A Review of the Clonal Selection Theory of Acquired Immunity

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***Abstract*-The clonal selection theory proposed by Nobel Prize winning immunologist Frank Macfarlane Burnet is a deceptively simple algorithmic process that describes the diversity of antibodies and is the foundation for the understanding of the acquired immune system of vertebrates. This paper provides a summary overview of the theory including its inception, main processes, and some interesting theoretical aspects.**

Keywords- Clonal Selection Theory, Clonal Selection Principle, Clonal Selection Algorithms, Artificial Immune System

I. INTRODUCTION

Cziko [19] on clonal selection theory:

"[…] *the immune system does not attempt to predict the antibody structure that will bind with an antigen, but rather uses a type of 'shotgun' approach that sends in a diverse army to meet the invaders*"

The above quote provides an apt general description of the acquired immune systems approach at defending the host organism from pathogens. It took nearly 100 years to define and verify a theory that accounted for the diversity of antibodies. Silverstein [5] suggests that like Darwin's theory of evolution, much of the specifics of the theory may have been out dated given scientific progress, although this is no reason to abandon the core principles, rather it emphasises the need to embrace the continually updated version of the theory.

This work provides a high-level survey of the development and core principles of the clonal selection theory of acquired immunity. This discussion is limited to the discussion of the clonal selection of B-lymphocytes and humoral immunity given that the development of the theory and much of the experimental and theoretical work on clonal selection has been completed in this context. Section II summarizes the clonal selection theory including the theories development (II.A), and a brief of the theory itself (II.B.) Section III discusses the selection aspect of the theory, and section IV discusses diversity mechanisms both in the initial repertoire of cells and in the on-going adaptation of the immune response. Section V summarizes some aspects and findings of theoretical models on the theory and finally section VI suggests additional aspects of the theory although not covered in this work. A glossary of relevant immunological terminology is also provided.

II. CLONAL SELECTION THEORY

"*Don't let conflicting and awkward 'facts' stand in the way of an esthetically satisfying theory whose fundamentals are consistent with the world model and with one another! And be suspicious of 'facts' that seem in the way of any coherent theory.*" Lederberg [21], pg 181.

A. *Theory Development*

Ada and Nossal [39] provide a detailed summary of the theories development and related confirmatory experiments, as does Silverstein [5]. See Forsdyke [9] for an account on the development of the theory highlighting the contributions of Ehrlich. See [20] for Lederberg's comment on clonal selection and 'elective theories', and [21] for his account on his part in the history of the development of the theory. Cziko [19] provides an interesting "selectionist's" account on the development of the theory, equating the theory and its development to Darwin's theory of naturals selection to which it bears a strong resemblance on both accounts.

1. Constructionist **'side chain theory'** (Ehrlich)
2. Instructionist **'antigen template theory'** (Pauling)
3. Selectionist **'natural selection theory'** (Jerne)
4. Accepted **'clonal selection theory'** (Burnet, Talmage, Jerne)
5. Seminal confirmatory experiments (Tonegawa, Nossal, Lederberg, many others)

Figure 1 - Terse overview of some theories and their contributors

Paul Ehrlich observed the explosive (exponential) generation of antibodies triggered by the primary exposure of antigen in blood. He proposed a 'side chain theory' (circa 1900) that blood cells naturally made side chains (receptors) capable of binding to all antigens. This constructionist theory proposed that the information and capability to detect all antigen was already possessed by the host (in the genome) and expressed by the antibodies. This model was ultimately rejected because it was demonstrated that antibodies could be produced in response to man made substances - something of which the antibodies had no prior knowledge. Further, the theory could not explain the improvement of the specificity of the response in the secondary and subsequent exposures to the same antigen.

The 'antigen-template theory' was proposed to suggest that antigen direct the response. In fact, this instructional theory proposed that the antibodies used the antigen as a template or model to which they modified themselves to provide a more effective complement. The emerging field of genetics suggested the invalidity of the theory given that specificity of an antibody could not be changed after its creation – that the process of gene to protein is one-way.

Niles Jerne [33] had great difficulty with template theory, listing a number of observations to which the theory could not account. The template theory proposed a one-to-one relationship between antibodies and antigen, although the experimental observations suggested that far more antibodies were produced than antigen in the primary response. Further, the template theory suggested the antibodies role ended after neutralizing the antigen, and observations indicated that antibodies have a short life span, thus failing to account for observed immunological memory – the improved specificity of the secondary response.

1. Secondary stimulus with the same antigen provokes a more active production of antibody than the primary stimulus
2. The change in character of antibody produced in response to repeated exposure to the same antigen. More avid antibody towards the antigen.
3. Exponential rise in the number of circulating antibody after the first exposure to antigen.
4. Continued production of antibody for long periods after exposure to antigen.
5. Surface of the antigen plays a dominant role in the specificity of antibody.

Figure 2 - The problems with 'template theory' outlined by Jerne [33]

Jerne proposed an alternative called the 'natural selection hypotheses' of antibody diversity. His proposed that the host contains a small pre-existing collection of randomly generated antibodies to start the process. In generating this pre-existing pool, self-antibodies are somehow neutralised before causing harm to the host. Antigen 'naturally select' antibodies of high affinity from which more antibodies of the same affinity are produced. The result of the antigen-antibody selection mechanism is the production of a lot more antibodies specific to the antigen, which presented an improved response of the host to the antigen. Interestingly he made the offhand suggestion that copying errors during the production of antibodies may result in an improved fit with the antigen.

In his work on antibodies and allergy, David Talmage [10] extended upon Jerne's natural selection hypothesis suggesting that Jerne's theory is an extension of Ehrlich's side-chain theory, and that replicating cells (not antibodies) were the main feature of the immune response. He suggested the idea that immune cells must specialise in producing antibody with the same specificity.

Frank Macfarlane Burnet [17] also extended Jerne's theory at the same time (and subsequently received priority) focusing on the "clone" perspective and the self-replicating cellular mechanisms during the immune response. He called his theory the 'clonal selection hypothesis' of antibody diversity. The missing link for Burnet was the pre-existing naive antibody population. Antigen select the best matching cells resulting in their replication. He suggested that each immune cell produces antibody with only one type of the same receptor. His theory attributed the exponential rise in the number of antibodies after infection to the exponential rise in the number of antibody producing cells due to clonal expansion of selected cells. It also explained the improved secondary response given the specialisation of receptors during expansion, although the mechanisms of receptor specialisation were only speculated, they were confirmed 20 years later.

Talmage [11] followed up on the theory providing a more detailed account, as did Burnet who later released a book on his theory [18]. Burnet continued his work on the study of immunological tolerance, to which his theory supported, and with Peter Medawar won the Nobel Prize in 1960 for work that provided the foundation for viable tissue and organ transplant. It is also interesting to note that it was Burnet who conceptualized the 'self nonself' abstraction of the immune system in his work on immunological tolerance [14], which like his clonal selection theory has remained a foundational concept of modern immunology.

Nossal and Lederberg [37] supported the one-cell-one-antibody assertion of the clonal selection theory providing first test and confirmatory evidence for the theory. As mentioned, Burnet speculated at the mechanisms for antibody diversification and specialisation in his theory. His prediction was confirmed approximately 20 years later by Tonegawa et al. [36,48] who investigated the fine tuning of antibody receptors called somatic hypermutation (B-cell differentiation) for which he later won the Nobel prize.

B. *Theory Summary*

For a treatment of the clonal selection theory of acquired immunity see any book on immunology, for example [15] and [38], Rajewsky [26] also provides a contemporary summary of the theory.

An antigen is a molecule which can elicit an immune response, an antibody (immunoglobulin) is a molecule which can bind to and neutralise an antigen and a B lymphocyte is a cell which can bind to antigen as well as producing antibodies. The physical surface of an antigen has a number of different structural features. An antibody and B cell have receptors which have a specificity (ability to bind) to only one surface feature of an antigen, although the feature an antibody binds to may represented on more than one different antigen.

A host posses a base pool of randomly generated[1] lymphocytes, which defines the hosts natural immunity. These cells are prepared during the embryonic stage of development. During this time, the immune system learns a tolerance for the tissues of the host organism and those lymphocytes that are self-reactive are removed from the pool (killed).

The repertoire of natural lymphocytes possesses the ability to bind to any antigenic stimulus (natural or synthetic) with some affinity. The introduction of an

[1] Although with an evolutionary bias

antigen triggers an immune response. The lymphocytes with the highest affinity for the antigen bind to the antigen triggering an immune response. Thus it is the features of the antigen that select the best matching lymphocyte from the hosts repertoire.

After a lymphocyte binds to an antigen it moves to lymphatic tissue (germinal centre) to begin the differentiation process. The function of this process is preferential proliferation to replicate a clone of cells to produce antibody capable of neutralising the triggering antigen. The lymphocyte proliferates and differentiates into two different cell types; plasma cells that continue to replicate and which release large numbers of antibody, and memory cells that like the parent cell act as a catalyst for the immune response.

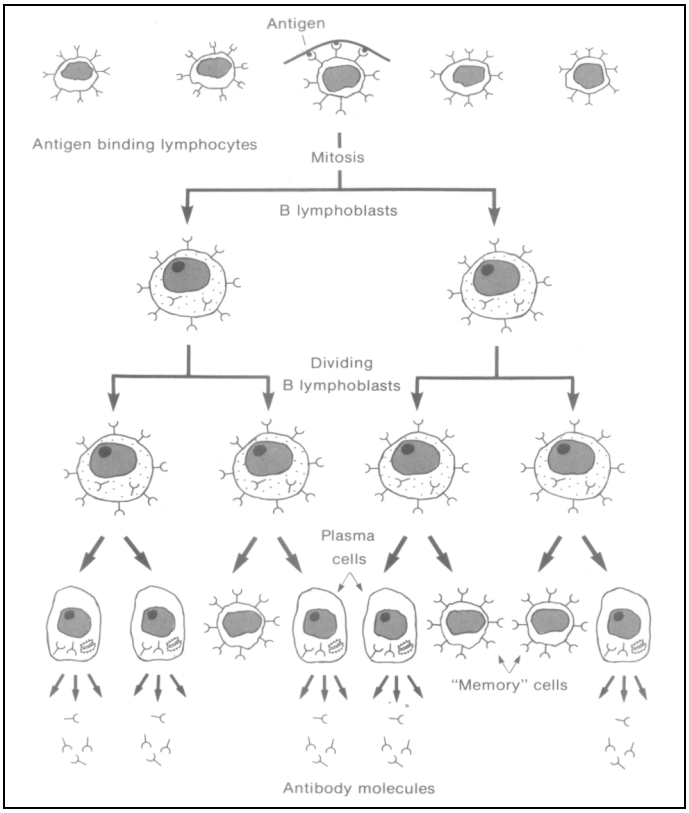

Figure 3 - Overview of the lymphocyte differentiation process of the clonal selection theory, taken from [23]

During the proliferation and differentiation process copying errors (called somatic mutations) may occur, although unlike the genetic mutations that occur in typical cell divisions (mitosis), the probability of mutation is much higher. The result is a clone of lymphocytes that have receptors similar to the parent cell, although vary slightly in their specificity to the triggering antigen. Thus, on subsequent exposures to the antigen, the host possess a larger repertoire of lymphocytes and antibodies capable of neutralising the antigen. Given the somatic mutations introduced during replication, some of the lymphocytes will contain an improved affinity for the antigen, thus improving their chance of being selected.

An antibody producing lymphocyte will only produce antibodies of the same affinity. This means that after the cell has formed (after mitosis) it may be considered an antibody factory where all antibodies will have the exact same affinity for an antigen surface feature. The fact that a surface feature may be represented by more than one antigen provides the basis for vaccinations. This provides a generalization capability where lymphocytes can be raised on one harmless antigen and be effective other more dangerous antigens that posses the same structures. The memory cells generated during the differentiation process may live for months and years which is much longer than typical lymphocytes that lives for hours or days, thus providing long term memory of the learned antigenic defence.

1. Small random (general specificity) initial repertoire
2. Negative selection of self-reactive lymphocytes in early embryo
3. Selection of best matching lymphocyte by antigen
4. Migration of lymphocyte to lymph tissue
5. Lymphocyte Differentiation
   a. Proliferation into plasma cells that produce lots of antibody
   b. Proliferation into memory cells (similar function to parent)
   c. Production of a small amount of antibody
6. Copying errors during proliferation modify the specificity of the cells receptors (and their antibody) to the triggering antigen, some possess improvements. Rate of mutation during this proliferation process is high relative to typical mitosis.

Figure 4 - Summary of the clonal selection hypothesis of antibody diversity

III. Selection

The selection of lymphocytes by antigen based on their affinity specifies an implicit negative selection of those lymphocytes that do not bind with the antigen. Put another way, lymphocytes suffer from cell death, although those that are selected by antigen are given an opportunity to proliferate and live on through their differentiated progeny.

Darden and Cain's [30] discussion the selectionist archetype for addressing adaptive problems in a philosophical biology context including Darwin's natural selection theory of evolution, clonal selection theory and selection theories of higher brain functions. They provide a general selectionist framework and demonstrate how each of the three cases fit in terms of the preconditions, interactions, effects, and long range effects. Cziko [19] also provides a selectionist account of the clonal selection theory, describing the production of antibody as a Darwinian microcosm, and an example of ontogenetic (life long learning) adaptation to supplement the adaptation provided by neo-Darwinian evolution.

IV. Lymphocyte Diversity Mechanisms

The genetics and chemistry of antibody diversity is beyond the scope of this work. This section provides a listing of the mechanisms that lead to the diversity of the B lymphocyte repertoire. These mechanisms are as follows:

Somatic mutation – also known as 'somatic hypermutation' (SHM), and 'somatic hyperpointmutation'. Along with genetic recombination, it was the first discovery made regarding the genetic component of antibody diversity [36,45,47]. It refers to

the point mutations of the genome that occur during the clonal expansion phase (when the cell moves to the germinal centre for differentiation [6,7]). The mutations are targeted at the genes that code for the receptors and occur at a rate about one million times higher than the mutation rates in other genes. Mutations include substitutions, additions, and deletions of genetic code. Specific sequences (motifs) within the receptor genes are targeted with high frequency, referred to as hot spots.

Genetic recombination – called 'secondary V(D)J rearrangements' refers to the recombination of the genetic code during the development of B lymphocyte cells [36,39,47,48]. This genetic recombination process accounts for the initial diversity of the lymphocyte repertoire and in the creation of new (naïve) B lymphocyte cells.

Receptor editing – refers to the genetic recombination of lymphocytes that are auto-reactive [27,31]. Cells that remain auto-reactive after this recombination are eliminated from the repertoire, in what is termed molecular selection of receptors. This likely occurs before somatic mutation, and in response to contact with antigen (similar to template theory). It has also been proposed that receptor editing may also provide an additional general diversity mechanism for so-called 'long jumps' in receptor affinity [3].

Class switching – also called 'class switch recombination' (CSR) [50,53] is the genetic recombination that results in antibodies changing their effectors (becoming different immunoglobulin types) although maintaining the same specificity for antigen.

Gene conversion – (GC) a genetic recombination and the main mechanism for diversification in chickens and other species rather than somatic mutation [28,53].

V. Some Models and Observations

There are many mathematical models of the clonal selection theory and related mechanisms, the discussion of which is too great for this work. Instead, the reader is directed to Perelson and Weisbuch [2], which provides a physicists review of the field of theoretical immunology. This section provides a summary of a few of the many interesting observations in that work.

The size of an organism defines the number of lymphocytes it may possess. The following provides a quantitative example of B-lymphocytes from a typical mammal – the mouse.

- Each B-cell has about 10^4 to 10^5 identical receptors on its surface
- The 'potential repertoire' of receptors that can be generated given the genetic basis is about 10^{11}
- The total number of lymphocytes is about 10^8 at any one time
- The expressed repertoire is about 10^7, meaning that there are on average 10 lymphocytes of a given specificity
- A lower limit on the potential number of antigen must recognise is in the order of 10^{16}

Some additional notes about clonal selection theory:

- Burnets non-mathematical theory of antibody diversity can be considered an algorithmic procedure
- Given the diversity of the expressed lymphocyte repertoire, whether a receptor detects an antigen can be viewed as a random event
- Learning in the immune system involves raising the population size of lymphocytes that have proven themselves valuable by detection antigen
- Learning involves biasing the expressed repertoire from random towards one that reflects the actual antigenic environment
- Increasing one clone, means other clones may have to decrease, although the total number of lymphocytes is not constant (not fixed resources, rather an upper bound). Clonal expansion results in some combination of the following effects
  1. Forget previously learned antigens
  2. Increase overall repertoire size
  3. Decrease randomly generated repertoire for detecting novel antigen

The aspects of an antigen that a lymphocyte can detect can be considered in aggregate and abstracted as an n-dimensional space or volume in which receptors exist as points within this space with a hyper-sphere of influence determined by their specificity. This formalism is called the 'shape space theory' and provides a way to model the generalized shape of a receptor-binding region of lymphocytes, and more interestingly the completeness of a lymphocyte repertoire. The theory predicts the probability that a given receptor will recognise a random antigen determinant with an affinity above a threshold as 10^{-5}. Given the predicted probability, for a repertoire to be complete the following three hypotheses must be satisfied:

1. Each receptor can recognise a set of different but related epitopes
2. The repertoire size is of the order 10^6 or greater
3. The receptors in the repertoire have shapes that are randomly distributed throughout the shape space

The first and second hypotheses have been confirmed by experimentation and observation. The third hypothesis is confirmed in a weak form given that it is expected that evolution has biased the genetic basis for receptor construction towards regions of the shape space that are more important to cover than others.

There are a number of differential equation models of B lymphocyte repertoires and models of the differentiation process that are accurate with regard to experimental observation. A characteristic of some of these 'affinity selection' models is that the number of memory cells and plasma cells are equal, and the number of cells created in an immunological response is proportional to the 'receptor occupancy' (affinity). There

are 'affinity maturation' models of somatic hypermutation that model the process as hill climbing (specifically an uphill walk on random landscape) with local optima. Some of these models show convergence properties that match observations and mutation schemes that turn on and off over time (variable mutation rates) to ensure selected receptors are not mutated out of existence. That is periods of mutation-free growth punctuated by periods in which there are busts of high mutation rates that attempt to improve receptor affinity for the stimulating antigen.

Finally, it is useful to mention some related wok on what is referred to as 'original antigenic sin' [49] (and observed in humans [44]). It refers to the concept of the immune system committing itself or specialising to a receptor such as on a virus, then having the virus mutate and change its surface epitopes. The affect is that the previously high affinity lymphocytes and antibody are now low affinity within the context of the mutated virus. Given the clonal selection and expansion of lymphocytes in the first exposure, the now low affinity receptors dominate the subsequent exposures, effectively out-competing other naïve lymphocytes that would be able to adapt to the new virus. This theory suggests the potential danger in a clonal selection strategy.

VI. Conclusion

There are many interesting aspects of the clonal selection theory that were not discussed that are more than likely to pertain to the development of a clonal selection algorithm, some of these areas include:

- Related and descendant theories such as the Jerne's 'idiotypic network theory' [34,35] and Matzinger's 'danger theory' [8,40-42].
- T lymphocytes are also subjected to clonal selection [16] and play a role in cell-mediated immunity (detection and neutralising of infected cells). Helper T cells generally express a surface marker called CD4, thus they also referred to as $CD4^+$ T cells. They play a role in triggering the differentiation process of B cells [32].
- The homing and migration of lymphocytes around the vascular and lymphatic systems to improve the chance of a cell encountering a specific antigen [1,13,51,52].
- The evolutionary (and other) bias in the generation of the initial repertoire of B lymphocytes [22,46], and
- The lifecycle and behaviour of memory B lymphocytes [4,12,24,25,43].

Glossary

A quick-reference glossary of immunological terms. See de Castro and Timmis [29] for a more complete and detailed glossary of immunological terminology suitable for an engineer or computer scientist.

Term	Summary Definition
Affinity	A measures of the quality of binding between an epitope and a paratope. The stronger the binding, the higher the affinity.
Affinity Maturation	The immune system equivalent of the Darwinian phrases of 'natural selection' and 'survival of the fittest' refers to the selective improvement of the affinity of B-cells.
Antibody	A 'Y' shaped protein. The two ends of the why contain a paratope that has specificity for one epitope. Can be free-float in solution (IgG, IgM), or attached to the surface of a B-cell (IgM, IgD). There are five types of antibody in mammals.
Antigen	A foreign molecule that can elicit an immune response
Auto-reactive	Refers to a receptor that has an affinity for self-molecules, that is cells of the hosts own tissues.
B-cell	A type of lymphocyte responsible for producing antibodies to combat antigen. They have an immunoglobulin attached to their surface (what is referred to as a B-cell receptor).
Clone	A group of genetically identical cells
Epitope	Part of an antigen that is recognised by antibodies, B-cells, and T-cells. Abstractly can be considered the three-dimensional surface features of an antigen
Helper T-cell	CD4+ (helper) T-cell (Helper T cells are the main group of lymphocytes that express the CD4 protein).
Immunoglobulin (Ig)	See antibody
Lymphocyte	A type of white blood cell, which includes B-cells, T-cells, and natural killer cells
Memory cell	A specialised B lymphocyte cell that is formed after a primary infection by an antigen. Typically very few are produced in the differentiation process compared to plasma and effector B cells. May persist for years or a hosts lifetime.
Paratope	Sequence of amino acids on a B cell (and T cell) that binds to a part of an antigens epitope. Area that undergoes mutations during somatic hypermutation.
Plasma cell	Large lymphocyte cells that releases large amounts of antibodies, created after a B-cell has been stimulated a helper T-cell. Secrets about 10,000 antibodies per second, typically living for days or weeks.
Receptor	Cell surface molecule that binds to particular proteins
Somatic Hypermutation	A process that occurs during B cell proliferation and differentiation in which areas of the genome that define the receptors expressed on B cells and their antibodies is genetically varied.
Specificity	The selective reactivity of an expressed receptor
T-cell	A type of lymphocyte, which includes (among others) CD4 + (helper) cells and CD8 + (cytotoxic) cells that kill virus, infected cells

Table 1 – Glossary of immunological terms when discussing clonal selection

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