ISJ 7: 146-148, 2010 ISSN 1824-307X

VISIONS AND PERSPECTIVES

Cytokine network in invertebrates: the very next phase of comparative immunology

D Malagoli

Department of Biology, University of Modena and Reggio Emilia, Modena, Italy

Accepted May 20, 2010

Abstract

Information on invertebrate cytokines has been growing impressively in the past five years. However, molecular characterization of newly discovered cytokines has not proportionally improved our understanding of the main reason underpinning their conservation among metazoans. One possible explanation is that cytokines have been conserved for the fundamental processes they control, but in mammals a single cytokine can hardly be considered as controller of complex reactions. In mammals, cytokines constitute a network of communication, and only this network can be considered the real controller of the effects that cytokines exert on immune or developmental functions. In all, the capability of constituting a network could represent the principal reason for cytokine evolution and conservation through diversification of metazoans.

Key Words: cytokines; innate immunity; invertebrates; evolution

Introduction

In mammals, cytokines are described as molecules responsible for the regulation of the maturation, proliferation, differentiation and survival of lymphocytes, macrophages and dendritic cells**,** and are further classified as lymphokines, monokines, interleukins and chemokines based on their origin and function (Corbellini, 2010). Among invertebrates (paraphiletic term but useful for the purposes of the manuscript), cytokines are present but much less characterized. No more than 6 years ago, the knowledge on invertebrate cytokines was limited and based on indirect evidence (Ottaviani *et al.*, 2004), and the very existence of possible counterparts of vertebrate cytokines was disputed (Beschin *et al.*, 2004). Recent experiments have introduced dramatic changes in this scenario, so that it could be said now we are moving into the "second age" of invertebrate cytokines.

The "second age" of invertebrate cytokines: state of art and future perspectives

In the last three years, several reports on invertebrate immunity have used the terms "putative cytokine", "cytokine-like" etc. (Malagoli *et al.*, 2007; Parrinello *et al.*, 2008; Roberts *et al.*, 2008; Zhang *et al*., 2008; De Zoysa *et al.*, 2009, 2010; Schikorski *et al*., 2009), but the factors that are commonly accepted as full-title invertebrate cytokines at present are Spätzle (Ferrandon *et al*., 2004) and Unpaired (Upd)-3 (Agaisse *et al.*, 2003) in *Drosophila melanogaster*, Insect chemotactic peptide (ICP) in the moth *Pseudaletia separata* (Nakatogawa et al., 2009) and Astakine-1 in *Pacifastacus leniusculus* (Söderhall *et al.*, 2005). These cytokines do not present traits of similarity with vertebrate cytokines, but seem to be widespread in different invertebrate models (An *et al.*, 2010; Hsiao *et al.*, 2010), and their receptor and the signalling pathway they elicit appear to be conserved among metazoans (Malagoli *et al.*, 2010). Interestingly, while the research for cytokine in invertebrates have been prompted mainly by evolutionary-aimed studies (Beschin *et al.*, 2004; Ottaviani *et al.*, 2004), very few considerations on the evolution of cytokines have been made after the discovery of full-title cytokines. It may appear a paradox, but more speculations on the possible history of cytokines can be realized starting from molecules that at present appear to be candidate cytokines. Indeed, some of those molecules, namely *Drosophila* Helical Factor (DHF) (Malagoli *et al*., 2007), *Ciona intestinalis* Tumor necrosis Factor (CiTNF) (Parrinello *et al*., 2008) and *Hirudo medicinalis* endothelial monocyte-activating polypeptide II (HmEMAP-II) (Schikorski *et al*., 2009) promise to give a significant contribution for the understanding of the evolution of cytokines.

DHF, or more simply HF as indicated in FlyBase (www.flybase.org), is a molecule with a predicted structure typical of the vertebrate helical

Corresponding author:
Davide Malagoli
Department of Biology,
University of Modena and Reggio Emilia,
Via Campi 213/D, 41125 Modena, Italy
E-mail: davide.malagoli@unimore.it

cytokines (Huising *et al.*, 2006). The discovery of HF (Malagoli et al., 2007) represented a significant contribution supporting the hypothesis that the molecular structure is a component of equal importance to gene and protein sequences in evolutionary studies (Malagoli and Ottaviani, 2007). CiTNF is another molecule in which sequence conservation is limited to key region of the molecule (Parrinello *et al*., 2008), and the same is true for HmEMAP-II (Schikorski *et al*., 2009). In these respects, it has to be remarked that differently from HF, there is still no information about the activity of CiTNF and HmEMAP-II in *Ciona* and *Hirudo*, respectively, because their discovery has not been followed by a detailed functional analysis, yet. Functional assays, based for instance on the utilization of native and recombinant molecules, are however necessary in order to unravel if the structure/sequence conservation corresponds to similar function.

However, even among the speculations on the evolution of cytokines, there is a fundamental aspect raised by the discovery of cytokines in invertebrates that has been neglected by comparative immunologists. In their efforts to isolate and characterize cytokines in invertebrates, researchers have been keeping their focus on the specific molecule under study. This led to very detailed and complete characterizations (Nakatogawa *et al.*, 2009), but some studies seemed almost concluded with the discovery and characterization themselves. The risk for the next future is to have a long list of new cytokines well-characterized in terms of molecular aspects, but very limited information on their implications for evolutionary biology.

The presence of cytokines in organisms with quite different evolutionary histories make it difficult to understand the basis of their conservation. The most obvious explanation is that these molecules are ancient signals that, regulating reactions fundamental for survival and homeostasis maintenance, have been conserved in structure and function. But if we step back to the principle reference of comparative biologists, i.e., mammals and, above all, humans, we can observe that the term "cytokine" indicates a soluble factor that necessarily acts together with many other cytokines in order to determine an effect (Corbellini, 2010). In mammals, the importance of cytokines relies principally on their capability of constituting molecular networks, and the functional meaning of a single cytokine is almost absent if we do not consider that molecule acting within a specific molecular context with many other players. Accordingly, human immunologists have articulated numerous and complex proposals to describe the interactions between different systems and mediators, e.g., the bow-ties (Ottaviani *et al.*, 2008).

Molecules identified as cytokines have been conserved in diverging metazoan taxa for hundreds million years, and it should be asked if this is due to their capacity to interact and constitute molecular networks, or to function as single signal molecules. The object of natural selection have been cytokines or cytokine networks?

Admittedly, only in *D. melanogaster* more than a cytokine has been identified at present, but if we consider the amount of data collected in the past five years and the possibilities offered today by bioinformatic approaches and the high throughput technologies, the discovery of several other invertebrate cytokines appears just a matter of time.

Now the complete molecular characterization of single factors is a reality in several invertebrate models, the very next phase of comparative immunology must be the identification of networks of cytokines as occurs in vertebrate species. The research for cytokine networks in invertebrate is a topic that directly involve comparative immunologists and evolutionary biologists as well. Even though there are several clues on the conservation of structure, receptors and signaling activities of invertebrate cytokines, if these molecules fail to constitute complex networks, should they still be called cytokines?

References

Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. Dev. Cell 5: 441-450, 2003.

An C, Jiang H, Kanost MR. Proteolytic activation and function of the cytokine Spätzle in the innate immune response of a lepidopteran insect, *Manduca sexta*. FEBS J. 277:148-162, 2010.

Beschin A, Bilej M, Magez S, Lucas R, De Baetselier P. Functional convergence of invertebrate and vertebrate cytokine-like molecules based on a similar lectin-like activity. Prog. Mol. Subcell. Biol. 34:145-163, 2004.

Corbellini G. Immunology: a historical perspective. In: Andrea Grignolio (ed), Immunology today: three historical perspectives under three theoretical horizons, Bononia Press University, Bologna, Italy, pp 35-52, 2010.

De Zoysa M, Nikapitiya C, Oh C, Whang I, Lee JS, Jung SJ, *et al*. Molecular evidence for the existence of lipopolysaccharide-induced TNF-alpha factor (LITAF) and Rel/NF-kB pathways in disk abalone (*Haliotis discus discus*). Fish Shellfish Immunol. 28: 754-763, 2010.

De Zoysa M, Jung S, Lee J. First molluscan TNF-alpha homologue of the TNF superfamily in disk abalone: molecular characterization and expression analysis. Fish Shellfish Immunol. 26: 625-631, 2009.

Ferrandon D, Imler JL, Hoffmann JA. Sensing infection in *Drosophila*: Toll and beyond. Semin. Immunol. 16: 43-53, 2004.

Hsiao CY, Song YL. A long form of shrimp astakine transcript: molecular cloning, characterization and functional elucidation in promoting hematopoiesis. Fish Shellfish Immunol. 28: 77-86, 2010.

Huising MO, Kruiswijk CP, Flik G. Phylogeny and evolution of class-I helical cytokines. J. Endocrinol. 189: 1-25, 2006.

Malagoli D, Conklin D, Sacchi S, Mandrioli M, Ottaviani E. A putative helical cytokine functioning in innate immune signalling in *Drosophila melanogaster*. Biochim. Biophys. Acta 1770: 974-978, 2007.

Malagoli D, Sacchi S, Ottaviani E. Lectins and cytokines in celomatic invertebrates: two tales with the same end. Inv. Surv. J. 7: 1-10, 2010.

Malagoli D, Ottaviani E. Helical cytokines and invertebrate immunity: a new field of research. Scand. J. Immunol. 66: 484-485, 2007.

Nakatogawa S, Oda Y, Kamiya M, Kamijima T, Aizawa T, Clark KD, *et al*. A novel peptide mediates aggregation and migration of hemocytes from an insect. Curr. Biol. 19: 779-785, 2009.

Ottaviani E, Malagoli D, Capri M, Franceschi C. Ecoimmunology: is there any room for the neuroendocrine system? BioEssays 30: 868-874, 2008.

Ottaviani E, Malagoli D, Franchini A. Invertebrate humoral factors: cytokines as mediators of cell survival. Prog. Mol. Subcell. Biol. 34:1-25, 2004.

Parrinello N, Vizzini A, Arizza V, Salerno G, Parrinello D, Cammarata M, *et al*. Enhanced expression of a cloned and sequenced *Ciona intestinalis* TNFalpha-like (*Ci*TNF alpha) gene during the LPS-induced inflammatory response. Cell Tissue Res. 334: 305-317, 2008.

Roberts S, Gueguen Y, de Lorgeril J, Goetz F. Rapid accumulation of an interleukin 17 homolog transcript in *Crassostrea gigas* hemocytes following bacterial exposure. Dev. Comp. Immunol. 32: 1099-1104, 2008.

Schikorski D, Cuvillier-Hot V, Boidin-Wichlacz C, Slomianny C, Salzet M, Tasiemski A. Deciphering the immune function and regulation by a TLR of the cytokine EMAPII in the lesioned central nervous system using a leech model. J. Immunol. 183: 7119-7128, 2009.

Söderhäll I, Kim YA, Jiravanichpaisal P, Lee SY, Söderhäll K. An ancient role for a prokineticin domain in invertebrate hematopoiesis. J. Immunol. 174: 6153-6160, 2005.

Zhang X, Luan W, Jin S, Xiang J. A novel tumor necrosis factor ligand superfamily member (CsTL) from *Ciona savignyi*: molecular identification and expression analysis. Dev. Comp. Immunol. 32: 1362-1373, 2008.