Molecular Immunology 69 (2016) 157–169

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Alternative adaptive immunity strategies: coelacanth, cod and shark immunity

Francesco Buonocore [a,*,1], Marco Gerdol [b,1]

[a] Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Largo dell'Università snc, 01100 Viterbo (VT), Italy
[b] Department of Life Sciences, University of Trieste, Via Giorgieri 5, 34127 Trieste (TS), Italy

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 31 August 2015
Accepted 1 September 2015
Available online 28 September 2015

Keywords:
Adaptive immunity
Coelacanth
Immunoglobulin
Major histocompatibility complex
CD4
T helper cells.

ABSTRACT

The advent of high throughput sequencing has permitted to investigate the genome and the transcriptome of novel non-model species with unprecedented depth. This technological advance provided a better understanding of the evolution of adaptive immune genes in gnathostomes, revealing several unexpected features in different fish species which are of particular interest. In the present paper, we review the current understanding of the adaptive immune system of the coelacanth, the elephant shark and the Atlantic cod.

The study of coelacanth, the only living extant of the long thought to be extinct Sarcopterygian lineage, is fundamental to bring new insights on the evolution of the immune system in higher vertebrates. Surprisingly, coelacanths are the only known jawed vertebrates to lack IgM, whereas two IgD/W loci are present. Cartilaginous fish are of great interest due to their basal position in the vertebrate tree of life; the genome of the elephant shark revealed the lack of several important immune genes related to T cell functions, which suggest the existence of a primordial set of T_H1-like cells. Finally, the Atlantic cod lacks a functional major histocompatibility II complex, but balances this evolutionary loss with the expansion of specific gene families, including MHC I, Toll-like receptors and antimicrobial peptides.

Overall, these data point out that several fish species present an unconventional adaptive immune system, but the loss of important immune genes is balanced by adaptive evolutionary strategies which still guarantee the establishment of an efficient immune response against the pathogens they have to fight during their life.

1. The gnathostome adaptive immune system

Although immunological studies have been focused for a long time on humans and mammalian models due to obvious medical implications, over the past few decades they have been extended to several non-mammalian vertebrates, providing important insights into the evolution and the origin of the adaptive immune system (Flajnik and Kasahara, 2010; Litman et al., 2005).

Detailed investigations have revealed some features common to the immune system of all vertebrates with jaws (gnathostomes), which include a highly complex set of specialized cells and the presence of a "memory" which guarantees the establishment of a rapid response against previously encountered pathogens. Specialized circulating cells, named lymphocytes, can recognize specific antigenic regions of the pathogens through cell-surface receptors, thereby giving rise to the immune response. This complex biological process involves many cellular and humoral components, proceeds through a series of tightly connected events, including clonal amplification, cellular differentiation and the production of antibodies (Burnet, 1959). Two major cell lineages, named T- (for thymus-derived) and B- (for bursa or bone-marrow-derived) lymphocytes, are involved in the immune response (Cooper et al., 1965).

T cell receptors (TCR) and immunoglobulin molecules (Ig), which are obtained through the recombination of variable (V), diversity (D) and joining (J) gene segments (Tonegawa, 1983), are certainly the most important classes of evolutionarily conserved molecules involved in the adaptive immune response across all vertebrates. The V(D)J recombination process, which takes place in T- and B-cells progenitors, has been studied in detail, unveiling the crucial role of two enzymes, the recombination-activating gene 1

* Corresponding author.
E-mail addresses: fbuono@unitus.it (F. Buonocore), mgerdol@units.it (M. Gerdol).
[1] These authors contributed equally to the paper.

http://dx.doi.org/10.1016/j.molimm.2015.09.003
0161-5890/

(RAG1) and the recombination-activating gene 2 (RAG2) (Gellert, 2002).

TCRs are heterodimeric molecules (αβ TCR and γδ TCR) which are exclusively bound to the cell membrane. αβTCRs recognize antigens after their processing by MHC class I and II molecules expressed on antigen-presenting cells, whereas the function of γδTCRs is independent from antigen processing and presentation by MHC molecules (Richards and Nelson, 2000).

Like TCRs, immunoglobulins (Ig) are heterodimeric molecules, composed by a light and a heavy chain. However, Igs can be either displayed on the cell membrane of B-cells and their precursors, or they can be produced as secreted molecules, originated by alternative spicing of mRNA occurring in differentiated B-cells called plasma cells (Litman et al., 2010). Different Ig isotypes have been identified in vertebrates to date: IgM, which is regarded as the most ancient antibody molecule and which shares similar functions in all gnathostomes (Flajnik, 2002); IgD, whose biological function is still not fully understood (Chen and Cerutti, 2010); IgW, originally discovered in cartilaginous fish (Berstein et al., 1996), but later also found in lungfish (Ota et al., 2003) and now considered to be orthologous to IgD (Ohta and Flajnik, 2006); IgY found in amphibians, reptiles and birds (Warr et al., 1995); IgG and IgE found in mammals and thought to derive from IgY-like ancestral molecules (Flajnik and Kasahara, 2010); IgA, found in mammals and its amphibian analogous IgX (Mussmann et al., 1996); other unusual immunoglobulins have a restricted taxonomic distribution, including IgT, only found in some bony fish (Zhang et al., 2010), IgF, only found in amphibians (Zhao et al., 2006), the camelid modified IgD molecules (Desmyter et al., 1996) and the structurally similar shark immunoglobulin new antigen receptor (IgNAR) (Greenberg et al., 1995). Moreover, a recent transcriptomic approach revealed a previously unsuspected variety of Ig isotypes in lungfish, which include IgN (which are related to IgW) and the enigmatic IgQ with a not convincing similarity to any of the known IgH isotypes (Zhang et al., 2014). It has been computationally estimated the possibly to generate up to 10^{14} different receptor variants in a single Ig locus (Flajnik and Kasahara, 2010).

This intricate defence system arose approximately 500 million years ago, probably in the now extinct placoderms, and the most evolutionarily ancient extant organisms in which this system is actually found are the cartilaginous fish (Cooper and Alder, 2006; Flajnik and Kasahara, 2010). New findings concerning invertebrate immunity and the interconnections between innate and adaptive immunity in vertebrates challenged the classical view of the metazoan immune response and even led some researchers to consider unnecessary the use of two different terms ("innate" and "adaptive") to describe immune responses (Boehm, 2012; Flajnik and Du Pasquier, 2004; Lanier and Sun, 2009).

2. Alternative adaptive immunity in agnathans

Recently, it has been evidenced that jawless vertebrates (comprising the agnathans lampreys and hagfish as living representatives) use a different form of adaptive immunity, which arose independently from that of jawed vertebrates. Indeed, even though they lack the molecular immunoglobulin repertoire of jawed vertebrates, lampreys and hagfish show some general immune responses that are typical of cellular immunity (Saha et al., 2010) and lymphocyte-like cells (Mayer et al., 2002). In a marked difference compared to gnathostomes, the antigen receptors involved in their immune responses are somatically derived variants of leucine-rich repeats (LRRs) that are termed variable lymphocyte receptors (VLRs) (Litman et al., 2010; Pancer et al., 2004, 2005). The diversity of VLRs is based on the insertion of LLR segments, which are organized into multiple flanking cassettes (about 850) within a single (in lampreys) or within two paralogous (in hagfish) non-functional germline VLR genes, resulting in the expression of a uniquely rearranged VLR sequence by individual lymphocytes (Alder et al., 2005; Pancer et al., 2005). The complexity of the rearrangements of the LRR segments within the VLR locus has been computationally estimated to possibly generate up to 10^{14} different receptor variants. The mechanism of VLRs assembling is based on sequence similarities between LRR segments (Nagawa et al., 2007) and it involves two activation-induced cytidine-like deaminases named CDA1 and CDA2 (Rogozin et al., 2007). Three different lymphocyte-like populations have been identified and characterized in lampreys and they seemingly display a correspondence to αβ T cells, B cells and to γδ T cells, respectively (reviewed in Kishishita and Nagawa, 2014).

3. Alternative strategies: some case studies

3.1. Coelacanth

3.1.1. Evidence from genomic analysis

Long thought to pertain to an evolutionary lineage extinct over 65 million years ago, the African coelacanth *Latimeria chalumnae* and its Indonesian sister species *Latimeria menadoensis* are dubbed "living fossils" due to their apparent morphological conservation compared to fossil records. Indeed, until 1938 only two Sarcopterygian taxa were believed to have survived late Devonian mass extinction, Tetrapoda and Dipnoi. The re-discovery of coelacanth off the coast of South Africa is still considered as one the most important scientific discoveries of the 20th century and paved the way to research and, often, to speculation concerning the key evolutionary position of this lobe-finned fish, whose origins can be traced back to ~400 Mya. Even though recent molecular evidence revealed that lungfishes are the most likely closest living relatives to modern tetrapods (Amemiya et al., 2013), coelacanths still represent an invaluable resource for understanding the evolutionary mechanisms underpinning the transition from aquatic to terrestrial life.

In 2013, the genome of the African coelacanth was completely sequenced, a major breakthrough which permitted to investigate many different aspects of vertebrate evolution (Amemiya et al., 2013). Among these, the evolution of the immune system was certainly one of the most significant, as extensive works specifically devoted at the analysis of the gene sets involved in innate and adaptive immune processes have been published shortly after the release of the genome sequence (Boudinot et al., 2014; Saha et al., 2014).

Overall, these studies concluded that the molecular immune repertoire of *Latimeria* spp. is, to a large extent, similar to that of other vertebrates and, in particular, that most immune-relate sequences share a closer similarity with tetrapods than with teleost fish, further supporting the position of coelacanths as a sister group to former taxonomic superclass. However, these investigations also highlighted some key distinctive features, with the absence of IgM, a key player of the adaptive immune system in all gnathostomes, definitely emerging as the most striking one.

Initially, the characterization of the coelacanth immunoglobulin heavy chain genes was undertaken by the analysis of BAC libraries obtained from *L. menadoensis*, permitting to identify two distinct loci containing V_H, D_H, J_H and C_H elements. However, the C_H segments of both loci were found to be remarkably similar to those found in shark and, in particular, in lungfish IgW. The two genomic loci were therefore named LmIgW1 and LmIgW2 accordingly. On the other hand, no similarity was found with teleost fish and tetrapod IgM C_H domains. The subsequent genome sequencing of *L. chalumnae* basically confirmed earlier findings, highlighting