

The gut: beyond immunology

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The immune system is characterized by the ability to distinguish self from non-self. The intestinal immune system bears this latter property but, furthermore, it must discriminate among nutritious and beneficial substances from toxic or harmful ones. Considering that the gut has to be colonized by commensal bacteria participating in digestion as well as in the control of pathogen microorganisms, it is not surprising that mucosal surfaces are the largest and probably the most exquisitely specialized immune system's compartment. This means that not only innate and adaptive immunity are present, but further, particular structures, cells, and mechanisms such as physical barriers, epithelia, Peyer's patches, M cells among others, which together are involved in the dynamic control of the homeostasis between gut and its flora. The present review deals with some popular conceptions about the digestive system with particular emphasis on the gut's immunology.

Key words: Mucosal immunity. Gut. Commensal flora. Yoghurt,

El intestino: más allá de la inmunología

El sistema inmunitario se caracteriza principalmente por la capacidad de distinguir lo propio de lo extraño. En el intestino, el sistema inmunitario no sólo comparte esta propiedad, sino que es, además, apto para seleccionar los componentes nutritivos y/o benéficos de aquellos que pueden ser nocivos. Si se considera que el intestino es colonizado por bacterias comensales que contribuyen a la digestión y al control del crecimiento de microorganismos patógenos, no es sorprendente que sea la superficie mucosa el más extenso y, acaso, el más sofisticado de los compartimientos del sistema inmunitario. Así, las respuestas innata y adaptativa se suman a estructuras, células y mecanismos exquisitamente especializados, tales

como el epitelio intestinal, las placas de Peyer, las células M, entre otros, que en conjunto son responsables del control dinámico de la homeostasis entre el intestino y su flora. La presente revisión versa sobre algunos conceptos populares sobre el aparato digestivo y hace hincapié en el papel del intestino como órgano inmunitario.

Palabras clave: Inmunidad de mucosas. Intestino. Flora comensales. Yogur.

Introduction

The gastrointestinal tract is a continuous tube that includes the mouth, esophagus, stomach, small intestine, and colon. It has 4 fundamental functions: *a)* to break foods down physically into small pieces; *b)* to reduce them chemically to their basic constituents –fatty acids, aminoacids, and sugars–; *c)* to absorb these molecules into the bloodstream; and *d)* to eliminate the undesirable residues. In a sense, the digestive tract is an inner extension of the body's exterior; it segregates nutrients from our true insides until those components are fit for our use. This is clearly an essential work, and practically all animals have some version of this tube. There are, however, anatomical developments that have given us the nutritional cushion to be warm-blooded, active, and cerebral animals. The cultivation and improvement of crops, cooking –which increase the digestibility of many foods–, and even the scientific investigation of nutrition, can all be considered cultural adaptations to the same end. Food and digestion are certainly an inseparable *duo* since the beginning of mankind. Thanks to the witty masterpiece on the appreciation of food, *The Physiology of Taste*, edited in 1825, Jean Anthelme Brillat-Sauvarin is now one of the immortals in the pantheon of gastronomy. On the other hand, digestion and particularly its final disposals have been matter of interest of popular customs. Thus, in Catalonia, during Christmas holidays, the Spanish *belén* (Nativity scene) has a special hallmark: a little guy called the *caganer* (“shitter”) hidden in the creche which is considered of good luck to those who find him. Therefore, among the sheeps, cows, shepherds, the Three Kings, baby Jesus, Virgin

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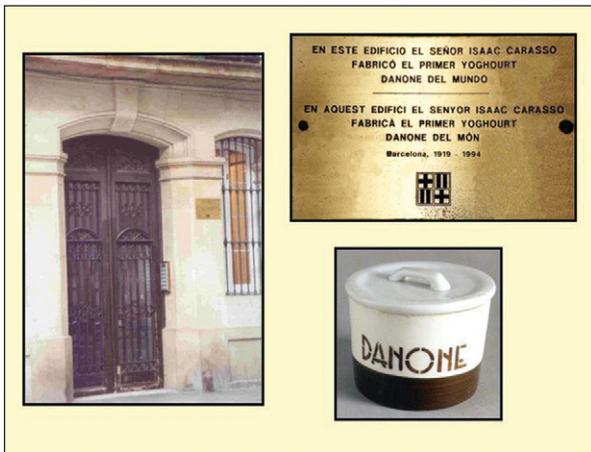


Figure 1. The human consumption of lactobacilli fermented milk dates from immemorial times, at least 2000 years, mainly in minor Asia and in the near Orient but particularly in the Balkans. Moreover, in the Bible, Abraham attributed his longevity and fecundity to “angel’s milk”. The term yoghurt has its origin from the turkish word “yogurt”, wich means long life. In spite of its long life, the first branded yoghurt was produced until 1919 in a modest factory located in carrer dels Àngels at Barcelone’s heart district. It was then that Isaac Carasso founded the Danone company, although the enterprise’s beginning in Spain was difficult, for at that time, yoghurt was considered a medicinal product. In fact during a gastroenteritis epidemic, yoghurt was prescribed to children of Barcelone’s popular neighborhoods. The royal family became aware of the benefits of yoghurt and thus Danone’s sales and its prestige increased. Carasso’s son, Daniel, was the one who made Danone a big enterprise first in Paris and later in New York. The commemorative plaque at Carrer dels Àngels literally reads in both, Spanish and Catalan: “In this building Mister Isaac Carasso elaborated the first yoghurt Danone in the world”.

Mary and Saint Joseph, is this little guy squatting with his pants around his knees taking a dump. Certainly, Jesus is the most saintly figure in the nativity scene, but the *caganer* is the most popular. According to the tradition, the *caganer* is a farmer engaged in one of the most primary and vital human function, to eat and then enrich the earth with his waste. Beyond this tradition there is an incommensurable number of jokes, sayings, and proverbs worldwide surrounding this theme. Digestion and related items have prevailed up to this century as a motif of inspiration for several artistic expressions. Thus, the notable Brazilian writer Rubém Fonseca in *Secreções, Excreções e Desatinos* (“Secretions, excretions and madnness”) (2002) introduces the new “science” of copromancy aiming at reading fortunes by means of stool examination, while the Belgian conceptualist Wim Delvoye’s sculpture *Cloaca*, exhibited during 2002 in the New Museum of Contemporary Art at Manhattan, was a complex installation that resembled the human digestive tract. Constructed with a variety of glassware, pumps and tubes, this artifact must be fed with nutritious meals twice a day. It then digests and twice a day eliminates its final product.

Scientists have not been excluded from this particular curiosity of the digestive system (*vide infra*).

The human digestive tract harbours bacterial populations most of them in the lower reaches of the intestine and colon. It is sterile at birth, and it is colonized gradually by microorganisms from the mother and from the environment. This colonization begins with lactic acid bacteria, enterobacteria and streptococci. From weaning onwards, anaerobic bacteria increase both in number and diversity until a flora similar to that of an adult is established. Since it is innocuous, it is considered commensal flora and further, whenever there is evidence that it is beneficial to its host it can be called symbiotic. However, commensal microorganisms might be potentially pathogenic. In fact, only minimal changes, e.g. a single mutation, are required to change from commensal to pathogen.

As it happens, misconceptions about the nature of the digestive tract and its bacteria are for one of the more durable claims to the secret to health. This was introduced by Elie Metchnikoff in the early 20th century, the century of immunology. Taking his lesson from Charles Darwin, Metchnikoff pointed out in his book *The Nature of Man: Studies in Optimistic Phylosophy* (1904) that evolution does not guarantee perfection, that most organisms survive in the world despite various imperfections and disabilities. And man is one species that is in particularly serious disharmony with nature. The pain of childbirth is one of many “disharmonies of reproduction”; both suicide and love of life in the face of necessary death are symptoms of the “disharmonies of self preservation”. Then, there are the “disharmonies of digestion”, the principal example being the colon, which is useful for animals that live on bulky plant foods, but which is “certainly useless in the case of man”. Worse than useless: positively harmful. For it harbours great number of bacteria, some of which produce toxins and slowly poison the body. According to Metchnikoff, the colon brings on our premature death.

Metchnikoff put his faith not in nature, but in science and its ability “to amend the evolution of the human life, i.e., to transform its disharmonies into harmonies”. And science, he later wrote in *The Prolongation of Life* (1908), offers several options in the case of the colon. Surgical procedures to remove or bypass it, though popular among doctors, were potentially hazardous, as was the frequent use of antiseptics. Metchnikoff opted instead for a treatment based on traditional culinary practice. He would recall the high number of centenarians in his native land. The only reasonable association he could find was the high consumption of lactobacilli fermented milk. Thus, he was the first scientist who analyzed such cultures under the microscope. It was then that he attributed to yoghurt –in a perhaps overambitious attempt to explain longevity– the ability to counteract gut colonization by pathogenic bacteria and to neutralize their

toxins. To give Metchnikoff his due, he accepted at the end that his theory was nothing more than that, a theory. He became convinced that other factors should be involved in longevity which required to be analyzed in depth. One of the greatest supporters of Metchnikoff's theories was Isaac Carasso, a merchant Jew from Salonika, who, in 1912, became interested in the industrial production of yoghurt. Because of political conflicts at the time (Salonika became part of Greece) he lived in exile first in Switzerland and later in Barcelona. It was in his apartment (*carrer dels Àngels*, close to the Ramblas neighborhood) where he founded an artisanal factory for yoghurt and, by 1919, he made the first commercial yoghurt (originated directly from cultures kindly provided by Metchnikoff). In honor of his first son Daniel, Carasso named his product Danone (Daniel, the one) (fig. 1). The popularity of Metchnikoff's ideas—not only in France, but particularly in the United States—unleashed a yoghurt-consuming frenzy that continues unabated to this day, not to mention the delight of Danone's shareholders. One early sign of Elie Metchnikoff influence in the United States is the title of a book published in 1929 by a doctor James Empingham, *Intestinal Gardening for the Prolongation of Youth*. In the end, yoghurt's reputation lies in its ability to assist in the maintenance of the intestinal flora's balance, rather than its effects on longevity.

Yoghurt, notwithstanding, it is not far-fetched to suppose that the host's immune system is the one which keeps this equilibrium. This is reasonable considering that each human eats approximately 2-3 tons of aliments in his life span; around 3×10^{15} microorganisms from between 300 and 400 different species inhabit the digestive tract; they add up to a combined weight of approximately 2 Kg and are in contact with some 400 m² of mucosal surface. Hence, which of our systems, if not the immune, could deal with such myriads?

It is worth mentioning that there are other, non-immunological factors (chemical, physical, and biological) contributing to the homeostasis among commensal flora and its host. Thus, mucus production by goblet cells distributed along the gut's epithelium, peristalsis as well as the ciliary function keep bacteria away from coming into contact with the gut's epithelium. Similarly, gastric acid, bile salts, lactoferrine, lactoperoxidase, and lysozyme all create an unfavourable environment for pathogen's growth. The gut's flora itself contributes to the fermentation of non-digestible residues from the host's diet and endogenous mucus, to the control of epithelial cell proliferation and differentiation, and to the protection against pathogens, the so called "barrier effect". Clearly, all of the above mentioned is not enough to maintain the host's integrity. For this to happen, both branches of the immune system are needed, namely the innate and the adaptive ones, which together bear the hallmark of a high degree of specialization characteristic of vertebrate species.

Intestinal immune system components

Gut epithelium

Physical barriers represent the first line of defense against the outside environment. The gut's epithelium is able to discriminate those nutritive substances from those which are not. This is possible due to the tight junctions present in the apical region of the epithelial cell membranes allowing the entrance of only those compounds of molecular weight less than 2 kD¹. Although *strictu sensu* it is not an immune tissue, the epithelium participates in the defense mechanism by producing soluble mediators that contribute to an anti-inflammatory microenvironment, e.g., prostaglandin E₂, cyclooxygenase-2, arachidonic acid metabolites, and anti-inflammatory cytokines such as transforming growth factor- β (TGF- β).

M cells

Intertwined in the epithelium covering the dome area, like a window lattice in a brick wall, are the M cells. These are characterized by poorly developed brush border and cytoplasmic pynocytotic vesicles allowing the uptake of macromolecules, particles and antigens². To all intents, this is the only way in which complex antigens have access to the gut's immune system. Antigen transport through M cells may be considered the first step of an intestinal immune response. Since M cells do not express class II molecules of the major histocompatibility complex in their membranes, they are unable to process and present antigens. Rather, M cells transfer the intact antigen directly to professional antigen-presenting cells (dendritic cells and macrophages), located in the epithelium or in the subepithelial dome area. Transit via M cells is highly selective, only those antigens that bind to glycoconjugate receptors placed in the cell surface can be internalized. A great variety of viruses and bacteria can access into the host through M cells, particularly reoviruses, the poliomyelitis virus and the *Coxsackie* virus, as do *Shigella flexneri* and *Yersinia enterocolitica*, among others³.

Gut associated lymphoid tissue (GALT)

GALT can be divided into: *a*) organized tissues responsible of the induction of the immune response, such as Peyer's patches, mesenteric lymph nodes and lymphoid follicles; and *b*) effector zones including lymphocytes uniformly distributed along the epithelium and the mucosa's lamina propria.

The Peyer's patches contain all the necessary elements to induce and regulate an immune response. There are

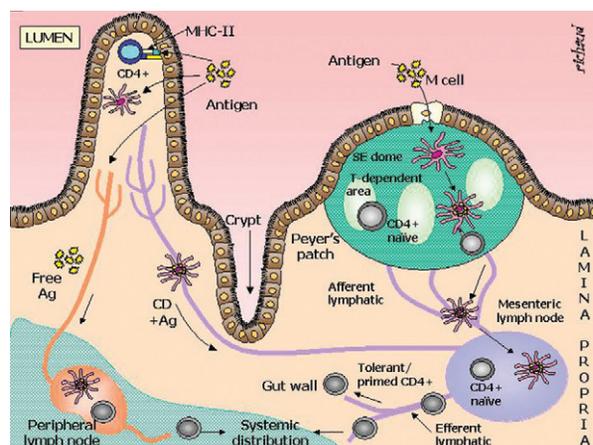


Figure 2. Gut anatomical components and immune response. Antigens (Ag) penetrate into the gut associated lymphoid tissue (GALT) through dendritic cells or via M cells. In the dendritic cell the antigen is processed so as to be presented to the CD4+ naïve T lymphocytes located in the Peyer's patches. Alternatively, dendritic cells containing antigen can migrate through the lymph vessels towards the mesenteric nodes where they stimulate CD4+ T cells as well (see text).

MHC-II: class II molecules of the major histocompatibility complex; SE: subepithelial. (Figure modified from Mowat⁵.)

B lymphocytes areas (B follicles) surrounded by T cells. Even though there is a high rate of cellular division in B follicles, the presence of plasma cells is rare compared to that of peripheral lymph nodes or the spleen. The area between the follicles and the gut mucosa's epithelium is rich in B and T lymphocytes as well as dendritic cells, strategically distributed so as to respond to the various antigens or particles transported through the mucosal epithelium above the Peyer's patches⁴. Activated lymphocytes in the GALT can migrate to the mesenteric lymph nodes draining the Peyer's patches, with the consequent induction of an immune response (fig. 2)⁵. It is then that they reach mucosal surfaces and extraintestinal tissues, even though the majority of the effector populations will remain in the lamina propria and in the gut's epithelium⁶.

Dendritic cells

There are 3 main dendritic cell subpopulations in the gut, and are classified according to their expression of surface markers. Thus, those expressing CD11c and CD8 α are termed "myeloids", which are the most abundant in the Peyer's patches (40%), the lamina propria (60%) and in the mesenteric lymph nodes (40%). Besides antigenic presentation to T cells, one of the main functions of this cell subpopulation is to contribute in the maintenance of the anti-inflammatory environment through interleukin (IL)-10 production. CD11c-CD8 α + cells, also known as "lymphoids", re-

present 35% of the dendritic cells in the Peyer's patches, 20% in the lamina propria and 35% in the mesenteric lymph nodes. Although these cells also produce IL-10 and present antigens, their main task is geared towards protection against parasites and allergens⁷.

The third subpopulation of dendritic cells is known as "double negative", as they do not express none of the aforementioned surface antigens. These cells are also relatively abundant in the Peyer's patches, lamina propria and mesenteric lymph nodes, although their precise function is as yet unknown⁸.

Certainly, these dendritic cell subpopulations can be found in other lymphoid tissues, such as spleen, but there they secrete IL-12 which participates in cell-mediated immune responses and inflammation.

The gut dendritic cells are ready to receive and process the antigens transported by M cells. They are, however, themselves able to capture antigens straight from the gut lumen thanks to the extension ability of their dendritic elongations between the junctions of epithelial cells.

Lymphocytes

The most abundant B lymphocyte subpopulation in the gut are the conventional B2 cells, CD19+CD5-. Around 70% to 90% of them produce immunoglobulin (Ig) A, from 10% to 20% synthesize IgM, and a small number produce IgE. The synthesis of these immunoglobulin isotypes is given by the interaction of helper CD4+ T cells besides the effects of TH2 cytokines, e.g. IL-10 and TGF- β , although this is not a *sine qua non* condition^{9,10}. In this regard, it has been recently described a T cell-independent mechanism for the synthesis of IgA by the peritoneal B1 lymphocyte subpopulation (CD19+CD5+)¹¹⁻¹⁴. This IgA, in contrast to "conventional" IgA, has the ability to recognize solely antigens of the commensal flora, and thus, it has been put forward as a primitive mechanism of gut immune response, almost not changed in evolutionary terms.

It is worthwhile mentioning that the IgA produced by the gut is unable to activate complement, neither by the classical nor the alternative pathway, thus, it is considered an anti-inflammatory immunoglobulin. Its main role is rather to preserve the integrity of the mucosal barrier against potential infectious agents, since the secretory component of IgA protects the mucous secretions from protease action. IgA, once secreted and transported to the lumen, can neutralize pathogens, bacterial toxins and enzymes. Furthermore, it can avoid the adherence of microbes to the epithelium, the absorption of antigens and the entry of viruses.

Practically all the T lymphocyte subpopulations are present in the gut. The memory CD8+ cells gather in non-lymphoid tissue, particularly the lamina propria,

awaiting an eventual encounter with the antigen¹¹. Within this group, it must be included the T $\gamma\delta$ cells, which have a dual function: on the one hand, they show an exquisite reactivity to the heat shock proteins present in the bacterial walls of the intestinal flora that leads to the production of inflammatory cytokines like tumor necrosis factor- α , IL-1 β , IL-8 and interferon- γ (IFN- γ), and, on the other, by expressing the CD40 ligand (CD154). Moreover, T $\gamma\delta$ lymphocytes are potent inducers of the isotype switching from IgM to IgA in B cells¹⁵. T $\gamma\delta$ cells can also acquire immunological memory and migrate to peripheral lymphoid tissue with great ease.

The most abundant T lymphocytes in the Peyer's patches, mesenteric lymph nodes and lymphoid follicles are the helper CD4+ T cells. In this anatomical region, the presence of three CD4+ T lymphocyte subpopulations is more conspicuous, namely the TH3 cells that synthesize TGF- β , the TR1 producing IL-10 and TGF- β and the CD4+CD25+, being this latter the responsible of the local tolerance to antigens from the commensal flora¹⁶⁻¹⁹.

There are also lymphocytes located in the epithelium, termed intraepithelial lymphocytes which have effective as well as immunological regulatory functions. Opposite to what occurs in the lamina propria, nearly 80% of intraepithelial T lymphocytes belongs to the CD8+ $\alpha\beta$ subpopulation with cytotoxic properties, whereas 13% are T $\gamma\delta$ ²⁰.

Gut immune response

Antigens penetrate into the gut through dendritic cells or via M cells. In the dendritic cell the antigen is processed so as to be presented to the CD4+ *naïve* T lymphocytes located in the Peyer's patches^{21,22}. Alternatively, dendritic cells containing antigen can migrate through the lymph vessels towards the mesenteric nodes where they stimulate CD4+ T cells as well. Depending on the antigen nature, the response is polarized to an anti-inflammatory (TH2) or a proinflammatory (TH1).

Antiinflammatory response

In physiological conditions, the diet products and normal flora components, e.g. bacterial lipopolysaccharides, enter into the lamina propria or the Peyer's patches after being captured by the dendritic cells. As has already been mentioned, in these places there is an anti-inflammatory microenvironment given by prostaglandin E₂ and cyclooxygenase-2 spontaneously produced by the mesenchymatose cells and the gut epithelium. Interestingly, the synthesis of these mediators is independent

on the presence of flora or of any inflammatory stimulus whatsoever^{23,24}. Even further, the high production of arachidonic acid metabolites represents a stable phenotype that is unique to the intestinal lamina propria. Anti-inflammatory cytokines, such as TGF- β and IL-10 (produced by epithelial cells) induce an incomplete maturation of the dendritic cells that have captured the antigen to be processed. This process takes place in the lamina propria or in the Peyer's patches. After antigenic presentation to the *naïve* CD4+ T cells, these differentiate towards the regulatory phenotype (CD4+CD25+) that produce IL-10 and IFN- γ and/or towards the TH3 phenotype which produce TGF- β .

As a consequence of this interaction IgA is produced by B lymphocytes and the *in situ* limitation of the response is performed by regulatory T cells. Therefore, systemic and local tolerance is achieved²⁵.

Inflammatory response

In the Peyer's patches or in the lamina propria, the presence of pathogen-associated molecular patterns (heat shock proteins, toxins) leads to local swelling. These antigens are recognized through toll-like receptors expressed by the mesenchymatose cells, macrophages and epithelial cells²⁶. As a result of the inflammatory process, the dendritic cells mature completely and produce IL-12. These cells migrate to the mesenteric nodes, where they present the antigen to the resident *naïve* T lymphocytes. The latter differentiates towards TH1 producing IFN- γ and/or towards TH2 secreting TGF- β , IL-4 and IL-5. These cytokines magnify even further the inflammatory process and induce the synthesis of IgA^{26,27}. The activated and differentiated cells spread to the periphery through the lymphatic vessels so as to communicate to the rest of the immune system the pathogenicity of the antigen. The migration of lymphocytes to secondary lymphoid organs is possible by the expression of integrins and cell adhesion molecules in the vascular endothelia, as well as by chemokines' action. As a rule, the cells return to repopulate the lamina propria and the mesenteric nodes in the guise of "centinels" prepared to a subsequent attack. Thus, the consequence is a local and systemic immunization⁵.

Both responses are not exclusive, they occur permanently and reflect the physiological immune dynamics.

Innate immunity *versus* adaptive immunity

According to the above, it would seem that it is the adaptive immune response the responsible for the balance between commensal flora and its host. This is arguable, however. One must only recall that the gut immune system is isolated from microorganisms by

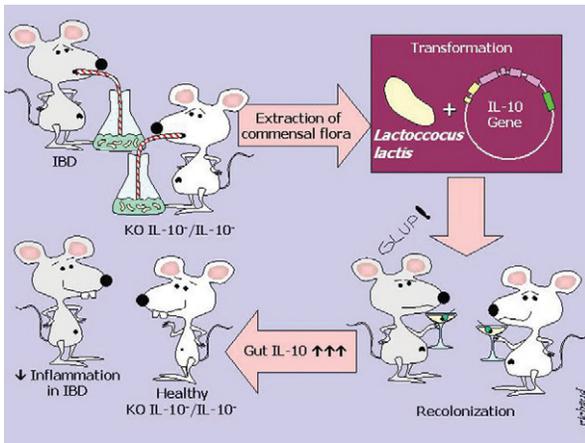


Figure 3. Authors' own conception of Steidler's and coworkers experiment. The balance between proinflammatory and anti-inflammatory cytokines has a crucial role in the maintenance of homeostasis. This was extraordinarily exemplified by colitis murine models in which the anti-inflammatory action of interleukin (IL)-10 produced by transformed commensal bacteria reduced or even avoided the pathological process. IBD: inflammatory bowel disease; KO: knock out.

physical and chemical barriers and by the anti-inflammatory environment present in the GALT. Moreover, within the commensal flora there are some species that benefit the host, such as *Bacterioides thetaiotaomicron*, which, on the one hand, induces the production of agiogenins (ribonucleases which inhibit bacterial growth) by Paneth cells. On the other, *B. thetaiotaomicron* protects the host tissues from the lytic action of the complement cascade stimulating the synthesis of CD59, which hinders the consolidation of the membrane attack complex²⁸. A vast array of molecules, such as the family of cytokines and chemokines, cell adhesion molecules, heat shock proteins, the toll-like receptors and nitric oxide, participate as effector and modulating molecules, both in the innate and in the adaptive immune response, even though their precise roles are yet to be defined²⁹.

Epilogue

It would seem, after this overview of the gut as immune organ, that everything that could be said has already been said. Nothing further from the truth. Suffice is to observe the increasing incidence of ailments relatively infrequent before the 20th century, e.g., inflammatory bowel disease, allergies, while concomitantly, infectious as well as parasitic diseases are on the wane. This is clearly the result of society "modernization", i.e., urbanization, small families, little or no animal contact, a high use of antibiotics, and an aseptic environment, to name but a few factors. By contrast, in developing countries we can see the opposite situation, with allergic and,

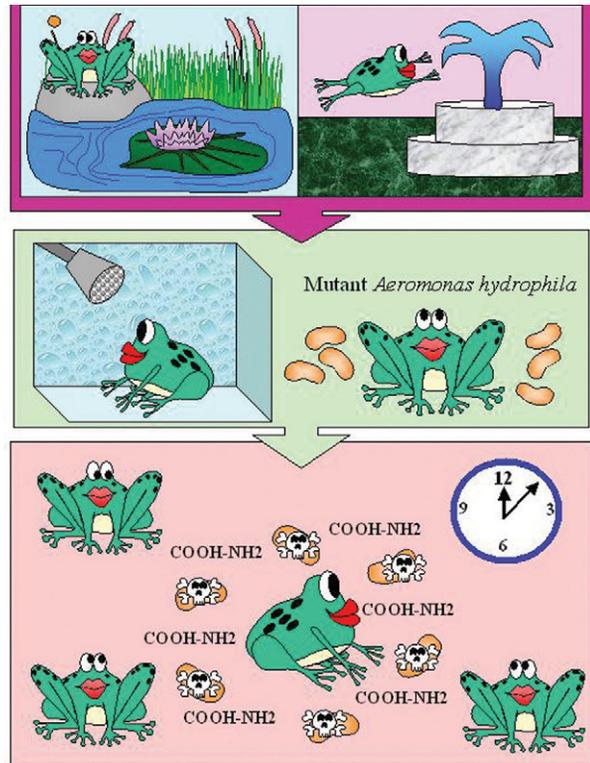


Figure 4. *Rana esculenta* is an ordinary frog easily found in the ponds and fountains of Rome. Simmaco and his team collected them in order to explain the molecular mechanisms that control the colonization of normal flora in the skin. Once collected and bathed, the frogs were exposed to a mutant strain of *Aeromonas hydrophila* and, in only 10 min, such bacteria were killed thanks to the antibiotic peptides naturally synthesized by the frogs' skin.

particularly, inflammatory bowel diseases being infrequent, while infectious and parasitic ones being common, if not rampant. The recently fashionable hygiene theory exemplifies the above³⁰. This dichotomy clearly shows that a balance must exist between commensal flora and the individual and, in turn, between the individual and its environment. A question that was not dealt with at the zenith of the immunology century –let alone at its beginning– was how the normal flora was controlled. The answer, as of today, is very simple: no one knows. This answer is no less disappointing for being simple. Our ignorance about the composition, origins, ecology, population dynamics, phylogeny, evolution and relationship with antigens of the intestinal flora is enormous, not to say unmeasurable. A whole century had to go by for some old questions to be reformulated. The gut physiology involves a great number of multidirectional interactions between immune cells, non-immune cells and the intestinal flora. These interactions have been studied thanks in part to experimental animal models.

An enlightening example is a recent study in which a murine model of inflammatory bowel disease and a

knock out mice for IL-10 (which, in the end develop ulcerative colitis) were used. *Lactococcus lactis* was extracted from the gut flora of both strains of mice. Employing genetic engineering, the gene that codifies for IL-10 was introduced into these bacteria, whereby they acquired the ability to produce the cytokine spontaneously³¹.

The bacteria so transformed were then reintroduced into the mice gut. The findings were surprising: in mice with inflammatory bowel disease the pathological process was reversed up to 50%, whereas in the knock out mice ulcerative colitis did not develop (fig. 3). In a similar paper, with a murine model of peanut allergy, it was shown that helminthic infection with *Heligmosomoides polygyrus* induced a considerable decrease in the production of allergen specific IgE, as well as a depression of the anaphylactic response³².

Both studies are themselves extraordinary elegant for proposing clear, concrete and precise hypotheses. The analysis of distinctly inflammatory pathologies, mediated by TH1 cytokines that can be antagonized by anti-inflammatory stimuli (Th2 cytokines, particularly IL-10) make evident the importance of equilibrium in the immune response.

The studies that deal with simple questions on physiological conditions are, however, the ones that have provided greater information about the control of the normal flora. Thus, Simmaco and Boman's team has worked during the last ten years on the innate immune mechanisms for the control of the commensal flora. After several studies with drosophila, they selected a frog (*Rana esculenta*) as a new experimental model, which has been seldom used in the laboratory but is abundant in the ponds, puddles, swamps and fountains around the city of Rome. Such a choice was the result of a rather simple reasoning: this ordinary frog, having a skin that is moist, sticky and attractive to microorganisms, must be subjected to countless antigenic challenges when jumping from one habitat to another, e.g. pond-fountain-puddle-swamp.

As if this wasn't enough for the study of innate immunity, the frog's skin is more readily accessible than mice's, let alone the latter's guts. It was then that they gave themselves to the Respighi-reminiscent task of collecting frogs from ponds and fountains. Once inside the lab, and after a good bath, the frogs were exposed to *Aeromonas hydrophila* (a natural denizen of the frog's dermis), but a mutant strain. Straight away, the frogs' skin began the synthesis of antibiotic peptides (until then, only analyzed in *Drosophila*), capable of destroying these bacteria in a lapse of 10 min (fig. 4). Not satisfied with only this result, another group of frogs, after the prescriptive bath, were smeared with a steroid unguent which blocked the synthesis of these peptides, and were subjected to the same process. As opposed to the first group, in these frogs the production of antibacterial

peptides was inhibited and their skin colonized with such mutant strain³³. This paper was worthy of an extraordinary leading article by one of the truly great immunologists from the second half of the 20th century³⁴. Give or take a couple of words, Klein mentioned that in the *Batracomiomaquia*, the war between frogs and mice (attributed by some scholars to Homer as a parody of his own *Illiad*), the conflagration starts when a mouse drowns after falling of a frog's back as it was being carried across the river, but not before swearing that he was to be avenged on the frogs. It was a close call for the frogs as, soon after the war started, they were about to be obliterated by the mice. It took the divine intervention of no less a god than Zeus to fend off the tragedy.

In the search for the best animal model for the study of immunology, mice are, undoubtedly, odds-on favourites. Regarding the control of the normal flora, however, the current score in this struggle is 1-0 to the frogs. The final score is today still uncertain, since the battle has just started. There still are many enigmas to decipher. It is enough to ask oneself: which adaptations does a microorganism require to form part of the microflora?; how do invertebrates control their normal flora?; which effects does the make-up of the normal flora have on the whole gamut of immunological mechanisms?; what is the contribution of the innate and specific immune responses?; are these responses permanently engaged with microflora antigens and, if that is the case, how do they avoid becoming paralyzed by constant exposure?; and, if not, what makes the immune system become involved?

Be all this as it may, it is clear that any live being that prides itself on having an immune system –no matter how primitive– must necessarily have the ability to cohabit with a commensal flora and to recognize when it can be harmful.

In spite of everything that has been said in this review, there are some special circumstances when the gut's immune system must not be our concern at all. That is the case of the *bécasse* (*Scolopax rusticola*, a caradriform bird with a long, thin and flexible beak), which has a high gastronomic value, particularly in France. This is the only bird that is cooked and eaten together with its intestine, since among its commensal flora inhabits the cestode *Amoebotaenia* spp., responsible of its exquisite and much-appreciated flavor³⁵. Before such a dish, does it really make sense to ask how or what controls the normal flora? Bon appetit!

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