



ABO and WCM biotypology: The secret of the holobiont

Marcello Menapace

PhD, MBA, MSc, Devonshire House, Manor Way, WD6 1QQ, Borehamwood, Hertfordshire, United Kingdom

Abstract

It has always been thought that we are just plain old human beings being composed of mere human eukaryotic cells and occasionally attacked by viruses and bacteria. But this vision is plainly wrong. We now know that we are a coherent mix of all kinds of life, running from unicellular microorganisms to pluricellular organisms, which form part and parcel of what can be called human being. We are “holobionts” and as such the multiplicity of cells and viruses belonging to all kingdoms of life collaborate in an equilibrium that if perturbed can cause disease. The ancient medicines (including Western Constitutional Medicine [WCM]) adopted this way of thinking and the ancient observers sagely imprinted their medicinal systems with this holistic view of life for which disease has to be contrasted with a return to equilibrium. And the ancient medicines used as primary tool the correction of the diet seen as the restoration of the equilibrium between the energies (microorganisms) in the body. More recently a new medicinal system the ABO groups has taken a stronger stand in the scientific arena through the experimental confirmation of its main mechanism of action by glycosciences (especially glycobiology). Necessarily, bacteria, fungi and other unicellular organisms can feed off on glycans which are both produced and absorbed by the human body (diet) and the suitable balance of diet will interfere in this cycle. It is now the time to capture all these advances in ancient and modern medicine and fuse them into a new medicinal system, the ABO-WCM biotypology, to better explain the reality of the holobiont and to completely transform modern science into a truly personalized approach.

Keywords: glycan, abo blood group, clustered saccharide patches, ABO antigens; glycotopes, holobiont, glycobiology, metaorganism

Introduction

The human body throughout the lifetime of individuals in health and disease states contains many different sites that are colonized by microbial communities, which vary significantly between individuals and is driven primarily by body habitat [22]. These microbial communities, termed microbiota are made up of several predominant bacterial phyla (composed of hundreds of bacterial genera and species), and collectively together outnumber human cells in the body by an estimated factor of 10 [23].

Newer state of the art techniques (contemporary genomic research and metagenomic analysis) has expanded our understanding of the presence and composition of microbes residing in diverse habitats in the human body, which was previously based on microbial culturing techniques [24]. Culture independent methods has revealed that as many as 90% of the microorganisms cannot be cultured by standard techniques (uncultured fraction) and this includes diverse organisms belonging to previously unanticipated microbial lineages only distantly related to the cultured ones [25, 26]. These novel methods utilizes 16S ribosomal gene-specific next generation sequencing (NGS) to extract bacterial DNA or RNA from organs and tissues thus evaluating the presence of microbes without culturing them [27]. We are quickly realizing that many microbial species, which are successfully adapted to the human body, cannot not be cultured but can be proven to colonize humans indirectly through next-generation DNA sequencing techniques [28]. An analysis of 27 different body sites, including the skin, nostril, hair and oral cavity revealed that distinct anatomical niches house unique

microbiomes and that each body site has distinct and dominant bacterial taxa [29]. Hence, body sites previously assumed to be sterile in healthy humans, have been shown repeatedly to be colonized by microbes of different phyla without apparent signs of disease [30].

The use of deep-sequencing technologies have allowed scientists to investigate sites once thought of as sterile, such as the stomach, bladder, and lungs, which have now been shown to harbor an indigenous microbiota [31]. Recently published results of the Human Microbiome Project (HMP) Consortium have provided the first reliable overview of the breadth of structure, function, and diversity of a possible healthy human microbiome across multiple body sites [32, 33]. As a result, there is growing recognition that perturbations in organ-specific microbiota (dysbiosis) are a constant and unavoidable feature of human diseases [34].

Human Body Habitats

The most well-known and vastly studied colonization site is the gastrointestinal tract (GIT), which is home to the most abundant commensal community in the entire human body [35].

There is a delicate and balanced ecosystem in the human intestine, in which 10^{14} bacteria dwell habitually forming the human gut microecology [36]. Several studies performed over the last 2 decades to catalogue microbial genes by metagenomic sequencing indicated that Firmicutes and Bacteroidetes are the dominant bacterial phyla of a healthy gut [37]. This highly metabolically active microbial community, called intestinal microbiota, living in the human intestines, plays a critical role in health and well-being of their host [38]. As for gut microbiota, this

result introduced the concept of tissue microbiota equilibrium as a potential factor in human health [39].

Epithelia

Skin

The skin microbiome is composed of highly specialized microbes, which are capable of enduring the heterogeneous conditions offered by the skin: *Propionibacterium* spp. Adapted to flourish in skin sites enriched in sebaceous glands, *Corynebacterium* spp to moist sites, while *Proteobacteria* spp. dominate in dry sites [40].

The composition of the lung microbiome includes principally microbes from the Bacteroidetes, Firmicutes, and Proteobacteria orders, which are spatially distributed in an uneven fashion so that it is greater near the trachea [29]. A similar microbiota to that of the lungs was found also in the oropharynx region (same phyla), and was more varied than the nostril microbiota with only two major phyla: Firmicutes and Actinobacteria [41]. Recently, it has even been demonstrated that microbes can be found deep in the dermis under the epidermis in contact with various cells below the basement membrane, communicating directly with the host [42].

Oral Cavity and Oesophagus

Streptococci and *Lactobacilli* are both members of the oral and oesophageal microbiota [43].

Blood

Researchers have recently described a human blood microbiome and tissue bacteria [44]. Particularly, the human blood of healthy individuals is not believed anymore to be sterile, but to contain its own microbiome (as community of pleomorphic microbes) [45]. This hypothesis was formulated about 50 years ago until it was finally prove in 1993 by the Bulgarian scientist Emil Kalfin demonstrating that microorganisms are multiplying in the red blood cells [RBC] of healthy people [46]. More recently, using 16S rRNA gene sequence amplification, such microbes have been discovered in different blood fractions, with most of the bacteria being located in the buffy coat and in red blood cells [47]. Almost surprising is the observation of cell-free bacterial DNAemia in both septic and healthy patient blood, with a significant difference in the taxonomic classification between the two groups with a predominance of bacteria of the order Bifidobacteriales in the healthy group [48].

And certain microorganisms are known to move from the tissue, where they reside, to the blood or other tissues (a process known as atropobiosis), utilizing a number of tricks, especially that of being dormant intracellularly [49]. These microbes can be effectively resuscitated at an optimal growing temperature of 43°C (at 37°C they were dormant), within 48 hours, were shown to be Gram stained, be cultured and represent 47 bacterial orders belonging to 15 phyla and 39 fungi orders belonging to 2 phyla [28]. Other researchers have found four phyla (namely, Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes), which were consistent irrespective of molecular method used (DNA vs. RNA), with the results of other published studies, representing the core blood microbiome [50].

Closely related tissues to the blood, such as the vascular endothelium, have been shown to be colonized by a selective microflora [30].

Internal Tissues

Several other human tissues are natural habitats of resident bacteria.

Gut

It is a known fact that in healthy mammals, commensal bacteria are anatomically restricted to either the intestinal lumen, the epithelial surface or within the underlying gut-associated lymphoid tissues (GALT) [51]. It seems that only select number of bacteria avoid multiple physical and biochemical mechanisms that maintains a separation from immune cells and colonize the interior of intestinal lymphoid tissues, without causing adverse inflammation or immune responses [52]. Among these microbes are *Alcaligenes* spp., *Achromobacter* spp., *Bordetella* spp., and *Ochrobactrum* spp., isolated from lymphoid follicles (ILFs), the interior of Peyer's patches (PPs), and the mesenteric lymph nodes (mLNs) of animals and humans [53]. Therefore, bacteria colonizing the GALT (and PPs) can create and maintain a homeostatic environment by inducing only weak antigen-specific immune responses (optimal IgA induction), without excessive inflammation [54].

Stomach

Another long thought sterile and inhospitable environment due to its highly acidic environment, the stomach is now known to harbour its own ecosystem of bacteria as demonstrated by microbes regularly sampled from healthy adults [55]. This paradigm shift was initiated by the discovery of a pathogen, *H. pylori*, in 1982, (the bacterium escapes gastric elimination, by producing ammonia from urea, that neutralizes acid), and later researches confirmed the existence a gastric-specific microbial community [56]. Microbiota taxonomic complexity and bacterial load is not very high in the stomach with about 101–103 CFU/ml and fewer aero-intolerant species (37 out of 110 total species present in the stomach) than in other parts of the gut [57]. Variations of microbial composition of the gastric ecosystem (dysbiosis) is the hallmark of disease in the stomach and many urease-producing bacteria (UB) and nitrosating or nitrate-reducing bacteria exist habitually in the stomach [58]. Their presence seems not to be without any effect of the physiology of the organ. It has been shown that *H. pylori* can cause transient hypochlorhydria (acid inhibition), which may contribute to gastrointestinal homeostasis by modulating gastrointestinal microbial composition [59]. The high variability of the stomach microbiota correlates with its pH, as the main environmental factor.

Brain

Microbiota have also been identified within the CNS, where an α -proteobacteria class was reported to be the major commensals persistent in the human brain regardless of immune status [30]. The presence of bacteria from intestinal or oral origin have been observed in atherosclerotic plaques with significantly higher levels of Proteobacteria and fewer Firmicutes [60]. The previous classical idea was that the brain, being a privileged organ, could only be infected by gut bacteria during septic conditions (encephalopathy), associated with neuroinflammation [61]. But the realization that the brain actually harbours distinct bacterial species has been quite a stir in the scientific community. Gram-negative bacteria have been reported as the predominant

bacteria found in normal human brains [62]. Both bacterial and viral genomes were detected in the normal brain specimens, with a predominance of α -proteobacteria (70%) with respect to other phyla [30]. Bacterial infection at the level of the brain has been postulated to be a co-cause of neurological diseases of proinflammatory type such as Alzheimer [63]. Indeed, Alzheimer brains displayed has a larger proportion of Actinobacteria species and a 5–10-fold more bacterial genomic sequences with respect to normal brains [27]. Bacterial genomes sequenced with NGS showed greater diversity (α -, β -, γ - and δ - classes of proteobacteria, actinobacteria and cyanobacteria) in normal than in multiple sclerosis patients' white matter [64].

Reproductive Tissues

Urogenital Tissues

The male reproductive tract has its own microbiome (located in the seminal vesicles) as confirmed by tests on seminal fluid [65]. Diverse kinds of bacteria were experimentally found in the human semen, with apparently no significant differences between sperm donors and infertility patients [66]. Moreover, bacteria are present in semen samples of men and can be transmitted to their female sexual partners [67].

The vagina itself is a habitus of bacteria and microbes, especially *Lactobacillus* (practically the only detected bacterium) [68].

The presence of bacteria that are not or cannot be routinely cultivated have also been recovered in samples of female urine (free from urinary tract infection) by voided, transurethral, and/or suprapubic collection methods [69].

Uterus and Placenta

The advent of next-generation metagenomics sequencing has allowed for the culture-independent characterization of the vaginal microbiome, which was shown to be dominated by *Lactobacillus* species in both nonpregnant and pregnant populations [70]. This is in line with traditional microbiological techniques (culture-dependent) resulting in the delineation of "normal" flora (defined as *Lactobacillus* predominant), and "abnormal" or "aberrant" vaginal flora (nonlactobacillus predominant) [33].

Up until recently, intrauterine infection of the placenta was thought to be the cause of preterm birth (PTB) and chorioamnionitis with placental/fetal colonization by vaginal bacteria, though it was revealed that oral bacteria of *Streptococcus* species and *Fusobacterium* species were major responsible [71, 72]. It has since been discovered that bacteria can be found within the placenta of term and preterm subjects [73]. Even the maternal basal plate of the placenta (the tissue layer directly at and below the maternal-fetal interface) from both preterm or term gestations (without clinical or pathologic chorioamnionitis) harbors Gram-positive and Gram-negative microbes, intracellularly [74]. Moreover, an in-depth metagenomic characterization of the placental parenchyma microbiome has previously been provided highlighting a unique niche of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla [75]. The placental parenchyma microbiome was altered in cases of PTB and chorioamnionitis with high abundance of both urogenital and oral commensal bacteria and significant variation in microbial metabolic pathways [76]. In healthy humans, the composition of the endogenous placental microbiome is distinct

from that of the vagina and resembles more the oral microbiome, exhibiting limited microbial diversity, which indicates that bacteria may perform specific functions [77]. This means that the foetus and hence the newborn develops in a non-sterile environment, where bacteria are present in the uterus and may seed the baby with a starter culture of bacteria received by their mother [33]. It has now become clear that mother, fetus, and different symbiotic microbial communities induce or constitute conditions for the development and reproduction of one another: from reciprocal scaffolding of developmental processes and mutual construction of developmental, and ecological niches [78].

Breast Tissue

In sites all around the breast of healthy volunteers, many species belonging to various phyla (principally proteobacteria) were recovered, confirming previous suspicion of microbes in the milk reaching the ducts from the skin [31]. The source of such bacteria was suggested from several studies to be from the mother's gastrointestinal tract [79]. An important study demonstrated that different bacterial profiles in breast tissue exist between healthy women and those with breast cancer, with higher relative abundances of bacteria that had the ability to cause DNA damage *in vitro* in the latter [80].

Conclusions

Finally, several studies revealed the presence of tissue microbiomes in diseased patients, whose conditions would not have been previously linked to bacterial infection. Bile duct tissues were observed to harbor a distinct microbiome, dominated by the Dietziaceae, Pseudomonadaceae and Oxalobacteraceae bacterial families in patients with cholangiocarcinoma [81]. In critically ill patients after stroke, common commensal bacteria were discovered to translocate into distant tissues and cause post-stroke infection and pneumonia [82].

All these studies demonstrate that microbes may well be resident in all tissues in healthy conditions as symbionts.

Holobiont

There is a new notion of self that is developing of late: the view that we, humans, are a dynamic and interactive community of human and microbial cells [83]. We, as humans, could be considered as hybrid organisms, consisting of both human (eukaryotic) and bacterial (prokaryotic) cells [84]. Humans are thus eukaryotic macroorganisms, i.e., hologenomic entities or vast collaborations of mutually competitive and co-dependent cellular ecologies [85]. This one single functional unit between the host and its associated microbial community is being defined as holobiont (or metaorganism) [86].

This innovative and pioneering idea is confirmed by the plethora of studies of interactions between normal human physiologic functions and the commensal microbes [87]. Our human body, indeed, hosts a vast array of different microbes belonging to phylogenetically very different orders, ranging from bacteria to fungi to viruses and protozoa [88]. The interactions between the host and the commensals or mutualists or parasites or even facultative opportunists (switching between commensalism and parasitism) are so well-adjusted as to maintain homeostasis [89]. This balanced ecosystem of different kingdoms (viribiota, mycobiota, etc.), when perturbed, loses harmony leading to dysbiosis and diseases [90]. Thus, the equilibrium (or homeostasis)

of the host with its intestinal microbiome is crucial to health^[91]. When the equilibrium is ruptured, endogenous or exogenous microbes, called pathobionts (temporarily benign microbes with the potential, under modified ecosystem conditions, to become key players in disease), take the upper hand and wreak havoc in the holobiont system^[92].

The fitness of the holobiont, the microbe-host system, relies on a varied set of molecular interactions with our mutualistic partners, ranging from food provision (humans) to useful metabolites (microbiota)^[93]. New concept and approaches of microbial ecology have emerged and are being applied to pathogens and the microbial communities in which they exist (the pathobiome)^[94]. The innate immune system has most likely been the first human physiologic system to be linked with the two-way regulation of and by the gut resident bacteria^[95]. The microbiome has been implicated in the modulation of the gut-brain axis (alteration of behaviour and regulation of central nervous system molecular changes) at a transcriptional level^[96]. Some symbionts can produce neurotransmitters as waste products (short chain fatty acids [SCFA] and γ -aminobutyric acid [GABA]), thereby manipulating the host and leading to what is now known as microbiota-gut-brain axis^[97]. Thus, gut resident microbes can influence indirectly the brain through immunologic and inflammatory activation, and induction of protein aggregation resulting in control over neurodegenerative diseases^[98]. Others produce metabolites such as niacin which reduces inflammation of the intestinal tract by preventing the development of colitis and colitis associated colorectal cancer^[99]. Similarly, amino acid catabolism of tryptophan leads to bioactive molecules, which are endogenous ligands of several receptors regulating immune and inflammatory responses and tight junction resistance and modulating neuroendocrine communication between the digestive and nervous system^[100]. Yet, other symbionts and pathogens alike produce a series of antimicrobial molecules (lantibiotics, bacteriocins and microcins, etc) that aid competition among different species^[93]. Gut microbiota metabolomics and metaproteomics has been associated with systemic effects such as inflammation, immune activation and bacterial or viral infection^[101].

Such incredible presence, almost ubiquitous in the human body, of microbial species has been studied to identify commonalities between subjects. Some researchers have proposed the existence of three distinct enterotypes with unique properties and capabilities, highlighting different metabolic responses to diet or medication to obtain a positive equilibrium (health equal to eubiosis) and avoid a disequilibrium state that fosters disease (dysbiosis)^[84]. This would tentatively explain nutrigenomic and pharmacogenomic differences between individuals as the association between microbiota and host genetic factors being influenced both by diet^[102].

Mobilome

Moreover, certain viruses (as part of the virome), bacteriophages (phages), live inside their bacterial hosts in a single ecological community and influence this ecosystem through microbial gene expression regulation and mobile genetic element processes^[103]. Phages are obligate parasites of bacteria and bacteria have many mechanisms of defence against bacteriophage infection^[104]. Phages are the most abundant biological entities on Earth, with 70% of bacteria being infected^[105]. Being the most abundant

microorganisms in the gut, these prokaryotic viruses, can propagate via lytic (direct killing) or lysogenic infection of bacteria, often with species-level specificity^[106]. Indeed, their abundance is known to be in the order of about 10^{15} phage particles in the human gut contains, suggesting that phages may modulate the gut microbiota^[107]. The phageome, therefore, greatly outnumbers both eukaryotic viruses (that infect human host cells) and human cells and display mostly lysogenic (temperate) behaviour by establishing a long-term association with its host (lysogen)^[105]. Lysogeny occurs through a merger with the host's bacterial genome as quiescent lysogenic 'prophages', which can be propagated vertically during prokaryotic cell division^[88]. During lysogenic conversion, temperate phages modify the genome of bacteria by incorporating in their bacterial genomes, as prophages, leading to lysogenic bacteria^[108]. When prophages are 'induced' (under stress conditions), they reinitiate their lytic cycle and kill bacteria^[90]. Phages, that inhabit mammalian microbiotas, live in each microorganism singularly or with others, can bind to polysialylated eukaryotic glycans and have a strong influence on shaping the microbiota^[109]. Phages, by binding to mucosal glycoproteins with their capsid Ig-like protein domain, may act as non-host derived immunity^[86]. Hence, phage infection can influence bacterial growth, microbial metabolic activity, their pathogenicity, antibiotic resistance and interspecies competition which consequently impact largely unrecognized aspects of mammalian health and disease^[88]. This occurs since bacteriophages engage in the horizontal gene transfer between bacterial populations, transmitting genetic elements for antibiotic resistance and disease pathogenesis (virulence)^[90]. Phages and bacteria coevolve together in an antagonistic race in the gut environment and this pushes the boundaries of diversification and differentiation of both^[91]. The result is a non-static population of bacteria and phages even in a stable environment, due to HGT.

There are other methods used by the microbiota to transmit information interspecies. These are known as mobile genetic elements (MGE) with their relative processes. Some MGEs include conveyance of novel bacterial phenotypes (competition, auxiliary metabolic capabilities and stress tolerance) and modulation of bacterial gene expression, during lysogenic conversion^[105]. Apart from phages, commensal bacteria can acquire genetic information through plasmids (via conjugation), transposons (Tn), integrons, insertion sequences (IS) and integrative and conjugative elements (ICEs), as well as up-take of naked DNA from the environment (natural transformation)^[110]. Transposons, or jumping genes, usually the carriers of additional information, can transfer from a plasmid to other plasmids or from a DNA chromosome to plasmid and vice versa^[112].

The mobilome is therefore defined as the set of all MGE of a cell^[113].

Metagenome

As it has just been stated, MGE constitute a genetic pool called the mobilome, the separation, identification and categorization of which remains a daunting task^[114]. MGE that include plasmids, transposons, integrons and bacteriophages generally associated with prokaryotic cells, also participate in the adaptation of a defined holobiont^[115]. MGE may also be referred to as the mobile metagenome, a reservoir of genetic information involved in key

aspects of community function (the core genome content of the collective microbial species comprising communities) ^[116]. But this mobile metagenome is not relegated only to prokaryotes, it includes also the host's eukaryotes.

Eukaryote HGT events have also been recently acknowledged: transfer of MGE from fungi, bacteria and even from other eukaryotic cells have been recognized ^[117, 118]. These events push our understanding of the interactions of MGE in the holobiont to a whole new level and may well be the cornerstone of future studies on human metagenome. The outstanding studies that have been performed recently confirm that HGT can occur in humans too ^[115]. Indeed, heritable HGT events (transfer of eukaryotic DNA to the human genome with subsequent inheritance by descendants) have been lately described in humans ^[119]. Because of the overwhelming number of bacterial cells in the human holobiont and of their many mechanisms to transfer DNA to the environment and to other organisms, there are still many opportunities and avenues for non-ubiquitous, bacteria to human HGT to occur ^[120]. Viral genomes have also been found in animal genomes. Examples include lentivirus in primates and integration of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes ^[121, 122]. The sequencing of the virome, the foremost gene pool on the planet, has demonstrated that viruses participate in the making of the metagenome as weavers or bulders ^[115, 123]. Viral genomic material can be endogenized in eukaryotic cells and these rearrangements participate in the process of generation of new structures, metabolic ways and gene regulation that result in adaptational changes ^[124]. Therefore, the metagenome seems to be the final result of the physiological and genetic (hologenome) integration of an organism and all of its associated symbiotic microbes, including parasites, mutualists, synergists, and amensalist, whether they are prokaryotes or viruses ^[125, 126]. The mapping the human microbiome is what is called metagenome and requires that not just the characterization of the microbes and their contribution to the biology of the host, but also the identification of their genetic repertoire (e.g., metabolomics and metatranscriptomics of their potential functions and metabolic capacity) ^[33].

Metaorganism

Viruses then should not be considered as always pathogenic since in the light of the holobiont, they share their fate with that of their hosts. The intimate association of the metaorganism with microorganisms often defined as symbiosis (as the sharing of environment and resources, including metabolic products and genes) cannot occur in the absence of viruses ^[127]. Their staggering number is so great to simply overshadow all other life forms we know of, combined, making them the most abundant in all biomes and ecosystems ^[128]. Their biodiversity is equally great as viruses are able to infect host species throughout all major branches of the cellular tree of life, from archaea to bacteria to eukarya, irrespective of their environmental niches ^[129]. Given their extremely variable size, morphologies, and genetic options for inheritance across generations, including adaptation and evolvability, viruses can strongly impact hosts affecting all levels of their biological organization, from host-genome composition to internal ecosystem function ^[130]. Ultimately, the study of viromes, through metagenome analysis, has allowed to understand how viruses demonstrate the ability to manipulate host cell biology through the expression of proteins that modulate

the immune response ^[131]. Viruses now are shown not to be just parasitic, but also to very often display definite commensal and mutualistic behaviour, given their sheer numbers in the holobiont. Viruses have now been recognized as symbiotic members of the host's consortia of microbes, parading any form of relationship with the host on a continuum between antagonism to mutualism depending on environmental changes ^[132]. A famous example is the endogenization of retroviruses in the placenta to produce placenta-specific transcripts (syncytins, domesticated retroviral enveloped proteins) that allow the fetus to survive against the mother's immune system ^[133]. Yet other benefits of virus latent infection are the provision of immunity to infection by bacterial pathogens, and commensal bacteria simulating beneficial function, such as gut architecture and lymphocyte function development ^[134, 135]. The holobiont then results from the staggeringly high and precise symbiotic interactions between the microbial associates, including viruses, and a host, which can impact important host traits ^[136].

Some such host traits comprise the neuro-immune system and all the functions defined and regulated by the brain-gut axis, in which intestinal microbiome plays a key role ^[137].

ABO Constitutions

The concept of ABO constitutions is the result of the difference between people of ABO group determinants, as HBGAs, which are essentially glycans ^[138]. The ABO blood group glycans star all glycoconjugates on and inside the cell membrane, not just on red blood cells but also on plasma proteins and on epithelial cells (alloantigens) ^[139]. But it was first discovered in red blood cells, thus the name of blood groups. ABO glycotopes are expressed on glycolipids, glycoproteins of mucins of the GI tract and on a variety of other human cells and tissues and in various body fluids and secretions ^[140].

The ABO gene, located on chromosome 9, encoding two glycosyltransferases (GT), confers to humans 4 blood groups: O (or H), A, B and AB ^[139, 141]. Hence, the ABH antigens are not primary gene products but they are the enzymatic reaction products of GT enzymes.

These different glycotopes may well be the likely cause of proven associations between ABO blood group and various types of disease from neoplastic to cardiovascular disorders ^[140, 142]. Even stronger seems to be the influence of the ABO blood group on the severity of several microbial and viral infections ^[4, 143, 144]. Thus, ABO constitutions are linked at least to susceptibility to disease as dictated by all ancient traditions which link constitutions to susceptibility.

More importantly, the ABO and Lewis (HBGA) epitopes expressed in the GI tract seem to actually shape the composition of gut microbiota ^[145]. Since ABO glycans are expressed on mucosal surfaces of intestinal epithelial cells (IECs), they are potential receptors for non-pathogenic and pathogenic microorganisms influencing immune and many other metabolic responses ^[146]. Virus also can differentially recognize HBGA displayed on IECs and on mucins in Secretor individuals ^[147]. Hence, phages bound to mucin glycoproteins could protect the epithelium against pathogenic bacteria invasion ^[90]. As certain viruses can bind differentially to HBGA, so can bacteriophages recognize specific mucin glycoproteins and bind to them with their capsid proteins, thereby influencing bacteria composition and regulate innate and acquired immunity ^[148]. Moreover, due to

narrow host specificity of phages, it has been noticed that just a few phages are common among individuals with the vast majority being subject specific [104]. This feature fosters the idea of genetic individualization of the phageome.

There are many other functions of the GI microbiome from the availability of carbohydrate-active enzymes (CAZymes) to the production of SCFAs [1, 89, 149]. Let's not linger on these extraordinary functions and move to the other type of constitutions

WCM Constitutions

The metaorganism is a complex system of intertwined relationships between all eukaryotic host cells and the microbiome (including bacteria, viruses, and other small eukaryotes), possibly centered around the mucus layer of our GI tract [86]. Hippocrates, already about 2500 years ago, noticed the importance of the gut when he allegedly said: ‘All disease begin in the gut’ [150]. Were ancient medicines on to something already thousands of years ago? Possibly so as only recently modern biomedicine has started to view the gut as a fundamental place of interactions between health and disease [151]. Not just in the West but also in the East was this information already present and circulated steadily among physicians of old. Indeed, TCAMs like Traditional Chinese Medicine (TCM) already knew the importance of digestive system thousands of years ago since the production of “Qi”, which is vital energy for whole body, largely rely on abdominal condition [152].

Hippocrates devised a system that would enable physicians to recognize humanity as divided into four types, associated with the four humours or elements: fire, earth, water and air. It was through the knowledge of human biology that Hippocrates by means of a process of scientific methodology (observation and experimentation) arrived at formulation of the four humoral theory as the congenital constituents of the human body (diathesis) [153]. Diathesis is therefore the endogenous chemical or biochemical individuality of each person that makes them unique. Each person has a unique combination of the four humours but one predominates over the others so that person can be grouped under the predominating humor, either bile (yellow, earth), blood (red, fire), black bile (water) or phlegm (blue, air). Each constitution of ‘idiosyncrasia’ defines, therefore, a grouping of the person into one of the four groups which provides the information that determines not just the human body’s composition, but also disease predisposition, and drug response [154]. Hence, each person, as for the ABO grouping, can be classified in one of the four biotypes (fire, earth, water and air) defined by Hippocrates. The classification can be seen in Table 1.

Table 1: The Four Humors

Qualities	Wet	Dry
Cold	Water	Earth
Hot	Air	Fire

In this classification (Table 1), the traditional humoral theory of ancient medicine as exposed by St. Hildegard or Bingen will be adopted. According to Hildegard, the humors were the traditional four fluids of blood, phlegm, bile, and melancholia and each person had a characteristic complexion or temperament which was determined by the humors [155]. Moreover, the humoral theory is the foundation of the temperamental theory in

psychology. According to Galen, the greatest Roman physician of all time who has adopted the humoral theory, each individual also has a temperament (a psychological character dependent on the four humours [156]. Each temperament—sanguine, phlegmatic, melancholic, and choleric—is the result of the prevalence of one of the four humors and then acquired more anatomical (Morgagni), fundamental organic (diathesis, e.g., Hutchinson) and constitutional (De Giovanni) and/or psychological (Kretschmer) connotations [157].

Even in the twentieth century, the concept of constitutions is fundamental in sports science. Anthropometric measurements are key to this classification called somatotyping. The somatotype is the expression of a genetic determinism as measured from a morpho-constitutional point of view and can be rated by assigning a three-numeral rating representing endomorphy, mesomorphy and ectomorphy [158]. Modern somatotyping is the result of a modification actuated by Heath and Carter of a unique method for the classification of human physique invented by Sheldon back in the 1950s [159]. Although the Sheldon somatotypes are based on three biotypes while the Hildegardian humoral biotypes are four, it would be easy to reconcile the four into three classification, as shown elsewhere [160].

The humoral theory of Hippocrates is now returning to the spotlight at the start of the twenty first century after having dominated the medical practice for over two millennia until the middle of the 19th century. This resurgence of the humoral theory occurred in order to accommodate the way health and disease is contemplated in the post-genomic era: the humoral theory views on heredity and homeostasis are in line with modern medicine [161]. Nevertheless, since this system of medicine (the use of humors or elements as in the Chinese or Indian tradition) is shared among all ancient traditions, it shall be used also in our system too. Though in novel ways.

The ABO WCM System

The two identified typologies are not mutually exclusive. It has been recently shown that an appropriate matrix can be created by the [160]. In sum, the two biotypologies, each consisting in the division of individuals into four distinct groups, will result into (four times two) eight distinct ABO-WCM biotypes. This is shown in Table 2.

Table 2: Classification according to the ABO-WCM System

ABO/WCM	Fire (F)	Earth (E)	Water (W)	Air (A)
A	FFA + FMA	EFA + EMA	WFA + WMA	AFA + AMA
B	FFB + FMB	EFB + EMB	WFB + WMB	AFB + AMB
AB (used C)	FFH + FMH	EFH + EMH	WFH + WMH	AFH + AMH
O	FFO + FMO	EFO + EMO	WFO + WMO	AFO + AMO

In Table 2, 8 basic biotypes are obtained. In each of the eight cells two forms (male and female) are defined. The standard way to read the ABO-WCM system is the following: the first letter represents the WCM humoral biotype (the first row of the table), the second letter represents the sex of the individual (male or female) and the last letter represents the ABO characteristics (where AB is the only one that does not follow the ordinary nomenclature as the letter C is used).

In this way a total of 16 individuals have been characterized.

This formulation of the system allows to take advantage of both the constitutional insights and dietary recommendations of the two form of medicines together. An example is shown in Table 3.

Table 3: Some Characteristics of Individuals AMA and FFO

Characteristics	Source	FFA	AMO
Biological strength	WCM	Strong	Weak
Muscular structure	WCM	Well-built (mesomorph)	Thin (ectomorph)
Metabolism	ABO	Slow	Fast
Predisposition (sports)	ABO	Hard training	Light training
Diet type ¹	WCM	Moist regimen	Dry regimen
Diet type ²	ABO	Mainly vegetarian	Mostly carnivore
Psychological trait	ABO	Tends to be relaxed	Tends to be active
Psychological trait	WCM	Action driven (somatonic)	Over-thinker (cerebrotonic)

This example is not meant to be exhaustive but just typifying.

1 = Following Galen's concept of temperaments, all foodstuff can be reduced to the four basic qualities [156].

2 = Similarly, BTD suggest a particular diet where foodstuff glycans are aligned with each body's own ABO blood typology [162, 163].

The example of Table 3 shows a comparison between two individuals belonging to different biotypes. The first FFA is a female fire biotype with a blood type while the second, AMO, is a male air biotype with an O blood type. According to the respective theories, the fire biotype is considered physically stronger than an air biotype (fire is considered as an athletic body type [mesomorph], while air is more an intellectual, smaller muscle mass [ectomorph]) [164]. To clarify, an air biotype (as for a water biotype [endomorphs]) would never be able to compete successfully in any kind of sports, given their low resistance and physical structural weakness. On the contrary, a fire (athlete) and earth (strong and large body structure like that of a body builder) biotype are naturally shaped (as mesomorphs) to be able to compete in athletic (fire) and strength (earth) sports. Temperamental aspects of each biotype are also characteristic of their particular constitution: as an air biotype (cerebrotonic) is more concerned in intellectual activities, while a fire (or earth) biotype are more somatonic. having complex traits associated with functional and anatomical predominance [156, 165].

But this is only half of the coin, the other half is the ABO constitution. The A blood type is more relaxed and doesn't withstand stressed conditions very well. Moreover, diet-wise the A blood type is more pescatarian than carnivore though would eat without a problem chicken, eggs and turkey. On the contrary, the type O is more carnivore and would do great with all types of meat, most fish and eggs and is capable of withstanding stress easily [162, 163]. The psychological features of the ABO groupings have been amply studied in Japan, where it is also embedded in the social system [166, 167].

These are a just few hints of what can be derived simply by classifying these individuals, actually each person, into these biologically and endogenously distinct groups. Hence, it can be seen that they are constitutional (based on their biochemical [internal] and physical [external, or phenotypical] structure). Of course, each individual then is also unique and other factors should be taken into consideration when holistically studying or

analysing a person. But, this classification system (the ABO-WCM system) can give us a lot of prima facie impressions of their internal genetic and biochemical make-up.

Conclusions

In conclusion, microbes are found all over the human body and they have a direct impact on the immune system, metabolism and homeostasis [7]. As a matter of fact, they may well be involved in almost any physiologic and metabolic activity of the human body [10]. The holobiont (theory of life, which is a fact and not a theory anymore) is the basis to understand the two systems of classification of human beings: the ABO and WCM biotypologies.

A first theoretical framework of BTD was proposed taking into account the beneficial or detrimental effects of glycans in foods, depending on one's ABO type [6]. The ABO blood group differentiates biochemically each person and imposes biological characteristics that have been studied for a long time in many medical fields, at least since its discovery in 1902. The resulting ABO constitutions is thus the first piece of the puzzle [168].

The other piece is the WCM constitutions. These constitutions are defined by the predominance of one of the four elements/humors that form each body, according to the Galenic tradition, which was derived from Hippocrates [156].

The Hippocratic concept of endogenously determined constitution (diathesis), as a form of disease-linked individuality represents a central tenet of modern molecular medicine [153], effectively merging old (WCM) and new (ABO) concepts and bringing them to the fore.

Both systems recommend that diet be the main intervention to rebalance the disequilibrium within the holobiont (to obtain eubiosis from dysbiosis).

But, diet should be based on the characteristics of the patient as identified by the doctors practising the TCAM and based on their knowledge of the constitutions of each individual [152]. All the ancient traditions are in agreement that disease is caused by a disequilibrium of the constitutive elements of the human body. In the western tradition, the infirmity is caused by the humors and their imbalance with respect to the body (the proper constitution of each person) which should be remediated by restoring a balance among the four humors and qualities [155].

The loss of the concept of constitutions occurring by the middle of the twentieth century was not due to its alleged unscientific foundations but to the general nature of modern medicine. Indeed, its decline in the face of a new medicine was due to the division of medicine into specialities: each discipline was by now so specific (with its own concepts, knowledge and methods) that it was technically impossible to support a holistic approach [154]. With this novel system that merges together the ancient western tradition (WCM) with the modern view of the holobiont (under the phenotypical manifestation of the ABO blood groups) it is possible to view humanity in a novel way. This new system avails itself of the great successes of both viewpoints and promises to completely transform modern science into a truly personalized approach.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

Not applicable. All materials are publicly available at the referenced online databases.

Competing interests

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