An Interview with Molecular Biologist, Prof. Marco Ruggiero MD, PhD:

Understanding the GI and Brain Microbiome and the Role of GcMAF in Harmonizing the Immune System with the Microbiome Populations

by Jacques Fernandez de Santos

Introduction

Marco Ruggiero was born in Firenze (Florence), Italy, in 1956. There he graduated from the School of Medicine in 1980. He has a PhD in molecular biology and a specialization in diagnostic radiology. He served in the army as lieutenant medical officer. From 1984 to 1986 he worked at the Laboratory of Cellular and Molecular Biology of Burroughs Wellcome, where he published a paper sponsored by Nobel laureate Sir John Vane. He subsequently worked at the National Cancer Institute of the National Institutes of Health (Bethesda, Maryland, US), where he performed research on oncogenes and signal transduction. He returned to Italy as professor of molecular biology at the University of Firenze until his retirement in 2014. In his 36-year scientific career, he published more than 150 peer-reviewed articles and was invited to participate in hundreds of congresses and conferences. As senior author, in 2011, he published a seminal paper on HIV infection and AIDS together with Prof. Peter Duesberg of Berkeley and Prof. Henry Bauer. Currently, his main research interests are in the fields of oncology, neurosciences, and immunotherapy, cooperating closely with the National Autism Conference and Autism One. Together with his wife, Dr. Stefania Pacini, he is the inventor of the probiotic yogurt "Bravo" and of powerful stimulants of the immune system based on macrophage-activating factors.

Jacques Fernandez de Santos: As reflected in your book, recently published with Peter and Drew Greenlow, *Your Third Brain*, there are three brains: the one inside the head, one in the GI tract, and the microbiome, an "organ" within organs disseminated throughout the gut and different parts of the body. How would you schematically define those three brains in lay terms?

Marco Ruggiero: When we wrote the book, we postulated the existence of three brains: two of these brains are "human"; that is, made by human neurons and glial cells in the brain inside our heads and embedded in the layers of the GI tract. These two human brains are interconnected and the flow of information is bidirectional; in lay terms, the brain inside our head influences the working of our GI tract and vice versa: the neurons in the GI tract influence the working of the brain inside the skull. The "third brain" was an intuition of mine, as explained in the book, and with this term I indicated the microbiome that is mainly, but not uniquely, located in the gut. In fact, the commensal microbes that constitute the microbiome produce substances, neurotransmitters, that influence both the neurons inside the skull and the neurons in the GI tract. There is a huge amount of scientific literature describing the interactions between the microbes and the function of the brain in health and disease. Thus, scientists now openly talk about "melancholic microbes" or "voices from within," referring to the alterations of the microbiome in psychiatric disorders such as depression or anxiety. Other scientists propose the development of "psychobiotics," which are sort of probiotics aimed at restoring the function of the third brain so as to rebalance our neurologic and psychological functions. It is also well assessed that experimental changes in the composition of the microbiome in the gut lead to changes in behavior that can be reversed by reconstituting the original microbiome. In sum, there is ample evidence for the existence of such a nonhuman "third brain," even if, as far as I know, we´ve been the first to use such a term to describe the role of the microbiome in influencing our neurological, cognitive, and psychological functions. In the past few months, however, after the publication of our commentary in *Frontiers in Neuroscience* (2015 Dec 22;9:485), I realized that there

is indeed a "fourth brain." The fourth brain is nothing other than the brain microbiome; that is, the array of microbes that live in symbiosis with our neurons and glial cells inside our heads. This is a completely novel concept that had never been described thus far. The idea for such a fourth brain derives from a paper that was published a few years ago by Canadian researchers looking for microbes in the brains of HIV/AIDS patients (*PLoS One.* 2013;8[1]:e54673). I guess that it was a surprise for them to find out that "in an organ widely assumed to be free of infectious agents in the absence of a specific disease process, autopsied and surgically-derived human brain specimens showed a restricted but distinct bacterial population in the present studies, which was composed of bacterial classes chiefly recognized in the physical environment, i.e., soil and water." In other words, in the brains of healthy people, there are the same microbes that are found in the environment and, consequently, the gut. It is interesting to notice that, according to the authors, the microbes travel to the brain carried by cells of the immune system that include macrophages. In fact, they write, "The brain is constantly surveyed by trafficking leukocytes (activated lymphocytes and macrophages), which provide a Trojan horse mechanism for microbial entry into the nervous system across the blood brain barrier." And, quite obviously, the presence of the microbes in the brain has an enormous influence on the functioning of the neurons and the glial cells up to the point that the authors are compelled to write, in a rather poetic fashion, "Since bacteria express multiple molecules … their capacity for influencing brain function is immense" – "immense" being an absolute superlative.

In 2013, however, the existence of another immunological pathway leading to an even closer interconnection between the brain and the immune system, and hence the microbes that are carried by immune cells, was unknown, and only in 2015 it became evident that the brain has a classical lymphatic system like any other organ. Thus, as we describe in our paper in *Frontiers in Neuroscience*, there is an even stricter relationship between the function of the immune system and the function of the brain inside our heads: in fact, cells of the immune system carrying microbes to and from the brain may travel through these newly discovered lymphatic vessels bypassing the blood–brain barrier. The identification of the fourth brain bears unimaginable consequences: we have to consider the microbes as cells of the central nervous system with a dignity equal to that of neurons and glial cells, but with two important differences. First of all, they are nonhuman and the information in their DNA is microbial and not human; this means that they are looking after their interest that may or may not be coincident with the human interest. Second, they change continuously as we interact with the environment and, most important, with food, quite at variance with neurons and glial cells. In simpler words, as a sort of a slogan, we could say that "the microbes that you have in your brain are the microbes that you have in your gut … and you want to have good microbes in your gut!" In addition, we may want to add that "you want to have a functioning brain lymphatic system .. . and active macrophages" that recirculate and balance the microbial populations in the brain and in the gut. Thus, now we have four brains: two human brains, one inside our head and one in the walls of the GI tract, and two nonhuman brains, again, one inside our heads and one in the mucosa of our GI tract. Most likely, as we keep on studying the brain microbiome, or the fourth brain, we shall come to the conclusion that, in essence, we have only one integrated brain that is composed by human and nonhuman cells distributed in the gut and inside our heads. However, since we need to classify things in order to study and comprehend them, I feel that this, probably artificial, subdivision in four brains may help us in coping with the complexity of this system.

JFS: Is that third brain a moving organ located in different parts of the body even though an important part of it is lodged in the gut?

MR: If we assume that the third brain is constituted by the microbes composing the human microbiome, then we have to conclude that it is scattered throughout the body, even if the greatest mass of the microbiome is located in the mucosa of the GI tract, from the mouth to the anus. At variance with the human neurons and glial cells that constitute the first and the second brain, the microbes constituting the third brain, just like the microbes inside our skull constituting the fourth brain, are much more variable and do not have a fixed location. In other words, the composition of our microbial third and fourth brains is highly dynamic and reflects our interactions with the environment, mainly but not uniquely through the consumption of food. Therefore, the third and the fourth brains are constantly changing in response to the changes in the environment and to our relationship with the environment itself. And, consequently, the influence of the microbial third and fourth brains on the human first and second brains is constantly changing. This rapid adaptation to the changes of the environment is instrumental in giving our integrated human/microbial brains the flexibility that they need to adapt and survive in the most diverse environments. However, this variability and flexibility of the microbial brains are not limitless; there appears to be a core microbiome, both in the gut and in the brain, that is fairly constant; and the study of such a core microbiome, and how to restore it in case of unbalances, is a major challenge in the field of microbiome and neuroscience research.

In addition, the emergence of the concept of an integrated human/microbial brain gives us the opportunity to reconsider the role of the immune system in determining our neurological or psychological activities. For example, if the immune system cells that carry the microbes to the brain inside our head are not functioning to the best of their capabilities, the microbiome inside the brain suffers alterations that influence the working of neurons and glial cells. Or, if the newly discovered brain lymphatic system is clogged by inflammation in the deep cervical nodes where the lymph from the brain drains, the recirculation of lymph in the brain is hampered, and therefore the immunological surveillance of the brain is impaired. This may lead to unbalances in the composition of the brain microbiome because pathogenic microbes from the gut or the environment cannot be controlled by the combined action of the healthy microbes of the brain microbiome and immune system cells.

JFS: Neurons and interstitial cells are in the brain, the GI tract, and apparently also in the heart. What do those facts mean in terms of a total new vision of the human body, and ways of carrying the information at all levels inside the body?

MR: With the identification of the microbial third and fourth brain, our vision of the flow of information in the human body drastically changes. First of all, it becomes difficult to define as "human" a body that contains 10 times more microbial cells than human cells and more than 100 times more microbial genes than human genes. The very definition of being "human" has to change, and we have begun to perceive ourselves as a complex ecosystem wherein the information is continuously and dynamically exchanged between the very many different living entities that constitute what we call our body; a body, however, that belongs as well to the myriads of microbes that contribute to all its functions.

In biology, the basic level of information from which all other types of information derive is located in the DNA under the form of genes. If we consider that our microbiome contains 100 times more genes than our human genome, we easily understand that the human information is rather marginal. If we assume that our behavior somehow depends on the information contained in the

DNA, it is clear that our behavior then mostly depends on the microbial rather than the human information; or, if you prefer, the microbial "will" rather than the human will.

In fact, the most primeval form of behavior, common to all living things, essential for life itself and from which all other forms of behaviors derive, is the eating behavior. A recent peer-reviewed paper clearly states that our microbes "manipulate host eating behavior to increase their fitness, sometimes at the expense of host fitness" (*Bioessays*. 2014 Oct;36(10):940–949). In other words, our most basic behavior is only marginally based on our supposed human free will, and it is the microbes that tell us what to eat so that they can increase their fitness. If we can reach an agreement and their fitness coincides with our human fitness, then we are in a state that is defined "health"; otherwise, our human part of the body is "sick" even though the microbial part of our body could indeed flourish. As in most things in this universe, when harmony prevails and interests coincide, there exists a "win–win" situation. Vice versa we end up in a state of continuous fight wherein only one part, the microbial, is bound to win. Although it may represent a serious blow to our human esteem, the fact is that when we die, it is only the human part of our body that dies, whereas the microbial part with all its information remains alive and well.

JFS: Is our memory actually located just in our first brain, or is it somehow disseminated in different parts such as organs like the heart? Is memory located throughout our body's cells and organisms of the microbiome?

MR: I began studying the molecular mechanisms of learning and memory in 1987 when, together with my good friend and colleague Renato Corradetti, we published three seminal papers on the molecular bases of memory (*Brain Res*. 1987 May 12;411[1]:196–199; *EMBO J*. 1987 Jun;6[6]:1595–1598; *Neurochem Int*. 1989;14[1]:1–9). In those days, researchers were convinced that the hippocampus was involved in the consolidation of information from short-term to long-term memory. Now, almost 30 years later and with the novel notions of a third and a fourth brain, we can safely assume that what we call *memory* is actually scattered throughout our body and resides both in our human neurons, as commonly accepted, and our microbial cells. In fact, it is now well established that the process of learning and memory involves changes in the DNA, in the process called *expression* that often goes under the name of *epigenome*. Therefore, since the changes in the epigenome occur both in our human neuronal cells and microbial cells, it is possible to say that our memory is dispersed and there is not an anatomical seat of the memory, something like the hard disk of a computer.

It is true that certain areas of the brain are dedicated to some specific functions that are related to learning and memory, but it is also true that, in certain cases, such areas can be removed and neurological functions can be maintained. If we wish to deal with metaphysics, we may even deduce that, although our human memory dies with the death of our human cells, our microbial memory persists after the death of our human body, and this concept bears an incredible number of implications that deal with matters such as life after death, the persistence of memory and consciousness after death, and so on.

JFS: In that sense, in the book *Your Third Brain*, it is mentioned that in fact we could live and function with 90% of the brain missing, as some cases have been detected and published in important scientific reviews, notably one case in Marseille (France). Have other similar cases been discovered? How could such a replacement work and be explained?

MR: The case that you are referring to was published in a prestigious medical journal, the *Lancet*, in 2007 (Jul 21;370[9583]:262). It described the case of a perfectly normal adult man from Marseille, a civil servant, married father of two children, who serendipitously discovered at age 44 that essentially 95% of his brain was absent and his skull filled with fluid that, unbeknownst to him, had accumulated since his childhood. This paper clearly demonstrated that we can live perfectly normal lives with only about 5% of our cerebral mass. Quite obviously, these results challenge all the notions of neuroanatomy, wherein even the most minute areas of the brain has an assigned function.

Interestingly, such a case is not isolated. Several years earlier, in 1992, authors from the Psychology Department of the Kalamazoo Regional Psychiatric Hospital in Michigan, US, had demonstrated that twins (homozygotic or heterozygotic) who had significantly different cerebral masses because one of the two had suffered from hydrocephalus, also had different intelligence. The oddity lies in the fact that those with drastically reduced cerebral mass had above-average intelligence! In other words, all the other variables being equal (as in the case of twins and particularly homozygotic twins), the size of the brain made the difference; however, contrary to common logic, the smaller the brain, the higher the degree of intelligence.

Quite curiously, these studies were not followed up, most likely because they challenge all the notions that we have about the functioning of the brain. However, since these studies are what in philosophy are called *black swans* (and here there are many black swans), we cannot escape the fact that our reductionist vision of localizing all neurological and psychological functions in the brain inside our head is, at best, very partial, if not completely wrong.

JFS: Mental states and decision-making, according to the book, are generated in the gut rather than in the first brain due to microbiota conditions; is that all? Is free will just generated in the gut, which could sound a bit reductionist, or do we still have a psychological margin independently from the microbiome or third brain?

MR: At slight variance with what I wrote in the book about a year ago, I now postulate that mental states and decisions are generated both in the gut and inside our skull, but mainly by the microbial brains located there. The issue of free will as we knew it died when it was discovered that the microbiome contains a number of genes estimated to be between 2 and 8 million; that's more than 100 times the number of human genes in our DNA; about 20,000. In fact, if we consider for a moment only the human part of ourselves, all our mental states and decisions ultimately depend on the information contained in our human DNA. It is the information in the DNA that tells the neurons how to connect and form the neural web of interactions that are at the basis of our consciousness and thinking. In addition, the interaction with the environment, the processes of learning and memory, our experiences that determine our future decisions – all these events are embedded in the DNA of the neurons in the form of epigenetic variances that can be transmitted through the generations with the epigenetic inheritance.

These are some of the reasons why it is postulated that the information at the basis of our neurological and psychological functions ultimately resides in our DNA. If we now put into the equation the existence of an enormous amount of information that is present in the microbial DNA, it becomes evident that our behavior is actually "manipulated" by the microbes, as pointed out in the paper quoted above. When the authors write "manipulate," they use the true meaning of the verb. However, it would be equally reductionist to state that the human part of ourselves has no role

and we are like puppets maneuvered by tiny microbes. The reality is that we are a complex society of different living beings in whom the behavior of each can be cooperative and altruistic or competitive and egotistic. In the first case, we live in a state of health; whereas in the second case, one class of living beings prevails and, as odd as it may seem, the human part is the most frail and impotent.

We may compare this complex society that is our body to the *rhizome theory* as proposed by the French philosophers Deleuze and Guattari. In conclusion, the concept of free will has to be extended to the "free will" of each individual microbe that is part of our body, since each individual microbe harbors genes that drive it to behave in the best way to guarantee its own fitness. If we accept this notion, then we can still maintain a concept of free will that is the algebraic sum of the free wills of all the beings living with us in this very moment.

JFS: Hundred of years ago we did not have the same type of diseases that we have today; for instance, autism affects 1 in 68 children today versus 1 in 10,000 in 1990. Why in the case of autism is this happening: is it vaccines and poor nutrition combined that harm the original microbiome and therefore the immune system?

MR: Although the cause of autism remains unknown to this date, I think that with the publication of our paper in *Frontiers in Neuroscience*, we have at least elucidated some important aspects of its pathogenesis; that is, the mechanisms that lead to the onset and progression of the symptoms. Changes in the nutritional properties of food, increased exposure to chemicals and other toxicants including electromagnetic radiations or radioactivity, excessive exposure to antibiotics and other drugs – all these factors may contribute to the observed increase in the incidence of autism spectrum disorders. In addition, a different way to diagnose and classify disorders of the development may also contribute to such an observed increase. There is, however, a point that is rarely taken into consideration and may prove rather relevant: the effects of low-intensity electromagnetic fields on the human microbiome and hence on the third and fourth brains. We elaborated on this point in a chapter that we wrote for the prestigious *Encyclopedia of Cancer*. In fact, even though the low-intensity electromagnetic fields to which we are constantly exposed appear to be fairly safe for our human cells, they appear to be detrimental for our microbial counterpart. In our chapter, we write:

The effects of electromagnetic fields on the human microbiome open a new perspective in assessing the risks for health and in preventing them. So far, most of the research was concentrated on assessing the effects of electromagnetic fields on those areas of the body that were exposed to their energies. In other words, all the effects of electromagnetic fields on human health were ascribed to their interaction with the human cells of our bodies. However, since we have learned that electromagnetic fields, even of minimal intensity such as the endogenous electromagnetic fields, modify the human microbiome, their effects might be much more complex and far ranging. In fact, microbes and the microbiome may amplify or mitigate carcinogenesis, responsiveness to cancer therapeutics, and cancer-associated complication (Garrett 2015) and, therefore, electromagnetic fields modifying the microbiome may interfere with all such cancer-related responses. It is foreseeable that the development of functional foods containing probiotics for the prevention and treatment of cancer will have to

take into account the effects of endogenous as well as exogenous electromagnetic fields on the human microbiome.

Quite obviously, all these considerations apply even more to the development of the brain (or at least of the human first brain inside our head).

JFS: It is currently proven that there is no good health without a healthy microbiome, or third brain. You and your team have been able after years of research to create a super food that stimulates strongly the immune system through the stimulation of macrophages and natural killer cells. The key component of this super food, amongst others, is the protein GcMAF (Gc protein-derived macrophage activating factor), which could be described simplistically as carrier for the vitamin D. How would you define the main properties and characteristics of this protein as well as the super food you have created as a whole?

MR: We began working on this "super food" that comes in the form of a fermented milk and colostrum product, a sort of a yogurt, about 5 years ago, when we were interested in developing a nutrition-based immunotherapeutic protocol. After hundreds of trials and errors, we eventually found the exact formula that is now in production, under the commercial name "Bravo." This product shows two features that render it unique: it contains the live microbial strains that reconstitute the healthy human microbiome, and it contains about 200 natural, newly formed molecules that contribute to health maintenance in a wide variety of ways.

The live microbial strains contained in Bravo are known to activate the immune system, stimulate macrophages, fight cancer, and help neurological and psychological processes; all these features contribute to the observed health effects of Bravo that have been published in a number of peer-reviewed papers (see, for example, *Anticancer Res*. 2014 Jul;34[7]:3569–3578).

The newly formed molecules, naturally occurring during the fermentation of colostrum and milk, exert a number of biological functions that may contribute to health. Such biologically active molecules are not present in milk or colostrum but are formed during the process of fermentation, thanks to the enzymes produced and released by the microbes that we carefully selected for such a purpose. Among these molecules, there are peptides that help control blood pressure, others that stimulate the immune system, others that show antimicrobial properties, others that show antithrombotic properties, others that favor the absorption of minerals, others that have antioxidant activity, and others that influence mood and perception. There is, however, one particular molecule that gained great attention in the recent past and that is naturally produced (in other words, it is not added to the product) in Bravo: GcMAF. This powerful stimulator of the immune system shows a number of interesting healthful properties and has proved effective in a variety of pathologic conditions even independently of its main action of stimulating the immune system.

JFS: This super food and its main component, GcMAF, have shown at the beginning of your studies years ago amazing results in pathologies such as AIDS or cancer proliferation. Can you please expand, before going to other pathologies, on the results observed on those important diseases?

MR: Probiotic yogurts had been known for years as very effective tools in the fight against HIV/AIDS or cancer, thanks to the combined action of the microbes and the products of their metabolism. In 2010, a Canadian research team led by Dr. Reid demonstrated that a probiotic yogurt very similar to Bravo increased CD4 cells in African HIV/AIDS patients and ameliorated their clinical situation in a manner quite comparable to that of conventional antiretroviral drugs without the side effects of the latter (*J Clin Gastroenterol*. 2010 Oct;44[9]:e201–e205). Two years later, the same research group demonstrated that such an effect on HIV/AIDS patients was not limited to those living in impoverished countries and presumably suffering from malnutrition, but was observed in Canadian patients as well (*Gut Microbes*. 2012 Sep–Oct;3[5]:414–419). Interestingly, the same research group observed that one African patient in the probiotic group experienced seronegativization; that is, while consuming the probiotics, she tested HIV negative after having been confirmed HIV positive before being assigned to the probiotic group, whereas no patients in the placebo group experienced such an occurrence (Gut Microbes. 2011;2[2]:80–85).

It is interesting to notice that all the papers mentioned above were published at about the same time that we were presenting our results at the Sixth International Aids Society Conference on HIV pathogenesis, treatment, and prevention, in Rome in 2011; at that conference, we observed that consumption of "our" yogurt brought about very similar results in terms of stimulation of the immune system. When almost identical results are independently produced by different research groups that report observations spanning from Africa to Italy and Canada, the probability that such results are not the product of serendipity are very high.

Nowadays it is well accepted that probiotics are one of the pillars of the nutritional immunotherapy of HIV and AIDS, since they not only strengthen the individual's immunity but also help reduce the systemic inflammation and improve the prognosis of HIV-infected individuals (*PLoS One*. 2015 Sep 16;10[9]:e0137200). The effects of probiotic yogurts such as Bravo on cancer patients are even more striking. For example, a recent review describes the effects of probiotic yogurt on the immune system and in cancer (*Endocr Metab Immune Disord Drug Targets*. 2015;15[1]:37–45), while another paper states that probiotic yogurt prevents cancer (*J Agric Food Chem*. 2015 Nov 4;63[43]:9381). When our Bravo yogurt was administered to advanced cancer patients in the context of a natural, nutrition-based, immunotherapeutic protocol, we observed significant results that were published in peer-reviewed journals, with one notable case wherein we even observed the reversal of a genetic marker of cancer (*Anticancer Res*. 2014 Jul;34[7]:3569–3578; *Anticancer Res*. 2015 Oct;35[10):5525–5532). In other words,

a cancer-causing gene (also known as oncogene HER2) that was highly represented before the nutrition-based approach was no longer present after the implementation of the protocol.

JFS: Also, later on, you and your team started studying the effects of this super food on autism in children and different neurodegenerative adult diseases, with very encouraging positive results. Why does it work, and to what extent have you observed net improvements in specific cases of autism independently of its etiology?

MR: Although we may have contributed to the understanding of the pathogenesis of autism, I am convinced that the etiology, that is, the cause(s), may be multiple and different in each individual. The results of supplementation with Bravo yogurt in the context of an integrated, nutrition-based immunotherapeutic protocol in autism have been widely described in a number of conferences and congresses, and there is ample scientific consensus that probiotics help heal the symptoms of autism. According to some authors, alterations of the gut microbiome could actually be the cause for autism (*Drug Metab Dispos*. 2015 Oct;43[10]:1557–1771), and therefore it is logical that restoration of the microbiome helps improve the symptoms. However, I think that the dramatic improvements which we observe in children consuming the Bravo yogurt in the context of an individualized protocol are due to the combination of at least two factors: the reconstitution of the microbiome in the gut and in the brain (the third and fourth brain) and the stimulation of the immune system, notably the macrophages, that is in charge of balancing the two microbial populations between the gut and the brain. Whatever the case, we are witnessing significant improvements that are being independently confirmed by doctors from all over the world.

JFS: The lymphatic system of the brain is a new and amazing revolutionary discovery, announced not even a year ago, that explains many of the discoveries made by your team regarding immune therapy in the last few years. What are the implications of this new discovery for your work, and therefore for all of us?

MR: The implications are enormous, particularly if we consider that the newly discovered brain lymphatic system now has to be associated with the very novel concept of the brain microbiome. The presence of commensal microbes inside the brain and their role as cells of the central (and probably also of the peripheral) nervous system calls for a complete reassessment of all the notions of neurobiology and neurology. As we describe in our paper in *Frontiers in Neuroscience*, the direct connection between the brain and the immune system may explain a number of observations that could not be explained before. For example, in 2002 a Brazilian researcher, Matarrazzo, described two cases of children who at first developed normally, but before age 3 developed autistic symptoms following the reactivation of a chronic otorhinolaryngologic infection; that is, an infection of the nose and throat. He then proceeded to administer adrenocorticotropic hormone as a means to reduce inflammation; and, in one case where the drug was prescribed

in the first months of the disease, the child was completely cured. The other patient, who was 2 years old when autistic symptoms appeared and was treated only 6 years later, showed a partial but definitive improvement with the treatment (*World J Biol Psychiatry*. 2002 Jul;3[3]:162–166). In 2002 no one could explain such wonderful results, but now we can interpret them in light of our publication in *Frontiers in Neuroscience*.

Chronic inflammation in the nose and throat clogs the deep cervical nodes, the nodes where the lymph drains from the brain. Therefore, the lymph cannot recirculate and toxins or toxicants accumulate in the brain, an occurrence that can be severely detrimental to the developing brain. In addition, the obstacle to of the lymph flow leads to accumulation of fluid inside the brain, and such an accumulation in a closed cavity (the skull) leads to the disruption of the connections between the neurons. Finally, such an impairment of the lymphatic drainage causes a relative immunodeficiency inside the brain, since the cells of the immune system, mainly the macrophages, cannot recirculate. Such an immunodeficiency may lead to an imbalance of the brain microbial population and to the chronic inflammatory status of the brain and the meninges that has been widely described in autism. As Matarrazzo demonstrated in 2002, if the inflammation in the nodes is fought at the early stages of autism progression, all these events do not occur or are rapidly reversed, and the symptoms of autism disappear. Most likely a similar mechanism is at work in a number of neurological conditions ranging from Alzheimer's disease to multiple sclerosis, wherein the involvement of the immune system is well assessed.

JFS: With your recent discoveries the excellent results observed in patients with autism and neurodegenerative diseases treated with therapeutic nutritional approaches can now be explained. Recirculation of the lymph in the brain through the lymphatic vessels could be the key. Is this observation on its way to be fully proved?

MR: The discovery of the brain lymphatic system brings about what is termed a paradigm shift; thus, now that a direct connection between the brain and the immune system has been established, the pathogenesis of many brain-based disorders is being revisited. We played our role in defining the importance of inflammation in the deep cervical nodes in autism, but other authors are proposing that a similar mechanism is at work in other neuropathologies such as Alzheimer's disease. Just a few months ago, researchers from the US, France, and Sweden described "the clearance systems of the brain as they relate to proteins implicated in Alzheimer disease pathology" with particular reference to the newly discovered lymphatic drainage (*Nat Rev Neurol*. 2015 Aug;11[8]:457–470). Only a few days ago, in 2016, based on the discovery of the brain lymphatic system, other researchers reevaluated the entire corpus of knowledge concerning brain immunology and went so far to title their academic paper "Get It Through Your Thick Head: Emerging Principles in Neuroimmunology and Neurovirology Redefine Central Nervous System 'Immune Privilege'" (*Nat Rev Neurol*. 2015

Aug;11[8]:457–470). However, as odd as it may seem, I and my team have been the only ones so far to merge the two observations of the brain lymphatic system and the presence of microbes in the normal brain into a single unitary concept: the brain microbiome, or the fourth brain. Such a concept is too novel to have been accepted by academia; however, there are some hints that other researchers share this intuition. For example, at the beginning of 2016, Irish researchers wrote a scientific paper with a rather unusual title that is fully consistent with all the notions that I have described so far: "The Brain's Geppetto-Microbes As Puppeteers of Neural Function and Behaviour?" (*J Neurovirol*. 2016 Feb;22[1]:14–21). In this paper, they write that "there is a growing literature showing active behavioural manipulation in favour of the microbe," and they conclude that "novel experimental approaches and theoretical concepts, such as the hologenome theory, are necessary to incorporate transgenerational epigenetic inheritance of the microbiome into evolutionary theories."

JFS: In *Your Third Brain*, you write extensively on the importance of correctly feeding the second brain (the GI tract) and the third brain or the microbiome: the second brain with healthful food, including a diet based on 60% high-quality protein, 20% carbohydrates, and 20% fatty acids, and the third brain with what you call "the dessert cup" – the probiotic product with GcMAF that you and your team have been studying for years. How do both healthful alimentation and your team's probiotic interact?

MR: The nutritional plan that I describe in the book is known under a plethora of different names: ketogenic diet, Paleo diet, caveman diet and so on. All these names refer to the fact that primitive people, before the age of agriculture, ate very little carbohydrates (carbs) and relatively high proteins and fats. Since this type of diet induces the accumulation of ketone bodies, it is also called ketogenic. Such a nutritional approach is recognized as effective in the complementary treatment of a variety of chronic conditions ranging from cancer to neurological disease including autism (*Eur J Clin Invest*. 2016 Mar;46[3]:285–298). For example, as far as cancer is concerned, this approach is consistent with the 90-year-old observation of Prof. Otto Warburg, who demonstrated how cancer cells depend solely on carbs, whereas normal cells could use also ketone bodies for their survival. Consistent with this approach, our research group has been working for about 30 years on the role of glycolysis (the metabolism of carbs) in carcinogenesis and other human diseases and we have published 13 peer-reviewed papers on the role of a byproduct of glycolysis (diacylglycerol) in cancer as well as in chronic conditions such as neurodegenerative diseases, chronic kidney disease, and cardiovascular disease. We observed that the low-carb-high fat/protein nutrition improves the response to the natural immunotherapy based on Bravo, as well as to all the specific therapeutic approaches for each individual neoplasia, since it makes cancer cells more susceptible to ionizing radiations, chemotherapy or immunotherapy, as we demonstrated several years ago (*Biochem Mol Biol Int*. 1995 Sep;37[1]:81–88). We designed our nutritional approach around Bravo yogurt in order to exploit its main features that stimulate the immune system and reconstitute the human microbiome

in addition to containing healthful fats and proteins with excellent nutritional value.

JFS: Is it possible that, just by consuming "the dessert cup," certain conditions can be improved in places such as Africa, where access to reliable sources of good protein might be difficult? In this sense, your probiotic product has been distributed for free in Malawi and some Asian countries to children and to people living with HIV/AIDS, with, it seems, very good results.

MR: Probiotic yogurts proved effective in a variety of conditions, in particular where malnutrition and immunodeficiency are present at the same time. In fact, they provide for an excellent source of protein, healthful fats, vitamins, and micronutrients. In addition, all probiotic yogurts contain live cultures that exert positive effects on human health as well as a wealth of proteins and peptides that modulate the immune system, favor the absorption of minerals, decrease inflammation and, in general terms, improve nutritional status. Our Bravo yogurt is peculiar in that it was designed taking into consideration the natural formation of powerful immune-stimulating molecules such as GcMAF. In addition, Bravo contains fermented colostrum, an excellent source of noble proteins and of proline-rich polypeptides (PRP). These molecules are also known as colostrinin and were independently discovered in colostrum and other sources, such as blood plasma, in the US and Poland. In this respect, they resemble the Gc protein, the precursor of GcMAF that also is present in colostrum and in blood. PRPs function as signal-transducing molecules that have the unique effect of modulating the immune system, turning it up when the body comes under attack from pathogens or other disease agents, and damping it when the danger is eliminated or neutralized. In other words, Bravo, which contains fermented colostrum, does not stimulate the immune system in a nonspecific way; it rebalances the immune system, and because of this it has also proved effective in those conditions wherein the immune system is overstimulated, such as with autoimmune diseases. The ferments to prepare the Bravo yogurt were donated to two refuge facilities for children, one in Malawi and one in Thailand, and the yogurt was prepared in loco using local cow milk. So far we have received very encouraging reports, and we hope to be able to help other communities as well.

JFS: Throughout your book, you expound extensively on the importance of proteases in order to assimilate the amino acids that compose the proteins we eat. In parallel, you have worked in the pharmaceutical industry, in the field of proteases. Those inhibitors are widely used to treat HIV infection. The official HIV/AIDS hypothesis states that this is due to the inhibition of proteases of the HIV virus, which once its replication is suppressed, allows the immune system to recover. Within this context, is there another explanation of why this proteases or integrase inhibitors work in immune depressed patients, and could those inhibitors be applied to immune deficiencies not related to HIV in general for short periods of time?

MR: I began working on the enzymes called proteases and their inhibitors. I was a

postdoctoral fellow at the Burroughs Wellcome Research Laboratories in North Carolina in the 1980s. With my supervisor, Dr. Eduardo Lapetina, I published a seminal paper that was presented to the National Academy of Sciences of the USA by the Nobel laureate Sir John Vane (*Proc Natl Acad Sci U S A*. 1986 May;83[10]:3456–3459). In this paper, we describe the effects of a protease inhibitor derived from a certain bacterium called Actinomycetes. This natural inhibitor is called *leupeptin* and has several interesting properties that may be exploited in the field of natural immunotherapy with particular reference to viral infections. In fact, many viruses, from the influenza viruses to HIV, rely on proteases to be activated and, because of this, protease inhibitors are effective antiviral agents. Leupeptin was demonstrated to be effective against the influenza virus (*Antiviral Res*. 2011 Oct;92[1]:27–36) but I am not aware of studies of its effects on HIV. However, natural inhibitors of proteases can be found in a number of other natural compounds and may have a role in the natural immunotherapy of viral infections. For example, it is well known that milk and milk-derived products such as Bravo contain inhibitors of the protease called *angiotensin converting enzyme*, and it is also well known that there are a number of peptides with antiviral properties in fermented milk products such as Bravo (*J Proteomics*. 2015 Mar 18;117:41–57). In addition to peptides with protease inhibitory activity, milk and its derivatives contain glycosaminoglycans, such as chondroitin sulfate, that are similar to protease inhibitors called kunins and are known to inhibit the binding of HIV to its target cells that are the CD4 lymphocytes (*J Nutr*. 1995 Mar;125[3]:419–424). It is interesting to notice that this molecule, commonly found in milk and its derivatives, has been demonstrated to be extremely efficient against HIV since the 1990s. In PubMed there are reports describing how a combination of natural substances that included chondroitin sulfate reduced viral load to nondetectable levels in 10 days, a result not even imaginable today with the most advanced antiretroviral drugs (*Posit Health News*. 1998 Fall[17]:7–11). The following year, in 1999, an Italian researcher working at the Centre for Virology of the Institute Spallanzani in Rome demonstrated that chondroitin sulfate was efficient not only against HIV itself, but also the opportunistic pathogens that are known to infect immunodeficient patients (*Antivir Chem Chemother*. 1999 Jan;10[1]:33–38). Curiously, none of the research involving natural protease inhibitors such as leupeptin or the inhibitors in milk has received much attention, while this could be an inexpensive and fully natural way to block viral infections and strengthen the immune system. However, I would not be surprised if in the future it is demonstrated that anti-HIV (antiretroviral) drugs also show other effects that may be responsible (momentarily) for the recovery of the immune system independently of their antiretroviral effects. This is a frequent occurrence in medicine; the positive effects of a drug on the outcome of the disease are at first ascribed to some mechanism that is later on demonstrated to not be completely correct. The positive effects remain, but it may be discovered that the reason why the drug worked is completely different from the one that had been previously hypothesized. If we assume that a competent immune system can fight the

supposed HIV infection, then it is reasonable to hypothesize that drugs, remedies, or supplements that strengthen the immune system will help in such a fight.

JFS: The dessert cup with the GcMAF protein is an intrinsic part of the vitamin D axis. Is it important to check vitamin D levels and in case of low levels, supplement before taking Bravo or GcMAF?

MR: The issue of how much vitamin D we need is a truly complex one. So complex that a few years ago, the famous nephrology journal *Kidney International,* of the Nature Publishing Group, invited me to write a commentary titled "Chronic Kidney Disease and Vitamin D: How Much Is Adequate?" (2009 Nov;76[9]:931–933). There I put forward the idea that the adequate intake of vitamin D has to be reevaluated. In fact, the current indications refer to the biological effects of vitamin D on bone and calcium metabolism, with particular reference to the prevention of rickets. However, such a limited interpretation of the biological effects of vitamin D is now obsolete, and even the designation of the molecule is erroneous. Today we know that it is not a vitamin but a hormone, and that it regulates practically all the aspects of cell physiology and therefore is involved in almost all types of disease. If you search for "vitamin D and cancer treatment" in PubMed, you will find more than 5000 published articles, and the same happens if you search, for example, for "vitamin D and cardiovascular disease," where you find more than 4000 articles. However, as of today, no one is able to state with certainty what the adequate amount of vitamin D is in each particular condition and whether the amount to treat a disease is the same as that to prevent the same disease. Things are even more complicated if we consider that each individual has a different response to vitamin D because of variations (polymorphisms) in the gene that codes for the receptor of vitamin D. We published several papers on this issue, and, for example, we demonstrated that women harboring a variation of the gene show a significantly higher risk of metastases and recurrences of breast cancer (*Oncol Res.* 1998;10[1]:43–46). There are physicians in Germany who inject intravenously very high amounts of vitamin D, in the range of 400,000 International Units (IU), in the course of cancer treatment and, interestingly, do not report adverse effects. Personally, I try to expose myself to the sun as frequently as possible since in 30 minutes of exposure my skin produced more than 10,000 IU of endogenous vitamin D. When I don't have this opportunity, I take the same amount of vitamin D as a supplement but, as I have written, the response and consequently the dosage vary in each individual. The response to any treatment involving stimulation or rebalancing of the immune system needs adequate amounts of vitamin D; and, in case of doubt, supplementation may be required. (In PubMed, "vitamin D and immune system" yields 3459 papers!)

JFS: Speaking of heparin, as it is widely mentioned in *Your Third Brain*, is your team still studying it in order to find ways to administer it to patients, since it appears to have very important properties and it is a very effective molecule to fight cancer cells by

inhibiting their replication?

MR: I began working on heparin in 1985 when I was a young post-doc at the Laboratory of Molecular Biology of the University of Firenze, Italy; and I published a paper in the *Biochemical Journal*, which was among the most famous scientific journals in those days (1985 Apr 1;227[1]:57–65). In that paper, we described a rather strange association between endogenous heparin and phosphatidylcholine, a major constituent of the cell membrane. While the use of heparin as an anticoagulant drug is very well assessed, the role of endogenous heparin and in particular in the bloodstream remains a mystery, one of the few mysteries in today's physiology. Back in the 1980s, we postulated that endogenous heparin might have had roles other than the well-known anticoagulant activity, and, given our interest in the biology of cancer, we begun studying whether heparin was a naturally occurring anticancer agent. We published the first paper demonstrating that it is indeed an anticancer agent in 1991, when we showed that heparin inhibited the proliferation of carcinoma cells (*FEBS Lett*. 1991 Apr 9;281[1–2]:141–144). Two years later, we demonstrated that such an anticancer effect was not limited to carcinoma cells, but it could be observed in a variety of cells transformed by different oncogenes, which are genes whose mutations are known to cause cancer (*Cell Biol Int*. 1993 Aug;17[8]:781–786). Therefore, we concluded that the role of the heparin that in the bloodstream is related to the natural control of cancer, the so-called anticancer surveillance, a term which indicates the physiological mechanisms that defend us from the cancer cells that constantly arise in the body. Rather obviously, the heparin that is commercially available cannot be used as an anticancer drug as it is, since it would cause bleeding because of its anticoagulant activity. Because of this, for the following 20 years, we kept on studying its physiological assembly in the human body with the goal of discovering the secret of how endogenous heparin can protect from cancer without causing bleeding. We eventually found out that the key lies in its association with plasma proteins. In other words, when the endogenous heparin associates with certain proteins in the plasma, the properties of the proteins and of heparin change dramatically and novel biological properties, such as the anticancer activity, arise. We also found out that heparin regulated gene expression, another apparent oddity considering that the DNA is negatively charged and heparin is the biological molecule with the highest concentration of negative charges. Therefore, according to the principles of electrostatics, DNA and heparin should repel each other. On the contrary, we demonstrated that heparin, once bound to certain proteins, dramatically changes its overall electrostatic behavior and is transported inside the cells, where it can interact with molecules such as DNA, therefore influencing the functioning of the genes (*Biochem Biophys Res Commun*. 1986 Oct 15;140[1]:294–301).

JFS: "Miraculous" molecules such as GcMAF apparently have many abilities, although the real one would to be the carrier for the good molecules doing the real job. Actually GcMAF could be replaced somehow, as the important aspect is its carrying ability.

Could you please further explain this important aspect to better understand this molecule?

MR: First of all, we have to understand what *GcMAF* means: it is a macrophage activating factor (and this describes its function) derived from a protein called Gc (from *group component*; that is, a component of human plasma). The Gc protein is also known as vitamin D binding protein because one of its functions is to bind vitamin D and carry it to the target cells. The functions of the Gc protein have been known for years, long before the observation that it also could activate macrophages. It was very well known that the Gc protein binds actin, a cellular protein that is released when cells die. Therefore, the Gc protein works as a scavenger that prevents the accumulation of actin coming from dead cells that could be very toxic. In addition, it was known that the Gc protein also binds fatty acids such as oleic acid. We could make an analogy and say that the Gc protein is a big truck that can carry away toxic waste (the actin from dead cells), as well as vitamin D and oleic acid to their cellular targets. The Gc protein also carries another molecule with a rather fancy name: alpha-N-acetylgalactosamine. This is a sugar that is present in milk (hence the name with the suffix -*galactose*), and it is bound to one of the amino acids of the Gc protein. We observed that the function of activating macrophages is due to a molecular triad that happens to be carried by the Gc protein. This molecular triad is composed of alpha-N-acetylgalactosamine, vitamin D, and oleic acid.

Continuing analogies with vehicles, now we can say that the Gc protein is like a taxi that carries a three-person surgical team to the hospital so that they can perform their duties. These three people/molecules are the alpha-N-acetylgalactosamine, vitamin D, and oleic acid that need to be carried all together at the same time if they are to activate macrophages. The kind of taxi, or whether it is a limousine or a piece of junk, is rather irrelevant as long as it can carry the team to the hospital. Now, imagine that GcMAF is nothing other than a taxi carrying the three-people team to the hospital; we have found a way to replace the Gc protein that derives from blood with another class of molecules called glycosaminoglycans. It is as if we have replaced the taxi that moves slowly in the traffic with a helicopter; it can carry the three-person team to the hospital much faster and more efficiently. In addition, let's assume that the team is needed in more than one hospital; here it means that more than one macrophage needs to be activated. With a regular taxicab, once they have finished in the first hospital, the three members board the taxi that, slowly and with the uncertainty of traffic, carries them to the next hospital. With the helicopter, the transfer of the surgical team from a hospital to the next will be much more rapid and efficient and a greater number of patients will benefit. When I invented this "helicopter" that is the next generation of GcMAF, I followed this very reasoning, and I had the advantage of having worked on glycosaminoglycan for the previous 30 years. This is the reason why this next-generation MAF (here the Gc prefix is no longer necessary) is so powerful and versatile.

JFS: Besides GcMAF-carrying ability, you mention many others, such as inhibition of breast cancer cells, as well as protection of human cells from damage inflicted by heavy metals. Once the vitamin D axis is reestablished, there is an awakening of macrophages and natural killers. Is that correct? Are there other mechanisms by which GcMAF function?

MR: Molecules such as the old GcMAF or next-generation MAF have multiple actions, for the very simple reason that they operate at the most basic genetic level and influence the working of a number of genes that in turn influence a great number of physiologic functions. This translates in a long list of possible clinical applications. Just to name a few, the next-generation MAF could be used to restore the immune system, which is rather obvious, but also to fight several chronic conditions wherein the use of vitamin D, oleic acid, and glycosaminoglycans has already proved effective. I am referring to neurological disorders such as Parkinson's and Alzheimer's diseases, multiple sclerosis, amyotrophic lateral sclerosis, and brain aging, or to dermatological conditions such as psoriasis. Another possible area of intervention is related to cardiovascular conditions, since all three members of this "surgical dream team" have been shown to be effective in the prevention and treatment of cardiovascular conditions – and of course cancer. We do not know as yet the minimum length of treatment with this novel, rather revolutionary MAF, but we can safely assume that once the underlying disorders have been addressed, a minimum maintenance dose should be all that is required.

JFS: Stimulation of nitric oxide looks like a significant ability of GcMAF molecule, as it has an important effect on the circulatory system. Could you expand on the importance of this aspect and how it relates to cardiovascular health?

MR: Nitric oxide (NO) is a very interesting molecule; it was even awarded the title of "Molecule of the Year" by the journal *Science* in 1992. In those days, its fame was mainly due to its role in the mechanism of action of Viagra. In fact, among many other actions, NO cause vasodilation, and the increase in blood flow in different organs may be exploited for different scopes. For example, it had been known for decades that nitrates that release NO are very useful in increasing the coronary blood flow and can solve an attack of angina pectoris. Increased blood flow in the cavernous bodies of the penis fights erectile dysfunction; Viagra essentially works by blocking the degradation of NO, thus favoring its action. Later on, it was discovered that NO also has other interesting functions that could be exploited in the treatment of proliferative diseases such as cancer. In fact, NO selectively kills cancer cells because it causes a type of damage to cancer cells' DNA that they cannot repair. In other words, NO has the same effect on the DNA of healthy cells and cancer cells, but the cancer cells are unable to repair the damage inflicted by the NO, whereas the healthy cells do have not such a difficulty. We discovered that macrophages, when they are activated by GcMAF or the next-generation MAF, release NO, and such a release can easily be

demonstrated by looking at the blood flow with a common ultrasound system (*Anticancer Res*. 2014 Jul;34[7]:3569–3578). Thus, we may have found something very close to the long-sought-after "magic bullet." In oncology, before the advent of the "smart bombs," *magic bullet* referred to a molecule that could kill only cancer cells, leaving healthy cells unharmed – at variance with what commonly happens with conventional chemotherapy. The principle is rather simple: we activate the macrophages with the old GcMAF or, even better, the next-generation MAF. The macrophages go on a "search and destroy" mission; that is, they look for cancer cells or cells infected by viruses. Once they have found such an abnormal cell, the macrophages bind to it and release NO that causes irreversible damage to the DNA of the cancer cell. As far as the role of the NO released by the activated macrophages in cardiovascular health is concerned, we must take into consideration that a constant, physiological activation of macrophages can be achieved with probiotic products such as Bravo, which in this way may contribute to the maintenance of health. In fact, Bravo not only contains naturally produced GcMAF, but it also has some live microbes such as *Lactobacillus rhamnosus* that are known to activate macrophages per se (*Microbiol Immunol*. 2012 Nov;56[11]:771–781).

JFS: Immune therapy started with Dr. William Coley at the end of the 19th century and the beginning of the 20th century. However, the 20th century has been the century of the pharmacology approach in medicine. Teams and researchers like yourself are now working in the 21st century on immune therapy, and have rescued this fundamental approach to the future of medicine. Bearing all this in mind, how do you forecast the future of medicine in the years to come?

MR: It is interesting that you mention the work of Dr. Coley, the father of immunotherapy, since such an approach is being rediscovered in these very days. In a publication in PubMed, aptly titled "Back to the Future," the author writes: "Cancer patients infected with various bacteria were reported, for at least two centuries, to have spontaneous remission. W.B. Coley, of what is now the Memorial Sloan-Kettering Cancer Center, pioneered bacterial therapy of cancer in the clinic with considerable success beginning in the late nineteenth century. After Coley died in 1936, bacterial therapy of cancer essentially ended. Currently there is much excitement in developing bacterial therapy for treating cancer using either obligate or facultative anaerobic bacteria." (*Methods Mol Biol*. 2015;1317:239–260). It should be noticed that Dr. Coley had spectacular results in very advanced cancer cases, results that we cannot even dream of with our most advanced therapeutic strategies. The problem with Dr. Coley's approach, however, was the lack of standardization and, because of this, the difficulty of reproducing the results that he consistently obtained on a large scale. Quite obviously, our knowledge of the mechanisms of cancer is now much more refined, at the molecular level, and we also know the role of the microbiome in health and disease; therefore, many researchers think that the time is ripe to rediscover Dr. Coley's observations and interpret them in light of today's knowledge, with the hope of finding a reliable

way to treat patients otherwise labeled incurable. Such an approach is becoming "mainstream," up to the point that the journal *Science* recently wrote: "A new class of cancer treatments that unleash the power of the immune system on tumour may depend on some unlikely allies (the microbes)" (2015 Nov 6;350[6261]:614–615). In my opinion, these words do justice to the work of Dr. Coley, since they clearly state that the microbes "unleash the power of the immune system" that in turn represents "a new class of cancer treatments." It has to be said that researchers are exploring rather innovative ways to exploit the abilities of microbes to fight cancer, and they are finding allies that are even more "unlikely": the viruses! In a paper titled "Combined Bacterial and Viral Treatment: a Novel Anticancer Strategy," the author argues: "An idea for a new combination therapy will be described herein. It is a proposition to combine viral and bacterial anticancer therapies and make them fight cancer in concert" (*Cent Eur J Immunol*. 2015;40[3]:366–372). It may come as a surprise to learn that a virus that could potentially be exploited to fight cancer is no one less than … HIV! As I write, together with John W. Anderson, in the *Encyclopedia of Cancer*, " … there exists a HIV accessory protein termed viral protein R (Vpr) that plays a key role in virus replication and also induces cell cycle arrest and apoptosis in various cell types including T cells, neuronal cells, and tumor cells." Thus, as odd as it may seem, a protein produced by HIV could be exploited as a beneficial antitumor agent in cancers. … In recent years, several investigators have studied the potential use of Vpr as an antitumor therapeutic. … "The antitumor properties of certain HIV proteins might even have been responsible for establishing a symbiotic relationship in humans. … " (*Encyclopedia of Cancer*. doi:10.1007/978-3-642-27841-9_562-4). These observations are consistent with a rather odd report from Columbia University describing how women carrying HIV, or at least its antibodies, appear to have a better 5-year survival rate (80%) when compared with the general population (70%) (*J Surg Oncol*. 2005 Jan 1;89[1]:23–27). All these results and the deriving considerations lead me to speculate that we may be on the verge of a revolution in cancer therapy and ready to rediscover and appreciate the long-neglected work of Dr. Coley.

JFS: Could this approach, at least in Western countries, counterbalance and refocus the dominance of pharmacological approach in modern medicine by giving more freedom to doctors in order to be able to use all the tools at their disposal?

MR: I truly think that rock-solid scientific evidence is accumulating and supporting those approaches once labeled "alternative." The microbial therapies of cancer are just an example of such a rapidly evolving field. Regrettably, laws and regulations follow much more slowly, and it may take years before such approaches are officially recognized by regulatory bodies. However, the use of Internet by patients is rapidly changing the scenario within which therapists are called to operate. In fact, it often happens that patients are more updated and prepared than therapists, and it is not infrequent to notice that patients go to their doctors asking for approaches that have solid scientific evidence although they may not yet be approved by regulatory bodies. Patients are often prepared to travel long distances

to have therapies performed when these are not available in their countries. Internet, low-cost flights, and globalization are changing also the way that patients perceive their quest for health, and the world of medicine has to adapt if it wishes to survive. The same reasoning applies to doctors as well. It is now commonplace to find European or American doctors in wealthy countries in Asia or the Middle East, free to practice innovative therapeutic approaches with great benefits for those patients who can afford the expenses. If Western medicine does not adapt itself, this phenomenon will only increase, and, sadly, only the wealthy will be able to benefit from the latest discoveries in medical science.

JFS: The lack of development of molecules such as GcMAF or heparin could be interpreted as "laziness" by the pharmaceutical industry. The production of such immune components has shown amazing results, being much less harmful that conventional treatments, and less toxic when those treatments need to be administered. Why? Is there a conflict of interest or simply a lack of interest from allopathic medicine and Big Pharma?

MR: Having worked in the pharmaceutical industry in the US and in Europe, I can say that, just like any other industry, the pharmaceutical industry is not driven by ideology but by profit. Therefore, so-called Big Pharma is not for or against alternative or complementary medicine; it simply follows the paths that lead to profit, and as of today the research and development of allopathic drugs has proved very effective in this respect. The responsibility for such a situation also lies in part in the alternative-practitioner community, which sometimes has a sort of ideological approach to medicine and health-related issues and is against the industrial approach a priori. It is also true that many natural remedies show little appeal to the pharmaceutical industry because it is difficult to patent them and therefore profit. However, I have the perception that things are changing and I am observing a phenomenon that I have already witnessed in the 1980s. Back then, Big Pharma was not interested in the emerging field of molecular biology and biotechnologies; it was focused on the old-fashioned approach of skilled chemists' synthesizing thousands of putatively helpful molecules in their labs. The community of molecular biologists, however, was composed mainly of hippie-type biologists, rather than chemists in their white coats, and they focused on the biological processes that lead to the onset of diseases, with the goal of exploiting such a knowledge to develop naturally based molecules aimed at restoring cells' physiology. Those scientists begun founding their microscopic companies, sometimes in little more than their garages, with the goal of producing one single antibody or one single biologically derived molecule. Thirty years later, such companies evolved into the gigantic biotech industries that we know today and lead the market in the research and development of remedies based on the knowledge of how cells function. I hope that such a transition will occur also for what is now defined as alternative or complementary medicine, and I see many signs that this is already occurring.

JFS: The final chapters of *Your Third Brain* raise a fascinating field ultrasonography and ultrasound. To what extent could this "remote control" science revolutionize the field of immune therapy?

MR: At variance with common perception, ultrasounds are not generated exclusively by machines such as those that are used in diagnostic ultrasonography, a branch of radiology that I mastered in the past. Ultrasounds are commonly generated in nature and are perceived by animals, as anyone who has a god knows very well. According to some theories, ultrasounds have shaped the universe as we know it, and they may have contributed to shaping the DNA that codes for life on this earth. Therefore, it is not surprising that there are genes in our DNA that are turned on by ultrasounds, and, by a leap of imagination, we could visualize someday having a remote control that lets us turn on or off genes, just as we zip between television channels from our sofas. And such a day may be closer than we think. Since 2013, we have published papers demonstrating that ultrasounds alter mental states and may be useful in the treatment of a number of diseases. Quite recently, we published two papers demonstrating how ultrasounds improve neuronal connectivity and how such an effect may be applied to neurological conditions such as autism (*Conf Proc IEEE Eng Med Biol Soc*. 2015 Aug;2015:8131–8234; *Biomed Signal Process Contr*. 22[2015]:44–53). These results of ours are consistent with previous observation by Hameroff and colleagues, who described how ultrasound could be used to address untreatable pain (*Brain Stimul*. 2013 May;6[3]:409–415), and more recent reports describing the potential use of ultrasounds in a series of neurological diseases (*Nat Rev Neurol*. 2016 Mar;12[3]:161–174). As far as of cancer immunotherapy is concerned, there are not very many studies demonstrating such an effect of ultrasound, probably because most researchers, including ourselves, were mostly interested in its effects on the brain. However, in some preliminary experiments that have not yet been published, we noticed that ultrasound at certain frequencies can kill cancer cells, leaving the healthy cells unharmed. This selectivity can be explained by taking into account the fact that cancer cells have a metabolism quite different from that of normal cells, and therefore the effects of the ultrasound seem to be detrimental to them. Interestingly, healthy cells not only do not suffer any damage as a consequence of ultrasound treatment, but they also seem to be stimulated in their functional activity. In other words, at least in vitro, ultrasounds seem to have a sort of "magic touch" on cells; they kill the cancer cells while benefiting the healthy cells at the same time. Although the precise mechanism of action still has to be determined, such effects are currently being exploited in the field of cancer immunotherapy. For example, in 2014, our Japanese colleagues and competitors in the field of GcMAF research published a paper demonstrating how the combination of GMAF injections and ultrasound therapy (designated sonodynamic therapy) was very effective in breast cancer treatment (*Anticancer Res*. 2014 Aug;34[8]:4577–4581). In the past few days, working together with one of the most respected medical doctors in the field of integrative medicine, we noticed that a careful application of ultrasound after specific immunotherapy significantly

amplified the effects of the immunotherapy itself. Since ultrasound is inherently safe and harmless, as demonstrated by some 50 years of diagnostic ultrasonography, I think that such an approach will become commonplace in a very short period of time.

JFS: What last message could you give to our readers?

MR: I wish to give the same message that I used to give my patients not long ago: every day we are witnessing a new boost in scientific discoveries that, at variance with the past, now can immediately be applied to the individual case. Therefore, diseases that yesterday were deemed incurable today can be successfully fought. We are learning so many things about the immune system, the microbiome, the relationship between our human and nonhuman brains, that yesterday's knowledge already is obsolete. Cancer, autism, and neurological or cardiovascular diseases can now be approached with a variety of options that offer realistic hopes to cases previously designated incurable. We live in a most exciting time, and it seems that there are no boundaries to what we may achieve in health and medicine if we are open to the progress of knowledge.

Jacques Fernández de Santos is an independent journalist based in Spain who has covered different countries, analyzing their economies, politics, and social issues. He has covered missions in countries such as Ukraine, Brazil, Jordan, Bulgaria, Kurdistan-Iraq, and Turkey. His last mission was in Morocco in 2015 (www.marco-hub.fr). He also occasionally undertakes scientific interviews with researchers such as Dr. Marco Ruggiero.