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Diversity of immune systems

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ABSTRACT

Explaining the diversity of immune reactions requires comparative immunology that takes a phylogenetic view; there is interest in uncovering the underlying mechanisms throughout the animal kingdom. Because of the complexities and diversities of individual phyla, comparative immunology can compare and therefore reveal and elucidate immune mechanisms between and within major groups. There exist two categories of immune responses; 1) non-specific, innate, natural, non-adaptive, and non-anticipatory; 2) induced, adaptive, specific, anticipatory responses. Non-specific mechanisms include the ubiquitous phagocytosis and encapsulation. In invertebrates, several leukocyte types possess membrane associated markers (e.g. β_2m, Thy-2, Lyt-, Lyt-2/3). Transplantation immunity, has revealed various degrees of specificity in sponges, coelenterates, annelids, insects, echinoderms and tunicates. Cytotoxicity is a crucial immunodefense function. Humoral immunity includes naturally occurring and inducible agglutinins, lysozymes, lysins, non-lysozyme bactericidins, and lysosomal enzymes. Humoral immunity in insects (e.g. cecropins and defensins) that lack specificity and memory, functions efficiently against microbial pathogens. Cytokine-like molecules, of invertebrates, may regulate host defense responses by an acceptable network similar to that of vertebrates. Communication and recognition molecules are universal since protozoans possess a pheromone (*Er*-1) that is related to IL-2, IL-1 and TNF-like activities are found in annelids, mollusks, echinoderms and tunicates. In the future molecular biology must be used extensively to dissect immunodefense more fully, a more modern approach in understanding one component of an organism's totality.

KEY WORDS: Immunity - Diversity - Comparative Immunology - Evolution - Molecular biology.

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INTRODUCTION

Explaining the diversity of immune reactions requires comparative immunology that takes a phylogenetic view; there is interest in uncovering the underlying mechanisms throughout the animal kingdom. We can assume, first of all, that all creatures can distinguish between self and non self

Evolutionary theory suggests that there may have been a universal stem cell that underwent differentiation because of environmental pressures. In fact, evolution of the immune system began in the sea with unicellular protozoans, as depicted in a somewhat fanciful cartoon showing growth of a young amoebae into a full fledged immune system (Langman, 1989). This depiction progresses from simple *self/non-self* recognition to infection of the amoebae by intracellular parasites, past phagocytosis to the emergence of receptors. This review recognizes that invertebrates are exceedingly diverse. Thus, it is not surprising to find diverse defense/immune responses whose effector mechanisms remain somewhat unexplored (Cooper *et al.*, 1992a). Both modern and classic analyses suggest that certain immuno/defense responses may be: 1) be analogous in invertebrates and vertebrates, revealing the general principle that immuno/defense is a strategy that evolved to ensure survival after attacks by microorganisms; 2) unique to invertebrates; 3) antecedents of vertebrate immunity; 4) solely those ascribed to mammals. Without question, the immune system is universal, but the extent of commonality and evolutionary origin is poorly understood.

Comparative immunologists analyze how immune systems may reflect what has evolved over millions of years: immune responses of extant are related to those of extinct species

Before serious study of the immune system is begun, one must have a clear understanding of how an organism and its immune system develop. In sexual reproduction, the fertilized egg is the point of origin for all the cells, tissues and organs that will comprise a fully developed organisms. In organisms all of the molecules synthesized by all of the different cells must act in unison just as all of the cells of the different organ systems must also do. This is why none of the systems that comprise an organism can be entirely isolated from the others.

The single cell origin of multicellular organisms also allows for other types of analysis that are based upon the stages of development between the fertilized egg and the juvenile. Darwin (1859) wrote that: «in the eyes of most naturalists, the structure of the embryo is even more important for classification than that of the adult. For the embryo is the animal in its less modified state; it reveals the structure of its progenitor. In two groups of animals, however much they may at present differ from each other in structure and habits, if they pass through the same or similar embryonic stages, we may feel assured

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that they have both descended from the same or nearly similar parents, and are therefore in that degree closely related. Thus community in embryonic structure reveals community of descent». One can find here the roots of what we inherited from Darwin in the field of comparative immunology (Cooper, 1982).

The foundation was thus laid for the prescient experiments of Metchnikoff (1892) on simple phagocytosis and its relationship to immunology. Observations from experiments on invertebrate phagocytosis served to splinter the monolithic field of immunology in the 19th century into the two new fields that deal with inflammatory responses and invertebrate immunology, a subdivision of the parent discipline, comparative immunology. To this day, classical immunologists still adhere to the structural and functional bipartite system, i.e., cellular/humoral, that was a direct result of Metchnikoff's observations.

Furthermore, closer examination of the immune system shows that one can consider phagocytosis in protozoans as being the evolutionary precursor to a functionally distinct immune system. In simple (unicellular) organisms, the process of phagocytosis is a means of food capturing and, by default, a means of defense since the only way for a single-celled organism to defend itself is to eat or be eaten! In multicellular creatures, however, the ability to have cell specialization has afforded the evolution of immune systems that have a clear dissociation between food acquisition and immnodefense functions.

We can subsequently assume that as animals evolved over time, there was an increase in the complexity of their *cells, tissues,* and *organs* resulting in the immune system becoming more distinct and complex. Of course, one can ask the question why develop an immune system at all? Simply put, immune systems are absolutely necessary for the survival of an organism and it must be able to fend off all the external and even internal attacks made against it. While external threats come from potentially pathogenic microoganisms, internal threats may come from mutated cells that, according to current theory, become cancerous and threaten the existence of the organism.

In other words, the immune system of multicellular creatures evolved to defend against the threat of infection posed by foreign invaders and to distinguish *self* from *non-self*. Having said this, it must be noted that the internally based threat is still sometimes an area of contention as it is not always clear that internal threats exist in lower invertebrates. The implication of this is that if lower invertebrates did not face internal threats, then how, why and when did higher organisms evolve the ability to counter this threat as they do today? One clear example of an invertebrate exposed to internal threats are the molluscs who have been discovered to have true lymphatic leukemias (Cooper *et al.*, 1992a).

There exist innate, natural, non-adaptive, non-anticipatory responses vs. induced, adaptive, and anticipatory responses

It is essential in our attempt to trace possible origins of immune responses to reveal those genealogical relationships which have been conserved, i.e. structure and not function. Function has been examined in attempts to define homology and analogy as convergence and divergence have been examined in order to account for functional invertebrate leukocytes. However, we must start by using deuterostomes if we are searching for vertebrate origins of the immune response, if we are to uncover genalogical relationships. Although there is unlimited technology such as biochemistry and molecular biology for revealing conserved structures at molecular, cell, and organ levels, we still have limited evidence of what may be recognized today as conserved structures.

Adaptive radiation, in the immunologic context, assumes that evolution is from a single, ancestral form («immuno defense stem cell») to a variety of different immunocytes, each of which is adapted and specialized in some unique way to survive in a particular habitat. This is evident in the multitude of leukocytic types which possess variable functions as those which we observe in invertebrates. *Divergent evolution* is adaptive radiation which gives rise to several different types of descendants adapted by various means to different environments. On the other hand, *convergent evolution*, which also occurs fairly frequently, represents two or more quite unrelated groups that may, in adapting to similar environmental conditions, develop similar characteristics. For example, this may be true for immunocytes, and more specifically lymphocyte-like cells (LLC's) of tunicates (Cooper *et al.*, 1992a, b).

Homologous/analogous structure

With respect to structure, homology refers to similarity in relationship to adjacent structure, in embryonic development, and in nerve and blood supply. In immunological terms, this may translate into the evolution of hemopoietic organs. The thymus is an appropriate example of such a relationship, as it appears in the various vertebrate classes (Cooper, 1973) and its possible pharyngeal antecedent in tunicates may be a precursor whose cells are capable of replication both with and without antigenic stimulation (Raftos *et al.*, 1990; Raftos & Cooper, 1991; Sawada *et al.*, 1994). In contrast, analogous structures are those structures which superficially serve similar or comparable functions but have quite different basic structures and developmental patterns. Consequently, the presence of analogous structures does not necessarily imply evolutionary relationships between the animals bearing them. This relationship between analogous structures holds true for functions of leukocytes in metazoans (perhaps those of protostomes/deuterostomes). To be able to sequester in-

fectious microorganisms is the *raison-d'être* of immune system and is a common strategy developed to preserve the species which may have entirely different roots.

Evolutionary function

In immunologic terms, sequence analyses have revealed a family of molecules (the Ig superfamily) that seem to reflect examples of both analogy and homology whose basic function is recognition (Cooper *et al.*, 1992a). Stewart, in discussing the evolution of the immune system, took the following position: «In evolution, structures, be they molecules, cells or organs, change continuously, so that homologies can be reliably recognized. Functions on the other hand, are not conserved: they evolve discontinuously. It is therefore vital to identify the object of evolutionary study as a structure, and not as a function» (Stewart, 1992). Because of the complexities of individual phyla due to their enormous diversity, comparative immunology can compare immune mechanisms between (e.g. insects and earthworms) and within major groups (e.g. insects and crustaceans). We can now define evolutionary steps or selection pressures as they may apply to immunological implications in single cell animals and complex viviparous mammals. Implications include recognition and discrimination, development of the histocompatibility system, allogeneic recognition and short-term memory, freely circulating and increased numbers of diverse blood cell types, cellular immunity, and immunosurveillance of self cells for those that are infected or cancerous.

What do we know?

Non-specific defense mechanisms include the ubiquitous phagocytosis and encapsulation. In invertebrates, several leukocyte types possess membrane associated markers (e.g. β_2m, Thy-1, Lyt-1, Lyt-2/3) whereas other cells possess markers that are exclusive for invertebrates (e.g. lectin and agglutinin). Transplantation immunity, a widely studied immune response has revealed various degrees of specificity in sponges, coelenterates, annelids, insects, echinoderms and tunicates.

Evolution of the cytotoxic response; a crucial immunodefense function

Although antibodies are vertebrate inventions, the cytotoxic cell immunity with limited TCR/MHC repertoire could have developed at an early stage of invertebrate evolution since it is still found in some invertebrates (Cooper *et al.*, 1992a). Williams (1987) has proposed that the Ig superfamily probably evolved from a heterophilic recognition system which underwent extensive diversification. This recognition system may have developed into an immune system derived from a cytotoxicity system which involved programmed cell death (apoptosis). Since natural cell cytotoxicity may be gaining favor as a possible link between invertebrate and vertebrate cell mediated responses, this view seems not farfetched. With the demonstration of graft rejection in invertebrates, a response accompanied by specificity and memory, the idea that the TCR/MHC co-evolution may have begun in certain invertebrates, becomes an interesting idea worthy of more intense scrutiny.

Cytotoxicity as observed *in vitro* constitutes a more refined model than experimentally produced transplants (that are rejected by cytotoxic cells) to test the extent of recognitive potentialities of invertebrates leukocytes. This would extend the analyses of host mechanisms devoted almost exclusively to graft rejection responses to those against allogeneic and xenogeneic targets. Some of our current efforts rely on the ability to translate the *in vivo* responses described above into *in vitro* assays of allogeneic and xenogeneic reactivity (Roch & Cooper, 1991; Cooper *et al.*, 1995; Cossarizza *et al.*, 1995a, b; Suzuki & Cooper, 1995a, b; Suzuki *et al.*, 1995; Quaglino *et al.*, 1996). Previously, such technical facilities have not been readily available for analyzing invertebrate systems. Our recent work has been directed toward developing *in vitro* assays (Cooper *et al.*, 1992b), and how these assays may add to general information on an overview of the immune response evolution (Cooper, 1992).

Humoral immunity: in vitro *release of lectins*

Cells of the immune system are known to synthesize and to secrete molecules that effect humoral responses. Arizza *et al.*, (1991) have actively researched lectins and provided the following information: «In invertebrates, cellular recognition has been attributed to a protein-carbohydrate molecular mechanism located at the cell surface. Sugar-binding proteins (named lectins or agglutinins) have been found on hemocyte surfaces (Amirante, 1976; Amirante & Mazzalai, 1978; Vasta *et al.*, 1984; Parrinello & Arizza, 1988) and they are present in the hemolymph of all the examined species, probably involved in nonadaptive immune recognition». In many cases, an opsonic function of humoral lectins has been demonstrated (Renwrantz & Stahmenr, 1983; Cheng *et al.*, 1984). In vertebrates, lectins have been identified in several tissues. They are present on the surface of lymphocytes and macrophages and can be involved in phagocytic functions as well as recognition processes in the immune response (White, 1986). Hemocytes of several invertebrates contain (Amirante & Mazzalai, 1978) and also presumably secrete lectins (Leippe & Renwrantz, 1988; Stein & Cooper, 1988).

Tunicates possess lectins in their blood, and the humoral lectins studied so far are specific for sialic acid and more frequently, for D-galactosyl residues (Parrinello & Canicattì, 1982, 1983; Vasta & Marchalonis, 1984). D-galactosyl-specific lectins can be present on the hemocyte surfaces of the tunicate *Ascidia malaca* (Parrinello & Arizza, 1988). Parrinello & Arizza (1989)

showed that in the blood of *Phallusia mamillata*, two different lectin types exist which have a similar specificity for lactose. Humoral lectins, isolated from the serum, consist of a major subunit (58 kDa) and a minor one (15 kDa), probably associated by disulfide linkages. Cellular lectins, isolated from sonicated hemocytes, are formed from two subunits similar in molecular weight (36 kDa and 35 kDa, respectively) which can occasionally be observed in the SDS-PAGE pattern of purified serum lectins. Neither lectins of the humoral type nor their subunits have been isolated from hemocytes. αa-Lactose specific lectins are released from the hemocytes of *Phallusia mamillata* during short-term cultures (Parrinello & Arizza, 1989). Two major conclusions were reached. First, the molecular weight of the subunits, the immunological cross-reaction, and the sugar specificity suggest that the released lectins are similar to those isolated from sonicated hemocytes. Second, because lectin release appears to occur independently of active protein synthesis, perhaps these lectins are preformed, stored in hemocytes and released if they are stimulated *in vitro*. It is, of course, assumed that the *in vitro* situation offers insight into the response that occurs *in vivo*.

Humoral immunity in insects (e.g. cecropins and defensins) which lacks specificity and memory does function efficiently against microbial pathogens

The primary structure and mechanisms of actions are elucidated, and the target organisms defined. Many genes that encode these peptides are cloned, and there is a high degree of homology with components that regulate mammalian acute phase genes. Invertebrate cytokine-like molecules may regulate host defense responses by an acceptable network similar to that of vertebrates. Communication and recognition molecules are universal since protozoans possess a pheromone (*Er*1) that is related to IL-2. IL-1, and TNF-like activities are found in annelids, mollusks, echinoderms and tunicates.

The need for communication between cells is clearly necessary in multicellular organisms. The ability to communicate exists within the immune system and between the nervous, immune and endocrine systems (Cooper, 1991). Cytokines provide an excellent example of molecules that comprise a medium of communication. We can therefore attempt to verify whether the roots of cytokine evolution may be found in single celled eukaryotes, and in so doing, reveal aspects concerned with cytokine evolution. We are faced with the origin of life within an aquatic milieu and the need to establish and maintain boundaries that ensure survival. Consequently, molecules that communicate between single cell organisms and between cells, tissues, organs and systems in multicellular organisms are essential. In the search among unicellular organisms, Luporini *et al.*, (1986) analyzed the molecular basis of cell recognition in the ciliate *Euplotes raikovi*. The results strongly suggest the existence of an evolutionary link between mammalian cytokines and ciliate pheromones.

Cellular communication evolved within the aquatic milieu: Cytokine-like molecules; IL-1 and IL-2

The need for organisms to fight infections probably first led to the evolution of receptors or recognitive mechanisms in primitive organisms before the development of receptors based on rearranging genes (Janeway, 1989a). It is assumed that vertebrates possess both systems, but two questions can be posed with respect to invertebrates: 1) Do invertebrates possess only the broad-based, nonclonally distributed recognitive systems wherein specificity evolved in order to recognize patterns on the surfaces of numerous microorganisms? 2) Do they possess antecedents of the receptor which is based on rearranging genes? There are more specialized functions being recognized in invertebrates. For example, the actions of cytokines, while not restricted to specific immunity, play important roles in non-specific immunity. As has just been reviewed, cytokines may also be involved in purely recognitive events as revealed in protozoans (Cooper *et al.*, 1992).

Some of these cytokine-like molecules such as IL-1 and IL-2 participate in the initiation of T-cell responses. These molecules are able to exert autocrine and paracrine effects in the regulation of responses (Cooper, 1991). Their source may be macrophages or lymphocytes but other types of cells, e.g. epithelial, endothelial and fibroblastic, also produce cytokines. It is therefore of special interest that invertebrates seem to produce cytokines (IL-1 like, IL-2-like, and TNF-like). However, a word of caution is needed here, as with the other topics with which we grapple: origins and relationships.

Janeway (1989b) also believes «that the requirement for a signal delivered from another cell, now called 'signal two' in studies of lymphocyte activation, predates the development of the rearranging receptors that deliver signal one». Pattern recognition is the basis and not the form of recognition which is associated with the adaptive immune response. Specificity is broad and non-clonally distributed, which will ensure that a response will occur (avoidance of non-responsiveness), and that it will discriminate *self* from *non-self*, but of the infectious variety (which is natural and life-threatening), not that which is human-induced using non-infectious antigens.

Concerning complex metazoans, Beck & Habicht have isolated and characterized 'primitive cytokines' from two invertebrate phyla (Echinodermata and Annelida) and one subphylum (Urochordata) (Beck & Habicht, 1986; Beck *et al.*, 1989; Beck *et al.*, 1990). They have identified IL-1α, IL-1β and TNF α/β TNF-like activity, the latter referred to as α/β until there is rather conclusive evidence clarifying which activity has been detected. In addition to its direct cytotoxic activity in the L cell assay, invertebrate TNF α/β can stimulate L cell cytotoxicity by synergizing with human recombinant IFNα, an activity

reported for human TNF β (Williams & Bellanti, 1984). That IL-1 and TNF have been identified in invertebrates suggests that we will find other cytokines that could be of phagocytic or T cell origin in these animals. IL-1 and TNF play key roles in regulating the vertebrate host defense system (Beck *et al.*, 1986) and other cytokines are involved in vertebrate inflammatory responses. In the cytokine network, IL-2 also occupies a central position since it can stimulate cells to release TNF, α and β, IL-1 and TGF β. IL-2 is also a mitogen for many cell types and activates both T cells and macrophages (Neta *et al.*, 1990). Since IL-2 has been found in lower vertebrates (fish and frogs) (Watkins & Cohen, 1987), it has been investigated as a molecule which has been conserved during evolution. Finally, there is evidence of an 'IL-2-like' activity in invertebrates as well (Beck *et al.*, 1989). This apparent universal occurrence of cytokine-like molecules underscores the need to broaden our views of the consequences of cellular communication or 'cross-talk'.

In solitary tunicates, the pharynx, a major hemopoietic site, is sensitive to tunIL indicated by significant incorporation of ^{3}H-thymidine

More recent information on cytokines comes from work on IL-1-like and IL-2-like molecules (Raftos *et al.*, 1991a, b, 1992; Beck *et al.*, 1993), which will affect tunicate pharyngeal cells that are composed of lymphocyte-like cells and granular amoebocytes. Since these cells are involved in the specific allogeneic and phagocytic reactions, and little is known about their regulation or control, cytokines may provide certain clues relative to regulation. A tunicate interleukin 1 (IL-1)-like fraction stimulates cell proliferation *in vitro* (Raftos *et al.*, 1991a; Sawada *et al.*, 1994). This fraction, designated tunicate IL-1β, has been isolated from tunicate hemolymph by gel filtration and chromatofocusing chromatography. Mitogenic responses to tunicate IL-1β were dose dependent and could be eliminated rapidly by removing tunicate IL-1β from the culture medium. A second tunicate hemolymph fraction had no effect on tunicate cell proliferation even though it exhibited IL-1-like activity in a mouse thymocyte proliferation assay.

TOWARD THE FUTURE...

Molecular biology is essential in dissecting immunodefense more fully

Modern immunology can now trace its molecules, cells, organs and functions that fight infectious diseases to prototypic ancestors in both the aquatic and terrestrial environments. It is not clear which environment exerted the greatest evolutionary pressure during the development of immunologic characteristics. Clearly immune responsiveness began as recognition even at the unicellular level when eukaryotes appeared. Multicellularity brought with it the simultaneous differentiation and specialization of multiple functions including immune competence. The idea of comparative immunologists adhering to the dogmatic deuterostome/protostome classification of complex metazoans may prove to be inadequate since this taxonomic scheme concerns embryologic origins of the mouth and anus. Other theories that relate to homology and analogy, convergence and divergence may help us to explain what are (for now) common, functional immune traits among both animal groups. Origins deciphered by amino acid and nucleotide sequences will resolve the dilemma of immunologic characteristics common to deuterostomes and protostomes. Precise characterizations and analyses of the resulting homologies and comparisons will yield fruitful information.

First, we may then deduce common origins as has been revealed, for example, in functional molecules and cells such as actin/myosin that govern motility. Second, in contrast, we may arrive at what are clear analogies. With respect to origins of immunity there seems to be a preponderance of characteristics associated with tunicates [those of lymphocyte-like cells; IL-1; (IL-2 like) molecules, putative members of the Ig superfamily (Thy-1; Lyt); lectins] that may prove to be homologous once stringent requirements of sequence analyses are fully described. The *self/non-self* view should be rescued from *ad nauseum* preoccupation with examining yet more species with respect to (for example) phagocytosis, graft rejection or agglutinin synthesis. Instead, we should direct our research at the membrane level of hemocytes. Immunodefense may be our last approach in understanding an important component of an organism's totality.

Unsolved problems: Some practical approaches using invertebrates and ectotherms as models

This review has mentioned a marine protochordate, the solitary tunicate, as an ideal animal model useful in deciphering the origin of vertebrate immunity. As in all multicellular organisms, there are two principle characters in organ systems: the cells and the molecules they synthesize and secrete. First, these two characters exert local functions (a single system) and may in some instances provide communication between systems. Although tunicates possess numerous immune cell types, the lymphocyte-like cell is becoming of more focused interest because of its greater role in specific immune responses *vis a vis* the other cell types that effect more generalized responses. Second, the cytotoxic response, whether between allogeneic effectors and targets or xenogeneic targets, is an example of a nonclonal type of immune response that could have been primordial. Cells are known to synthesize and secrete soluble mediators which act as communicators within the immune system itself (lectins) or they may act as communicators that bridge the immune with other systems (nervous, endocrine). Considering the dearth of investigators, these

findings are remarkably timely. Still, the greatest shortcoming is the lack of more biochemical and molecular work. Sequence analyses would do much to reveal the extent of homology or analogy of cells and the molecules they synthesize and secrete. The horizon is becoming more recognizable as the future work of the labs cited indicated. Once the sequence information is available, we can then conclude with a measure of confidence that our cells and their products are related to those of vertebrates.

Kinship between invertebrate and vertebrate immune systems is problematic since invertebrates do not possess variable-region molecules (VRM), e.g., Igs and TCRs. Development of these molecules was probably a crucial event in the evolution of primordial vertebrates (Matsunaga, 1985; Matsunaga *et al.*, 1987; Matsunaga & Dahl, 1989; Marchalonis & Schluter, 1990; Cooper *et al.*, 1992a). Their invertebrate precursors undoubtedly had a more than adequate defense system: witness the numerous extant species. Retaining this ancestral immunodefense system (non-specific, innate, natural), the vertebrate line built upon it to evolve the modern immune system (specific, induced, adaptive). To ensure the perpetuation of the latter in the face of countless numbers of antigens associated with rapidly mutating microorganisms, this new VRM system was redeployed to trigger pre-existing effector mechanisms of defense against potentially destructive pathogens. Finally, there was probably still a further development, i.e. immunoglobulins, and antigen presentation by T-cell receptors in relation to class I and class II MHC components.

In contrast to this argument, Stewart has proposed that the VRM did not evolve to fight infection. Rather, he suggests «that the role of the first VRMs was to function as membrane-bound receptors; this would enable lymphocytes to interact with each other and so control their own growth (Stewart, 1992)». Langman does not fully concur with Stewart and cautions: «I would suggest that it is not the 'completeness' and high rate of variability that is of central importance». Finally, a VRM without a selectable function cannot be maintained. Thus if the central self-regulating VRM envisaged by Stewart were so essential, the *scid* mouse should be an unregulated bag of anarchic cells (Langman, 1992)». Actually, the presence of a Thy-1-like component on tunicate lymphocytes conforms to the first stage in the evolution of molecular structures (Stewart, 1992). According to his scheme, among invertebrates, tandem duplication of a *one* domain structure (110 amino acids; membrane-bound receptor, e.g. Thy-1) led to a *two* domain structure. Although we may ponder the proposed function of a primordial VRM system, it is essential to define the animal group in which it may exist, (perhaps a protochordate despite the enormous distance, yet persistently proposed similarity based upon embryology) and how much of the immune system qualifies to be a true antecedent.

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