Review paper

Convergent evolution of acquired immunity

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Abstract

An acquired immunity is connected with clonal proliferation of antigen-specific immune cells leading to the formation of memory cells responsible for efficient response to successive encounter of the same antigen but not to an unrelated one. Recently it turned out that such ability emerged independently at least three times in the course of evolution of the animal kingdom. 1) An acquired immunity of human and other jawed vertebrates (fish, amphibians, reptiles, birds, and mammals) is based on lymphocytes, each of them with particular set of TCR or BCR antigen receptors, which vast diversity (up to 10^{18}) is dependent on somatic DNA rearrangement of limited numbers v(d)j genes belonging to immunoglobulin superfamily (IgSF). 2) Only in 2004-2006 it was evidenced that an acquired immunity exists also in jawless vertebrates (hagfish and lampreys), but evolved independently as vast diversity (10^{14}) of their VLR lymphocyte receptors is connected with somatic DNA rearrangement of leucine-rich repeats (LRRs), unrelated to IgSF. 3) Quite recently (2005-2007) it was evidenced that the fruit fly, Drosophila melanogaster, is able to survive the lethal dose of injected pathogen after preceding "vaccination" with the sublethal dose of the same pathogen, but not the unrelated one. Variability of IgSF-type Dscam receptors (Down's syndrome cell adhesion molecule) (up to 18,000 isoforms) on insect immunocytes is created by alternative splicing. In conclusion, recent achievements of evolutionary immunobiology indicate that an acquire immunity emerged by convergence from various elements of pre-exiting innate immune systems of particular taxonomic groups.

Key words: *antigen receptor diversity, immune memory, immune specificity, jawed vertebrates (gnathostomes), jawless vertebrates (agnathans), Drosophila, Dscam molecules, evolution of immunity.*

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Introduction

Our immune system consists off the innate and acquired defence mechanisms. The former refers to various cellular and humoral components we are born with thus they are ready for efficient protection already after the first encounter of antigens. The latter refers to highly specific responses performed by the lymphocytes and their products, antibodies, which are preceded by the time-consuming clonal lymphocyte proliferation and maturation to memory cells and effector cells. For this reason the lymphocyte-mediated immunity is inefficient after the first encounter of the antigen but improves with repeated exposure to infection resulting in gradual adaptation to microbe-rich environment, thus lymphocyte-mediated immunity is traditionally called and adaptive immunity. However, from the evolutionary point of view, both components of the immune system, an innate and acquired immunity, have an adaptive value [1]. Both of them participate in the maintenance of homeostasis when threatened by external pathogens and other molecules recognised as non-self, i.e. they enable an individual to survive in and adapt to a microbe-rich environment. Therefore the term "the adaptive immunity" applies for the both components of immune response which are intimately linked. It is worth however to consider the acquired immunity as a separate entity added to pre-exited innate immunity [1]. The hall-mark of acquired immunity is a clonal selection of antigen-specific cells and the increase in memory cells after priming what makes the subsequent response faster and more efficient. To fulfil these requirements the vast diversity of anticipatory antigen receptors is necessary.

The processes underlining an acquired immunity are considered as evolutionary traits added to the pre-existing innate immune system. For decades it was believed that it

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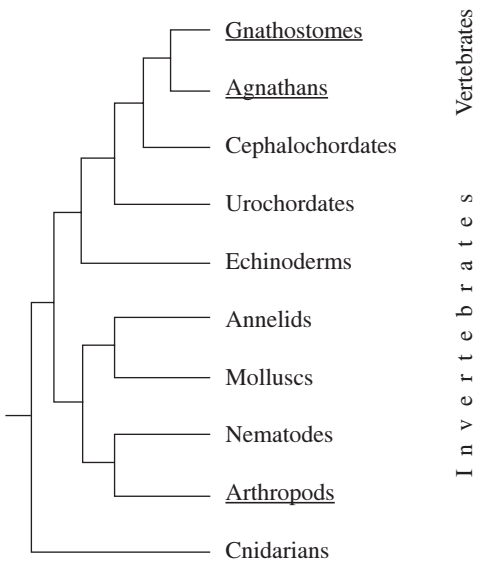

Fig. 1. Simplified phylogenetic tree of metazoans; an acquired immunity emerged independently by convergent evolution in various animals as evidenced in underlined taxons. Description in the text

has happened only once, in the ancestors of vertebrates with jaws (gnathostomes) (for a review see [2]). In the last decade the presence of aquired immunity has been documented also in vertebrates without jaws (jawless vertebrates, agnathans) (for a review see [3]) while in the last years an aquired immunity has been shown convincingly in the representative of arthropod invertebrates, namely in the insect, the fruit fly, *Drosophila melanogaster* (see [4, 5]) (Figure 1).

The gnathostome acquired immune system

The principal elements of acquired immune system of jawed vertebrates (gnathostomes, i.e. fish, amphibians, reptilians, birds, and mammals) are T- and B-lymphocytes. Progenitors of these cells rearrange different sets of variable (V), diversity (D) and joining (J) gene segments to generate the vast diversity (up to 10^{18}) antigen binding regions of membrane-bound TCR or BCR receptors, respectively, and antibodies (Ig) being a soluble form of the latter. The membrane bound BCRs and secreted antibodies recognise exposed determinants of intact antigens, while TCRs as a rule recognise peptide fragments of antigens presented by other cells within cell surface molecules encoded by the major histocompatibility complex (MHC) class I and class II genes. All of these key molecules are members of immunoglobulin superfamily (IgSF). Immunobiologists have concentrated phylogenetic exploration on the identification of genes for TCR or BCR/Ig lymphocyte receptors, RAG-1 and RAG-2 genes (recombination- activating genes whose products initiate the somatic V(D)J rearrangement), and MHC class I and class II genes. All of these genes have been identified in every gnathostome species that has been carefully examined. It turned out that the same basic construction of the acquired immune system is conserved throughout the gnathostome radiations. An apparent lack of a full constellation of IgSF family genes and molecules mentioned here in the animals from other phylogenetic groups has led to conclusion that an aquired immunity based on IgSF genes is restricted exclusively to contemporary jawed vertebrates, i.e. in fish, amphibians, reptilians, birds, and mammals. This suggested that the phylogenetic roots of our acquired immune system based on somatic diversification of V(D)J genes were formed in early ancestors of jawed vertebrates (e.g. see [2]).

The agnathan acquired immune system

The failure to identify the cardinal elements of gnathostomian acquired immunity in their nearest living relatives, agnathans, was puzzling, especially since acquired types of immune responses (accelerated rejection of secondary skin allografts and delayed-type hypersensitivity) have been reported in hagfish and lampreys, the only living representatives of jawless vertebrates [6, 7], which possess the lymphocyte- and plasmocyte-like cells [8, 9]. Very recently it turned out that agnathans have an acquired immune system that is based on a different type of genetic units than that of gnathostome vertebrates. Instead of the immunoglobulin-type antigen receptors of jawed vertebrates, jawless hagfish and lampreys have variable lymphocyte receptors (VLRs), which consist of leucine-rich repeat (LRR) molecules. Somatic diversification of VLR gene occurs through a multistep assembly of LRR modules randomly selected from a large bank (perhaps 1500-2400) of flanking LRR casettes [10-12]. Each lamprey lymphocyte typically expresses a unique VLR gene in monoallelic fashion. The potential VLR repertoire estimated to be 10^{14} for the lamprey and 10^{17} for the hagfish [12] is essentially equivalent to that of antibody repertoire. Only the number of lymphocytes therefore limits the diversity of the anticipatory repertoire. This is true for the actual lymphocyte-receptor repertoire of both agnathans and gnathostomes. The available repertoire is limited by the presence of less then 10 million lymphocytes in lamprey larvae and in jawed vertebrate representatives like the zebrafish [12]. It turned out that lamprey can use soluble VLRs for specific recognition of antigens in a humoral response. Alder et al. [12] injected lampreys with a particulate immunogen, namely the anthrax spore coats (exosporium) of *Bacillus anthracis* at weekly intervals and examined cellular and humoral responses. Compared with unstimulated animals, the fraction of large VLR-positive lymphocytes increased during the 8-week stimulation period from 9 to 93% in the blood, from 11 to 90% in the kidney, and from 7 to 76% in

the typhlosole, the major haematopoietic tissue in the larvae. Plasma VLR concentrations in 8-week immunised animals were increased by 8- to 10-fold over preimmunisation levels. VLR specificity was proved by selective reactivity with *B. anthracis* versus *B. subtilis* spores, a related bacterium used as a control. These data indicate that lampreys are capable of specific humoral response to athrax exosporium by producing increasing levels of specific VLRs. These data strongly suggest convergent evolution of remarkably different strategies for generating anticipatory lymphocyte receptors in jawless and jawed vertebrates.

The acquired immune response of *Drosophila*

Until recently, an acquired immunity was thought to be restricted to vertebrate animals, which represent only less than 4% of contemporary metazoan species. However, recent studies conducted by Pham et al. [5] on the fruit fly, *Drosophila*, demonstrate that also invertebrate immune system can remember antigenic challenge and mount a specific immune response. The authors vaccinate the flies against *Streptococcus pneumoniae*, bacteria that can cause pneumonia in people. Priming *Drosophila* adults by injecting a sublethal dose of *S. pneumoniae* into the haemolymph protects the flies from an otherwise lethal second challenge with *S. pneumoniae* but not with *Salmonella typhimurium*, *Listeria monocytogenes* or *Mycobacterium marinum*, i.e. microbes pathogenic to *Drosophila*. And vice versa, priming with sublethal doses of these microbes does not protect from *S. pneumoniae* challenges. Thus the protection is very specific and persists through the life of the fly, which displays a life span of only 5 weeks under experimental conditions. These results suggest the existence of immune specificity and memory of the fly immunity, the characteristics attributed so far only to the vertebrate adaptive immunity.

Pham et al. [5] demonstrated also that the defence against *S. pneumoniae* is dependent on phagocytes. For another set of experiments they used flies with impaired phagocytosis by preinjection with latex beads which are taken up by phagocytes and saturate their phagocytic capabilities. While 3000 bacteria were required to kill normal fly, only 20 bacteria kill a fly with impaired phagocytic properties. Moreover, *S. pneumoniae* primed flies lacking phagocytosis died at the same time as naive flies. The authors concluded that a priming dose of *S. pneumoniae* allows phagocytes to recognise and kill *S. pneumoniae* more efficiently during a subsequent challenge.

Another hallmark of vertebrate acquired immunity is the extensive somatic diversification of immune receptors. Watson et al. [13] presented evidence that *Drosophila* immune-competent cells, hemocytes, have the potential to express more then 18,000 isoforms of the IgSF receptors Dscam, originally identified as the homolog of the Down syndrom cell adhesion molecules. The number of Dscam forms per single hemocyte is in the range 14 to 50, a large diversity relative to a single specific antigen receptor expressed by a lymphocyte. Treatment of hemocytes with anti-Dscam antibodies impaired their phagocytic properties. Perhaps Dscam is a signalling receptor or co-receptor during phagocytosis. Secreted isoforms are present in hemolymph. Perhaps diverse secreted Dscam isoforms are involved in opsonizing invading pathogens in the hemolymph.

What is most important, Watson et al. [13] evidenced that Dscam molecules are extensively diversified by alternate splicing, e.g. somatically. Moreover, the molecular diversity of Dscam transcripts generated through a mechanism of alternative splicing is highly conserved across major orders of insects, which represent an estimated 60% of metazoan species.

Convergence of insect Dscam and vertebrate antigen receptors

The diversity of vertebrate antigen receptors and insect Dscam molecules is reached by convergence, i.e. alternative ways of reaching similar goals [14]. A convergent emergence of a vast variability of antigen receptors had happened independently at least three times in metazoan animals, namely in gnathostomes, agnathans and phylogenetically distant insects (arthropods) (Figure 1), using different molecular patterns: IgSFs for gnathostomian TCRs/BCRs and insect Dscam, and LRR for agnathan VLRs.

Extensive germline and somatic modifications of conventional innate receptors have been reported recently in various taxonomic groups of animals, exemplified by fibrinogen-related proteins (FREPs) in a mollusc, variable region-containing chitin-binding proteins (VCBPs) in a cephalochordate, and novel immune-type receptors (NOTRs) in bony fish [15, 16]. They also may provide the basis for alternative mechanisms of receptor diversification and the convergent emergence of acquired immune systems in various animals. As evidenced by comparison of normal and lymphocyte-deficient mice [17], combination of innate and lymphocyte-mediated immunity is associated with energy savings, thus the addition of the acquired immune system to the innate immunity seems not to be constrained by energetic costs. The functioning of at least three different strategies for antigen receptor diversification recognised so far in phylogenetically distant taxons points to the fitness value of anticipatory acquired immunity not only in relatively large and long-living vertebrates but also in small short-living flies. Watson et al. [13] assumed that in a case of many invertebrates with the relatively short life span, the diversity of immune receptors is less important ontogenetically but rather enhances the adaptive potential of animal populations to changing environmental and pathogenic threats.

Acknowledgments

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