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The neuro-immunological interface in an evolutionary perspective: the dynamic relationship between effector and recognition systems

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1. ABSTRACT

The evolutionary perspective indicates that an immune-neuroendocrine effector system integrating innate immunity, stress and inflammation is present in invertebrates. This defense network, centered on the macrophage and exerting primitive and highly promiscuous recognition units, is very effective, ancestral and appears to have been conserved throughout evolution from invertebrates to higher vertebrates. It would seem that there was a “big bang” in the recognition system of lower vertebrates, and T and B cell repertoires, MHC and antibodies suddenly appeared. We argue that this phenomenon is the counterpart of the increasing complexity of the internal circuitry and recognition units in the effector system. The immediate consequences were a progressive enlargement of the pathogen repertoire and new problems regarding self/not-self discrimination. Probably not by chance, a new organ appeared, capable of purging cells able of excessive self recognition. This organ, the thymus, appears to be the result of a well known evolutionary strategy of re-using pre-existing material (neuroendocrine cells and mediators constituting the thymic microenvironment). This bricolage at an organ level is similar to the effect we have already described at the level of molecules and functions of the defense network, and has a general counterpart at genetic level. Thus, in vertebrates, the conserved immune-neuroendocrine effector system remains of fundamental importance in defense against pathogens, while its efficiency has increased through synergy with the new, clonotipical recognition repertoire.

2. INTRODUCTION

2.1. The enigma of the immune system

Often the fundamental and basic questions and problems of different disciplines are neglected, while scientists concentrate on more restricted and well defined aspects. Even in the discussion of experimental papers, referees sometimes suggest removing the more general part of the discussion as this is only considered speculation. With few exceptions, biologists dislike speculation, believing it a sort of weakness in comparison to strong experimental data. In immunology, which was started by Jenner, and continued by Pasteur, Koch, Boehringer and others as applied biomedicine and microbiology, the result is that some basic questions have been neglected. This original sin has never been forgotten, and this imprinting has been and still is dominant. It is true that the non-medical part of immunology also had its pioneer, i.e. Metchnikoff, but his immunological perspective did not become dominant.

From an evolutionary point of view, immunology appears to present what has been called an enigma or "big bang", i.e., the sudden appearance of adaptive clonotipical immunity from lower vertebrates onward. In mammals, adaptive immunity reaches its maximum level, and, depending on the species, the immune system comprises two extraordinary repertoires of $10^{11\text{-}15}$ T and B lymphocytes, each mounting a receptor of different specificity. The usual interpretation of this phenomenon is that such a high variability in the molecular structures devoted to the recognition of epitopes gives a tremendous advantage in terms of recognition specificity. Consequently, it is assumed that such a sophisticated capability to recognize very small differences in the "shape" of antigenic molecules should guarantee a more specialized type of reaction, resulting in a more effective immune response. If this tenet were true, a much higher capability to cope with all sorts of infectious, potentially damaging agents should be present in vertebrates, and particularly in higher vertebrates, in comparison with invertebrates. As bacteria, fungi and viruses are the most important pathogens, we should expect that vertebrates have a tremendous advantage in terms of their ability to survive infections. However, this is apparently not the case, while the innumerable invertebrate species have survived remarkably well for millions of years despite the lack of a clonotipical immune system ! Thus, even taking into account other variables, such as, possibly, high reproductive capability, the following open questions are inevitable. What is the real advantage of a

clonotipical adaptive immune system? What is the selective pressure for the emergence of such a complex system ? Is this system really devoted to defense against external pathogens ? How have hosts and pathogens interacted to produce the present immunological scenario?

Usually it is assumed that pathogens are pathogens, forgetting the very simple fact that pathogens become pathogens only when there is a host to colonize, and only when this phenomenon gives the pathogens an evolutionary advantage. The first bacteria (chemotrophic) where devoid of pathogenicity simply because there were no hosts to colonize!

The relationship between hosts and pathogens is very complex and the history of their interaction is far from being clear. For instance, it is known that mammalian viruses usually can infect mammalian cells and become pathogenetic only for mammalian hosts. Moreover, their pathogenicity depends on receptor molecules on target cells. In any case, pathogenicity is the net result not only of the capability to enter into the body of the host, but also of the pathogen's ability to mount a reactive response. In this sense, it is not paradoxical to assume that the immune response itself is a basic component and an integral part of the pathogenetic pathway leading to body damage and, eventually, the death of cells and of the entire body. Bacteria- and virus-induced apoptosis is an example of the ancestral relationship between host and pathogen interaction.

3. THE IMMUNE-NEUROENDOCRINE EFFECTOR SYSTEM: THE ROLE OF THE MACROPHAGE

The above questions apparently have no convincing answers, and, indeed, we have the impression that immunologists have underestimated these problems, probably because they go to the core and challenge the role and the biological meaning of the immune system. Another more trivial explanation is that the scientific interest of most immunologists has been restricted to mammalian immunology, while other types of immune responses, such as those present in invertebrates, have been largely neglected and ignored.

We believe that a possible answer to these questions can be found by changing the paradigm and adopting a different perspective. In particular, the assumption that in vertebrates the adaptive clonotipical immune system is the most important system responsible for the defense against pathogens is an oversimplification. In the last 20-25 years, data have been published on the relationship between the immune system, on one hand, and the neuroendocrine system on the other. Profound functional and structural relationships between these systems have been shown (1-4). We have contributed to this topic with work on the evolutionary relationship between the immune and neuroendocrine systems, and studies of immune responses (cell migration and phagocytosis), stress response and inflammation throughout evolution from invertebrates to vertebrates. Our major findings are that all these phenomena appear to be mediated by a common pool of well conserved molecules, and that the macrophage is the main cellular actor. The central role of this cell is not arbitrary and suggests that these three types of responses are indeed deeply interconnected (5-7).

Even invertebrates are capable of very sophisticated immune and neuroendocrine performance. For instance, they are able to accept autografts and to reject allo- and xenografts (8, 9). Moreover, they are able to recognize foreign stimuli and build up integrated responses. The most striking phenomenon is that antigens or stress provoke an overlapping set of responses which include immune responses, stress and inflammation, concomitantly. Thus, these reactions, apparently aimed at the neutralization of stimuli perturbing body homeostasis, are ancestral. We have suggested that immune responses, stress and inflammation have been intermixed and interwoven since the beginning of evolution.

Indeed, we and others have found that molecules involved in the stress response in higher vertebrates, and particularly the products of pro-opiomelanocortin (POMC) and cytokines, are important actors in biological and immune responses all along the evolutionary path. A similar phenomenon has been described for other molecules, e. g. nitric oxide (NO), involved in inflammatory reactions (5-7, 10).

Immunoreactive (ir) adrenocorticotropin hormone (ACTH), irß-endorphin, irα-melanocyte-stimulating hormone (MSH), ircorticotropin-releasing hormone (CRH) molecules and ircytokines (IL-1, IL-2, IL-6, TNF) have been found in the immunocytes with phagocytic activity of several invertebrate species (7). Moreover, irACTH and irß-endorphin have been demonstrated in immunocytes of both lower and higher vertebrates. With regards the biological role of these molecules, we have shown that ACTH, ß-endorphin, CRH and cytokines, such as IL-1, IL-2 and TNF, are capable of affecting cell migration, and, apart from ß-endorphin, also of increasing phagocytic activity (7). Moreover, CRH, ACTH and cytokines are able to provoke the release of biogenic amines from phagocytic immunocytes (5).

In conclusion, our studies indicate that a simplified type of stress response appears to be present early in evolution, and the basic mechanisms are probably very well conserved, as the key mediator molecules are the same, i.e. CRH and ACTH, and the series follows the same order and pattern, i.e. CRH---> ACTH---> biogenic amines. In our invertebrate model, all the phenomena occur in immunocytes, a cell type we have proposed as "an immune-mobile brain", capable of both immune and neuroendocrine responses (5). The conservation of these ancestral types of stress response is probably the reason why the mammalian lymphocyte is still capable of responding to CRH and of releasing ACTH (2, 4).

We have also described the presence and biological role in invertebrates of another important molecule, NO, which is considered a chemical mediator of inflammation (10). We found that nitric oxide synthase (NOS) is present in phagocytic immunocytes (11). Following stimulation of the animals with both *Escherichia coli* or LPS, the immunocytes express an NOS inducible form. This enzyme activity is comparable to that observed in mammalian cells and is inhibited by the same NOS inhibitors. In addition, the role of NO in defense mechanisms has also been reported in invertebrates. The immunocytes of two molluscs, *Mytilus edulis* and *Viviparus ater*, produce a bacteriocidal substance that has been indirectly identified as NO (12).

On the basis of these findings, one cell from the macrophage lineage would appear to be the main actor in a coordinated series of events - immune responses, stress and

inflammation - elicited by a variety of stimuli that can damage the body. These reactions, sharing cells and mediators and overlapping each other, can be seen in a unitarian perspective. Immune response, stress and inflammation are as an integrated network, in which according to the type and the intensity of the stimulus, local conditions and anatomical constraints, and the evolutionary level of the species, one response can prevail over the other (7).

4. THE INVERTEBRATE RECOGNITION SYSTEM

As described in the previous paragraph, a very efficent network of defense based on the macrophage and on its capability to perform innate immune responses, such as chemotaxis and phagocytosis, and neuroendocrine responses, such as a proto-type stress response, has developed in invertebrates. The macrophage is also capable of secreting other molecules, such as NO and cytokines, capable of triggering an inflammatory response. An additional type of immune response against foreign intruders, cytotoxicity, is concomitantly present in invertebrates (13, 14). Thus, the efferent part of the defense network and its capability to kill foreigners and pathogens appear to be well developed and highly efficient in invertebrates. However, in order to activate this defense machinery, invertebrates should be able to recognize threatening agents and to discriminate between self and not-self. We and others have shown that this last basic requirement is present in invertebrates, even if the molecular basis of the discrimination is still unclear (15). In any case, it is reasonable to assume that the recognition in invertebrates is gross and far less sophisticated than vertebrates. Nothing similar to the specific clones of B and T lymphocytes is apparently present in invertebrates, and the ancestor molecules of Ig, TCR and MHC have not been clearly identified (16).

Even if very few data are available, we can speculate that the recognition system in invertebrates is set to recognize structural commonalities. As suggested by Medzhitov and Janeway (17), innate immunity is based on recognition of invariant modules in different bacterial and viral species. In this sense, the limited sensitivity of the recognition system is mirrored by the limited number of molecular structures which are recognized. Paradoxically, from an invertebrate point of view, we can say that there are, in effect, very few different bacteria or viruses, making the small number of recognition units (an extremely restricted repertoire) perfectly adequate for the small number of foreign modules to be recognized. In other words, the recognition capability of invertebrates is optimal and does not need an enlarged repertoire of recognition units, because a common module is recognized in the different pathogens. The limited capability of the intercellular communication system in invertebrates corresponds to an immune recognizing system with only a limited number of fundamental modules common to bacteria and viruses, and is optimized for a gross, but efficient discrimination between self and not-self. The organisms which do not fit the "visibility field" of this communication system (receptors) involving the cells of host cannot exert any pathogenic effect. Nevertheless, the reaction triggered by these limited number of recognition units is complex and efficient, as is based on the ancestral defense network described above (innate immunity, stress and inflammation).

This hypothesis can explain, at least in part, the above enigma, i.e., the fact that invertebrates survive quite well in dirty water and in polluted environments full of pathogens. Thus, a peculiar type of promiscuity allows invertebrates to recognize different pathogens. In this way, invertebrates are fully protected against a variety of pathogens, because they recognize all as one of a limited group and elicit an effective, standard type of response.

5. THE RELATIONSHIP BETWEEN RECOGNITION AND PATHOGENS

The promiscuous and gross recognition units in invertebrates probably underwent a process of increasing refinement in vertebrates, allowing the latter a finer recognition of self and not-self molecules. The relationship between pathogens and organisms changed when more sophisticated recognition units appeared in lower vertebrates. In these animals new, more complex circuitry and relationships between cells and organs probably emerged. We can speculate that receptors capable of a finer recognition appeared in most cells of the body in lower vertebrates and that they underwent a progressive refinement in higher vertebrates and mammals, allowing the recognition of a larger repertoire of ligands, including potential pathogens. Concomitantly, rudimentary clonotipical recognition units emerged, enlarging the ability to recognize more complex molecular structures and mount reactive responses. The difference between bacterial and viral species was progressively appreciated, and the number of potentially damaging agents increased with the increase in the receptor number and specificity. If this hypothesis is correct, we can assume that the progressive increase in the complexity of the lymphocyte repertoire is concomitant with increased receptor recognition. The selective pressure explaining the expansion of the clonotipical immune system in vertebrates is probably to identify in the enlargement of the repertoire of molecular structures recognized by receptors present on different cell types, including lymphocytes. We can envisage a sort of paradox where the progressive complexity of the recognition systems progressively increases both the number of different pathogens which are "seen" and the defense capability in proportion to the number of clonotipical recognition units present in the immune system. During evolution, organisms may then become progressively sensitive to an increased number of bacterial and viral species, which would exert a pressure to enlarge the lymphocyte repertoire, and so on. If pathogenicity of a variety of microorganisms is the consequence of the emergence and progressive enlargement of receptor specificity and the concomitant appearance of the clonotipical immune system. In other words, the increasing complexity of organisms is probably responsible for the increasing number of potentially damaging agents and the concomitant need to set up more sophisticated mechanisms to cope with these.

Thus, the reason for a clonotipical immune system would be the increasing complexity of the organisms, which, in turn, requires a more sophisticated type of circuitry and recognition units. It is interesting to

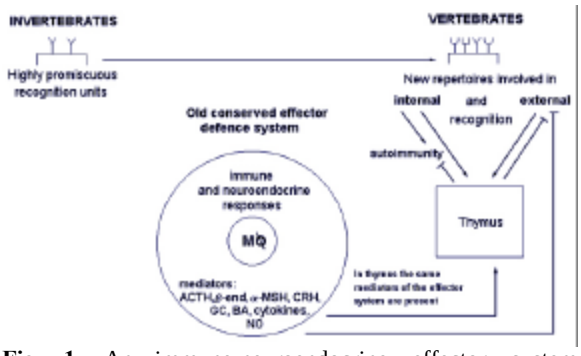

Fig. 1. An immune-neuroendocrine effector system integrating innate immunity, stress and inflammation is present in invertebrates and conserved throughout evolution. This defense network, centered on the macrophage, exploits primitive and highly promiscuous recognition units which underwent an explosive enlargement and refinement from lower vertebrates onward. A new organ, the thymus, concomitantly appeared to cope with new problems of self /not-self recognition. Abbreviations: ACTH, adrenocorticotropin hormone; ß-end, ß-endorphin; α–MSH, α–melanocyte-stimulating hormone; CRH, corticotropin-releasing hormone; GC, glucocorticoids; BA, biogenic amines, NO, nitric oxide.

note that the increase in the complexity of the immune recognition system is a combinatorial one.

6. THE INCREASING COMPLEXITY OF THE RECOGNITION SYSTEM

As we argued in previous paragraphs, the effective arm of the immune system is well conserved throughout evolution, while the recognition system has become much broader and more sophisticated. If we exclude that the main driving force of this change is the need to cope with pathogens, the main evolutionary reason for the expansion of the recognition system may be extra immunological. It has been suggested that this primordial system of variable region molecules, related to the cell adhesion molecules, developed with the first vertebrate by an endogenous, self-organizing process, the principal function of which was to contribute to the integration of the internal molecular environment (18). We have provided evidence in favor of the hypothesis that an integration of immune and neuroendocrine functions was even present in invertebrates and led to an integrated defense system (innate immunity, stress and inflammation) (7). As far as we can see, there was no need to create other effector systems, and, indeed, these original mechanisms have been conserved and are still operating in all vertebrates. In fish, three, new, major immunological phenomena occur: the appearance of a new immunological organ, i.e. the thymus; the set up of a lymphocyte repertoire and the formation of antibodies; and the appearance of MHC. Extending Steward's suggestions (18), we can illustrate the critical changes which occurred from invertebrates to the first vertebrates as in figure 1. We assume that the initial driving force to enlarge the recognition system was the need for better recognition of cells and molecules in the effector defense system, so as to improve integration of their constitutive circuits and achieve better regulation and connection with the primitive recognition system and its receptors.

This expansion of the recognition units generated two additional problems: on the one hand, the increased capability to recognize external molecules improved the global efficiency of the defense network, but paradoxically increased the number of potential pathogens, on the other, the danger of self-recognition and self-destruction emerged. Strategies to avoid this possibility became urgent, and a new organ was created to perform both tasks, i.e. purge recognition units from cells capable of excessive self-recognition and maintain those capable of reacting to foreign molecules. This organ is the thymus, which, not by chance, is a neuroendocrine organ presenting all the components to perform a local immune/inflammatory/stress response (19, 20). All the cellular and molecular components to build such a new organ were present in invertebrates, suggesting that evolution made use of a well known strategy of re-using pre-existing material. This phenomenon can be considered an example of bricolage at an organ level, similar to that which we and others have described throughout evolution at the level of molecules and genes (7, 21, 22). Thus, we can speculate that a well established cascade of integrated biochemical events was re-used to set up the thymic microenvironment and to perform new tasks, such as the new type of discrimination between self and not-self, which occur from the first vertebrate onward. In particular, it is interesting to emphasize that in vertebrates, there is evidence suggesting that glucocorticoids, the final effectors of stress response, play a major role in the fine tuning of the thymic T cell repertoire by regulating positive and negative selection (23).

7. THE INTEGRATION BETWEEN THE OLD EFFECTOR SYSTEM AND THE NEW REPERTOIRES OF RECOGNITION UNITS

As depicted in figure 1, during evolution, the old effector mechanisms merged with the new sophisticated recognition system. In this way, the efficiency of the ancestral, conserved defense machinery was greatly increased, as it could utilize an extensive and very specific repertoire of clonally distributed recognition units. Examples of this synergistic merging include bacteria opsonization and antibody mediated cytotoxicity.

This scenario predicts not only that stress and inflammation are an integral part of defense reactions, but that they are activated and finely regulated during the clonotipic immune responses in vertebrates.

Thus, the final version of the defense system in vertebrates appears to be the combination of two systems: the first is represented by the network of innate immunity, inflammation and stress constituted by the same basic ingredients and mediator molecules that have been well conserved during evolution; the second is the joint T and B cell repertoires. The latter grew from a few genes and gene-segments to create a large repertoire of receptors by recombination mechanisms. In this way, the efficiency of a strong ancestral effector system is combined with the sophistication of recognition repertoires able to appreciate very small differences in the three-dimensional forms of molecules from the internal and external worlds.

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