

LETTER TO EDITOR

Evolutionary aspects of allorecognition**L Ballarin¹, L Du Pasquier², B Rinkevich³, J Kurtz⁴**¹*Department of Biology, University of Padua, Padua, Italy*²*Zoology and Evolutionary Biology, University of Basel, Basel, Switzerland*³*National Institute of Oceanography, Haifa, Israel*⁴*Institute for Evolution and Biodiversity, University of Münster, Münster, Germany**Accepted September 8, 2015*

Dear Editor,

Although frequently neglected, allorecognition phenomena exhibit suites of effector mechanisms, altogether featuring one of the main biological characteristics of living organisms. A recent workshop organized by the Münster Graduate School of Evolution at the University of Münster, Germany, focused on the evolutionary aspects of allorecognition and the potential links to immune systems.

The term allorecognition, generally defined as the capability of non-self recognition between conspecifics, encompasses apparently unrelated phenomena on various biological organizations, such as: i) recognition between different strains within a single bacterial species (Gibbs *et al.*, 2008); ii) mating type recognition in protozoans (Luporini *et al.*, 2006); iii) recognition between cell lineages in the multicellular reproductive structures of slime molds (Hirose *et al.*, 2011); iv) intraspecific hyphae recognition in fungi (Glass *et al.*, 2000); v) self-incompatibility in plants (Takayama and Isogai, 2005); vi) graft rejection in metazoans (Karp and Meade, 1993; Bilej *et al.*, 2010; Eckle *et al.*, 2013); vii) self-sterility in many animals; viii) colony specificity in marine colonial organisms.

Evolutionary considerations can provide a unifying framework to identify communalities of these systems and to shed light on their relation to immune systems, and in particular the evolution of specific recognition and memory within innate and adaptive immune systems (Du Pasquier, 2005; Kurtz, 2005; Litman *et al.*, 2005). Allorecognition systems seem to be an important evolutionary force that helps in shaping the wide diversity of contemporary immune systems providing animals with some useful tools, such as the receptor variability and polymorphism that are required for the efficient distinction between non-self and self (de Boer, 1995; Dionne, 2013).

Indeed, colony specificity has been widely described in colonial invertebrate taxa with sessile modes of life, such as sponges (which can be assimilated to colonial organisms), cnidarians, bryozoans, tunicates and algae. In these organisms the survivorship of recruiting propagules is highly dependent on the ability to compete for the available substrate with other sedentary organisms, including conspecifics, and 'natural transplantations' frequently occur when colonies physically contact each other. In the latter case, either a fusion of conspecific colonies into a larger chimeric entity or a non-fusion reaction, that prevents colony fusion, occurs, depending on the presence or absence of shared alleles at a limited number of highly polymorphic fusibility/histocompatibility loci (Sabbadin *et al.*, 1992; Rinkevich, 1993; Cadavid *et al.*, 2004; Nicotra *et al.*, 2009; Voskoboynik *et al.*, 2013).

Larger colonies deriving from fusion between conspecifics have undoubtedly some ecological advantages with respect to smaller colonies, such as enhanced competitive capabilities, improved survivorship following attacks by predators, shortened onset of reproduction and augmented fecundity, as a great number of zooids contribute to gamete production, or benefit in terms of resource sharing (Buss, 1982; Grosberg, 1988). Fusion between conspecifics leads to chimerism, where cells of different genotypes are commonly intermingled in the new developing biological entity. In spite of the low attention reserved to this phenomenon by the scientific community, chimerism is widespread in nature and documented as well in various non-colonial taxa, including mammals (Rinkevich, 2011). During evolution, it could have played a role in shaping the immune systems, by selecting allorecognition mechanisms whose elements could have been recruited afterwards in other types of immune responses.

In contrast to the aforementioned benefits that may incur to chimeras, a competition between somatic and/or germ cell lineages of different origin may develop within chimeras, leading to somatic or germ cell parasitism, in which one of the cell lineages dominates and parasitizes the whole

Corresponding author:

Loriano Ballarin

Department of Biology

University of Padua

Via Ugo Bassi 58/B, 35100 Padua, Italy

E-mail: loriano.ballarin@unipd.it

chimeric colony (Magor *et al.*, 1999; Rinkevich, 2011). Hence the need for histocompatibility systems, as an acquired response to prevent within-organism conflicts, and the appearance of genetic systems limiting fusion to genetically related and kin colonies (Dishaw and Litman, 2009; Czárán *et al.*, 2014; Gilbert, 2015).

The high degree of polymorphism of genes involved in allorecognition ('antigens' and receptors) is encountered in all organisms so far studied with many cases of convergence (Dionne, 2013). This stresses the selective value of allorecognition, that may have played a role in the generation of specific immune systems of many Metazoa, and which reached its highest complexity in vertebrates with the appearance of adaptive immune systems.

Those listed above were some of the major issues discussed on last July 7th-8th, at the workshop organized by the Münster Graduate School of Evolution (Westfälische Wilhelms-Universität Münster, Germany), entitled: "Evolutionary aspects of allorecognition: from intraspecific conflicts to links with adaptive immunity" and intended for PhD students.

The workshop included three lectures held by L Ballarin, fellow at Münster University (*General aspects of allorecognition: cells, molecules and physiological responses*), B Rinkevich (*Chimerism: will two walk together except they have agreed? (Amos, 3:3)*) and L Du Pasquier (*Analogies and homologies in the somatic generation of immune repertoires of Metazoa*). The workshop also included a 'knowledge café' session.

The 'knowledge café' was an invaluable scientific exercise that allowed the direct involvement of PhD students with the puzzles and the open questions associated with allorecognition, the origin of the polymorphism in genes involved in allorecognition, and the relationships between allorecognition and specificity in immune responses of invertebrates and vertebrates alike. The scientific discussions were not terminated at the end of the workshop but they continued, following activities like exploiting the web, future planned round tables and the collection of the emerging ideas and opinions in evolutionary aspects of allorecognition.

General aspects of allorecognition: cells, molecules and physiological responses

L Ballarin

Allorecognition is a widely diffuse phenomenon in nature. Shared feature of allorecognition are: i) the variability of the recognition proteins, related to the high polymorphism of the respective genetic loci; ii) the induction of an inflammatory response, characterized by the recruitment of immunocytes upon the recognition of allogeneic molecules, the degranulation of cytotoxic cells and the release of cytokines; iii) the induction of cytotoxicity which, in invertebrate, frequently occurs as a consequence of the activation of the melanin-producing enzyme phenoloxidase (PO) and the production of reactive oxygen species. In the compound ascidian *Botryllus*

schlosseri, colony specificity, a type of allorecognition present in sessile, colonial invertebrates, manifests itself as rejection of genetically incompatible colonies, with the appearance of a series of necrotic, melanic spots along the contact border. Cytotoxic morula cells (MCs), constituting the majority of circulating hemocytes, are the first cells to sense non-self molecules diffusing from the alien colony. Upon their recognition, MCs degranulate and release immunomodulatory molecules able to recruit other immunocytes in the contact region. They also release PO, which is responsible of the observed cytotoxicity. MCs also synthesize C3 precursors and store amyloid fibrils inside their granules, which poses the question of the role of C3 and amyloid in ascidian inflammation. Since the synthesis of melanin is controlled by α -MSH, produced by phagocytes, another unresolved questions concern the role of other immunocytes, *i.e.*, phagocytes, in colony specificity and the presence of a cross-talk among different immunocyte types in ascidian inflammation.

Chimerism: will two walk together except they have agreed? (Amos, 3:3)

B Rinkevich

While immunity in all multicellular organisms (animals and plants alike) is highly efficient in dealing with parasites, in many taxa it fails to combat chimerism between conspecifics. Indeed, natural chimerism is widely documented in nature, appearing in about ten phyla of protists, invertebrates and plants, vertebrates and mammals, including humans. As a matter of fact, when appearing, chimerism serves as important ecological and evolutionary tools in metazoans' life history portraits, dictated costs and benefits for the genotypes involved. Including in the list of benefits are the increase of genetic variability, size-dependent ecological qualities that are improved following chimerism (affecting growth rates, reproductive outputs, survivorship, competitive exclusion benefits, increasing tolerance against environmental drivers), the development of synergistic complementation, the assurance of mate location, and more. Major costs include the threat of somatic and germ cell competition and parasitism, sexual sterility, the development of diseases (including cancers and autoimmune diseases), and organ malformations. Clearly, natural chimerism is an evolutionary driven phenomenon. The major questions that will be asked are, why chimerism first appeared? What are the evolutionary benefits that support its existence, and why do not all multicellular organisms present scenarios for chimerism?

Analogies and homologies in the somatic generation of immune repertoires of Metazoa

L Du Pasquier

The immune systems of Metazoa are under pressure to diversify repertoires of recognition structures that enable individuals to survive in a

diverse and rapidly changing pathogenic and competitive environment.

During evolution various solutions that meet this demand have been selected within the different phyla. First, large families of germline genes encoding the receptors can be generated by multiple duplications. Some of these are used in allorecognition, for example polymorphic Immunoglobulin superfamily members encoded by the *alr 1* and *alr 2* histocompatibility loci of *Hydractinia* that involve homologous interaction between cell surface molecules of the same family that end up, launching alloaggression reactions. Second, several phyla use combinatorial associations to somatically generate, repertoires of large numbers of specific receptors (that exceed the number of genes encoding them!) that can provide adaptive individual responses during ontogeny. The associations can be between peptides (within the families of leucine rich repeats, immunoglobulin domains, lectin domains, etc.). The combinatorial associations can also take place during genesis of the effector cells populations at the level of nucleic acid segments. At the RNA level they can involve splicing like in the mysterious receptor called DSCAM in arthropods, the role of which in immunity is far from being clear, but the diversity of which is amazing.

At the DNA level they can involve somatic rearrangement with combinatorial association of exons (VDJ recombination), conversion, mutation and generate repertoires of receptors with different adaptive characteristics that can be analogous, but not necessarily homologous to each other. Somatic modifications (*i.e.*, at the DNA level) can be encountered in echinoderms (183/333 molecules), molluscs (FREP molecules) and vertebrates (molecules of the leucine-rich repeat or immunoglobulin superfamilies) but are not due to homologous mechanisms but to analogy and convergence. The most recent discovery of such an analogy is that of the adaptive immune system of agnathans that, in three lymphocyte lineages similar to $\alpha\beta$, $\gamma\delta$ T cells and B cells of gnathostomes, makes use of leucine-rich repeats receptor genes, to generate somatically the repertoire of their immunoreceptors. They do it with the help of a member of the cytidine deaminase family, an enzyme homologous to AID, also involved in somatic generation of Immunoglobulin superfamily receptors repertoires in gnathostomes.

Two aspects that can profoundly differentiate responses among Metazoa and that need to be elucidated are:

1) how selection of repertoires is achieved, whether MHC analogues will be discovered in agnathans.

2) whether specific cell proliferation is induced after encounter with a non-self epitope in any invertebrate. This will condition whether secondary responses can actually be attributed to classical immunological memory or to persistence of ongoing responses or to yet unknown mechanisms.

Because of the involvement of MHC, the answers to these two questions will perhaps help

placing allorecognition in the context of the evolution of adaptive immune systems.

Acknowledgements

Authors wish to thank the students Ferro D, Hamley M, Knoblich K, Kutzer M, Talarico M, Wieczorek B, Wohlleben A, who took part in the discussions, as well as Dr Kloke V for her invaluable support and Santander Universities for funding.

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