cells and T cells in the body recognize any one foreign epitope. These few cells then must rapidly multiply in order to produce enough cells to stage a defensive attack against that particular epitope which usually takes several days. This duration of time lets the pathogen to cause considerable harm and hence innate immunity is very essential as the first line of defense [5].

2 Complement System

The complement system is part of the innate immune system and forms the basis of antibody mediated immunity. Apart from defending against bacterial infection, it has other physiologic activities such as bridging innate and adaptive immunity, causing inflammatory response and disposing off immune complexes. Complement was first identified as a heat sensitive principle found in the blood serum that complemented antibodies in destroying bacteria [5]. There are more than 30 complement proteins in the serum. The ways in which the complement system is activated are known as pathways. There are three pathways of activation namely classical, lectin and alternative pathways. The initiation of the complement cascade in classical pathway is caused by the binding of an antibody to an antigen on the surface of the target cell. The alternative and lectin pathways provide non-specific innate immunity which does not require any antibody [5, 13, 15, 16]. All the pathways of activation produce a key enzyme called $C3$ convertase. $C3$ convertase cleaves $C3$ into $C3a$ and $C3b$ [5, 13, 15, 16]. Normally the larger fragment is designated with a $b$ attached to the component name and the smaller is designated with an $a$ attached. All of these three pathways essentially converge at a particular stage in the cascade and produce a common complex called $C5$ convertase which starts a new set of biochemical reactions ultimately attacking and lysing the target cells. This terminal process is called the membrane attack pathway or the lytic pathway, as it is responsible for lysis [5, 13, 15, 16]. The membrane attack complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane [5, 15, 16].

3 Implementation Overview

The software model has been implemented using object oriented C++ language on Red Hat Linux 9.0. It has a multithreaded architecture based on pthreads [10]. The graphics are displayed using Simple DirectMedia Layer (SDL) version 1.2 [8]. An XML file has been used to hold different types of complement components, keeping in mind that the system will evolve over time. The Iksemel parser [1] has been used to parse this XML file. The resulting software model is able to simulate an effective defense mechanism by the immune system with the aid of the complement system against a bacterial attack. This model explores the different pathways of the complement system and is able to model disease scenarios. The software model is integrated into SIMISYS version 0.3 [6] and is given the title SIMISYS version 0.4. It includes a graph, depicting the results of the simulation being displayed on a small panel on the screen. A console output gets displayed simultaneously indicating the activated pathway, all the complement system objects involved in it, the bacteria concentration, etc., on the terminal. The model allows the user to be able to zoom in and zoom out using the respective keys on the keyboard. There are three different screens, each representing a different region of the human body. These are the main screen representing the tissue, a second screen representing the activities by the lymphnode object and a third screen displaying the activities carried out by the liver object. The T key on the keyboard enables one to toggle from one screen to another. Most operations carried out in this modeling are string based operations. Pattern recognition through substring recognition and matching of the epitope or signature of each object is the main idea used for movement, opsonization and immune complex clearance mechanisms. The software model includes programs, which implement the complement system as a vital part of the immune system. In this model, a three dimensional
Grid acts as a place holder for the biological entities and molecules. Each grid cell or position can have a pointer to a biological entity like a human cell or a bacterial cell and to a complement system object. Each complement system object references the grid through a grid pointer and the grid keeps track of complement system object present within its cells through a cs pointer. A grid pointer is used by each entity to point to the grid position that it is situated in. Using the grid pointer, a cell can check its neighboring grid positions for other entities, or inquire about the chemicals, and antibodies present within its own position. A back end pointer (bePtr), present in each grid cell points to the entity that is in it currently. The class GridWrapper encapsulates a three dimensional area of grid cells. It contains information about a grid along with the memory allocated to it. The GridWrapper class is helpful while simulating more than one grid area. Every entity and complement system object contains a pointer to a GridWrapper class giving them access to the data in the world to which they belong.

4 Software Specifics

The software modeling of the complement system has been performed by modifying and enhancing SIMISYS version 0.3 [6]. The new version, SIMISYS 0.4 has many new classes providing additional functionality. The proteins and glycoproteins, which constitute the complement system, are mainly synthesized by the liver. Hence a class liver has been created. This class is responsible for creation of complement object instances and placing them under the GridWrapper representing tissue. A DOM parser is used to parse the XML file which holds the configuration of different types of complement system components to be created along with the count of each such component as type integer. Instances of class complement object, which are created by the liver object, are placed at different positions randomly in the Grid area representing the tissue. The main functionality of these complement object instances is to inspect for entity. If an entity of type Bacteria is found, based on its status whether antibody-coated or hasMannoseGroups is set to true. If Bacteria membrane hasLPS, the corresponding pathway namely classical, lectin or alternative and the corresponding initial component namely C1 or MBP or C3b gets activated by setting its respective status to ACTIVE. The complement object then appends a string representing the name of current type of component to the cellMembrane attribute of the Bacteria object, which is also a string. This is to indicate the presence of the particular complement system object and thus aiding in the activation of the next type of complement system object in cascade. Then it frees itself from the Grid. Now the complement system object references the Grid through the Bacteria object, so that wherever the Bacteria object moves, the complement system object moves along with it. In all the cases, only if the MAC-FORMED flag of the complement system object is set to true, then the status of Bacteria object is set to DEAD. Some basic methods of class complement system include move(), live(), setStatus(), getType(), cleave(), releaseAnaphylatoxin(), and die().

In SIMISYS 0.4 some new entities have been introduced to enforce the required functionality. These include the KupfferCell, Erythrocyte, Mastcell and EpithelialCell. All of these entities are created using BasicCell as their super class except for KupfferCell which, has been created as a type of Macrophage. This version of SIMISYS still maintains the cellular automata approach of one grid cell can hold one biological cell and one or more identified molecules [6]. Modifications have been made enabling a biological cell or an entity and a complement system object to occupy a single grid cell at any given time. Class erythrocyte has been introduced mainly for the purpose of immune complex clearance [4]. The CR1 receptor is a 4-bit string assigned to each erythrocyte object. It has a value of 1100 which is complement of the C3b receptor value. This CR1 receptor is represented using a character array as shown in Figure 2. If the circulating immune complexes are not cleared, they get deposited in the tissue and cause excessive tissue damage. When an erythrocyte finds a BasicCell object whose status is Antibody-Coated or DEAD or LYSED, it calls the complement() method and tries to find the complement of its CR1 on the cellMembrane of the found BasicCell object. A
The complement of the bit string receptor or signature is found when the 0s of the source signature tallies with the 1s of the destination signature as shown in Figure 3. The complement, the erythrocyte object sets BasicCell objects status to CLEARED, bePtr to NULL, grid pointer of the BasicCell to setOccupied(0) and lastly the gPtr to NULL. This is to make sure the dead Bacteria object does not occupy any Grid place of tissue. It then sets an IC pointe to this BasicCell object, sets its status to CARRIER and move towards the BloodVessel which finds these erythrocyte instances in its immediate vicinity and translocates them to the liver Grid area. The phagocytic Kupffer cells [9] in the liver destroy the immune complexes carried by the erythrocytes.

5 Results

The notations used to label the linear graphs representing different objects are given in Figure 4. Figure 5 illustrates the behavior of the complement system against a simulated bacterial attack through all the pathways. Water is a constituent of the grid.

Because of hydrolysis of C3, it is cleaved to C3b. Some Bacteria instances with hasMannoseGroups flag set to true and many with hasLPS flag set to true are created randomly. As a result both lectin and alternative pathways get activated immediately without any antibodies required. The first reaction to bacterial invasion, in any injury, is a general inflammatory reaction. This can be seen by the number of erythrocyte and Neutrophil instances shooting up to indicate more blood and hence more immune cells around the infected area. This happens after MastCell instances sense a particular concentration of anaphylatoxin and degranulate to produce Histamine which makes the BloodVessel object morePermeable to allow more erythrocyte and neutrophil instances.

Blood vessels in the area of the infection widen to increase the supply of white blood cells that fight infection. The THelper objects get activated and stimulate the BCell objects to produce antibodies. Antibody production and hence the antibody coating of the Bacteria objects activates the classical pathway. All the three pathways are activated. The complement system objects lyses the bacteria cells directly or chemotactically attract phagocytes which destroys the bacteria cells by opsonization. Due to the combined defense mechanism of the immune system and the complement system the Bacteria entity in this situation is more susceptible and hence its graph starts declining as their number drop.

Lectins are proteins that recognize and bind to specific carbohydrate targets. The lectin pathway is activated by binding of mannose binding protein to mannose residues on the surface of microorganisms [1]. The results in Figure 5 demonstrate a situation where only the lectin pathway components namely MBP, MASP1, MASP2, C4, C2, C3, C5, C6, C7, C8 and C9 are present. Since all the bacterial cells do not display mannose residues on their surface, some Bacteria objects are created with hasMannoseGroups flag set to true. As a result the lectin pathway is not so effective in eliminating the Bacteria but the number of Bacteria objects is kept under control by not letting them to multiply rapidly.

Alternative pathway components are the created in the simulation without creating any other component. Figure 7 illustrates the alternative pathway whereby C3b is being loaded assuming that C3 has been cleaved because of hydrolysis. The result it good as the alternative pathway gets activated through...
the initial component \text{C3b}. The \text{hasLPS} flag of the \text{Bacteria} entity triggers this activation. Even though there is a slight increase in the \text{Bacteria} entity concentration initially, later the concentration reduces drastically thus establishing the effective mechanism of the alternative pathway in the lysing of bacterial cells.

Classical pathway is activated by antibody-antigen complexes. Figure 8 depicts the results of only classical pathway being activated. This scenario is created with classical pathway components being loaded into the grid and creating maximum number of \text{Bcell} and \text{THelper} objects specified. The antibodies are loaded into the grid as well and directed against the bacterial cells. This suggests that the probability of all the \text{Bacteria} objects in the simulation getting coated with antibody is more. Hence the result is good as there is a huge drop in the number of \text{Bacteria} instances.

C3 is a key component in the complement system which generates C3b. It is an abundant serum protein. C3b enhances phagocytosis and enables immune complex clearance. The result in Figure 9 shows a situation where C3 is not created in the simulation. The increase in the count of \text{Bacteria} objects is obvious and can be seen in the graph. Patients with C3 deficiency have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC. The majority of patients with C3 deficiency has recurrent bacterial infections and may have immune complex diseases [5, 13, 14].

The graph shown in Figure 10 depicts the secondary complement deficiency in \text{systemic lupus erythematosus} [3, 2] disease scenario. The autoantigens from apoptotic \text{epithelialcell} objects drive the \text{Macrophage} objects to engulf them and present this autoantigenic signature to the \text{Tcell} and \text{Bcell} objects, which get activated, and start proliferating. Hence autoantibodies are produced. This can be seen by the linear graph of antibodies going up even without the involvement of \text{Bacteria} entity. As a result of production of autoantibodies immune complexes are formed with healthy and apoptic epithelialcell objects. Immediately after the immune complexes are formed, the complement system get activated through the classical pathway. Because of excessive consumption of \text{complementsystem} objects the linear graph representing \text{complementsystem} goes on declining, leading to an impaired clearance of immune complexes. This increase in the concentration of immune complexes can be depicted from the graph.

Late complement system components namely C5, C6, C7, C8 and C9 are required for the formation of membrane attack complex and hence the lysis of target cells. Deficiencies of late complement components [12, 7, 11] that constitute the membrane attack complex result in a significant increase in susceptibility to
neisserial infections. The graph in Figure 11 shows an
everse increase in the number of Bacteria objects when concentration of C5 component is reduced to 0. The count of the Bacteria entity reaches its maximum within no time because of no lysing activity by the complementsystem objects. Future work will involve workign with biological models of disease for verification and software implementation on a parallel cluster of computers for greater model and simulation realism.

6 Conclusions

This paper has discussed the implementation of a software system called SIMISYS 0.4 that adds a detailed software model of the Complement System to an existing software model of the immune system. The current system models the three complement activation pathways. It is also able to model disease scenarios where the complement system plays significant role.

References