Did immune system antibody diversity evolve?

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Neo-Darwinism claims that mutations coupled with natural selection have evolved various immune systems existing in life today from simple to the very complex system in humans. However, the various adaptive immune systems in the animal kingdom are all equally complex, yet with major discontinuities between them. It is improbable that one evolved into another. All multicellular organisms have an elaborate innate immune system, and many animals have one of the two known arms of the adaptive system that produces enormous antibody diversity to destroy specific pathogens. Although very different, all known immune systems are highly effective in dealing with pathogens. No evidence exists for the evolution of the immune system, which appears to be irreducibly complex.

The immune system is one of the most well characterized, yet complex biochemical systems in the animal body, but researchers still have much to learn about its design and how it functions.[1,2] Two basic types of immune systems exist, *innate* and *adaptive*.

Innate immunity is a germline encoded system found in all multicellular plants and animals, including humans.[3] This system's genes are expressed without major modification, and its receptors can recognize specific features of most pathogens, allowing it to respond to pathogens it has never encountered before.[3]

The adaptive immune system consists of two arms. Cell-mediated immunity against intracellular pathogens is carried out by white blood cells called T lymphocytes while humoral immunity against extracellular pathogens and toxins is mediated by antibody-producing B lymphocytes. The humoral adaptive immune system is able to produce enormous antibody diversity and 'adapt' to fight specific pathogens. Humoral adaptive immunity, found only in more advanced life forms, is the topic of this paper.

Affinity maturation and somatic hypermutation

The first step required for adaptive humoral immune defense involves B cells expressing membrane-bound antigen receptors (antibodies; figure 1) with a variety of specificities. When a foreign antigen enters the body that 'fits' with an existing antibody, the resultant antibody-antigen interaction causes the antigen-specific B cell to proliferate, a process termed clonal expansion. As the immune response progresses, the closeness of the fit improves. This process is called affinity maturation. An antigen is exposed to millions of antibodies, but usually only a few have a sufficient affinity to trigger a primary immune response. The antibodies produced in a primary response interact with the antigen following a normal distribution curve with low to high affinity binding. The majority of the antibodies will be of intermediate affinity. The higher the affinity of the antibody, the better it will bind with the antigen.[4]

The V(D)J somatic recombination system

During B cell development in the bone marrow and prior to antigen encounter, the three major gene families (V for variable, D for diversity, and J for joining) that make up the variable region of the immunoglobulin molecule (see figure 1) undergo somatic recombination (figure 2).[4]

Each of these gene families form a composite gene comprised of a unique combination of randomly selected V, (D) and J gene segments. Thus, V_L and J_L gene segments can join in any combination in the light chain, and V_H, D_H, J_H gene segments can join in any combination in the heavy chain. Variable region recombination occurs primarily in three steps: 1) 'looping out', 2) excision and 3) ligation and is mediated by recombinases, exonucleases and DNA ligases or nucleotidyl transferases.

Somatic recombination is controlled by two enzymes, RAG1 and RAG2 (recombination-activating genes), discussed later. Only one gene segment from each of the V, D and J types is used. If there are 200 different types of V_H gene segments, 20 of D_H and 4 of J_H, then the total

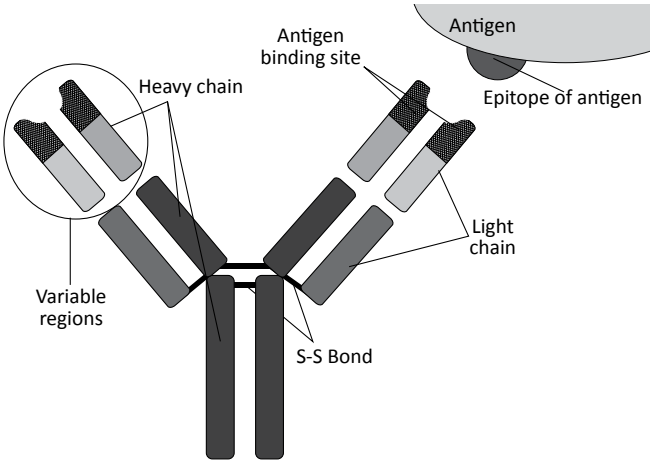

Figure 1. Basic structure of an antibody. Each antibody binds to a specific antigen in a lock and key fashion.

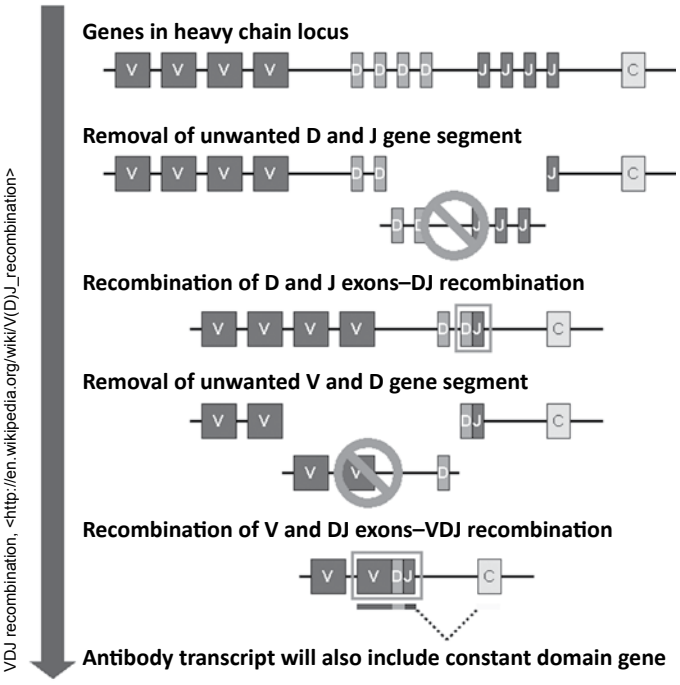

Figure 2. Representation of V(D)J recombination process of immunoglobulin heavy chains. Both heavy and light chains of human antibody molecules (and B cell receptors) contain both *variable* (V) and *constant* (C) regions. Three gene families make-up the variable region: Variable (V) genes, Diversity (D) genes and functional Joining (J) genes.

combination of the these segments alone produces 200 x 20 x 4 =16,000. For V_L and J_L another 1,500 possible K chains would result (300 x 5 = 1,500). All of these combinations are estimated to produce over 24 million different antibodies that recognize the large majority of significant antigens.[5]

Normal genetic recombination is very accurate, but a special design in the V(D)J system deliberately produces additional genetic diversity in the immunoglobulin molecule within constrained limits. The V(D)J recombination process is imprecise and some nucleotides can be lost from the ends of a segment, and up to a half dozen can be randomly inserted by the enzyme *terminal deoxynucleotide transferase*. Further antibody diversity arises because the mechanism for joining the variable region segments allows up to four-base flexibility at the V/D and D/J junctions. This feature is called *junctional flexibility*. Together, V(D)J recombination with its imprecise joining, junctional flexibility and the random combinations of heavy and light chain variable regions, as well as somatic hypermutation after antigen encounter, can give rise to over 10^{11} different antibody specificities in humans.[6] The V(D)J recombination system is remarkably similar in almost all vertebrates.

In addition, somatic hypermutation occurs throughout the V region of the antibody, but is not completely random. Mutational 'hotspots' exist in the hypervariable regions. Mutations that occur in the hypervariable regions (or CDRs) of the antibody affect binding to its antigen, and only B cells with improved 'fit' will be selected for clonal expansion. Antibodies that have lost affinity to the antigen are balanced by those that have increased affinity. As a result, only cells producing antibodies that have a higher affinity for the antigen are selected when new antigens are introduced, because those B cells that respond less well are unlikely to be cloned and those that do respond will be more likely to be cloned.[7] One antigen-specific B cell clone can produce a whole series of different subclones, most of which are eliminated, so that the majority of remaining clones will have an increased affinity for an antigen.

The RAG1 and RAG2 enzyme system

The two major enzymes used in the V(D)J recombination system in all jawed vertebrates are products of the recombination activating genes RAG1 and RAG2.[8] RAG proteins cleave recombination substrate sequences, producing blunt signal ends that can be joined to form precise signal joints and imprecise coding sequence joints which contribute to the antigen receptor diversity.[1,9]

RAG proteins are currently speculated to have evolved from a transposase enzyme used by the mobile genetic machinery known as the 'transposition' system.[10,11] Transposons are 'discrete mobile DNA segments that have been found in virtually every genome examined.'[10] Janeway *et al.* noted this theory of the origin of antibody diversity postulates that 'Invasion of a putative immunoglobulin-like gene by a transposable genetic element … conferred on the ancestral gene the ability to undergo somatic gene rearrangement, and thus to generate antibody diversity.'[12]

The evidence for the evolution of the RAG1 and RAG2 system from the transposase system is based solely on the classic homology argument applied to the molecular level. The closest homologue to the RAG genes are found in the sea-urchin genome.[13] The fact that a section of the RAG1 enzyme that cleaves the DNA between a signal sequence and the adjacent coding sequence is similar to transposases encoded by transposon genes[14] is not direct evidence that the RAG1 enzyme evolved from it. Actually, only an approximately 600 amino acid core region of RAG1 required for its enzyme function is statistically significantly similar to the *Transib* transposase.[15]

An alternate explanation for this homology is that many similarities exist between the V(D)J recombination process and mechanisms used by both the transposition and the retrovirus systems to shuffle their DNA around. In all three cases, the cleavage produces a blunt signal end and, also, a coding end that contains a closed hair pin structure. Therefore the chemical mechanisms used in some transposition systems and in the retroviral integration system can be assumed to be very similar to that used in the V(D)J recombination system.

Many similarities exist in the biochemistry of the components of these systems that allow them to do very similar things—they both shuffle DNA segments around. So it is not surprising that they exhibit similar biochemistry. We would expect systems that shuffle DNA around would have many similarities regardless of what DNA segments

are moved around. Enzymes that have similar functions usually have similar shapes, and shape is determined by amino acid sequence. No doubt as other similar systems are studied, more similarities and differences will be found.

The supposition that the RAG1 protein and the antigen receptor genes evolved from an ancestral transposon is 'speculation'.[16] Litman *et al.*, in the most detailed summary of immune phylogeny noted the *only* evidence is the similarity of a key section of the transposase enzyme and, for this reason, used the terms 'probably' and 'might have' five times in the first page of their review, and scattered similar terms throughout their review.[13]

Proving evolution by biochemical homology is flawed due to the same problems that exist in proving evolution by morphological homology. Biochemical homology is better explained by similarity of function. For example, 'Significant similarities exist in the catalytic amino acids of *Hermes* transposase, the V(D)J recombinase RAG, and retroviral integrase superfamily transposases' because similar steps are required for both V(D)J recombination and mobile genetic element recombination.[17]

A study by Kapitonov and Jurka found evidence that the catalytic core region of RAG1 is 'significantly similar' to the *Transib* superfamily in genomes of animals that include hydra, soybean rust, silkworm, dog hookworm, yellow fever mosquito and sea urchin.[18] A plausible evolutionary scenorio needs to explain *how* the proteins evolved in the first place and why the significant differences that exist arose. Differences include the chemistry of the reactions involved in the RAG1 and RAG2 system, and also in the system Van Gent *et al.* studied, the MUA-IN family of transposons.[19]

Another study by Zhou *et al.* on the insect *hAT* elements identified similarities in the mechanisms of the Hermes transposase and the RAG and retroviral integrase superfamily transposases.[17] However, Kapitonov and Jurka conclude that there exists 'no significant sequence identity between *hAT* TPases and RAG1', and therefore it is very improbable that the RAG system evolved from a *hAT* transposon.[20]

The assumption that the viral system evolved first, and therefore the V(D)J immune system evolved from the retroviral integrase system, is also problematic because viruses are believed to have evolved from cells! Furthermore, similarity of structure alone is not evidence that one evolved from the other, or that they have common ancestors. Many structurally similar proteins have two or more very different unrelated functions that could never have evolved one from the other. Therefore, they are postulated to have evolved by convergent evolution, a concept used to attempt to 'explain' their close similarity within a Darwinian framework.

This 'explanation' does not support the Darwinian worldview. Very similar enzymes are involved in the very different roles of metabolism and transcription regulation, but this observation does not prove that a metabolic enzyme evolved from a transcription enzyme or vice versa.[21] Many very similar proteins have very different functions in the body and no one has claimed that one evolved from the other. In addition, a single gene can be involved

> '… at several points in development to carry out similar functions but in different contexts; for example, the genes coding for the NF-AT factors intervene in both the immune response and in the development of the heart valves, and some memory-formation genes also control the storage of sugars in the organism.'[21]

Irreducible complexity

The immune mechanisms for generating antibody diversity do not provide evidence of Darwinian evolution, but rather support the concept of irreducible complexity and thus intelligent design.[22,23] Mill's reasoning is that the antibody formation system is 'much too complex to be accounted for by a simple transposase gene transfer from a virus or bacterium to a vertebrate organism.'[24] He concludes that

> '… the system of antibody formation clearly qualifies as "irreducibly complex" as defined by Behe: i.e., "a simple system composed of several well-matched interacting parts that contribute to the basic function wherein the removal of any one of the parts causes the system to effectively cease functioning." The interacting parts in this case would be the many immunoglobulin gene segments, the recognition factors, and the enzymes required for translocation of the different gene segments. In addition, they would necessarily include mechanisms for formation of the immunoglobulin surface receptor, which is critical to the production of adequate amounts of antibodies.'[24]

Evidence that adaptive immunity is an irreducibly complex system is fairly straightforward in humans. This is demonstrated by immune disorders, such as AIDS (loss of function in Helper T-cells), X-linked agammaglobulinemia (XLA; a deficiency in the enzymes BRIGHT and BTK, which leads to an inability to produce the level of protective immunoglobulins required—affected people develop repeated infections), and auto-immune diseases (a failure to differentiate self from non-self). All these examples support the conclusion that the failure of a single vital part of the system leads to catastrophic failure of the entire system, which is a primary demonstration of irreducible complexity.

Immune system diversity and evolution

It 'has been known for at least 50 years that … adaptive immunity appears abruptly in the cartilaginous fish.'[25] Each experimental and conceptual breakthrough has found that even in the 'primitive' jawed vertebrates the immune system is very similar to that system used in 'advanced' mammals.[26] Furthermore, 'All jawed vertebrates, beginning with cartilaginous fish, rearrange their V(D)J gene segments to

assemble complete genes for the antigen receptors expressed by T and B lymphocytes.'[27]

Jawless fish

It was once believed that all 'jawed fish can mount an adaptive immune response', but jawless vertebrates, such as hagfish and lampreys, 'lack all signs of an adaptive immune system. They do not have organized lymphoid tissue, they lack primary immune responses, and most importantly, they do not exhibit immunological memory.'[25] As Mestel wrote:

> 'Sharks mark a great divide in the evolution of immunity: before them, not a trace of antibodies or other pivotal immune proteins; after them, all elements are in place. … In groups that evolved before them, scientists have found no trace of either antibodies or three other pivotal immune proteins: T-cell receptors (TCRs), MHC proteins, and RAG proteins. Yet all four proteins are present in the shark, as well as in bony fishes, amphibians, reptiles, birds, and mammals. This sudden appearance is anything but scientifically satisfying. "Nobody wants to believe that the immune response started in its complete form in sharks … . But it's been very difficult to find evidence to the contrary."'[28]

Recent genetic research has found that a 'universe of novel and dynamic immune mechanisms exists among the invertebrates.'[29] Jawless fish, such as lampreys and hag fish, *can* generate an adaptive immune response comparable to antibodies in terms of function. Although jawless fish are considered primitive and 'ancient', their 'very different' system also uses a very complex mechanism to achieve receptor diversity.

They lack all the hallmarks of the adaptive immune system in jawed vertebrates: immunoglobulin, T-cell receptors, the recombination activating genes RAG1 and RAG2 for V(D)J rearrangement and MHC class I and II molecules.[30] Nevertheless, the jawless fish immune system functions by a 'presently undetermined mechanism' that appears to be as complex as the vertebrate immune system.[31] The system used, though, is so different from that used in jawed vertebrates that jawless fish are postulated to have a completely separate evolutionary history. Pancer and Cooper concluded that about '500 mya two types of recombinatorial adaptive immune systems appeared in vertebrates.'[32]

Jawless fish use completely unrelated genes known as *variable lymphocyte receptors* (VLRs) to generate an immune response.[31] The jawless fish system uses VLRs that consist of leucine-rich repeat (LRR) modules which produce somatic diversification by a multistep assembly of LRR modules 'randomly selected from a large bank of flanking cassettes'.[33] The researchers estimate that 10^{14} unique receptors are possible in this system.

This newly discovered system is of little help in determining the *origin* of the vertebrate immune system. The 'two radically different systems' are believed to have evolved separately in agnathans and gnathostomes in a relatively short time after the Cambrian explosion.[34]

Sea urchin and lancelet immune systems

The recent sequencing of the echinoderm sea urchin genome has found an 'unexpected sophistication' in this algae-eating life form, once labelled 'primitive'. Its immune system is actually more complex than the vertebrate system.[35] Even in the Nematode worm *legans*, the innate immune system is 'well developed'—but very different to that found in vertebrates.[1]

As many as 5% of the sea urchin genes are involved in immune functions. Many of these genes are very similar to their corresponding vertebrate genes. These toll-like receptor genes may even achieve gene diversification in a way similar to the adaptive system by recombination, deletion, gene conversion and meiotic shuffling of alleles followed by unequal crossovers.[36] This research has reduced the 'boundaries between germline-encoded innate receptors and the somatically variable adaptive immune receptors of vertebrates' because its 'simple' innate immune system, we now realize, is far more complex than previously thought.[36] The sea urchin even has interleukin and tumour necrosis factor genes previously known only in chordates.

The simplest immune system, the innate system in a lancelet, has recently been discovered to be much more sophisticated and 'modern' then previously believed. The study found that the lancet innate system 'produces a key immune system protein that is similar to, but much hardier than, the version found in people.' They live in a marine environment teeming with bacterial and viral pathogens and chemical threats, yet are extremely adept at defending themselves. The most primitive immune system known has been found to 'share genes and proteins remarkably similar to ours.'[37]

Conclusion

The voluminous research on the evolution of the adaptive immune system describes in enormous detail both the similarities and differences between the immune systems of a wide variety of animals, but does not provide evidence for the evolution of these irreducibility complex systems. The complex, designed processes used to produce antibody diversity and then to fine tune the adaptive immune response are not evidence of Darwinian evolution, but rather of intelligent design.

Recent work has also shown that innate immune systems formerly thought to be very primitive are far more complex than once believed, blurring 'traditional distinctions between adaptive and innate immunity.'[38] Various phyla use 'a remarkably extensive variety of solutions to meet fundamentally similar requirements for host protection.'[37] The large discontinuity between the various means of generating immune system diversity in the animal kingdom makes it highly unlikely that one system could have evolved into another.

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