How Evolution Tells Us To Induce Allotolerance

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Abstract

Modern immunology, in many ways, is based on 3 major paradigms: the clonal selection theory (Medawar, Burnet; 1953/1959), the pattern recognition theory (Janeway; 1989), and the danger/injury theory (Matzinger, Land; 1994). The last theory holds that any cell stress and tissue injury including allograft injury, via induction of damage-associated molecular patterns, induces immunity including alloimmunity leading to allograft rejection. On the other hand, the concept precludes that “non-self” per se induces immunity as proposed by the two former theories.

Today, the danger/injury model has been largely accepted by immunologists, as documented by a steadily increasing number of publications. In particular, overwhelming evidence in support of the correctness of the model has come from recent studies on the gut microbiota representing a huge assemblage of “non-self.” Here, harmless non-injurious commensal microbes are protected by innate immunity-based immune tolerance whereas intestinal injury-causing pathogenic microbes are immunology attacked.

The ability of the immune system to discriminate between harmless beneficial “non-self” to induce tolerance and harmful life-threatening “non-self” to induce immunity has apparently emerged during evolution: Protection of innate immunity-controlled beneficial “non-self” (eg, as reflected by microbiotas but also by the fetus of placentals mammals) as well as immune defense responses to injuring/injured “non-self” (eg, as reflected by plant resistance to biotic and abiotic stress and allograft rejection in mammals) evolved under pressure across the tree of life, that is, in plants, lower and higher invertebrates as well as lower and higher vertebrates.

And evolution tells us why the overall existence of protected microbiotas really makes sense: it is the formation of the “holobiont,” - a metaorganism - that is, the host plus all of its associated microorganisms that - in terms of a strong unit of selection in evolution - provides that kind of fitness to all species on earth to successfully live, survive and reproduce. In other words: “We all evolve, develop, grow, and reproduce as multigenomic ecosystems!

Regarding reproduction, another impressive example of active immunologic protection of “non-self” refers to pregnancy in placentals mammals that emerged about 400 millions of years ago. Similar to “non-self” microbiotas, pregnancy in placentals mammals reflects an evolution-driven phenomenon on the basis of innate immunity-controlled tolerance induction to semiallogeneic non-injuring/non-injured “non-self” aiming to ensure reproduction!

Altogether, the lesson learned from evolution of how to avoid allograft rejection is clear: prevent allograft injury to induce allotolerance, in other words: create a “transplant holobiont.”

Key words: Innate immunity, Evolution, Immunologic tolerance
Background

The danger/injury model

Modern immunology in many ways is based on 3 major paradigms. The 2 traditional models (clonal selection theory and pattern recognition theory) both state that microbial “nonself” induces immunity, which is “the science of self/nonself discrimination.”

More recently, the danger/injury theory states that injury induces immunity. The latter model was developed from 2 sources. At 20 years ago, (1) its discovery by our group during a clinical trial in kidney transplant patients provided convincing evidence that tissue injury (referred to in that article as allograft injury) induces immunity (alloimmunity-mediated allograft rejection), and (2) its publication by Polly Matzinger as a self-coherent chain of arguments resulting in the stringent conclusion that the self/nonself discrimination theory of immune responses must be inappropriate.

After the discovery of the innate immune system in the late 1990s, the danger/injury model was modified several times. Today, its core refers to the scenario that pattern recognition receptors (PRRs) expressed on or in cells of the innate immune system recognize microbe-associated molecular patterns (MAMPs) and danger/damage-associated molecular patterns (DAMPs). The DAMPs (a term coined by those 2 groups in the early 2000s) are endogenous molecules that normally are hidden from recognition by the immune system, but are induced by any tissue injury or cell stress. As reviewed previously, DAMPs are released from dying cells or damaged extracellular matrix as high mobility group box 1 (HMGB1), nucleic acids, extracellular adenosine triphosphate (ATP), and hyaluronan; they also can be exposed on stressed cells such as major histocompatibility complex class I chain-related proteins (MICs) and injury-induced altered-self antigens (neoantigens) which bind to natural immunoglobulin M (IgM), thereby activating the complement cascade.

According to current theories, cell stress/tissue injury-induced DAMPs, following recognition by PRRs, trigger innate immune pathways, resulting in a “sterile” inflammatory response. In the presence of nonself antigens such as microbial or allogeneic antigens, dendritic cells (DCs) become activated to elicit an adaptive immune (alloimmune) response (Figure 1). The key event of an emerging adaptive alloimmune response resulting in allograft rejection is the DAMPs-induced maturation of PRRs-bearing, donor- and recipient-derived, immature DCs to allostimulatory human leukocyte antigen (HLA)/non-HLA-presenting DCs. The latter DCs are able to migrate to the secondary lymphoid tissue of the recipient to activate naïve alloreactive T lymphocytes. This has been reviewed previously.

The danger/injury model also inherently precludes that nonself per se induces immunity, but in contradistinction, induces immunologic tolerance (Figure 1). As reviewed previously, in the absence of injury, presentation of harmless nonself antigens under noninflammatory subimmunogenic conditions, promotes generation of tolerogenic DCs (tolDCs) that are able to induce regulatory T cells (Tregs) dedicated to promote induction of immunologic tolerance.

The mammalian gut microbiota

The danger/injury model currently has been widely accepted by immunologists. Recent new insights into the role of the mammalian intestinal microbiota in maintaining immune homeostasis - in terms of a fascinating inversion of the self/nonself discrimination view of immunology - have underscored...
the correctness of the concept. The new paradigm in immunology has been shifted to the recognition that it is not the molecular structure of pathogens that activates the immune system, but rather, their perturbation of and damage to generic cellular processes. More than 100 trillion harmless commensal microorganisms (total, 1.5 kg) colonize the human oral cavity and gastrointestinal tract, outnumbering human cells by a ratio of 10:1. The composition and activity of this resident nonself play crucial roles in shaping the metabolic and regulatory networks that define good human health. Its effect on human wellbeing, which cannot be overemphasized, is mediated by a tremendous repertoire of microbial genes (microbiome) that perform myriad functions beneficial to the host, such as the development of gut-associated lymphoid tissues (GALTs), as reviewed previously. The microbiota play a pivotal role in the development of T cells, both within and outside the intestine, by selectively expanding and activating different T cell subsets under normal and/or pathologic conditions. In turn, intestinal innate immune responses that are induced by commensal populations regulate the composition of the mammalian microbiota, pointing to a complex interplay between the host immune system and the microbiota that is necessary for gut homeostasis. Toll-like receptors (TLRs) are crucial for maintaining tolerance to and shaping the assemblage and composition of commensal microbiota. Moreover, the mammalian gut innate immune system is able to discriminate (under the control of DCs and regulated by innate immune PRRs including TLRs and NOD-like receptors [NLRs]) between harmless nonself such as food antigens and commensal bacteria (to induce tolerance) and harmful nonself such as pathogenic bacteria, viruses, and fungi (to induce immunity). The extraordinary homeostasis is maintained by the generation of tolDCs to induce Foxp3-expressing Tregs, thereby maintaining mucosal tolerance and, in contrast, generation of immunostimulatory DCs to promote induction of Th1 and Th17 cells, thereby mounting immune responses, as reviewed previously (Figure 2). Hence, nonself antigens of gut commensals are not simply ignored, but rather, they trigger an active immunosuppressive process to maintain intestinal homeostasis. Accordingly, the immune system appears to be more a “bouncer at a nightclub,” rather than a defensive army to keep our organism “pure” from microbes. The immune system actively tolerates and recruits, farms, and protects the nonself symbionts that do not cause injury.

**Figure 2.** Scenario Model of the Complex Interplay Between the Mammalian Immune System and the Microbiota

**Abbreviations:** cT cell, conventional T cell; DAMPs, damage-associated molecular patterns; DC, dendritic cell; immunostimul., immunostimulatory; MAMPs, microbe-associated molecular patterns; PRRs, pattern recognition receptors; Th1, T helper 1 cell; Th17, T helper 17 cell; Treg, regulatory T cell

The innate immune system is able to discriminate, under the control of dendritic cells, and regulated by innate immune PRRs, between (1) harmless “nonself” (commensal bacteria) to induce Treg-mediated tolerance and (2) harmful nonself (pathogenic bacteria, viruses, and fungi) to induce Th1/Th17-mediated immunity. The extraordinary homeostasis is maintained by the generation of tolerogenic DCs to induce Tregs, thereby maintaining mucosal tolerance and, in contrast, generation of immunostimulatory DCs to promote induction of Th1 and Th17 cells, thereby mounting immune responses. Note: Presentation of microbial antigens by DCs to T cells is illustrated in terms of signal 1; signal 2 refers to costimulatory molecules, and signal 3 refers to secretion of Th1-/Th17 cell polarizing cytokines.
The danger/injury model in light of evolution

Tolerance to/protection of nonself in the absence of dangerous injury

Evolutionary biologists tell us of the ubiquity of nonself microbial associations that is not peculiar to mammals including humans. Rather, tolerance to/protection of harmless nonself is the normal condition for all creatures across the tree of life including plants, sponges, lower and higher invertebrates, and lower and higher vertebrates. Symbiosis is the rule and not an exception in the plant and animal kingdom. This recognition of biologists began with the work of Woese and Fox who, in the late 1970s, opened a new research frontier by producing sequence-based measures of phylogenetic relations, revealing the deep evolutionary history shared by all living organisms. This innovative concept led to a rapid development and application of molecular sequencing technologies, which allowed biologists for the first time to recognize the true diversity, ubiquity, and functional capacity of microorganisms. This recognition, in turn, has led to a new understanding of the biology of plants, animals, and bacterial kingdoms that have coevolved and coadapted in response to environmental selective pressures over hundreds of millions of years, thereby allowing emergence of microbiomes. Animals, for example, can no longer be considered individuals in any sense of classic biology: anatomic, developmental, physiologic, immunologic, genetic, or evolutionary.

Bacteria and the origin of animals

To properly cope with this issue, we first must understand the periods when nonself associations among bacteria and animals first evolved. The last common ancestor of plants and animals may have lived approximately 1 billion years ago. As reviewed previously, animals diverged from their protistan ancestors 700-800 million years ago, 3 billion years after bacterial life originated, and 1 billion years after the first appearance of eukaryotic cells. Thus, the current relations between protists and bacteria, from predation to obligate and beneficial symbiosis, probably were operating already when animals first appeared. Sponges (animals of the phylum Porifera) harbor hundreds of bacterial species. As early animals further diversified, animal-bacteria interactions continued to shape evolution in new ways, such as training and educating ancient animals to establish sophisticated metabolic pathways and efficient innate immune pathways that allowed immune-mediated host defenses as observed in sponges, cnidarians, and insects.

The long history of shared ancestry and alliances between animals and microbes is reflected in their genomes. As reviewed previously, analysis of the large number of full genome sequences presently available reveals that most life forms share approximately one-third of their genes, including those encoding central metabolic pathways. Many animal genes are homologues of bacterial genes, mostly derived by descent, but occasionally by gene transfer from bacteria. In 37% of the 23,000 human genes, there are homologues in the Bacteria and Archaea, and another 28% of the genes originated in unicellular eukaryotes. Among these homologous genes are some genes whose products provide the foundation for signalling between extant animals and bacteria, thereby allowing communication between microorganisms and their hosts.

Tolerance to/protection of beneficial nonself microbes

In accordance with the long history of shared ancestry and alliances between animals and microbes, evolutionary biologists provide increasing evidence suggesting that induction and maintenance of tolerance to/protection of harmless (beneficial) nonself (exemplified by resident microbiotas) reflect an evolution-driven paradigm in immunology that is controlled actively by components of the innate immune system. There are 2 examples from evolution that are addressed here.

As shown in studies on transgenic Hydra polyps with an altered antimicrobial peptide (AMP) repertoire or silenced TLR/MyD88 activity, components of the innate immune system of the cnidarian Hydra (a 3-cm small animal) are involved in maintaining homeostasis between the animal and its nonself resident microbiota. Toll-like receptor-controlled innate immune pathways lead to secretion of AMPs with regulatory functions in host-microbe homeostasis to protect beneficial and coevolved microbes, whereas bactericidal peptides may kill pathogenic microbes (Figure 3).

As argued in relation to observations from Hydra, AMPs appear to be key factors for host-bacteria coevolution. Thus, the role of TLRs in controlling the resident microbiota could date back to the earliest
multicellular organisms, because humans and Hydra share molecules involved in the TLR signaling cascade such as the Toll/interleukin-1 receptor (TIR) domain.

Similarly, innate immune recognition proteins that recognize bacteria-derived MAMPs control and maintain resident microbes in *Drosophila melanogaster*. Protective immune tolerance to the commensal microbiota is maintained via the peptidoglycan recognition protein SC2, a negative regulator of IMD/Relish innate immune signalling. In contrast, this pathway, when triggered by another set of recognition receptors (PGRP-LE and -LC), leads to production of AMPs that kill injury-inducing pathogenic microbes (Figure 3).19-22

Evolution tells us why the overall existence of microbiotas under the control of the innate immune system makes sense. According to the hologenome concept of evolution,23 our bodies must be understood as “holobionts” whose anatomic, physiologic, immunologic, and developmental functions evolved in shared relations between different species. The formation of the “holobiont,” a metaorganism, (the host [“self”] plus all its associated and integrated microorganisms [nonself]) provides – in terms of a strong unit of natural selection in evolution – that kind of fitness to all species on earth to successfully live, survive, and reproduce. We all develop, grow, and evolve as multigenomic ecosystems, armed with an immune system directed against sterile and infectious injuries. In fact, one may conclude that we have never been individuals!

Tolerance to/protection of semiallogeneic nonself, the fetus in placental mammals

Formation of host (self)-microbiota (nonself) holobionts is 1 example of how evolution works. Another example of active immunologic protection of nonself is pregnancy of eutherian placental mammals, which can be regarded as a host (self)-semiallogeneic (nonself) holobiont that emerged during the Devonian era approximately 380 million years ago,24 in an era when evolution already had created host-microbiota holobionts millions of years before placental mammals appeared. Emergence of tolerance to a nonself fetus in placental mammals

Figure 3. Proposed Evolutionary Model of Microbiota (oversimplified diagram based on first reports from the literature)

Abbreviations: AMP, antimicrobial peptide; DAMPs, damage-associated molecular patterns; MAMPs, microbe-associated molecular patterns; MSU, monosodium urate; PGRP, peptidoglycan recognition protein; PRRs, pattern recognition receptors; TLR, Toll-like receptor

Active control of microbiota composition by PRRs of the innate immune system, exemplified by studies on *Drosophila melanogaster* and *Hydra* polyp. In *Hydra*, a protective response to commensal populations is supposed to be provided by TLR-controlled innate immune pathways leading to secretion of AMPs with regulatory functions, whereas DAMPs such as monosodium urate and foreign RNA have been shown to elicit a strong innate immune defense response, as reflected by upregulation of the bactericidal peptide periculin-1. In *Drosophila*, a protective immune tolerance to the commensal microbiota is reportedly maintained via the PGRP-SC2, a negative regulator of IMD/Relish innate immune pathway, whereas another set of recognition receptors, the PGRP-LE and PGRP-LC, trigger the IMD/Relish pathways leading to production of bactericidal AMP that can kill injury-inducing pathogenic microbes; cell death-associated DAMPs such as proteases presumably operate as ligands of PRRs (as shown in other sets of studies).19-22

In the Interest of Evolution

Tolerance to noninjurious nonself in favor of an individual’s fitness and efficient reproduction; scenario model of the creation of a “triple holobiont” of pregnant women.

Figure 4. In the Interest of Evolution
may reflect the flexibility in evolution to use, as a blueprint, the more ancient innate immunity-controlled mechanisms to induce tolerance to commensal microbes, creating a “triple holobiont” in pregnant woman (Figure 4). Allogeneic pregnancy represents a physiologic situation in which tolerance to paternal alloantigens is critical for successful reproduction of placental mammals, serving survival of a species as a stringent imperative of evolution. Multiple mechanisms that operate during pregnancy to limit responses of maternal alloreactive T cells to fetal alloantigens have been described, but induction of Tregs appears to play a key role. There is compelling evidence in favor of an evolutionary selected mechanism of Treg-cell–mediated maternal-fetal tolerance gained during evolution of eutherian mammals. In analogy to the capacity of Tregs to mediate tolerance against nonself antigens of the microbiota, extrathymic generation of Tregs in placental mammals may have arisen to mitigate maternal-fetal semiallogeneic incompatibility, a scenario primarily driven by the evolutionary pressure to enforce maternal-fetal tolerance. Recent studies in human pregnancy revealed a significant increase of highly suppressive HLA-DR+ Tregs in the decidua.

In accordance with this scenario, there is increasing experimental data suggesting that the induction of Tregs during pregnancy is controlled by the innate immune system via generation of tolDCs, a mechanism that also is described for composition and maintenance of the mammalian microbiota. As reviewed previously, during normal pregnancy, most human and murine decidual DCs, under the effect of pregnancy-associated hormones, present a tolerogenic phenotype and mainly produce interleukin 10. In line with this, spontaneous abortions in humans and mice are associated with an increased number of immunostimulatory DCs. The DCs are highly susceptible to hormonal stimulation by expressing receptors for progesterone, estradiol, human chorionic gonadotropin, and luteinizing hormone. Accordingly, hormonal stimulation of activated bone-marrow–derived DCs resulted in most studies in an impaired upregulation of major histocompatibility complex class II molecules and costimulatory molecules associated with a reduced capability to secrete proinflammatory cytokines. In addition, a role for PRRs in controlling these innate immune mechanisms has been addressed.

Immunity/resistance to nonself in the presence of dangerous injury
As mentioned above, the danger/injury model includes the proposal that injury, via induction of DAMPs (and not nonself per se) elicits immunity. There are examples from evolution in support of this proposition, and 3 examples will be addressed briefly in the following subsections, the first dealing with plants.

Evolutionary role of DAMPs in plants
Plants are complex organisms. Their sessile lifestyle forces them permanently to monitor their surrounding hostile environment to identify dangerous stimuli and stressors that originate from attackers. Since plants do not have an adaptive immune system to defend against all those enemies, they initiate defense by relying solely on a sophisticated innate immune system that bears similarities with animal innate immune systems, but which probably evolved independently. The plant defense system recognizes microbial elicitors, such as MAMPs, herbivore-associated molecular patterns (HAMPs), and DAMPs that are released upon tissue injury caused by pathogenic microbes and other nonmicrobial inciting conditions. As reviewed previously, prominent examples of plant-derived DAMPs include cell wall fragments of larger plant molecules that are formed upon damage, such as oligogalacturonide fragments, resistance-inducing oligosaccharides, cell-wall-derived pectins, and endogenous proteins such as systemin and volatile organic compounds (VOCs). Certain immune responses of plants that are directed against herbivores can indirectly defend plants by changing the behavior of the natural enemies of the herbivores. An indirect defense is the emission of herbivore-induced plant volatile compounds that are specific VOCs that attract predators and parasitoids of insect herbivores to the attacked plant.

Plant biologists previously believed that this indirect defense response was directed against nonself, such as the mucus of the caterpillar. However, this is incorrect. Early studies were performed on a mechanical artificial caterpillar (Mec Worm) that could cause permanent damage during a prolonged wounding period. The use of this device mimicked the spatiotemporal feeding patterns of living herbivores that cause the lima bean (Phaseolus lunatus) to release a blend of VOCs as an indirect
defense response that resembled what was observed after insect feeding on the same plant. These experiments demonstrated that the effect of mechanical wounding (that is, sterile injury) on the induction of defense responses during herbivore feeding was substantial. More recent studies extended these early observations. Flame wounding or applying leaf extract or solutions of sucrose or ATP to slightly wounded lima bean leaves induced an indirect defense mechanism.

Evolutionary role of DAMPs in invertebrates

Another evolutionary observation in support of the concept that injury induces immunity refers to studies on the cnidarian Hydra polyps that were either exposed to monosodium urate for 24 hours or to foreign dsRNA introduced by electroporation, both known as typical DAMPs released from dying mammalian cells. Exposure to these 2 DAMPs elicited a strong innate immune response, reflected by a significant up-regulation of the expression of periculin-1, a novel antimicrobial bactericidal peptide of Hydra (Figure 3). In addition, there is increased evidence in support of the theory that the injury-inducing immunity concept has evolved in Drosophila melanogaster. In most gram-positive bacteria and fungi, recognition of MAMPs by extracellular PRRs of D. melanogaster leads to secretion of microbial/fungal cytotoxic proteases that operate as danger signals to trigger the Toll pathway via activation of the endogenous Toll ligand Spätzle. Spätzle can be regarded as a protease-induced DAMP that, in cooperation with MAMPs, elicits an innate immune response against pathogens. This theory is supported by recent reports that showed that wounding, which leads to a sterile injury-induced innate immune response in the fly, is associated with upregulation of serine protease activity.

Further support of the concept includes more recent studies in D. melanogaster that showed that cell-death-induced production of DAMPs, such as certain proteases, leads to a Toll signalling pathway through a persephone-mediated proteolytic cascade that cleaves the Toll ligand Spätzle (Figure 3). A more recent report referred to the range of wound-induced immune responses that can be modelled in flies. These wounding models have revealed the most immediate signals leading to immune cell activation, and highlighted a number of complex signalling cascades required for subsequent injury-associated inflammatory responses in flies. These recent results from studies in insects indicate that a Toll-like receptor-triggered defense pathway, elicited by injury-induced DAMPs, is highly conserved in invertebrates.

Role of DAMPs in parturition

Tolerance to/protection of semiallogeneic nonself, the paternal antigens, in placental mammals may represent an active evolution-driven process of the innate immune system, and not just ignorance of foreign antigens. However, evolution in reproduction continues when the fetus is mature, such as in disrupting the tolerant state. At term, evolution has solved the problem by again harnessing the function of the innate immune system by using injury-induced DAMPs. There is increasing evidence indicating that human preterm or term parturition reflects a process of disruption of the fetal tolerance state by an infectious or sterile-injury-induced maternal innate immune response.

Human parturition reportedly is initiated by an inflammatory innate immune response elicited by DAMPs derived from fetal or amniotic fluid such as HMGB1 in preterm gestation or fetal DNA at term. These DAMPs, presumably induced by mechanical stretch associated with oxidative stress to the myometrium (possibly hypoxia-induced), enter the uterine vessel circulation (a common physiologic mechanism at term prior to labor) to reach innate immune cells within the uterine wall. Thus, in term parturition, creation of sterile uterine inflammation mediated by proinflammatory mediators produced by DAMP-activated infiltrating leukocytes causes uterine contraction, labor, and delivery, as reviewed previously (Figure 5). Therefore, this is an evolution-driven event that mirrors the vital interest of evolution. It is an injury-induced innate immune process that initiates human parturition, aiming at successful reproduction of the human species, and is a process that may be interpreted as the innate immune-mediated rejection of semiallogeneic nonself. This scenario is reminiscent of injury-induced innate/adaptive immune-mediated allograft rejection.

Outlook

Does knowledge and information about evolution have an effect on currently applied organ transplant? Can the facts emerged during evolution be applied
to new strategies and efforts to prevent allograft rejection and induce allotolerance?

To properly answer this question, we must understand the periods when nonself associations between bacteria and animals evolved. We must realize that organ transplant was introduced and developed by surgeons 100 years ago, a small period compared with the 800 million years when animals evolved. Thus, there is no reason to assume that, during this ultrashort period, evolution could change its ancient pattern to actively tolerate and protect noninjurious nonself under the control of innate immunity, such as in the human gut microbiota and the mammalian fetus. Furthermore, during this ultrashort period, evolution did not create an extra new immune defense system against allografts, that inherently represent tissue injury-associated nonself, that is different from the immune system that functions as an evolutionarily evolved, very efficient host defense against infectious pathogens that also are tissue injury-associated nonself.

Hence, tolerance to nonself in the absence of injury, and immunity to nonself in the presence of injury are evolution-driven paradigms in immunology. Therefore, allografts are not rejected because they are foreign nonself. Allografts are rejected because they are injured organs. The lesson for transplant clinicians, to be learned from evolution, about how to avoid allograft rejection is plausibly clear. It is important to prevent allograft injury to induce allotolerance and create a “transplant holobiont.” There are various strategic tools to approach this goal as has already been discussed elsewhere.3,7

References


